Aquaculture 505 (2019) 263–270

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Methods to prevent and treat biofouling in shellfish aquaculture

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ARTICLE INFO

Keywords:
Biofouling
Shellfish
Acetic acid
Citric acid
Treatment
Aquaculture

ABSTRACT

Fouling organisms in bivalve aquaculture cause significant economic losses for the industry. Husbandry strategies to reduce biofouling can involve avoidance, prevention, and treatment. In this way, the type of rope used to collect spat or grow bivalves may prevent or reduce fouling by particularly harmful species but remains largely untested. Further, while a range of eco-friendly control methods exist, their effect on widespread, common biofoulers is poorly known. We tested biofouling accumulation and spat collection for seven commercially used ropes, and evaluated treatments of ambient and heated seawater, acetic and citric acid, and combinations of both applied across a range of exposure times to two commercially grown shellfish (*Mytilus galloprovincialis* and *Ostrea angasi*) and three biofouling species (*Ectopleura crocea*, *Ciona intestinalis* and *Styela clava*). Rope types differed significantly in terms of fouling rates and spat collection, with specific rope types clearly advantageous, despite not being used commercially in our study area. Treatments proved variably successful, with *E. crocea* highly susceptible to all treatments, *Ciona intestinalis* moderately susceptible, and *Styela clava* relatively resistant. Excluding *S. clava*, efficacious treatments were attainable that did not adversely affect shellfish. Combining heat and acid treatments were more successful than individual treatments and provide a useful avenue for further trials. This study provides baseline evidence for treatment efficacy that will tailor longer-term, field trials to validate and streamline biofouling treatments in shellfish aquaculture.

1. Introduction

Biofouling poses a significant problem for the shellfish aquaculture industry; an estimated 14.7% of annual operating costs is spent on biofouling control (Adams et al., 2011; Lacoste and Gaertner-Mazouni, 2015). Biofouling can also affect spat collection, reduce stock growth and saleability, further affecting farm productivity and profitability (Daigle and Herbinger, 2009; Sievers et al., 2013; Lacoste et al., 2014). These impacts drive persistent, resource intensive biofouling control efforts across the industry. Husbandry strategies to reduce the impact of biofouling fall under three broad categories: avoidance, prevention and treatment (Fitridge et al., 2012; Sievers et al., 2014; Sievers et al., 2017).

The most effective biofouling management strategies will incorporate all three. Even if highly successful treatments can be designed, costs are still incurred, and the high cost of fouling removal means that biofouling is often allowed to develop to damaging levels before farmers remove it. Further, most treatments that have been tested thus far show some harm to cultured stock, and many treatments offer little benefit to stock survival, flesh weights or growth rates (Sievers et al., 2013). Therefore, preventative measures are becoming an important area for new research (as in preventing parasites in fin fish aquaculture; Bui et al., 2019). For example, the type and colour of culture or spat-collecting rope may influence the severity and species composition of biofouling communities, allowing strategic use, particularly when tailored in concert with knowledge of spatial and temporal fouling patterns (Sievers et al., 2014).

Although useful to reduce fouling or avoid particularly harmful species, some biofouling will inevitably develop. On-site treatment methods which remove biofouling effectively, cheaply, easily and with minimal environmental impact are, thus, essential. Several such treatment methods have been tested experimentally, including exposure to air (Darbyson et al., 2009; Hillock and Costello, 2014), freshwater (Forrest and Blakemore, 2006b; Forrest and Blakemore, 2006a; Denny, 2008a; Denny, 2008b), heat (Carver et al., 2003; Guenther et al., 2011), and organic acids and bases (Forrest, 2007; LeBlanc et al., 2007; Piola et al., 2010; Rolheiser et al., 2012). In addition, novel antifouling compounds are being tested against shellfish biofoulers (Cahill et al.,

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https://doi.org/10.1016/j.aquaculture.2019.02.071
Received 2 January 2019; Received in revised form 31 January 2019; Accepted 26 February 2019
Available online 27 February 2019
0044-8486/

2013a; Cahill et al., 2013b). Many are recommended for use in the aquaculture industry (Fitridge et al., 2012; NSPMMPI, 2013), but industrial application has been met with varying success because the morphology, life history and biomass of biofouling organisms may mean that they react differently when exposed to the same treatment (LeBlanc et al., 2007; Piola et al., 2008; Sievers et al., 2017), and because treatment costs are often greater than profit gains.

Although the effectiveness of some sequential treatments have been explored (e.g. freshwater immersion for at least 2 h followed by exposure to air for at least 12 h; Gunthorpe et al., 2001), empirical testing of combined treatments (i.e. applied simultaneously) and their effect on fouling and culture organisms remains largely unaddressed. Combining treatments may cause them to act synergistically (Shin et al., 2006), thus, this approach has particular appeal as (1) it may be more effective against a broader range of taxa, (2) the effective concentrations and temperatures of treatments are likely to be lower, and (3) the effective exposure times are likely to be shorter. Parallels exist in other industries, such as the food industry, where bacteria such as *Escherichia coli*, *Listeria monocytogenes* and *Salmonella typhimurium* are controlled by combined treatments of acetic acid and heat (Shin et al., 2006), and in industrial settings, where the growth of the hydroid *Cordylophora caspia* is controlled in cooling water intakes via combined heat and chlorine treatments (Rajagopal et al., 2002).

We examined biofouling accumulation and spat (*Mytilus galloprovincialis*) collection for seven commercially available mussel ropes. We also tested the effectiveness of heat, acid and a combination of heat and acid against the Australian blue mussel *Mytilus galloprovincialis*, the commercially grown Australian flat oyster *Ostrea angasi*, and three common fouling species that widely affect mussel culture operations worldwide; the solitary tunicates *Ciona intestinalis* and *Styela clava*, and the hydroid *Ectopleura crocea*. We tested treatments against mussels and oysters because when treating for fouling, the culture species inadvertently get treated too.

2. Methods

2.1. Rope type

Several different rope types available in straight filament or looped filament designs that are currently in use across the industry. Seven industry-approved mussel ropes were obtained from three commercial rope manufacturers: 'Extreme Catch and Hold', 'Cut Loop' (both from Quality Equipment Ltd., NZ), 'Hatchery Rope' (black spat), 'Aqualoop', 'Christmas Tree' and 'Super Christmas Tree' (Donaghys Pty Ltd., NZ) and 'Spat Rope' (green spat; Whittam Ropes Pty Ltd., Australia; Supplementary Fig. 1).

Ropes were cut into 20 cm lengths, with 18 replicates per rope type. The ropes were randomly arranged and fixed within six purpose-built PVC frames, and were deployed in December (early summer) at two similar commercial mussel leases (Kirk Point – Werribee: KPW; and Clifton Springs; CS) for 8 w in Port Phillip Bay, Australia. For information and location of sites, see Sievers et al. (2014). Ropes within frames were held vertical at a depth of 5 m simulating commercial mussel lines. Upon retrieval, each rope sample was weighed, and the weight of a clean, wet weight rope was subtracted to provide a total wet fouling biomass. Ropes were stripped of all fouling and the fouling sorted to the lowest possible taxonomic level. Wet and dry fouling weights were attained for each taxonomic group, or number of individuals was counted. Dry weights were calculated following drying at 60 °C for 48 h. The stripped ropes were then processed for spat retention by soaking in a 10% solution of chlorinated bleach for two minutes (to dissolve organic material and facilitate mussel detachment) and shaking vigorously to remove the spat. The solution was then rinsed with running water through a 500 and 100 μm sieve stack to divide the mussel spat from other fouling and detritus. The age or size of mussels at primary and secondary settlement is not standardised (Alfaro and Jeffs, 2002), but collecting spat in the size class of > 100 to < 500 μm makes it more probable that only those mussels which are undergoing either settlement stage are sampled. The contents of the 100 μm sieve were then washed into a Bogorov counting chamber and the total number of mussel spat counted.

2.2. Treatments

All organisms were locally sourced from farms or piers and maintained in individual 2 L flow-through (1 L/min) beakers for 24 h at approximately 20 °C and 34 ppt (water in continuously pumped into the lab from Port Phillip Bay, Australia where individuals were collected). Treatments were conducted in 1 L beakers containing either ambient freshwater, ambient seawater, seawater heated to the desired temperature, acid solution made up to the desired concentration in seawater at ambient temperature, or a heated acid solution. To undergo treatment, organisms (n range: 3–11; Supplementary Table 1) were removed individually from their original beaker and dipped into the treatment beaker for the desired time using a hand net. Immediately following treatment, shellfish and fouling species were immersed in a bowl of fresh seawater (same as flow-through water) to rinse, returned to their original beakers and allowed to recover for 48 h. After the recovery period, survival was assessed under a dissecting microscope. Alive individuals were those observed to be siphoning water, able to close their values, responded to touch and/or were not mouldy. Any assessed as dead were kept for a further 48 h to confirm death. We found no individuals that changed status after this time.

2.2.1. Organic acids

Individuals (for *E. crocea* individuals consisted of colonies of 10–20 heads) were subjected to 10, 30 and/or 60 s dips in dilute acid solutions: 99.85% glacial acetic acid (Merck Australia Pty Ltd) diluted with seawater to 2% and 5%, and 99.5% anhydrous citric acid (Ward McKenzie Pty Ltd) diluted to 2%, 5% and 10%.

2.2.2. Heat

Organisms were subjected individually to 10, 30 and/or 60 s dips in seawater heated to 40, 50 or 60 °C.

2.2.3. Treatment combinations

Organisms were subjected individually to 10, 30 and/or 60 s dips in acetic acid solutions (2%, 5%) and citric acid solutions (2%, 5%, 10%) heated to 40 °C or 50 °C. Acid solutions were prepared as above.

2.3. Statistical analysis

For all analyses SYSTAT v 13.0 was used, and normality and homogeneity of variance were examined using Shapiro-Wilk tests and visualisation of residual plots, respectively. The effect of rope type on fouling development and mussel spat collection was analysed using two-way ANOVAs with location and rope type fixed factors. Total fouling weight and spat collected were fourth root transformed, *E. georgiana* and *S. taeniata* data were cube root transformed, and *E. crocea* and *C. intestinalis* data were square root transformed (*C. intestinalis* only settled in, and was analysed for, one location using a one-way ANOVA). The efficacy of treatments on fouling and culture species was determined using Fisher's exact tests comparing each treatment-duration combination to the appropriate control group (ambient temperature seawater for 10, 30 or 60 s dips). Replication for different species differed due to logistical constraints (see Supplementary Table 1).

3. Results

3.1. Rope type

Fouling accumulation and spat collection varied among the seven

Table 1
Results of a two-way ANOVA comparing the effects of site (Clifton Springs or Kirk Point) and rope type on total fouling accumulation, mussel spat collection, *Ectopleura crocea* weight, *Spirobranchus taeniata* weight and *Electroma georgiana* numbers, and one-way ANOVA for *Ciona intestinalis* numbers. Only Clifton Springs was analysed for *C. intestinalis*. All factors were treated as fixed. Boldface *p*-values are significant at < 0.05. Error MS is listed underneath the *p*-values.

Factor	df	Total fouling***		*Mytilus* spat***		*Ectopleura**		*Spirobranchus***		*Electroma***		*Ciona**	
		F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Site	1	1753.93	< **0.001**	3.67	0.058	621.73	< **0.001**	2.51	0.116	554.10	< **0.001**		
Rope	6	101.32	< **0.001**	9.77	< **0.001**	0.41	0.870	4.99	< **0.001**	1.61	0.150	5.57	< **0.001**
Site*Rope	6	7.03	< **0.001**	2.45	**0.029**	1.69	0.129	1.90	0.087	0.72	0.638		
Error MS	112		0.029		0.131		0.734		0.093		0.774	Error df = 56	1.366

* Square root transformed.
** Cubed root transformed.
*** Fourth root transformed.

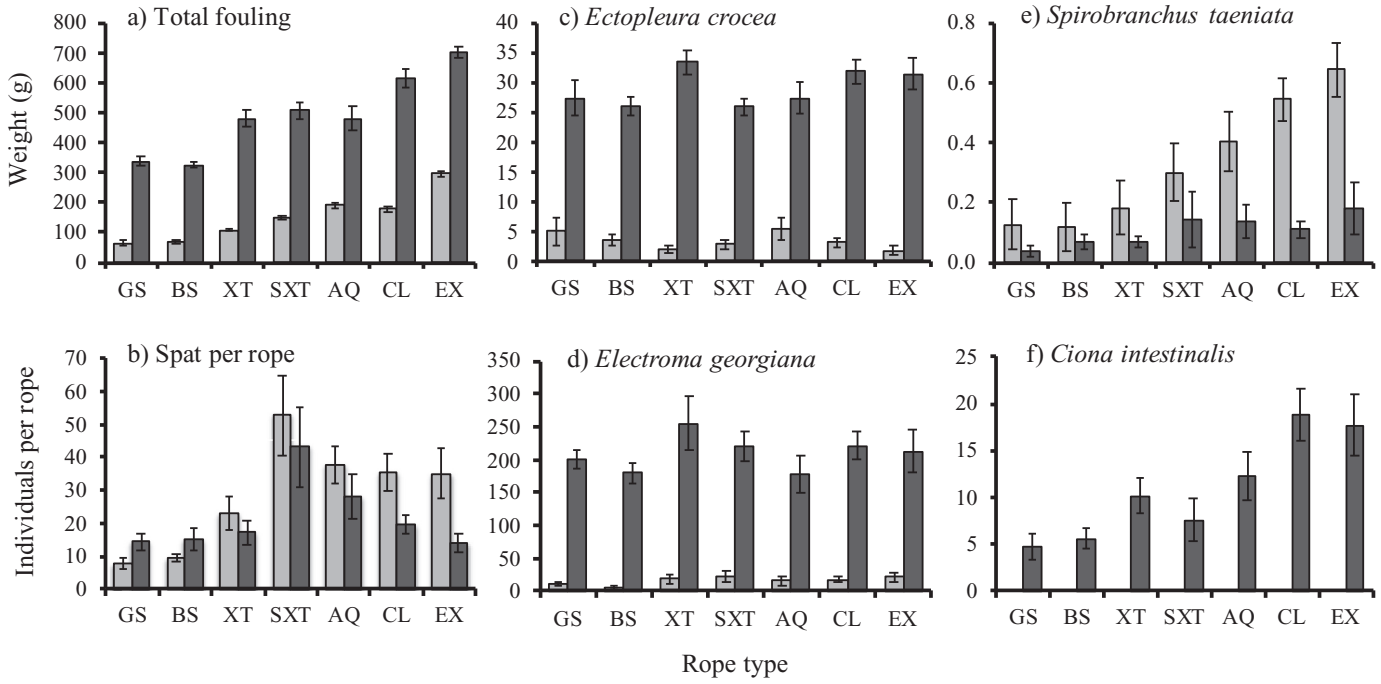

Fig. 1. Wet weight of total fouling accumulated (a), number of spat collected (b), dry weight of *Ectopleura crocea* (c), number of *Electroma georgiana* (d), dry weight of *Spirobranchus taeniata* (e), and number of *Ciona intestinalis* (e) per 30 cm rope section for the seven rope types. GS: green spat; BS: black spat; XT: christmas tree; EX: extreme catch and hold; CL: cut loop; AQ: Aqualoop; SXT: super christmas tree. Light grey bars: Kirk Point/Werribee; Dark grey bars: Clifton Springs. Bars represent mean ± standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rope types tested and between the two locations, with significant interactions between the factors (Table 1). The black and green spat ropes proved most successful against fouling accumulation, followed by the Christmas Tree, Super Christmas Tree, Aqualoop, Cut Loop and Extreme Catch and Hold (Fig. 1). However, in terms of mussel larvae retention, the black and green spat ropes performed relatively poorly, while the best rope was Super Christmas Tree, which attracted almost five times more larval recruitment than the black spat rope (the rope currently used by industry; Fig. 1). Variability among rope types was similar between sites, with fouling loads significantly higher at CS and spat collection typically higher at KPW, although this latter difference was less substantial (Fig. 1). In terms of individual fouling species, *E. crocea* and *E. georgiana* were far more abundant at CS but showed no preference for any of the ropes (Fig. 1, Table 1). The number of *S. taeniata* varied among rope types, matching the pattern observed for total fouling weight (Fig. 1, Table 1). *C. intestinalis* was only present at CS and varied in number among rope types, with green and black spat ropes experiencing relatively low settlement, and Cut Loop and Extreme Catch and Hold the highest (Fig. 1, Table 1).

3.2. Treatments

3.2.1. E. crocea

All treatments were highly successful against *E. crocea* causing 100% mortality, with the exception of a 10 s immersion in ambient 2% citric acid which caused only 60% mortality (Fig. 2).

3.2.2. C. intestinalis

Immersion in 40 °C seawater for 10 or 30 s induced some mortality (66%), but a longer exposure to this temperature for 60 s, or temperatures of 50 °C and higher for any length of time, caused 100% mortality (Fig. 2). Acetic acid was very effective against *C. intestinalis*; besides ambient 2% acetic acid for 10 or 30 s (66% mortality), complete mortality in *C. intestinalis* was observed following all acetic acid treatments. Immersions in 2% citric acid for 10 s at ambient temperature resulted in no mortality, with incremental mortality rates as temperature increased (66% at 40 °C and 100% at 50 °C). A 10 s 5% citric acid immersion resulted in 33% mortality at ambient temperature, and 100% mortality at 40 and 50 °C (Fig. 2).

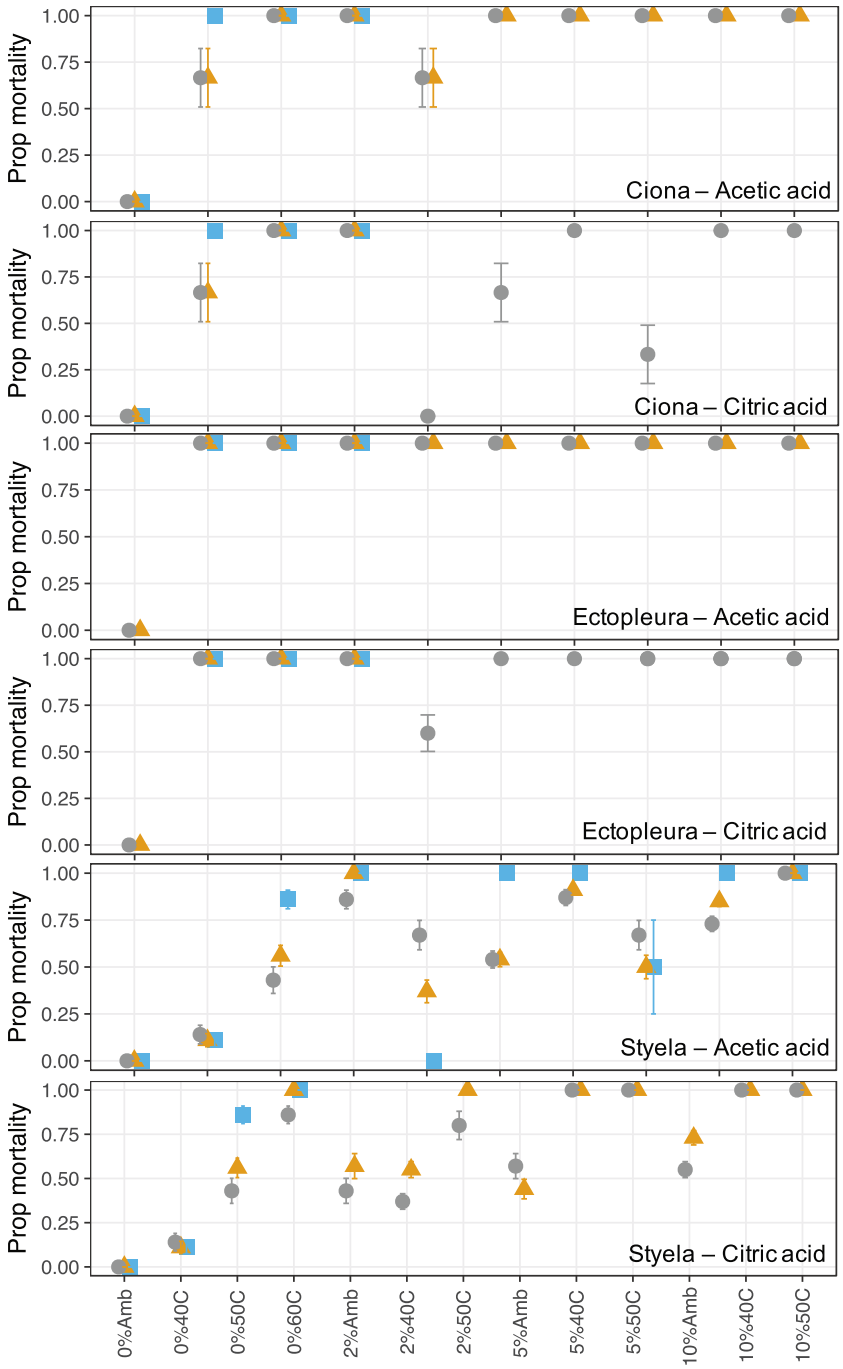

Fig. 2. Percentage mortality of *Ciona intestinalis, Ectopleura crocea* and *Styela clava* when exposed to ambient seawater, heated seawater, acetic acid, citric acid and acid-heat combinations. Dip duration: 10 s = grey circles, 30 s = orange triangles, and 60 s = blue squares. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2.3. *S. clava*

Low mortality was recorded for all 40 °C seawater treatments (~12%). As temperature increased so did mortality, with 50 °C causing 40, 70 and 86% mortality after 10, 30 and 60 s, respectively. Similarly, 60 °C resulted in 86, 100 and 100% mortality after 10, 30 and 60 s, respectively (Fig. 2). At ambient temperature, 2 and 5% acetic acid solutions killed approximately half of the tunicates. Immersions in 2% acetic acid heated to 40 °C for 10 and 30 s were effective at killing 54% while a 60 s dip killed all tunicates. A similar pattern was observed for 5% acetic acid at 40 °C; nearly 100% mortality was observed in the 50 °C treatments regardless of duration or acetic acid concentration. Like acetic acid, under ambient temperatures, citric acid killed approximately half of the tunicates regardless of duration or concentration. Again, as temperature increased so did mortality, with all 40 and 50 °C treatments at 5 and 10% citric acid achieving 100% mortality (Fig. 2).

3.2.4. *M. galloprovincialis (30 mm)*

Treatments at 40 °C for 10, 30 or 60 s, and at 50 °C for 10 s showed little to no mortality of small mussels (Fig. 3). Treatment for 30 and 60 s at 50 °C and any length of time at 60 °C resulted in 100% mortality. The only significant mortality in the acetic acid treatments occurred when temperatures reached 50 °C, with slightly higher mortality at the higher acid concentration. Results from the citric acid trials suggest, similarly

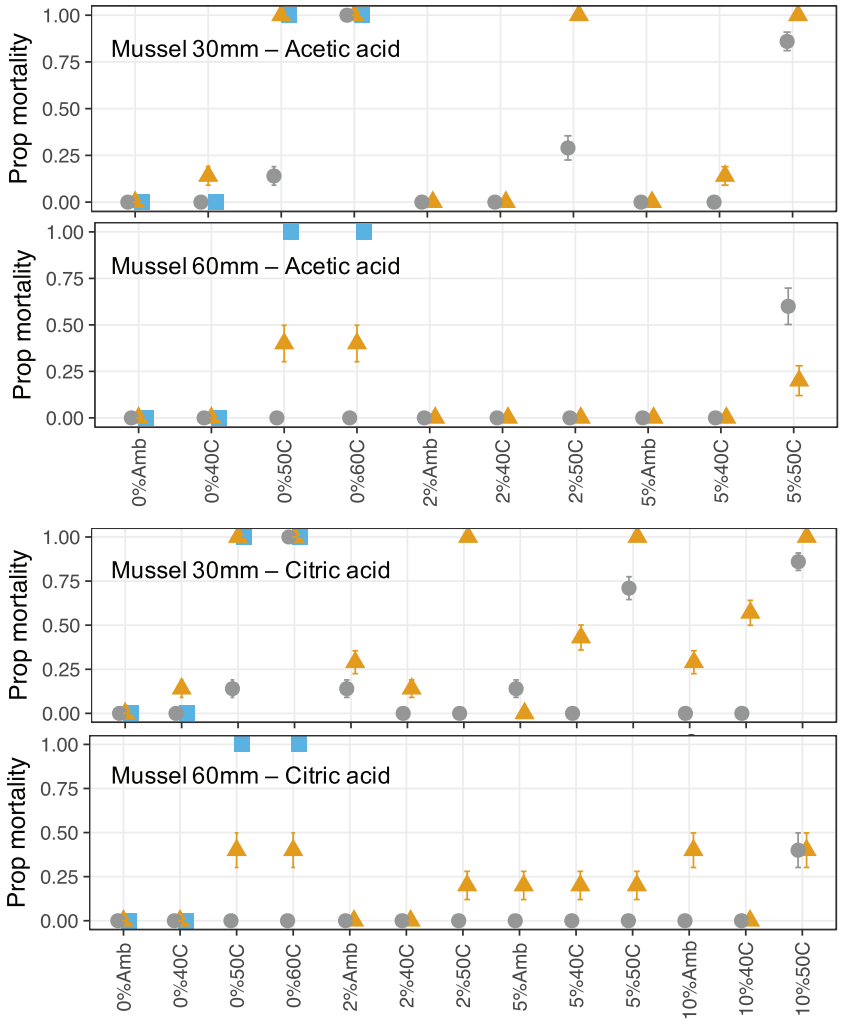

Fig. 3. Percentage mortality of 30 mm and 60 mm mussels (*Mytilus galloprovincialis)* when exposed to ambient seawater, heated seawater, acetic acid, citric acid and acid-heat combinations. Dip duration: 10 s = grey circles, 30 s = orange triangles, and 60 s = blue squares. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to acetic acid, that only at higher temperatures does mortality significantly increase. At 40 °C, 10 s dips did not kill any mussels, but 30 s dips resulted in some mortality, with few differences among the citric acid concentrations used. Notably, low mussel mortality was observed after a 10 s immersion under ambient conditions at 2 and 5%, but not at 10% (Fig. 3).

3.2.5. M. galloprovincialis (60 mm)

Larger mussels were unaffected by exposure to 40 °C seawater for any length of time, but showed some mortality at 30 s and complete mortality at 60 s for both 50 and 60 °C treatments (Fig. 3). Acetic acid induced mussel mortality at 50 °C. Citric acid appeared to have some impacts, with 20–40% mortality occurring when exposure time was 30 s or higher.

O. angasi (15 mm).

Heated treatments only induced oyster mortality at 50 and 60 °C; 100% mortality occurred at both temperatures following a 30 or 60 s immersion, while a 10 s dip killed 40% of oysters for the 60 °C treatment (Fig. 4). Acetic acid caused mortality at 50 °C regardless of concentration. Citric acid was by far most detrimental at 50 °C, with 100% mortality at this temperature for 30 s regardless of concentration (Fig. 4).

3.2.6. O. angasi (50 mm)

Similar results were found for small and large *O. angasi*; 50 and 60 °C for 30 s or longer killed all oysters (Fig. 4). Only 50 °C for 30 s resulted in 100% mortality for the acetic acid treatments, with all other treatments causing no mortality. Similarly, total mortality was only found for 50 °C for 30 s for all citric acid treatments. Some oyster mortality was observed under ambient conditions following a short 10 s immersion in 10% citric acid, but not at this concentration for longer immersions or at 40 °C (Fig. 4). For output of all Fisher's tests and sample sizes for all species, see Supplementary Table 1.

4. Discussion

A multitude of factors effect mussel larval settlement and post-mortality (see Brenner and Buck, 2010), and surface area and structure influence the success of spat collector ropes (Walter and Liebezeit, 2003; Filgueira et al., 2007). Increased surface area reduces competition for space and food, and increased structural complexity provides mussel spat with greater protection from predators (Walters and Wethey, 1996). Rope material likely also influences the strength of byssal thread attachment and, thus, the proportion of settled spat reaching first harvest (Lekang et al., 2003). In agreement, ropes with greater complexity and surface area collected more spat in our study. However, these ropes were also the most heavily fouled, likely due to

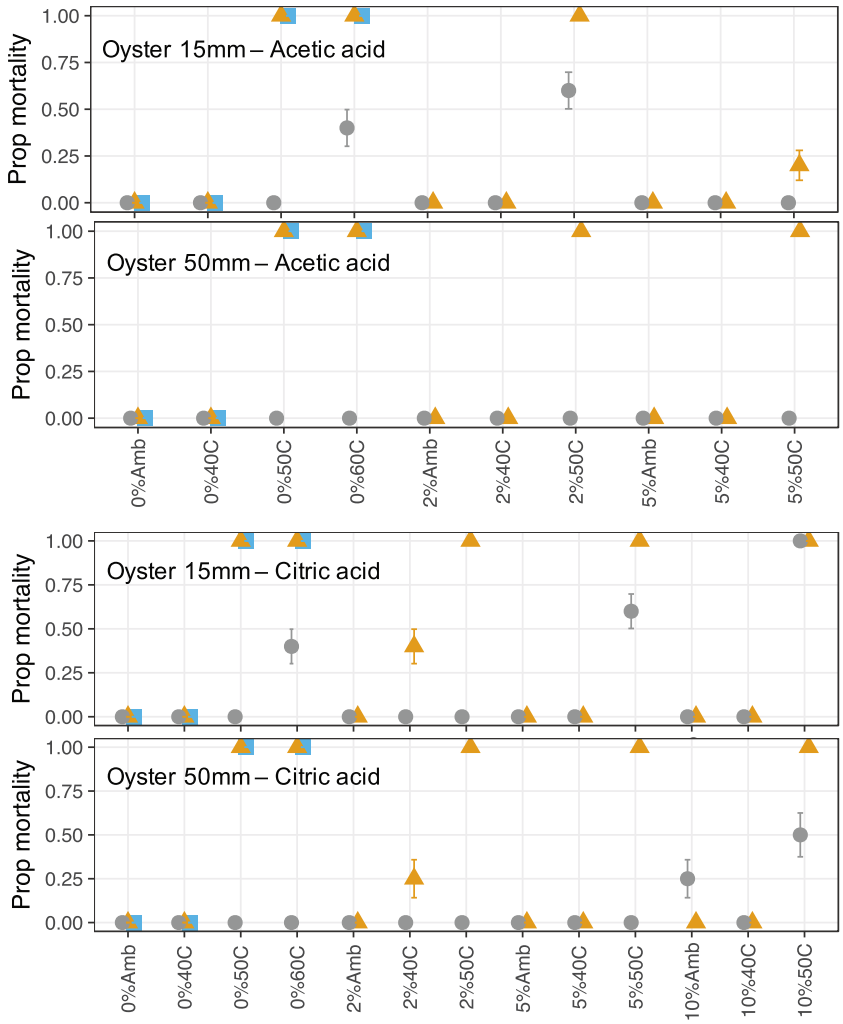

Fig. 4. Percentage mortality of 15 mm and 50 mm oysters (*Ostrea angasi)* when exposed to ambient seawater, heated seawater, acetic acid, citric acid and acid-heat combinations. Dip duration: 10 s = grey circles, 30 s = orange triangles, and 60 s = blue squares. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the same physicochemical properties that made the ropes more efficient spat collectors. Differences in fouling loads were largely driven by *S. taeniata* and *C. intestinalis*, two common species capable of affecting farm productivity (Cyr et al., 2007; Sievers et al., 2013; Sievers et al., 2014). Finally, considerable differences in fouling existed between the two sites, suggesting that monitoring of fouling can be a useful in the toolbox against biofouling (Sievers et al., 2014). We have no evidence why fouling loads differed between sites; both are similar depth, farmed in the same way and in similar locations within the bay (Sievers et al., 2014).

Despite the low level of fouling that accrued on the currently used black spat ropes, switching to Aqualoop or Super Christmas Tree would increase spat collection by 3–5 times, with less than a 2-fold increase in biofouling biomass within our study region. These results suggest that preventative measures can be useful in the toolbox against biofouling for shellfish farmers, but given spat collection also declined, a holistic approach to biofouling management is needed.

Typical biofouling removal protocols (e.g. air-drying or fresh-water baths), while at times effective at biofouling mitigation, are time consuming and labour-intensive. We found that short-term heat exposure deleteriously affected each of the tested fouling organisms. Even a relatively low temperature of 40 °C was enough to cause mortality in the hydroid *Ectopleura crocea*. Hydroid colonies in general are particularly susceptible to heat, with thermal shock treatment causing mortality or seriously affecting normal growth and development (Kroiher et al., 1992; Schmich et al., 2007). Exposure to 40 °C also killed high proportions of the ascidian *C. intestinalis*, with essentially no effect on oysters or mussels. Heat treatment may thus be a practical and efficient method to tackle hydroid biofouling in situ in mussel aquaculture. Importantly, however, this technique can lead to some shellfish mortality (Carver et al., 2003), as found here when temperatures reached 50 °C. Finer-scale data on mussel mortality at different temperatures for different lengths of time would allow more highly tailored treatments. Mussel susceptibility to heat treatments may also vary at different times of the year (e.g., applying 50 °C heat to winter-conditioned as opposed to summer-conditioned mussels), and in relation to other environmental stressors (e.g., poor food supply). In addition, mussels from different locations would likely have different thermal tolerances and, thus, recommended treatments may not be suitable across significant geographical distances.

Both spray and immersion techniques have been implemented using acidic and alkaline chemicals. In mussel culture, weak acetic acid solutions have been successfully trialled against biofouling including soft-bodied tunicates and algae (LeBlanc et al., 2007; Denny, 2008a; Piola et al., 2010). However, some mussel mortality may be experienced (Carver et al., 2003; LeBlanc et al., 2007) and the application of acetic acid may also affect non-target organisms and hamper naturally occurring biocontrol (Paetzold et al., 2008). Here, ambient 2% and 5%

acetic acid solutions were both highly successful against *E. crocea*, and a 5% solution brought about 100% mortality in *C. intestinalis*. The soft-bodied composition of these two species likely increases their vulnerability. In contrast, *S. clava* with a thicker, leathery outer test was more resistant. Citric acid tended to impart either similar or slightly lesser impacts on biofouling than acetic acid, but it caused slightly more mussel and oyster mortality than acetic acid. Caution must therefore be exercised when using this substance and further investigations into its effect on stock mortality are needed before its commercial implementation.

Combining heat and acid treatments were often more effective than heat or acid treatments in isolation. For example, combining 2% acetic acid with seawater heated to 40 °C increased the percent mortality of *C. intestinalis* from 66% to 100% after either 10 or 30 s exposure times, and heating 2% acetic acid to 40 °C and lengthening the exposure time to 60 s was > 90% more effective against *S. clava* than either heat or acetic acid when used in isolation. Therefore, depending on the biofouling species present, this approach can reduce the temperatures and chemical concentrations needed, and thus lessen the cost of biofouling management and reducing the risks to farmers.

In summary, we found several short-term treatments for treating mature mussels highly effective. *E. crocea* was highly susceptible to all the treatments tested. In terms of cost, time and efficacy, the recommended treatments to use against fouling by this hydroid – dependent on farming capabilities and logistical constraints – would be a 10 s dip in either 40 °C seawater, or an ambient 2% acetic acid solution. *C. intestinalis* was similarly susceptible, with 10 s immersions in either a 40 °C 2% acetic acid solution or an ambient 5% acetic acid solution the best option. All these recommended treatment options when tested against small and large mussels and oysters resulted in no mortality. *S. clava* was hardier and more variable in its response to treatment, and if present in large numbers on mussel lines would require harsher treatments. The mildest treatment that achieved 100% mortality was a 40 °C 2% acetic acid solution for 60 s. This treatment, however, led to some mussel and oyster mortality and is thus not recommended. For heavy infestation by *S. clava*, traditional line-stripping and re-socking may be most effective.

There are several caveats and considerations that require considerable attention when discussing the potential commercial application of antifouling treatments methods. Firstly, our analysis of mortality was restricted to short term monitoring (48 h) within a laboratory setting. Although this is an important preliminary study on the effectiveness of heat, acid and combined treatments against biofouling, and the tolerance of commercial mussels and oysters, further research is required before commercial application regimes can be suggested and implemented. For example, the removal of foulers into the lab may affect their capacity to withstand treatments, so in situ trials should be conducted. Further, growth and condition of treated stock should be compared with untreated stock in the long-term, and the overall yield at the end of the grow out cycle compared. Additional experiments in the field should also look at longer exposure times. Although we intentionally used short exposure times as these are more time effective for farmers and realistic in the field, longer exposure times may allow for lower temperatures and acid concentrations, with benefits for stock and operator safety. Similarly, the method of commercial application also warrants strong consideration and experimentation. For example, we need to test whether heated seawater and acid treatments are better implemented via aerial spraying (where water temperature and acid concentration can be applied at a constant temperature and concentration) of shellfish stock and infrastructure lifted from the water, or by running them through vessel-based/land-based heated seawater/acid baths (where water temperature and acid concentration would be more difficult to maintain). Finally, expanding our treatments to hard-bodied biofouling species is important, as these may be differentially affected. Although these are critical knowledge gaps, our treatment methods are currently applicable for treating equipment and infrastructure on- and off-site, with cost, ease of application, accessibility to product type, and environmental impacts dictating the choice.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2019.02.071.

Acknowledgement

The project was funded by the Fisheries Research and Development Corporation (FRDC) (project no: 2012/202).

References

Adams, C.M., Shumway, S.E., Whitlach, R.B., Getchis, T., 2011. Biofouling in marine molluscan shellfish aquaculture: a survey assessing the business and economic implications of mitigation. J. World Aquacult. Soc. 42, 242–252.
Alfaro, A.C., Jeffs, A.G., 2002. Small-scale mussel settlement patterns within morphologically distinct substrata at ninety Mile Beach, northern New Zealand. Malacologia 44, 1–15.
Brenner, M., Buck, B.H., 2010. Attachment properties of blue mussel (*Mytilus edulis* L.) byssus threads on culture-based artificial collector substrates. Aquac. Eng. 42, 128–139.
Bui, S., Oppedal, F., Sievers, M., Dempster, T., 2019. Behaviour in the toolbox to outsmart parasites and improve fish welfare in aquaculture. Rev. Aquac. 11, 168–186.
Cahill, P.L., Heasman, K., Hickey, A., Mountfort, D., Jeffs, A., Kuhajek, J., 2013a. Screening for negative effects of candidate ascidian antifoulant compounds on a target aquaculture species, Perna canaliculus Gmelin. Biofouling 29, 29–37.
Cahill, P.L., Heasman, K., Jeffs, A., Kuhajek, J., 2013b. Laboratory assessment of the antifouling potential of a soluble-matrix paint laced with the natural compound polygodial. Biofouling 29, 967–975.
Carver, C.E., Chisholm, A., Mallet, A.L., 2003. Strategies to mitigate the impact of Ciona intestinalis (L.) biofouling on shellfish production. J. Shellfish Res. 22, 621–631.
Cyr, C., Myrand, B., Cliche, G., Desrosiers, G., 2007. Weekly spat collection of sea scallop, Placopecten magellanicus, and undesirable species as a potential tool to predict an optimal deployment period of collectors. J. Shellfish Res. 26, 1045–1054.
Daigle, R.M., Herbinger, C.M., 2009. Ecological interactions between the vase tunicate (Ciona intestinalis) and the farmed blue mussel (Mytilus edulis) in Nova Scotia, Canada. Aquat. Invasions 4, 177–187.
Darbyson, E.A., Hanson, J.M., Locke, A., Willison, J.H.M., 2009. Settlement and potential for transport of clubbed tunicate (Styela clava) on boat hulls. Aquat. Invasions 4, 95–103.
Denny, C.M., 2008a. Development of a method to reduce the spread of the ascidian Didemnum vexillum with aquaculture transfers. ICES J. Mar. Sci. 65, 805–810.
Denny, C.M., 2008b. Development of a method to reduce the spread of the ascidian *Didemnum vexillum* with aquaculture transfers. ICES J. Mar. Sci. 65, 805–810.
Filgueira, R., Peteiro, L.G., Labarta, U., Fernandez-Reiriz, M.J., 2007. Assessment of spat collector ropes in Galician mussel farming. Aquac. Eng. 37, 195–201.
Fitridge, I., Dempster, T., Guenther, J., de Nys, R., 2012. The impact and control of biofouling in marine aquaculture: a review. Biofouling 28, 649–669.
Forrest, B.M., 2007. Fouling pests in aquaculture - issues and management options. NZ Aquaculture 12–13.
Forrest, B.M., Blakemore, K.A., 2006a. Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. Aquaculture 257, 333–345.
Forrest, B.M., Blakemore, K.A., 2006b. Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. Aquaculture 257, 333–345.
Guenther, J., Fitridge, I., Misimi, E., 2011. Potential antifouling strategies for marine finfish aquaculture: the effects of physical and chemical treatments on the settlement and survival of the hydroid Ectopleura larynx. Biofouling 27, 1033–1042.
Gunthorpe, L., Mercer, J., Rees, C., Theodoropoulos, T., 2001. Best practices for the sterilisation of aquaculture farming equipment: a case study for mussel ropes. In: Marine and Freshwater Resources Institute Report. 41 Marine and Freshwater Resources Institute, Queenscliff.
Hillock, K.A., Costello, M.J., 2014. Tolerance of the invasive tunicate Styela clava to air exposure. Biofouling 29, 1181–1187.
Kroiher, M., Walther, M., Berking, S., 1992. Heat shock as inducer of metamorphosis in marine invertebrates. Roux's Archives of Developmental Biology 201, 169–172.
Lacoste, E., Gaertner-Mazouni, N., 2015. Biofouling impact on production and ecosystem functioning: a review for bivalve aquaculture. Rev. Aquac. 7, 187–196.
Lacoste, E., Le Moullac, G., Levy, P., Gueguen, Y., Gaertner-Mazouni, N., 2014. Biofouling development and its effect on growth and reproduction of the farmed pearl oyster Pinctada margaritifera. Aquaculture 434, 18–26.
LeBlanc, A.R., Davidson, J., Tremblay, R., McNiven, M., Landry, T., 2007. The effect of anti-fouling treatments for the clubbed tunicate on the blue mussel, Mytilus edulis. Aquaculture 264, 205–213.
Lekang, O.-I., Stevik, T.K., Bomo, A.M., 2003. Evaluation of different combined collectors used in longlines for blue mussel farming. Aquac. Eng. 27, 89–104.
NSPMMPI, 2013. National Biofouling Management Guidelines for the Aquaculture Industry. Australian Govenrment Department of Agriculture, Commonwealth of Australia, Canberra.
Paetzold, S.C., Davidson, J., Giberson, D., 2008. Responses of Mitrella lunata and Caprella spp., potential tunicate micropredators, in Prince Edward Island estuaries to acetic acid anti-fouling treatments. Aquaculture 285, 96–101.
Piola, R., Dunmore, R., Forrest, B., 2008. Evaluation of marine response tools: spray

treatments. In: MAF Biosecurity New Zealand Technical Paper No: 2010/23. Cawthron Institute, Nelson,NZ June 2008.
Piola, R.F., Dunmore, R.A., Forrest, B.M., 2010. Assessing the efficacy of spray-delivered 'eco-friendly' chemicals for the control and eradication of marine fouling pests. Biofouling 26, 187–203.
Rajagopal, S., Van der Velde, G., Van der Gagg, M., Jenner, H.A., 2002. Laboratory evaluation of the toxicity of chlorine to the fouling hydroid Cordylophora Caspia. Biofouling 18, 57–64.
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, 53–60.
Schmich, J., Kraus, Y., de Vito, D., Graziussi, D., Boero, F., Piraino, S., 2007. Induction of reverse development in two marine hydrozoans. Int. J. Dev. Biol. 51, 45–56.
Shin, J.H., Lee, S.Y., Dougherty, R.H., Rasco, B., Kang, D.H., 2006. Combined effect of mild heat and acetic acid treatment for inactivating Escherichia coli O157:H7, Listeria monocytogenes and Salmonella typhimurium in an asparagus puree. J. Appl. Microbiol. 101, 1140–1151.
Sievers, M., Fitridge, I., Dempster, T., Keough, M.J., 2013. Biofouling leads to reduced shell growth and flesh weight in the cultured mussel Mytilus galloprovincialis. Biofouling 29, 97–107.
Sievers, M., Dempster, T., Fitridge, I., Keough, M.J., 2014. Monitoring biofouling communities could reduce impacts to mussel aquaculture by allowing synchronisation of husbandry techniques with peaks in settlement. Biofouling 30, 203–212.
Sievers, M., Fitridge, I., Bui, S., Dempster, T., 2017. To treat or not to treat: a quantitative review of the effect of biofouling and control methods in shellfish aquaculture to evaluate the necessity of removal. Biofouling 33, 755–767.
Walter, U., Liebezeit, G., 2003. Efficiency of blue mussel (Mytilus edulis) spat collectors in highly dynamic tidal environments of the lower Saxonian coast (southern North Sea). Biomol. Eng. 20, 407–411.
Walters, L.J., Wethey, D.S., 1996. Settlement and early post settlement survival of sessile marine invertebrates on topographically complex surfaces: the importance of refuge dimensions and adult morphology. Marine Ecol. Prog. Ser. 137, 161–171.