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Tackling a critical industry bottleneck: developing methods to avoid, prevent & treat biofouling in mussel farms

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Executive Summary

Background

Biofouling negatively affects shellfish production through several pathways, including: 1) reducing natural mussel spat settlement rates; 2) preying upon mussel spat and juveniles; 3) competing for food with mussels; and 4) smothering established mussels. These problems are well documented in the culture of other bivalves such as oysters and scallops, where water flow is restricted to such an extent by fouling organisms that the availability of food and growth of stock are impeded (Claereboudt et al. 1994; Taylor et al. 1997). However, the effects of fouling organisms in long-line mussel culture remain poorly known (LeBlanc et al. 2003). Various native ascidians, hydroids, tunicates, macroalgae and seastars are common biofoulers across the mussel farming industry in Australia's southern waters. In Victoria, as in other parts of the world, introduced species are also emerging as key pests.

At present, Australian farmers deal with biofouling reactively, with treatment strategies implemented only after outbreaks have occurred. Current treatment protocols are largely based on a 2001 study in Victoria investigating measures to reduce the risk of moving noxious aquatic species via aquaculture stock or equipment (Gunthorpe 2001). Individual farmers have tried several methods on an ad-hoc basis to try to manage their fouling loads but they do not have the time or resources to carry out rigorous scientific testing and trials. Similarly, they are not aware of the basic biology or life history of the fouling species they are dealing with, and have no documented monitoring program in place to assess when fouling episodes are to be expected, and what species to be on the lookout for. Effective strategies to control biofouling must integrate information over the complex of biofouling species and their various effects. As fouling will always develop on mussel lines, it is important to develop and test cheap, easy to implement on-farm treatments that are effective against a range of biofouling species that do not affect mussel production.

Aims/objectives

In this project, we aimed to develop information to enable mussel farmers to more effectively recognise, avoid, prevent and treat biofouling outbreaks. This was to be achieved through a range of field and laboratory experiments to:

- measure the effects of key biofouling species on mussel spat survival and grow-out;
- test farm management methods that will discourage and/or avoid biofouling episodes;
- test the effectiveness of existing and new biofouling treatment methods to develop cost-efficient, implementable, on-farm treatments; and
- develop integrated biofouling control strategies and produce a handbook for farmers.

Methodology

To determine the impacts that key fouling species have on mussels, experimental ropes seeded with different sized mussels were inoculated with one of three fouling species (*Ectopleura crocea*, *Ciona intestinalis* and *Styela clava*). After two months, ropes were retrieved and various mussel morphometric parameters recorded to ascertain any growth or condition reductions from smothering or competition from these fouling species. In addition, various laboratory experiments investigated the capacity for *E. crocea* to consume mussel larvae of different ages and thus sizes.

Three main farm management methods were assessed for their ability to reduce fouling loads: rope type, stocking density and line depth. Seven different commercially used rope types were assessed for their ability to capture spat and the level of fouling accumulation. Ropes were fixed to PVC frames and deployed at two mussel farms. After eight weeks ropes were taken back to the lab and weighed to estimate fouling biomass. Biofouling was then sorted into taxonomic groups and dry weights recorded after

desiccation. Mussel spat were removed from the ropes using a chlorinated bleach solution, sieved and counted. To assess the effects of stocking density on biofouling rates, experimental mussel ropes were stocked at low (200 mussels/m), medium (400 m/m) or high (800 m/m) densities and deployed at two mussel farms. Every 4 weeks, one quarter of the ropes were retrieved and mussel retention, mussel growth, fouling biomass and individual fouling species biomass determined. To assess fouling at different depths, PVC settlement plates were deployed at 5 m and 10 m depth at Pinnace Channel mussel farm and left to accrue fouling. In addition, fouling patterns were assessed at three farms over a 20 month period using similar plates. Percent cover of fouling species was calculated using a grid-point intersection method.

A range of different treatment options were tested against common fouling organisms as well as mussels in the lab including immersions in: freshwater, heated seawater (40, 50 and 60 ^{o}C, acetic acid (2 and 5%), citric acid (2, 5 and 10%), hydrated lime (4 and 6%), and multiple combinations of heat and acids/lime. For most treatments, 10, 30 and 60 s durations were used and following a 24 h recovery period any mortality was recorded. A subset of the treatments that were both most successful and cost-effective for farmers (40 ^{o}C, 2% acetic acid and 40^{o}C, 5% acetic acid and 6% hydrated lime) were up-scaled and tested on commercial mussel rope sections. Entire sections, with the natural fouling communities, were treated and allowed to recover for 1 w before mussel survival and fouler (identified to the lowest possible taxa) survival was recorded.

Compiling the data obtained in the current studies into an easy to read handbook for farmers enables them to make informed decisions regarding biofouling control, based on the level and composition of the fouling, and their own personal farming capabilities. The handbook will be a waterproof flip style book with identification pages and information on which treatments are most effective against the different species.

Results/key findings

All three fouling species significantly reduced mussel growth over a two month period; fouled mussels were 2.1 – 4.4% shorter than control mussels. Flesh weights were also significantly reduced by 13 and 21% in small mussels fouled by *E. crocea* and *C. intestinalis*, respectively. Bivalve larvae were found to provide 7.8% of the diet of *E. crocea*, and consumption rates were affected by mussel life stage and density.

Out of the seven rope types tested the ‘green’ and ‘black’ ropes accrued the lowest amount of biofouling. However, these two ropes also collected the least mussel spat. The ‘super xmas’ rope collected the highest spat and medium levels of fouling relative to all other rope types. No single rope type simultaneously maximised spat and minimised fouling.

A low initial mussel density resulted in the least amount of biofouling but also the lowest mussel retention with between 20-70% of mussels lost. Conversely, high initial densities saw high levels of fouling and high levels of retention. Plates at 10 m depth were significantly less fouled (40%) than plates at 5 m. Some species showed depth preferences, with the notorious fouler *E. crocea* only found on plates held at 5 m depth.

Of the six most abundant fouling species that covered settlement plates, three (*C. intestinalis*, *P. taeniata* and *Mytilus galloprovincialis*) exhibited temporally narrow settlement periods, typically at a single location. Other species (corophiid amphipod tubes, *D. listerianum* and *E. crocea*) exhibited variable settlement, with no discernable patterns emerging. The various treatment options tested differed in their success against fouling organisms and bivalves. Freshwater immersion was unsuccessful at inducing mortality in any of the species. Excluding this, two important fouling species, *E. crocea* and *C. intestinalis,* were killed by almost every treatment regardless of duration, concentration or temperature. *S. clava* was slightly more resistant, with citric acid treatments the most effective. Mussels and oysters only showed any significant mortality when temperatures were 50 ^{o}C and above, irrespective of whether acid or lime was added. When experiments were scaled up to entire mussel ropes the fouling communities did not include the above tested species. However, the treatments had similarly mixed success depending on the fouling species. Mussel survival remained high for all treatments except 5% acetic acid, which resulted in

~10% mortality. Both treatments with acetic acid killed all of the colonial tunicate *Diplosoma listerianum*, and these treatments and the lime treatment successfully killed the bivalve *Electroma georgiana*, the seastar *Asterias amurensis*, the sponge *Sycon* sp. and polychaete worms. The problematic tubeworm *Pomatoceros taeniata* was not affected by any treatment.

Implications for relevant stakeholders

The knowledge gained from the experiments developed and trialled here is invaluable to Australian, and indeed international, mussel aquaculturists. This is the first time manipulative inoculations of mussel ropes with known fouling species have been done, with clear evidence of compromised mussel growth and thus farm productivity. The production of an on-board handbook will enable farmers to quickly identify any biofouling and make an informed decision on how to manage it.

Recommendations

- Implementation of an on-farm biofouling monitoring programme to better understand local fouling patterns and processes could aid in avoiding periodic fouling episodes.
- Further investigation of depth as a tool to mitigate biofouling, and the effects of depth on mussel growth and condition. These studies should be completed at a range of temporal and spatial scales.
- Further exploration of density as a tool against biofouling and the long-term effects of density on mussel retention, growth and condition.
- Conduct long-term studies of rope type on fouling throughout the production cycle and fouling seasons. On current information, switching to aqualoop or super christmas tree rope types would increase spat collections by 3-5 times, with less than a 2-fold increase in biofouling biomass.
- Heated seawater and/or acetic acid at the appropriate temperature and concentration, respectively, can be quite successful at killing particular fouling, without harming culture stock.

Keywords

Biofouling*, Mytilus galloprovincialis, Ectopleura crocea, Ciona intestinalis, Styela clava, Pomatoceros taeniata,* aquaculture, mussel farm

Introduction

Background

Biofouling in shellfish aquaculture

Biofouling on aquaculture infrastructure and stationary stock develops through a well known ecological process. Macrofouling derived from algal spores and propagules and the larvae of marine invertebrates develops rapidly within days to weeks. Whilst there are some circumstances where biofouling is beneficial, or at least, does not affect production (e.g. enhanced shellfish growth: (Dalby & Young 1993); increased primary production of phytoplankton (food) for shellfish: (Lodeiros et al. 2002; Ross et al. 2002; LeBlanc et al. 2003); protection against predation:(Wahl et al. 1997; Manning & Lindquist 2003); facilitated settlement of commercially farmed shellfish: (Hickman & Sause 1984; Fitridge 2011)), biofouling is primarily deleterious to the cost effective production of shellfish.

The impacts of biofouling on shell surfaces and equipment fall into five major categories: i) *Physical damage* by invasive organisms (endoliths) that bore into the shell or epibiotic calcareous tubeworms growing on the shell surface, affecting aesthetics; ii) *Mechanical interference* of shell function due to colonisation of shells, particularly around the hinge and lip, affecting feeding ability and susceptibility to predators; iii) *Biological competition* for resources such as food and space; iv) *Environmental modification* due to colonisation of culture infrastructure, leading to reduced water flow, waste build-up, decreased oxygen levels and reduced food availability. In addition, biodeposition and the spread of non-indigenous organisms can have deleterious effects on surrounding natural ecosystems; and v) *Increased weight* from biofouling biomass on stock and equipment (e.g. panels, nets, ropes and floats), leading to greater production costs associated with extra maintenance requirements and loss of stock and equipment.

The significant impacts that biofouling has on the viability and profitability of shellfish aquaculture has necessitated a long and persistent effort in biofouling control. Historically, the aquaculture industry has borrowed antifouling technologies from other marine industries with the focus on chemical antifouling technologies. However, many of the chemicals and heavy metals involved are recognised as dangerous in the environment, with detrimental effects on the survival and growth of shellfish and implications for product marketing. This has prompted an effort to prevent or mitigate biofouling in shellfish culture through alternative methods. Consequently, biofouling control remains one of the most difficult and costly production issues facing the industry. Methods to avoid or mitigate the effects of biofouling in shellfish culture fall into five broad categories: i) *Natural avoidance* to prevent settlement and growth of biofouling; ii) *Physical removal* including scrubbing, brushing, chemical dips and sprays; iii) *Biocontrol* using natural species; iv) *Coatings* on shells; and v) *Control and protection for equipment* using antifouling coatings and organic biocides.

Biofouling in shellfish aquaculture is a significant management issue resulting in increased operational expenses and deleterious impacts on the species being cultured. Surprisingly, for an issue with such high impact in a growing global industry, sparse information exists on its effects and costs. Given the limited choice of products currently available, quantitative studies on the spatial and temporal variation of fouling species, and the effects of husbandry techniques and farm management on fouling development, are essential to assist the industry to choose the most cost effective and practical methods for fouling control, both now and into the future.

Status of the Australian Mussel Aquaculture Industry

Blue mussels (*Mytilus galloprovincialis*) are grown in Australian waters across Victoria, South Australia, Western Australia and Tasmania. Yearly production is in the order of 3000 tons representing a value of over $8 million, with Victoria, Western Australia and South Australia the largest producers. While production in some states is stable, Victoria's mussel industry, in particular, has experienced a sharp decline in production since a peak in 2003. Unlike many of Australia's fisheries and aquaculture industries, this decline is not rooted in a lack of access to suitable areas for production. Indeed, in 2007, the Victorian government doubled the area of coastal space available for the mussel industry by gazetting a range of new aquaculture zones. As a result of these new allocations, Victoria’s annual mussel production was expected to rise from its then

current 2000 t to 6000 t within five years (Love & Langenkamp 2003). Instead, farmers reported a 70% decline in production over the five years from 2003 (from 2000 t to 600 t). This has been related to consecutive years of poor mussel spat recruitment, leading to a lack of stock for grow-out, and lowered mussel growth and survival in the grow-out phase. The interaction of a range of biofouling species and mussel farms lies at the very heart of the production decline, and represents a serious bottleneck for not only the Australian mussel industry, but mussel industries all around the world.

General Effects of Biofouling on Mussel Aquaculture

The effects of fouling organisms in long-line mussel culture remain poorly known (LeBlanc et al. 2003). A range of biofouling species are believed to affect mussel productivity, including ascidians, sponges, bryozoans, macroalgae, polychaetes and hydroids (Lesser et al. 1992; de Sa et al. 2007). Many species are native to the farmed region, such as the hydroid *Amphisbetia bispinosa*, which heavily fouls farmed mussel shells in New Zealand (Heasman & de Zwart 2004), and the tunicate *C. intestinalis*, which is a significant competitor for food in mussel culture operations in its home range of the NE and NW Atlantic coast of Canada and the USA (Lesser et al. 1992; LeBlanc et al. 2003). Colonies of the NE Atlantic endemic hydroid *Tubularia* sp. (= *Ectopleura* sp.) heavily colonise mussel culture equipment in that region of the USA (Hampson et al. 1999; Getchis 2006). However, aquaculture is a known vector of introduced species (Carlton 1999; Hewitt et al. 2004) and an increasing number of non-indigenous species are finding their way into mussel culture operations. For example, *C. intestinalis* has detrimentally affected mussel harvests in its introduced realm of New Zealand and South Africa (Getchis 2006). Similarly, the clubbed tunicate *S. clava*, which hails from the NW Pacific, has been detrimental to mussel operations on the eastern Atlantic coast of Canada (Bourque et al. 2005). In all cases, economic losses for farmers have been substantial in terms of lost stock (due to the weight of the fouling pulling mussels from the dropper lines) and the extra cleaning required to remove the fouling organisms from the mussel shells (de Sa et al. 2007), or because of smothering of stock (Bourque et al. 2005).

Specific Biofouling Problems within the Australian Industry

Various native ascidians, hydroids, tunicates, marcoalgae, polychaetes and seastars are common biofoulers right across the mussel farming industry in Australia's southern waters. In Victoria, as in other parts of the world, introduced species are emerging as key pests. Farmers report that in the past five years, three invasive species have emerged as problematic; the hydroid *E. crocea,* the tunicate *C. intestinalis* and the seastar *A. amurensis*. All of these species are listed as National priority pests based on their invasion potential and impact potential (Hayes et al. 2005). *E. crocea* is present in Victoria and Tasmania and affects production by deterring mussel spat settlement through providing unsuitable settlement structure, actively preying on settlement-ready spat and smothering mussels during on-growing (Fitridge & Keough 2013). *C. intestinalis* is present in all mussel industry states and farmers report that it causes several production issues including smothering of stock and competition for space. Both the South Australian and West Australian mussel industries are within the potential invasion range of *A. amurensis* (Bax et al. 2006). Larval seastars settle on mussel spat collection lines and actively prey on mussel spat for several months prior to when spat are stripped from their settlement ropes and re-socked. The scale of this effect on production is unknown. Other introduced species present in Australian waters likely to cause devastating impacts if they establish in mussel farms are the tunicate *S. clava* and the macroalga *Undaria pinnatifida*.

Current Management of Biofouling in Australia

At present, Australian farmers only deal with biofouling through the implementation of treatment strategies after outbreaks have occurred. Current treatment protocols are largely based on a 2001 study in Victoria which investigated measures to reduce the risk of moving noxious aquatic species via aquaculture stock or equipment (Gunthorpe 2001). The study focussed on ropes with spat and attempted to develop effective treatments to remove four exotic species from mussel ropes: *A. amurensis* (Northern Pacific seastar), *Sabella spallanzanii* (a fan worm), *U. pinnatifida* (a golden-brown macroalga) and *Carcinus maenas* (a shore crab). A combined treatment regime consisting of a tap water immersion followed by overnight air drying and a detergent immersion followed by overnight air drying was found to control all the pest species and was cost effective and environmentally benign. At present, however, of these four species only *A. amurensis* is a problem for mussel farmers in Port Phillip Bay.

Individual farmers have tried several methods on an ad-hoc basis to try to manage their fouling loads but they do not have the time or resources to carry out rigorous scientific testing and trials. Similarly, they are not aware of the basic biology or life history of the fouling species they are dealing with, and have no documented monitoring program in place to assess when fouling episodes are to be expected, and what species to be on the lookout for, both now and in the future.

Understanding, Avoiding, Preventing and Treating Biofouling at Mussel Farms

Effective strategies to control biofouling must integrate information over the complex of biofouling species and their various effects. While present control involves reactive treatments, an alternate strategy in managing biofouling could be based on the ability to accurately predict the occurrence of fouling episodes (Cyr et al. 2007). Prediction may enable avoidance of biofouling by manipulating the setting out of mussel ropes in areas, depths or times with low settlement probabilities. In addition to predicting outbreaks, there is a need to expand knowledge of the specific effects each biofouling species has upon mussel production and the timing at which negative effects manifest in the production cycle. This will enable the industry to determine when to step in and treat. Finally, as some fouling will always develop on mussel lines, it is also important to develop and test cheap, easy to implement on-farm treatments that are effective against a range of biofouling species, without compromising mussel production.

Need

Biofouling has emerged as the main bottleneck to production in the mussel farming industry. For example, since 2003, mussel production has declined by approximately 70% in Victoria. Concurrent with this decline has been the rise of several problematic biofouling species, including the invasive hydroid *E. crocea*, the invasive Northern Pacific sea star *A. amurensis*, and several ascidian and algal species. Many of these biofouling taxa are common across Victorian, South Australian, Western Australian, Tasmanian and New South Wales mussel farms.

A clear need exists to develop methods to avoid, prevent and treat biofouling to reduce costs and improve production. Typically, biofouling management accounts for 30-40% of production costs. Current biofouling removal methods (stripping of lines or fresh-water baths) are time consuming and labour-intensive. As a consequence, biofouling often develops to damaging levels before farmers are able to remove it.

Farmers require knowledge of the timing, location and depth of key fouling species so biofouling outbreaks can be avoided. Further, there is a need to test whether the type of equipment used (e.g., rope type and colour) or its arrangement (dropper spacing and dropper depth) may reduce biofouling. As some biofouling will inevitably develop on mussel lines, new biofouling treatments that are cheap, easy to use and effective must be tested.

Objectives

1. Measure the effects of key biofouling species on mussel spat survival and grow-out.

This objective forms a critical part of the overall objectives of this project, as it determines the physical effects of fouling species on mussel growth and condition at different stages in the production cycle. This enables farmers to see that some fouling species can be not only aesthetically problematic but can also be having direct impacts on farm productivity.

2. Test farm management methods that will discourage and/or avoid biofouling episodes.

The purpose of this objective was to determine whether changes in current farm management methods could lessen or deter biofouling episodes. The management tools we have focussed on are those that can be most simply applied by farmers including rope type, stocking density and line depth.

3. Test the effectiveness of existing and new biofouling treatment methods to develop cost-efficient, implementable on-farm treatments.

Worldwide, the mussel industry has tried several methods to manage fouling loads but treatments are invariably site and biofouling species specific. This objective has been achieved by establishing and trialling laboratory tests using existing and newly developed treatment methods, and up-scaling the most successful of these to larger on-shore scale trials, allowing the most beneficial and appropriate methods for on-farm treatment to be determined.

4. Develop integrated biofouling control strategies and produce a handbook for farmers.

This objective has been the focal point of the work in this project; to combine all of the biofouling strategies into a simple handbook which farmers can use as a reference point to enable them to make decisions regarding biofouling control, depending on the level of fouling and their own personal farming capabilities.

Methodology

Objective 1: Measure the effects of key biofouling species on mussel spat survival and grow-out.

Through previous observations and direct communication with stakeholders, we chose four key biofouling species (the hydroid *E. crocea,* the colonial tunicate *Botryllus schlosserii,* and two solitary tunicates, *S. clava* and *C. intestinalis*) to determine their impact on juvenile and adult mussels. These species represent two established fouling species (*E. crocea* and *C. intestinalis*) and two species found in Port Phillip Bay that pose significant threats to the mussel industry if they become established (*B. schlosseri* and *S. clava*). Previous work suggests that *E. crocea* uses mussel larvae as a food source. Therefore, data were collected during the spat recruitment season to determine the trophic ecology of *E. crocea* in the farm environment. A desktop study investigated the prey capture and digestion rate of another key problematic organism, the seastar *A. amurensis,* on various sizes (and therefore ages) of mussels, to determine their possible impact as predators in the production cycle.

Smothering/competition for food/space

Mussel ropes 35 cm long were seeded with either small (approx. 45 mm) or large (approx. 56 mm) mussels, and inoculated with key fouling species (*B. schlosseri, S. clava, E. crocea and C. intestinalis*). The fouling organisms were randomly attached to mussel shells using Selleys® Quick Fix™ cyanoacrylate glue (Lemarie et al. 2000; Ross et al. 2001). Eight *S. clava*, 10–12 *C. intestinalis*, 10-12 *B. schlosseri* colonies (approx. 2 cm^2 in area) or eight *E. crocea* colonies (25 feeding polyps per colony) were used per replicate mussel rope. These numbers were assessed from the literature to represent a low – medium fouling scenario. The *S. clava* and *B. schlosseri* experiments were run concurrently, using five inoculated and five control ropes for each mussel size. The *C. intestinalis* and *E. crocea* treatments were run at the completion of the *S. clava* / *B. schlosseri* experiment, using ten inoculated and five control ropes for each mussel size. Experimental rope sections were cable tied to weighted ropes and deployed in Port Phillip Bay for two months (Figure 1). Treatment and control rope sections were carefully gardened of any additional fouling by hand every two weeks.

Figure 1. Rope sections inoculated with *Ciona intestinalis* (left image) and *Ectopleura crocea* (right image).

Following collection after two months, 15 mussels were randomly chosen from each rope and morphometric parameters were measured using vernier callipers to the nearest 0.5 mm. Mussels were cooked at 91–93 °C for 4 min, dissected and the wet and dry weights of the shell and flesh obtained using a balance accurate to

0.001 g. Dry weights were obtained following a 24 and 48 h desiccation period at 60 °C for shells and flesh, respectively. Condition was calculated with dry weights, using the formula: $weight_{COOKEDMEAT}$ / ($weight_{COOKEDMEAT}$ + $weight_{SHELL}$)*100. This index is not affected by prior freezing (Davenport & Chen 1987) and similar indices based on ratios of dry flesh weight to dry shell weight are effective at monitoring bivalve condition (Crosby & Gale 1990). Due to expected strong correlations and redundancy among shell parameters, a composite measure, $(length*width*depth)^{1/3}$, was used to represent overall mussel size, and along with the commercially important length and flesh weight parameters, was statistically analysed.

Predation on mussel larvae

Colonies of *E. crocea* were collected from two aquaculture zones, Clifton Springs (CS) and Kirk Point/Werribee (KPW), every three weeks during the spat collecting season (August to October) and preserved immediately. Concurrent oblique plankton tows were also taken. In the laboratory, the stomach contents of *E. crocea* were assessed and compared with the composition of the plankton, to determine how their diet compares with the plankton available in the water column.

Laboratory feeding experiments were also conducted with mussel larvae of different ages (Figure 2) and densities, to determine the rate of mussel larvae prey capture and digestion by *E. crocea*. Colonies of *E. crocea* were placed in containers seeded with mussel larvae at one of three densities: low (approx. 200 larvae L^{-1}), medium (approx. 400 larvae L^{-1}) or high density (approx. 800 larvae L^{-1}). The experiment was repeated using larvae of different ages: five day old trochophore larvae, 12 day old veliger larvae and 22 day old larvae that were nearing settlement. Each experiment ran for four hours, representative of approximately one feeding cycle. At the completion of the experiment, hydroid stomachs were dissected to assess the number of *M. galloprovincialis* larvae consumed.

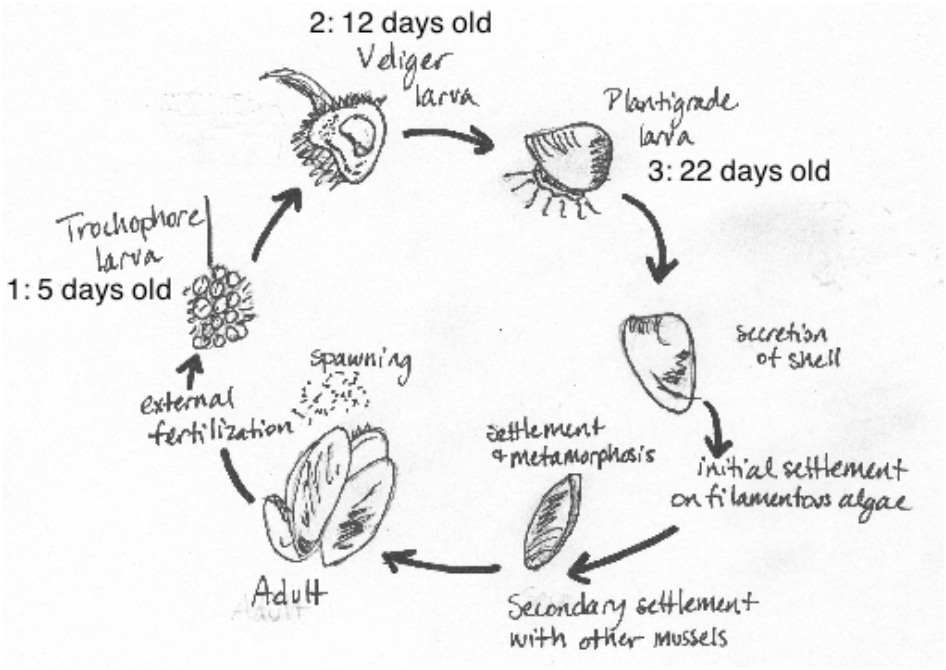


Figure 2. Life cycle of the blue mussel *M. galloprovincialis* showing the three different larval stages (1: Trochophore; 2: Veliger; 3: Plantigrade) offered to *E. crocea* during feeding trials (image modified from Clark University © 2004 http://www.clarku.edu/departments/biology/biol201/2004/ckammererburnham/questions.htm)

Seastar predation on juvenile and adult mussels

A. amurensis, the Northern Pacific seastar, was first recorded in Australian waters in the mid-1980's, in southeast Tasmania. It is believed to have arrived via shipping, with the same vector thought to be

responsible for its subsequent translocation across the Bass straight to Port Phillip Bay, on southern mainland Australia (Ross et al. 2003).

A search of the literature was carried out to seek peer reviewed publications concerning the relationship between *A. amurensis* and mussel prey, in terms of the response of seastars to mussel size and the attack behaviour displayed by the seastar. Publications were sourced from peer reviewed journals including Marine Ecology Progress Series, Aquaculture, Marine Biology, Estuarine Coastal and Shelf Science, Journal of Experimental Marine Biology and Ecology, and Papers and Proceedings of the Royal Society of Tasmania.

Statistical analysis

All analyses were performed using the software package SYSTAT v13.0. Box plots and plots of residuals were prepared to assess the data for homogeneity of variances and normality (Quinn & Keough 2002). One-way ANOVA was used to compare morphometric parameters and the weights of fouled and unfouled (control) mussels after cultivation for two months. Planned comparisons were subsequently used for the *C. intestinalis* and *E. crocea* experiment, to compare each fouling species to controls for each mussel size.

Data on diet and predation of mussel spat were all log transformed. Temporal differences in the relative abundance of common prey items in hydroid stomachs and in the plankton were assessed separately using a 2-factor ANOVA, where site and time were both fixed effects. Variation in the predation of mussel larvae by *E. crocea* in the laboratory was examined using a 2-factor ANOVA, where mussel density and age were both fixed effects.

Objective 2: Test farm management methods that will discourage and/or avoid biofouling episodes.

The purpose of this objective was to determine whether changes in current farm management methods could lessen or deter biofouling episodes. This included an assessment of the efficacy of seven different industry rope types as fouling deterrents and mussel larvae attractors, the influence of stocking density on the composition and severity of fouling and overall mussel retention, and the impact of dropping lines to depth as a successful management tool in deterring recruitment of fouling species.

Rope type

Several different rope types are currently in use across the industry. They are available in straight filament or looped filament designs. Seven industry-approved mussel ropes were attained from three commercial rope manufacturers: ‘Extreme Catch and Hold’, ‘Cut Loop’ (both from Quality Equipment Ltd, NZ), ‘Hatchery Rope’ (black spat rope), ‘Aqualoop’, ‘Christmas Tree’ and ‘Super Christmas Tree’ (Donaghys Pty Ltd, NZ) and ‘Spat Rope’ (green spat rope; Whittam Ropes Pty Ltd, Australia; Figure 3).

Figure 3. Photographs of the seven different rope types investigated.

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 at two mussel leases in the bay (three frames per lease) for approximately 8 w. 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 ^{o}C for 48 h. The stripped ropes were then processed for spat retention by soaking each rope section 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 & 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.

Density

Small mussels (approx. 15 mm in length) were collected and seeded onto 20 cm sections of mussel spat rope at three different densities: light (200 mussels/m), medium (400/m) or heavy (600/m). Mussels were held in place along the length of the mussel rope sections by monofilament sleeves, or ‘mussel sock’. There were 24 replicates of each density. Three rope sections (one of each density) were fixed with cable ties to each of 24 weighted 5 m dropper lines. Twelve dropper ropes were deployed at each of two mussel leases in Port Phillip Bay for 16 w. Every 4 w, three ropes (and therefore three replicates of each density) were removed and taken back to the lab. Each rope was stripped of all of its fouling and the fouling sorted to the lowest possible taxonomic level. Wet and dry fouling weights were attained for each taxonomic group, dry weights being

calculated after drying at 60^{o} C for 48 h. The mussels attached to each rope section were counted and a random selection of 15 individuals measured using vernier callipers to the nearest 0.5 mm.

Depth

Roughened PVC recruitment plates (20 cm × 20 cm) were secured to bricks with cable ties, and suspended from mussel backbone lines at the deepest production area in Port Phillip Bay. Each dropper rope held one plate in a horizontal downwards facing orientation. Ten replicate dropper ropes were deployed at 5m depth in the water column (current industry depth range for mussel lines), and ten at 10m depth on a submerged line. Plates were deployed for 8 weeks to accumulate fouling. Upon retrieval, percentage cover of fouling was estimated by overlaying a 10 × 10 grid on each plate and identifying the species underneath each of the 121 intersection points (excluding the outer 0.5 cm perimeter of the plates to avoid any edge effects). Fouling organisms were identified to the lowest possible taxon.

Biofouling patterns

In addition to the specific requirements of this study, we documented the spatial and temporal variability in mussel spat and biofouling settlement at three mussel farms within Port Phillip Bay over a 20 month period. Black PVC plates (11 cm × 11 cm) were sanded on one side, screwed to a 20 mm PVC pipe plate holder with stainless steel bolts and suspended from mussel backbone lines on weighted dropper ropes. Each dropper rope held two plates (at a depth of 2 and 4 m) in a horizontal downwards orientation to avoid excessive sedimentation. Five dropper ropes were deployed along a single commercial backbone mussel line at each farm. Plates were retrieved and replaced every 5–16 weeks (9.1 ± 0.6 w, mean ± SE) from July 2011 to April 2013, with all three sites sampled at similar times, which were assigned into collection periods for analyses. All plates were lost at Pinnace Channel (PC) during collections 4 and 5, resulting in a period of 12 weeks between plate retrieval (collection 3) and plate deployment (collection 6). After each collection, plate holders were scrubbed clean to avoid any small-scale immigration of spat or foulers onto plates. The percentage cover of mussel spat and fouling organisms was estimated by overlaying a 10 cm × 10 cm grid on each plate and identifying the species underneath each of the 121 intersection points. This method excludes the outer 0.5 cm perimeter of the plates to avoid any edge effects. Organisms were identified to the lowest possible taxon, with most identified to species level.

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.

The effect of rope type on fouling development and mussel spat collection was analysed using two separate 2-way ANOVA with location and rope type fixed factors. Fourth root transformations were used on both variables and Tukey's tests were done to compare differences between individual rope types.

The effect of initial mussel density was examined on total fouling accumulation (log-transformed), mussel retention (untransformed), *E. crocea* weight (cube-transformed) and *P. taeniata* weight (log-transformed). Three way ANOVA was used with site, initial density and time all treated as fixed factors, except for *P. taeniata* which only settled in the final time period, and so this factor was excluded from analyses.

The influence of depth (fixed factor) on fouling accruement was examined with a one-way ANOVA comparing plates at 5 m depth to plates at 10 m depth. Various transformations were required to satisfy normality and homogeneity of variance: total fouling and *E. crocea* – no transformation; amphipod tubes – square root; *D. listerianum* and *E. georgiana* – log; and *Anthopleura aureoradiata* – cubed root.

Due to the loss of plates at Pinnace Channel during collections 4 and 5, the data were non-orthogonal. Consequently, the percentage cover of the entire fouling community, amphipod tubes, *D. listerianum* and *E. crocea* was analysed using two subsets of the data, with an ANOVA examining differences throughout time and among farms for the eight collection periods when all three farms were sampled, and another comparing Clifton Springs and Kirk Point for the entire survey period. A single factor ANOVA compared locations for species that exhibited distinct peaks in cover during a single collection period (*C. intestinalis, M. galloprovincialis* and *P. taeniata*). Depth (fixed factor) was not significant for any of the species ($p > 0.05$) and was excluded as a term in subsequent analyses because its inclusion would have lead to missing cells.

For all analyses, time and location were fixed factors, and type III SS was used as some replicate plates were lost at various times and locations, resulting in an unbalanced design (Quinn & Keough 2002). Arc-sine or log transformations had little effect on improving variance homogeneity or normality, so raw data were used in all analyses.

Objective 3: Test the effectiveness of existing and new biofouling treatment methods to develop cost-efficient, implementable, on-farm treatments.

The effectiveness of existing and new biofouling treatment methods were tested against the survival of cultured mussels (*M. galloprovincialis*) and three common fouling organisms (*C. intestinalis, S. clava* and *E. crocea*; Figure 4). In addition to the requirements of this objective, some treatments were also performed on the oyster *Ostrea angasi*, a new shellfish being farmed in Port Phillip Bay alongside mussels and potentially subjected to the same biofouling management issues. The treatments consisted of: 1) freshwater immersion; 2) eco-friendly acids and alkaline solutions; 3) heat; 4) treatment combinations and 5) larger on-shore treatment trials.

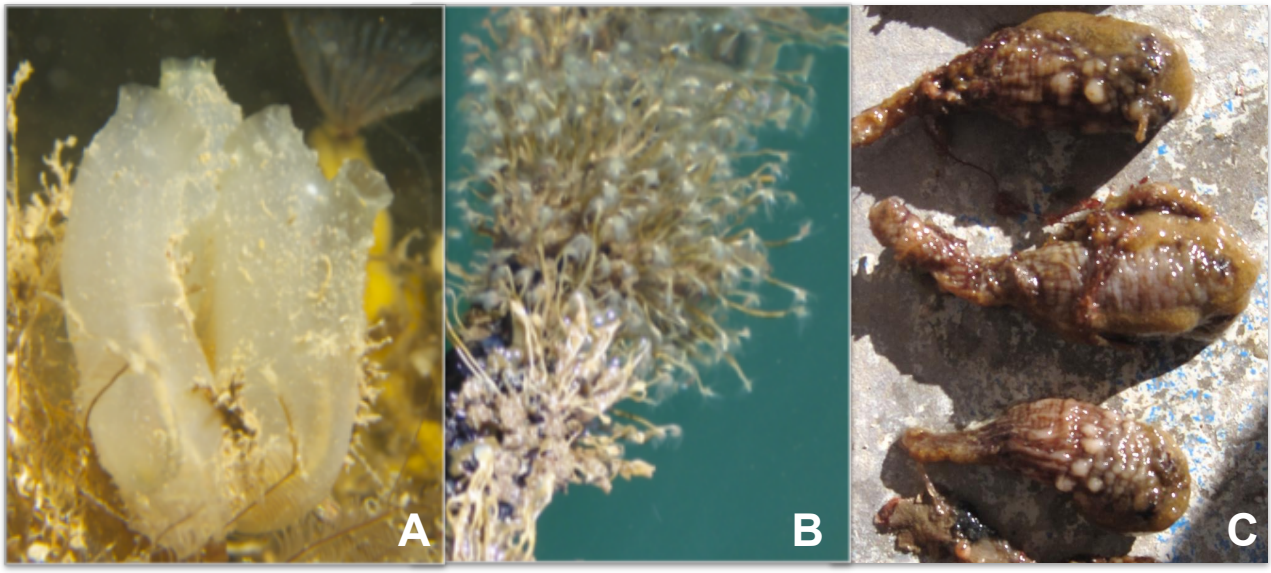


Figure 4. The fouling organisms *Ciona intestinalis* (A), *Ectopleura crocea* (B) and *Styela clava* (C).

Freshwater immersion

Fouling species and shellfish (juvenile and adult mussels and oysters) were collected from farms or piers and maintained in 2 L flow through beakers for 24 h. They were assessed using a dissecting microscope to ensure they were alive and functioning normally. Any dead individuals were removed. The organisms were subjected individually to 10, 30 or 60 s dips in freshwater; these exposure times are substantially shorter than the recommended overnight immersion time currently employed, but short-term immersions were thought to be more practical from the farmer's perspective for on-board treatment options. After exposure to freshwater, the shellfish and fouling species were re-immersed in a bowl of ambient filtered seawater and returned to their beakers and allowed to recover for 48 h. After the recovery period, their viability was assessed under a dissecting microscope to determine mortality rate.

Eco-friendly acids and alkaline solutions

Acid: Fouling species and shellfish (juvenile and adult mussels and oysters) were collected, assessed and maintained as above. The organisms were subjected individually to 10, 30 and/or 60 s dips in dilute acid

solutions: glacial acetic acid diluted with seawater to 2 and 5%, and 99.5% anhydrous citric acid diluted to 2%, 5% and 10%. After exposure, the shellfish and fouling species were re-immersed in a bowl of ambient filtered seawater, returned to their beakers and allowed to recover for 48 h. After the recovery period, their viability was assessed under a dissecting microscope to determine mortality rate.

Alkaline: Available fouling species and shellfish were collected, assessed and maintained. Unfortunately, it was not possible to obtain oysters or the hydroid *E. crocea* for this experiment and therefore they will not be reported on. The organisms were subjected individually to either a 60 s dip in diluted hydrated lime solutions (4 and 6%) made from a 90–95% preparation of calcium hydroxide (Cement Australia Pty Ltd), or exposure to air for 30 s followed by 60 s dips in each concentration of lime, then a further 30 s air dry. After treatment, the shellfish and fouling species were re-immersed in a bowl of ambient filtered seawater, returned to their beakers and allowed to recover for 48 h. After the recovery period, their viability was assessed under a dissecting microscope to determine mortality rate.

Heat

Fouling species and shellfish (juvenile and adult mussels and oysters) were collected, assessed and maintained as already discussed. The organisms were subjected individually to 10, 30 or 60 s dips in seawater heated to 40^{o}C, 50^{o}C or 60^{o}C. After exposure, the shellfish and fouling species were re-immersed in a bowl of ambient filtered seawater, returned to their beakers and allowed to recover for 48 h. After the recovery period, their viability was assessed under a dissecting microscope to determine mortality rate.

Treatment combinations

Fouling species and shellfish (juvenile and adult mussels and oysters) were collected, assessed and maintained as already discussed. The organisms were subjected individually to 10, 30 or 60 s dips in acetic acid solutions (2%, 5%) and citric acid solutions (2%, 5%, 10%) heated to 40^{o}C or 50^{o}C. After exposure, the shellfish and fouling species were re-immersed in a bowl of ambient filtered seawater, returned to their beakers and allowed to recover for 48 h. After the recovery period, their viability was assessed under a dissecting microscope to determine mortality rate.

On-shore treatment trials

Four of the most successful or practical treatments identified from the previous experiments were trialled on a larger scale at the Victorian Shellfish Hatchery, Queenscliff, Victoria. Commercial mussel ropes with stock and associated biofouling were obtained from a mussel lease and brought back to the laboratory. The ropes were laid out and cut into 25 sections, each 50 cm in length. Each section was encased within a loose fine mesh bag in order to contain all of the mussels and fouling, yet still allow adequate water exchange. Bagged sections were then suspended vertically in a 2000L aerated aquarium tank filled with recirculated ambient seawater and left to acclimatise for 24 hours (Figure 5). The following day, rope sections were exposed to one of four treatments: 1) 60 s immersion in seawater heated to 40 ^{o}C; 2) 30 s immersion in a 5% acetic acid solution; 3) 30 s immersion in a 2% acetic acid solution heated to 40 ^{o}C or 4) air dried for 30 s, dipped for 60 s in a 6% hydrated lime solution, and air dried for a further 30 s. Rope sections were returned to the aquarium tank immediately following treatment and left to recover for one week. After the recovery period, each section was stripped of fouling and mussels, ensuring to include any material that had fallen into the bottom of the mesh bag. Mussels were counted and recorded as dead or alive. Fouling organisms were sorted to the lowest possible taxonomic level and their viability assessed under a dissecting microscope to determine mortality rate.

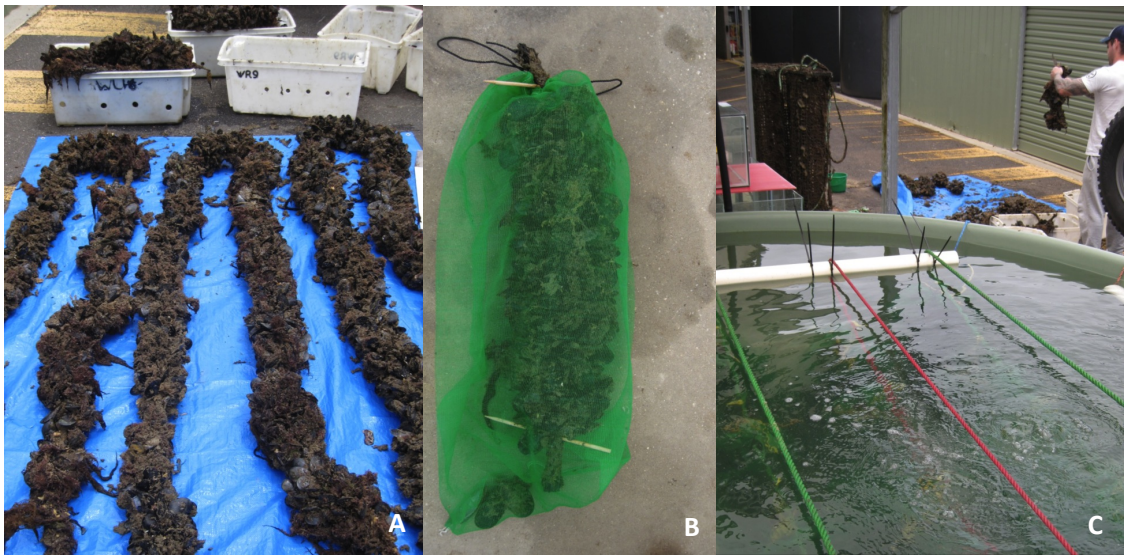


Figure 5. Fouled commercial mussel ropes (A); bagged experimental section (B); sections suspended within a 2000L aquarium tank post-treatment (C).

Statistical analysis

The efficacy of the treatments tested on individual fouling and culture species was determined using Fisher's exact test comparing each treatment-duration combination to a control group. N-values for different species differed due to logistical constraints. The number of individuals in control groups was: five for *E. crocea*, nine for *C. intestinalis,* eight for *S. clava*, seven for 30 mm *M. galloprovincialis*, five for 60 mm *M. galloprovincialis*, five for 15 mm *O. angasi* and four for 50 mm *O. angasi.*

One-way ANOVA was used to compare mussel and fouling survival/abundance one-week post treatment for the on-shore rope treatments. Planned comparisons were subsequently used to compare each treatment type to the control. Treatments were treated as fixed factors, and the proportion of mussels alive was exponentially transformed and *D. listerianum* and *Amphisbetia operculata* abundance was log transformed in order to achieve normality and heterogeneity of variances.

Objective 4: Develop integrated biofouling control strategies and produce a handbook for farmers.

The main purpose of this objective was to bring all aspects of the project together in order to develop the most effective biofouling strategies for farmers. Central to this was the production of a handbook outlining the species to be aware of, and methods to deal with outbreaks. The handbook is intended to be a simple to use 'flip guide', allowing easy identification of key fouling organisms and outlining a range of options for treatment or management. Printed on waterproof card, it is intended to be an 'on board' tool for farmers; a handy reference during all stages of the production cycle.

An additional phase of this objective was the fostering of industry ties with Canadian researchers, in order to learn from international experience with similar fouling species in the mussel industry. Prince Edward Island, in Eastern Canada's Gulf of St Lawrence, is a hotspot for mussel fouling. The PEI mussel industry is, with the support of various local and federal government funding agencies and academia, leading the development of methods to mitigate the impact of non-indigenous tunicates on shellfish operations. Dr Isla Fitridge travelled to Canada to meet with key personnel at the Department of Fisheries and Oceans Canada (Chris Mills, Fisheries & Aquaculture Officer), the PEI Department of Fisheries, Aquaculture and Rural Development (Aaron Ramsay, Brian Gillis, Kim Gills), and the University of PEI (Jonathon Hill), to discuss the Canadian approach and undertake site visits of affected mussel and oyster leases. The field components

of the visit allowed *in situ* control strategies to be witnessed directly. A study trip report was produced. Both of these outputs are presented in the section '**Project Materials Developed**'.

Results

Objective 1: Determine the impacts of key biofouling species on mussel spat survival and grow-out.

Smothering/competition for food/space

Unfortunately *B. schlosseri* died within a few days of inoculation and deployment, and subsequent re-inoculations were unsuccessful. The tunicate therefore had to be omitted from this experiment. *B. schlosseri* also experienced dieback at other locations around the bay at this time so this was a natural phenomenon which could not be foreseen. The other fouling organisms rarely died throughout the study period, but any that did were replaced with new individuals.

Inoculating experimental mussel ropes with *C. intestinalis*, *E. crocea* and *S. clava* significantly reduced growth in both small and large mussels (Figure 6, Table 1). After cultivation for two months, small control mussels had grown 9.2 ± 0.6 mm in length. In comparison, small mussels inoculated with *C. intestinalis* and *E. crocea* had only grown 6.8 ± 0.4 and 6.9 ± 0.7 mm, respectively (Figure 7, Table 1). These reductions in growth translate to mussels that were 4.0 and 3.9% shorter than control mussels for the *C. intestinalis* and *E. crocea* treatments, respectively. Small mussels also had a 21 and 13% lower meat yield when inoculated with *C. intestinalis* and *E. crocea*, respectively, compared to the control mussels (Figure 8, Table 1). Mussel condition was significantly lower in small mussels fouled by *C. intestinalis*, but not in mussels fouled by *E. crocea* (Figure 9, Table 1).

In the *C. intestinalis* and *E. crocea* experiment, large mussels experienced relatively slower growth rates. Control mussels grew 2.7 ± 0.7mm in length, whilst mussels inoculated with *C. intestinalis* grew 0.3 ± 0.4mm and mussels inoculated with *E. crocea* grew 1.1 ± 0.6mm (Figure 7, Table 1). These reductions in growth translate to mussels that were 3.2 and 2.1% shorter than the control mussels for the *C. intestinalis* and *E. crocea* treatments, respectively. Control and fouled mussels in the *S. clava* experiments grew 7.7 ± 0.7 and 4.6 ± 0.5mm, respectively (Figure 7, Table 1), translating to fouled mussels being 4.4% shorter. Although large mussels exhibited a similar trend to small mussels, with fouled mussels experiencing a 9, 14, and 9% reduction in meat yield for the *S. clava*, *C. intestinalis* and *E. crocea* treatments, respectively, these reductions were not statistically significant (Figure 8, Table 1). In addition, the condition of large mussels was not significantly affected by fouling by these three species (Figure 9, Table 1).

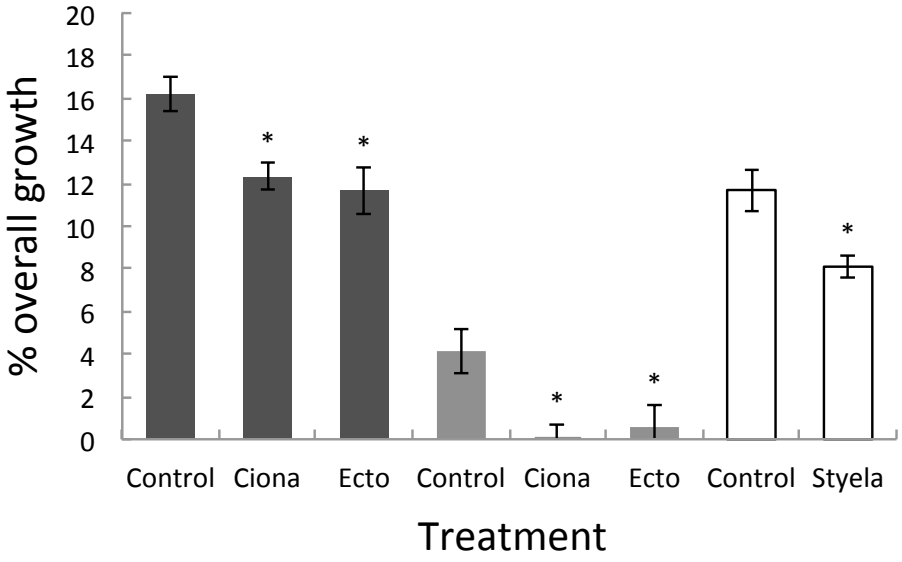


Figure 6. Mean (±SE) percentage growth of fouled and unfouled mussels after a two month cultivation period. Percentage growth was determined by change in overall size calculated as (length*width*height)$^{1/3}$. Control = unfouled mussels; Ciona = mussels fouled by *C. intestinalis*; Ecto = mussels fouled by *E. crocea*; and Styela = mussels fouled by *S. clava*. Dark grey bars = experiments using small mussels (N = 15) and light grey (N = 12) and white bars (N = 10) = the two separate experiments using large mussels. * = a significant difference in planned comparisons between control and fouled treatments (p < 0.05).

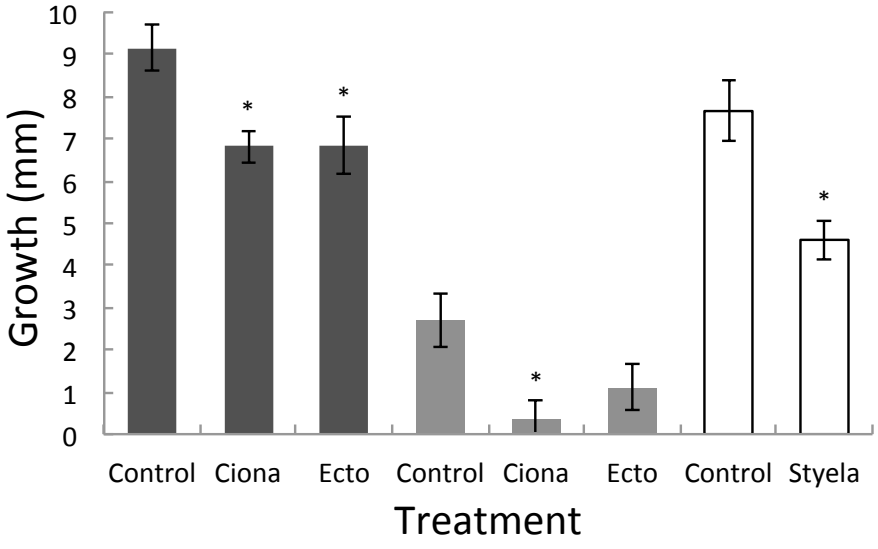


Figure 7. Mean (±SE) growth of fouled and unfouled mussels, in terms of shell length in mm, over two months. Control = unfouled mussels; Ciona= mussels fouled by *C. intestinalis*; Ecto = mussels fouled by *E. crocea*; and Styela= mussels fouled by *S. clava*. Dark grey bars = experiments using small mussels and light grey and white bars = the two separate experiments using large mussels. N-values as in Figure 1. * = a significant difference in planned comparisons between control and fouled treatments (p < 0.05).

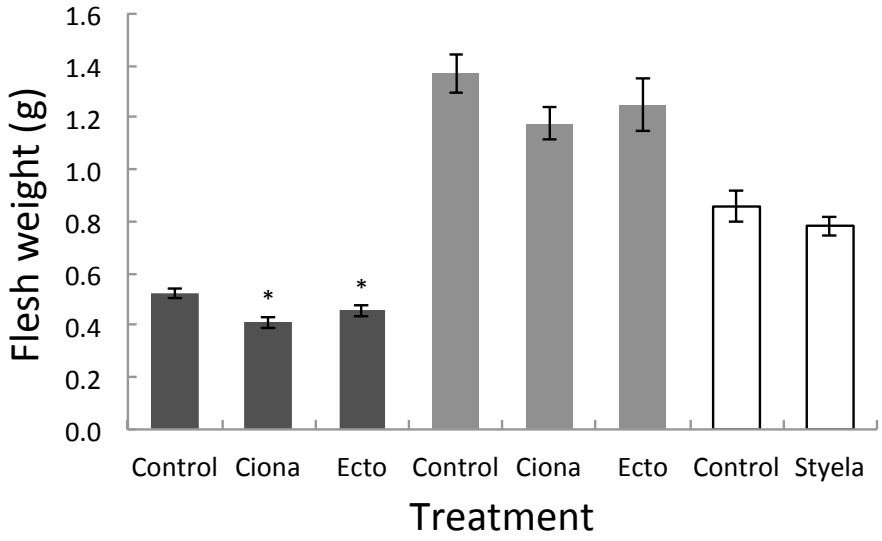


Figure 8. Mean (±SE) flesh weight of fouled and unfouled mussels after a two month cultivation period. Dry flesh weights in g were obtained after drying for 48 h at 60 °C. Control = unfouled mussels; Ciona = mussels fouled by *C. intestinalis*; Ecto = mussels fouled by *E. crocea;* and Styela = mussels fouled by *S. clava*. Dark grey bars = experiments using small mussels and light grey and white bars = the two separate experiments using large mussels. N-values as in Figure 1. * = a significant difference in planned comparisons between control and fouled treatments (p < 0.05).

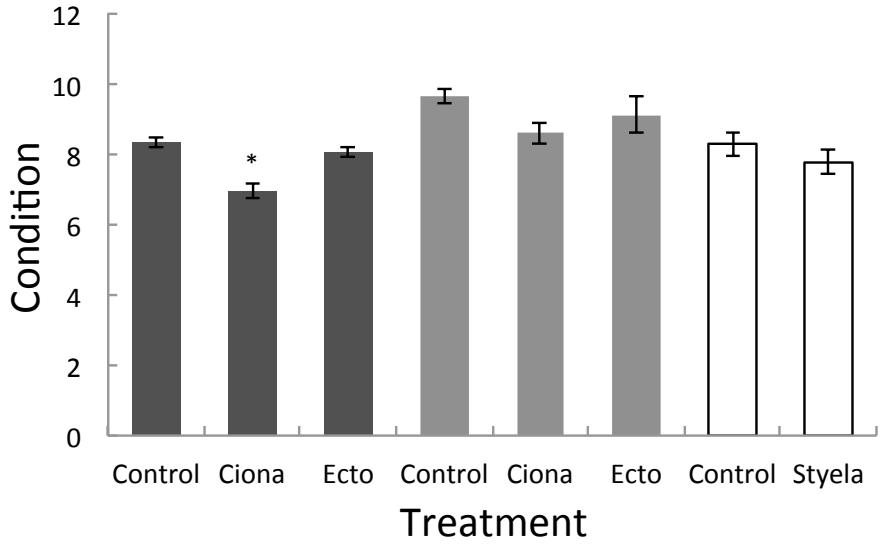


Figure 9. Mean (± SE) condition of fouled and unfouled mussels after a two month cultivation period. Condition is calculated from dry weights using the formula: $weight_{COOKEDMEAT}$ / ($weight_{COOKEDMEAT}$ + $weight_{SHELL}$) * 100. Control = unfouled mussels; Ciona = mussels fouled by *C. intestinalis*; Ecto = mussels fouled by *E. crocea*; and Styela = mussels fouled by *S. clava*. Dark grey bars = experiments using small mussels and light grey and white bars = the two separate experiments using large mussels. N-values as in Figure 1. * = a significant difference in planned comparisons between control and fouled treatments (p < 0.05).

Table 1. Results of one-way ANOVA comparing fouled mussels to control mussels after a two month cultivation period.

		Small				Large			
	Source of variation	df	MS	*F*	p	df	MS	*F*	p
C. intestinalis	Size	1	5.87	10.13	**0.008**	1	5.66	11.86	**0.007**
	Residual	12	0.58			9	0.48		
	Length	1	13.76	8.94	**0.011**	1	10.81	8.74	**0.016**
	Residual	12	1.54			9	1.24		
	Flesh weight	1	0.03	14.55	**0.002**	1	0.074	2.83	0.127
	Residual	12	0.002			9	0.026		
	Condition	1	0.66	6.11	**0.029**	1	2.29	4.29	0.068
	Residual	12	0.11			9	0.53		
E. crocea	Size	1	7.75	13.38	**0.003**	1	3.59	7.51	**0.023**
	Residual	12	0.58			9	0.48		
	Length	1	13.25	8.60	**0.013**	1	5.07	4.10	0.073
	Residual	12	1.54			9	1.24		
	Flesh weight	1	0.01	5.32	**0.040**	1	0.03	1.16	0.310
	Residual	12	0.002			9	0.026		
	Condition	1	0.12	1.08	0.320	1	0.58	1.09	0.323
	Residual	12	0.11			9	0.53		
S. clava	Size					1	6.18	10.02	**0.013**
	Residual					8	0.62		
	Length					1	23.31	12.74	**0.007**
	Residual					8	1.83		
	Flesh weight					1	0.014	1.14	0.317
	Residual					8	0.012		
	Condition					1	0.36	0.78	0.404
	Residual					8	0.47		

Note: Analyses were conducted for overall mussel size (length*width*height$^{1/3}$), shell length, dry weight, and condition, for small and large mussels. Results of planned comparisons comparing the three individual fouling species to controls are shown. Five control and five fouled replicates were used for the small mussel and the *S. clava* experiments. Four of each were used for the large *C. intestinalis* and *E. crocea* experiments. Boldface values are significant at $p < 0.05$.

Predation on mussel larvae

E. crocea colonies captured an assortment of prey items (Figure 10). Crustaceans were the most important prey items in relation to their availability in the plankton, comprising almost 70% of the diet of *E. crocea* compared to their planktonic contribution of just over 21%. Many of the crustacean prey items consisted of appendages or partly digested body parts that could not be identified. Of those that could be recognised, copepods were by far the dominant group (20.7%), followed by cladocerans (3.2%), crab larvae (2.8%) and nauplii (2.5%). Bivalve larvae and diatoms were also common prey items, making up 7.8 and 19.2% of the hydroid diet, respectively. Crustacean fragments were found in over 51% of hydranths. Copepods and bivalve larvae were also important prey items, being present in 13 and 7% of hydranths, respectively. In contrast, common planktonic food items readily available, such as tinntinnids, invertebrate eggs and dinoflagellates, were not present. Generally, the top five key components of both the plankton and the hydroid diet (taking into account their percentage contribution to the diet, the percentage of hydranths containing those items as prey and the proportion they contributed to the plankton) were considered to be copepods, bivalve larvae, diatoms, nauplii and cladocerans.

The planktonic abundance of all five taxa varied significantly over time (Figure 11), and between sites for nauplii (greater numbers at CS) and cladocerans (greater numbers at KPW). The interaction of site x time was significant for all five taxa (Figure 11, Table 2). At both sites, diatoms decreased in abundance towards November, whilst cladocerans peaked at this time; copepods were lowest in abundance in early September. Nauplii remained fairly stable in abundance over time at CS, but showed troughs in early September and November at KPW. Bivalves increased in abundance over time at CS, but dropped in abundance at KPW by November. In contrast, the abundances of all five taxa as prey were less variable than their numbers in the plankton, although site was significant for two taxa: more bivalves were consumed at CS, and more cladocerans at KPW (Figure 11, Table 2). Diatom consumption varied in time, being least abundant in diets in the September samples. All factors affected the presence of copepods, which peaked at both sites in hydroid stomachs in early September and were three times more abundant in hydroid stomachs at KPW compared to CS. Of the 450 hydranths examined in total, 287 or 63.8% contained at least one prey item. The maximum number of items recorded in one hydranth was 18.

In 4 h laboratory feeding trials, hydroid consumption was affected by mussel life stage and density, and their effects were not independent (Table 3). The older plantigrade mussels (22 days old) were consumed at the highest rate at all densities, and greatest predation occurred when mussel larvae were introduced to experimental containers at the highest density of 800 larvae L^{-1} (Figure 12). At low mussel densities, no trochophores or veligers were eaten, and predation occurred only when the hydroids were offered plantigrade larvae. At medium densities, *E. crocea* consumed larvae of all ages, but predation of trochophore (five days old) and veliger (12 days old) mussel larvae was low compared to plantigrade larvae. At high densities, a few five-day-old mussel larvae were consumed, but predation increased on 12-day-old veligers, and was greatest on 22-day-old plantigrade larvae (Figure 12).

Table 2. Statistical analysis of the effects of site and time on the abundance of the top five prey and plankton items. In all cases, the MSresidual is listed at the bottom of the p column. Bold face values are significant at p < 0.05.

Source	df	Bivalves p	Copepods p	Diatoms p	Nauplii p	Cladocera p
Plankton						
Site	1	0.277	0.383	0.152	**<0.001**	**0.012**
Time	3	**0.008**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Site x Time	3	**<0.001**	**<0.001**	**0.020**	**<0.001**	**0.013**
Residual	40	0.135	0.080	2.579	0.851	2.217
Prey						
Site	1	**0.007**	**0.001**	0.772	0.150	**0.002**
Time	3	0.054	**<0.001**	**0.002**	0.104	0.302
Site x Time	3	0.127	**<0.001**	0.896	0.336	0.621
Residual	40	0.057	0.130	0.124	0.018	0.023

Table 3. Statistical analysis of predation by the hydroid *E. crocea* on *M. galloprovincialis* larvae at different densities (low, medium and high) and life stages (5, 12 and 22 days old). MSresidual is listed at the bottom of the p column. Bold face values are significant at p < 0.05.

Source	df	p
Density	2	**<0.001**
Mussel age	2	**<0.001**
Denisty x mussel age	4	**0.017**
Residual	621	0.040

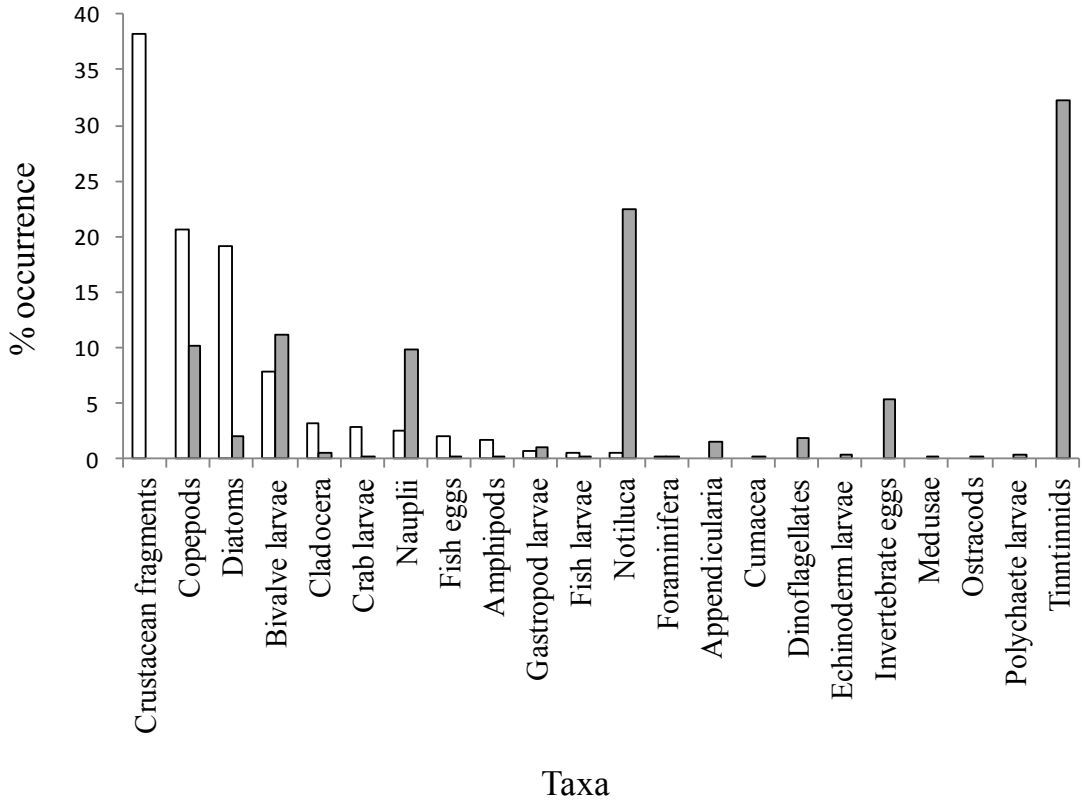


Figure 10. The percentage contribution of various taxa to the composition of the plankton (grey bars) and *E. crocea* stomach contents (white bars). Data are pooled across sampling times and farm locations. Note that crustaceans were readily identifiable as individual taxa in the plankton, but some could only be identified as crustacean fragments in hydroid stomachs.

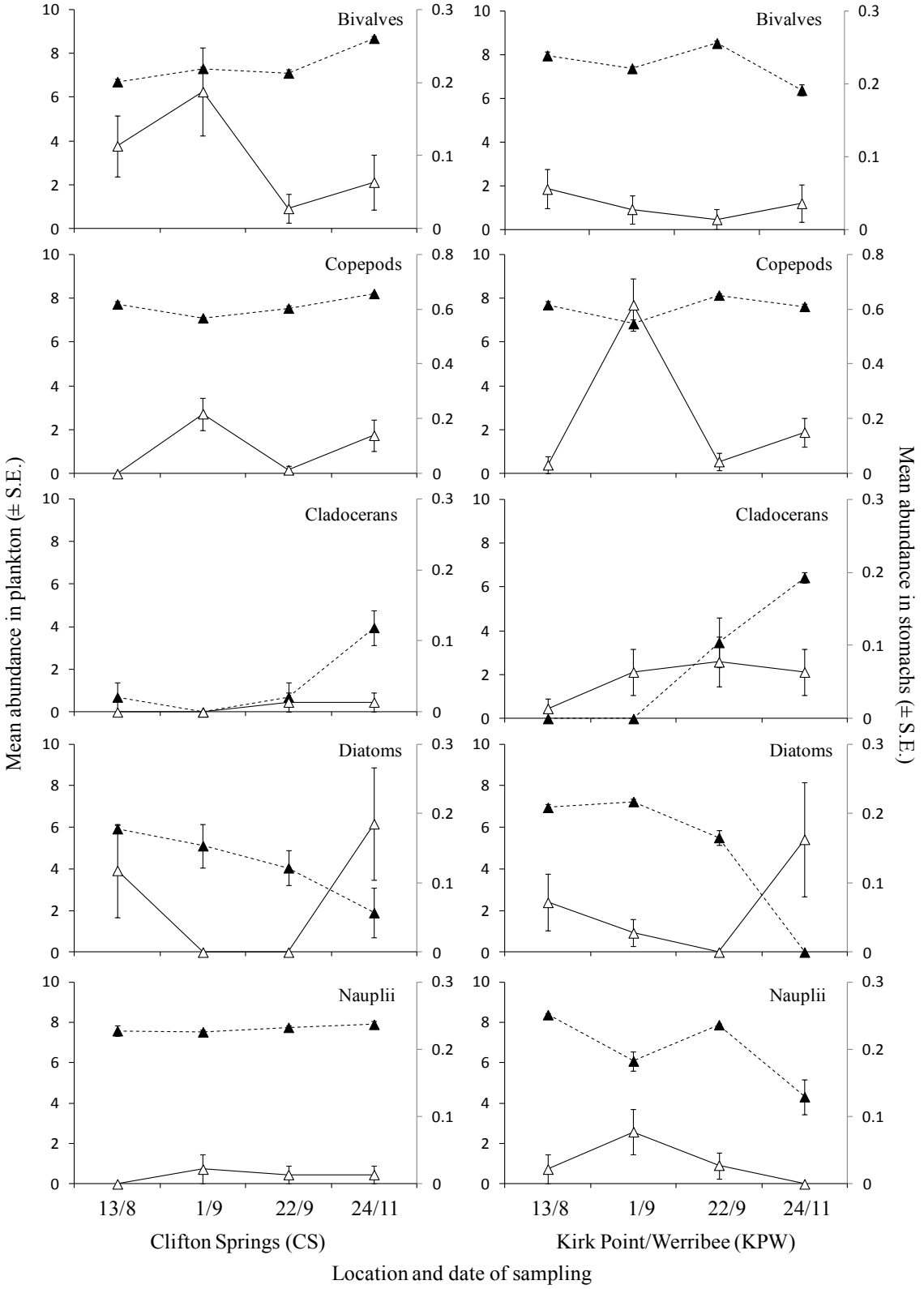


Figure 11. Mean abundance (±S.E.) over time of the five primary groups of plankton in plankton samples (filled triangles and dashed line, left axis) and in hydroid stomachs (open triangles and solid line, right axis), at CS (left hand charts) and KPW (right hand charts). Note that prey and plankton Y-axis scales differ.

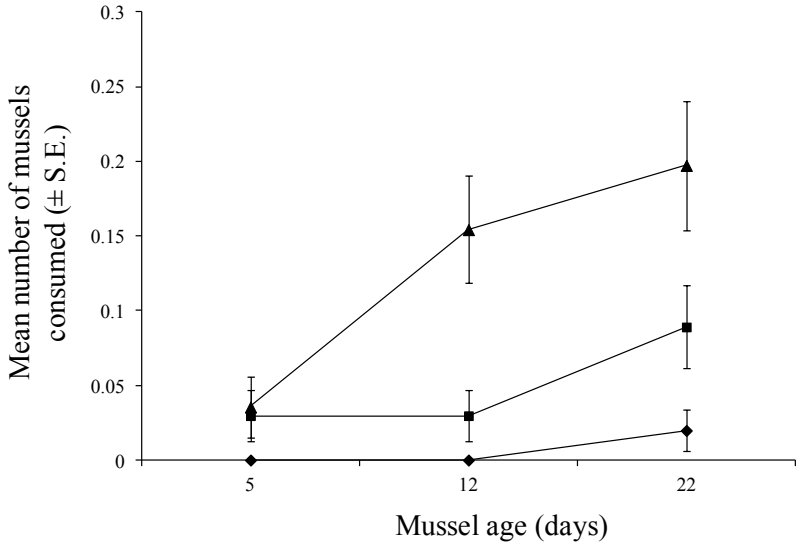


Figure 12. Mean number of *M. galloprovincialis* larvae (log transformed, ± S.E.) at different densities (low, medium and high) and life stages (5, 12 and 22 days old) consumed by the hydroid *E. crocea* during the course of one feeding cycle (4 h). ♦ = low density (200 larvae L^{-1}), ■ = medium density (400 larvae L^{-1}), ▲= high density (800 larvae L^{-1}).

Seastar predation on juvenile and adult mussels

Whilst *A. amurensis* has clear food preferences, it is a generalist predator able to consume other prey when preferred prey becomes rare (Ross et al. 2002). Since arriving in Australia, it has become dominant as a soft sediment invertebrate predator and is considered a threat to both native communities and commercial shellfish species (Ross et al. 2003; Ross et al. 2006). There are few studies on the relationship between seastars and mussel prey in terms of the response of seastars to prey size and the attack behaviour they display (Norberg & Tedengren 1995). The size of mussel prey eaten by *A. amurensis* can differ according to the quantity of suitably sized prey in the environment and the seastars' level of satiety. However, *A. amurensis* is thought to generally prefer juvenile mussels approximately 20 – 30 mm in length (Lockhart & Ritz 2001b). This is regardless of the availability of larger mussels which may provide them with greater energy profitability, or the size of the seastar where individuals have reached a radius of >50 mm. This correlation between selective feeding by *A. amurensis* and prey selection is thought to be due to the physiological character of the mussels at different ages (Kim 1969).

The decline and rarity of bivalves > 5-10 mm in habitats in Tasmania where *A. amurensis* is present suggests that intensive foraging by *A. amurensis* may be affecting the age structure of prey populations, preventing adult populations from establishing (Lockhart & Ritz 2001a; Lockhart & Ritz 2001b; Ross et al. 2002). Their prey capture rate is approximately one mussel per day, but can increase to several mussels per day depending on their level of satiety. *A. amurensis* eats the most accessible prey first, spending minutes to hours orientating individual mussels in preparation for consumption. They employ a combination of methods to feed on shellfish, pulling the shells using their tube feet to exert enough force to try to create a gap between the shells or widen naturally occurring gaps and weak spots. Once a gap has been established, which may be as little as 0.1 mm, they insert their stomach folds into the gap. The targeted predation of juvenile mussels has important consequences for natural mussel beds, which may provide source populations of spat for commercial mussel farms. Mussel survival into larger sizes is truncated by predation, therefore reducing the pool of reproductive adults and limiting the spat supply. The preference of *A. amurensis* for bivalve molluscs makes it a substantial pest for fisheries and aquaculture (Morrice 1995; Byrne et al. 1997). Intensive foraging by *A. amurensis* on juvenile mussels in commercial mussel farms could result in less stock on-growing to market size, leading to reduced profitability. Larval seastars settling on spat collectors may eventually prove to be problematic if they remain on ropes until they are big enough to prey on mussels. However, if seastars manage to infest mature growout ropes and predation is focussed on younger, smaller mussels, this may

reduce competition for resources including food and space with stock, thereby improving the marketable yield of older mussels (O'Neill et al. 1983). The tangible impacts of seastar predation at different production stages requires significant further experimentation, and currently, it seems that *A. amurensis* is not particularly problematic for mussel farmers in Port Phillip Bay.

Objective 2: Test farm management methods that will discourage and/or avoid biofouling episodes.

Rope type

The black and green spat ropes proved most successful against fouling accumulation, followed by the christmas tree, aqualoop, super christmas tree, cut loop and extreme catch and hold (Figure 13). However, in terms of mussel larvae retention, the black and green spat ropes performed 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; Figure 14).

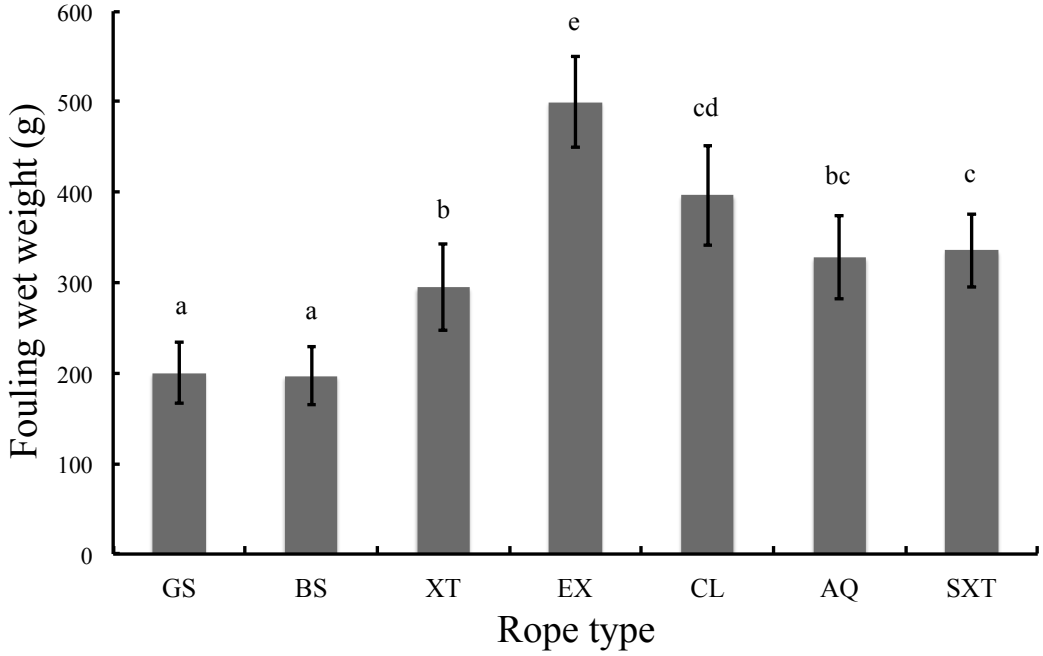


Figure 13: Rope type and fouling accumulation shown as wet weight in grams. GS: green spat; BS: black spat; XT: christmas tree; EX: extreme catch and hold; CL: cut loop; AQ: Aqualoop; SXT: super christmas tree. Bars represent mean ± standard error. Letters indicate significant differences between rope types based on Tukey's tests.

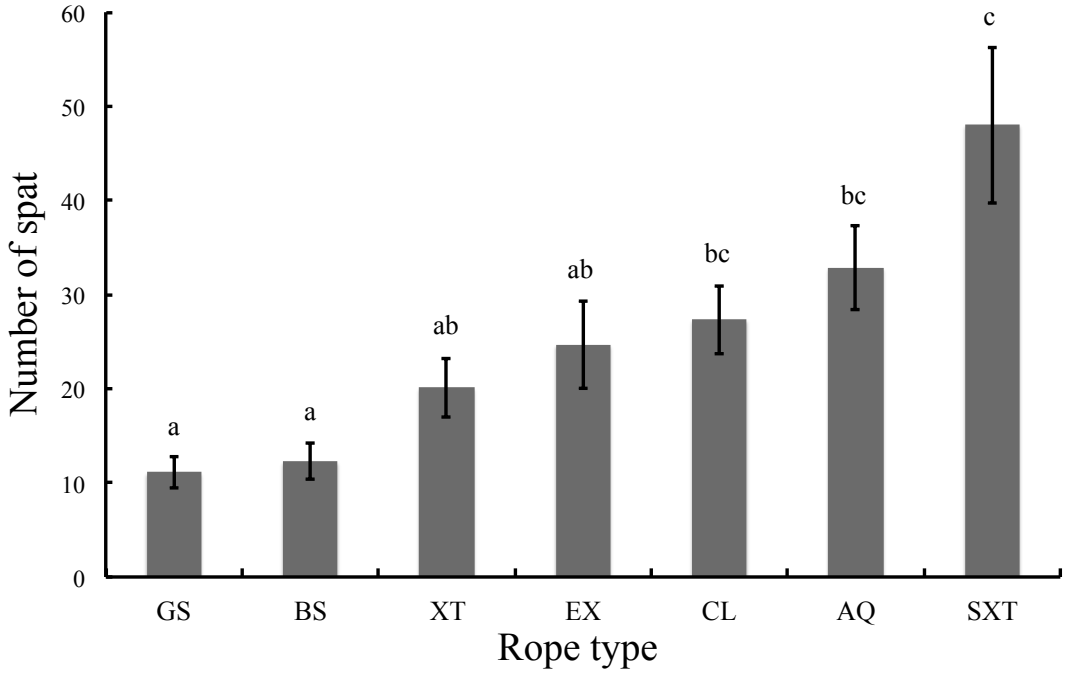


Figure 14: Mean (± standard error) number of spat for each rope type. GS: green spat; BS: black spat; XT: christmas tree; EX: extreme catch and hold; CL: cut loop; AQ: Aqualoop; SXT: super christmas tree. Letters indicate significant differences between rope types based on Tukey's tests.

Density

Accumulation of biofouling varied amongst density treatments with a strong site by treatment by time interaction (Table 4). Low density (200 mussels/m) mussel ropes were the least fouled, followed by medium (400/m) and high (800/m; Figure 15). This was true for both sites, regardless of the number of days immersed. However, low stocking density also exhibited the worst mussel retention, with losses of between 20 and 70% being experienced (Figure 16). Retention was high and fairly consistent between medium and high densities, with little differences between sites despite there also being a strong 3-way interaction (Table 5).

E. crocea settled primarily on ropes with low and medium initial mussel density, and was higher at KPW, with a significant site by time interaction (Figure 17; Table 6). The tubeworm *P. taeniata* only settled on mussels during the final four weeks of experimentation (Figure 18). Settlement was seemingly higher at CS, although no significant difference between sites or among initial densities were observed (Table 7).

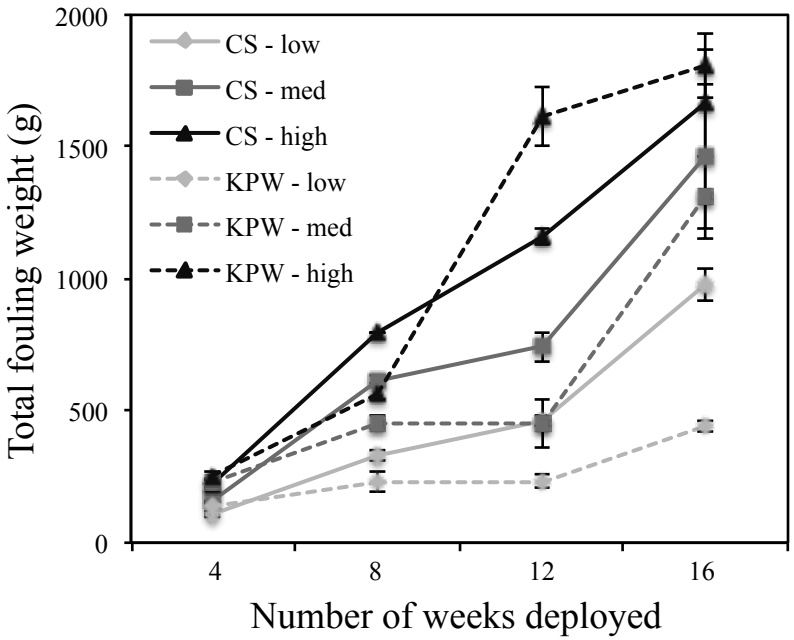


Figure 15: Mean total fouling weight (± standard error) accumulated on ropes stocked at either low (200 mussels/m), medium (400 m/m) or high (800 m/m) densities deployed at Clifton Springs (CS) and Kirk Point Werribee (KPW) for 4, 8, 12 or 16 weeks.

Table 4: Results of a three-way ANOVA comparing the effects of site (Clifton Springs or Kirk Point), density treatment (low: 200 mussels/m; med: 400 m/m; or high: 800 m/m) and time (4, 8, 12, or 16 weeks deployment) on fouling accumulation. All factors were treated as fixed.

Source	df	MS	*F*	p
Site	1	0.025	1.151	0.289
Treatment	2	5.159	235.388	<0.001
Time	3	12.343	563.172	<0.001
Site*Treatment	2	0.165	7.551	0.001
Site*Time	3	0.431	19.680	<0.001
Treatment*Time	6	0.050	2.294	0.050
Site*Treatment*Time	6	0.076	3.463	0.006
Error	48	0.022		

Note: Log transformed data

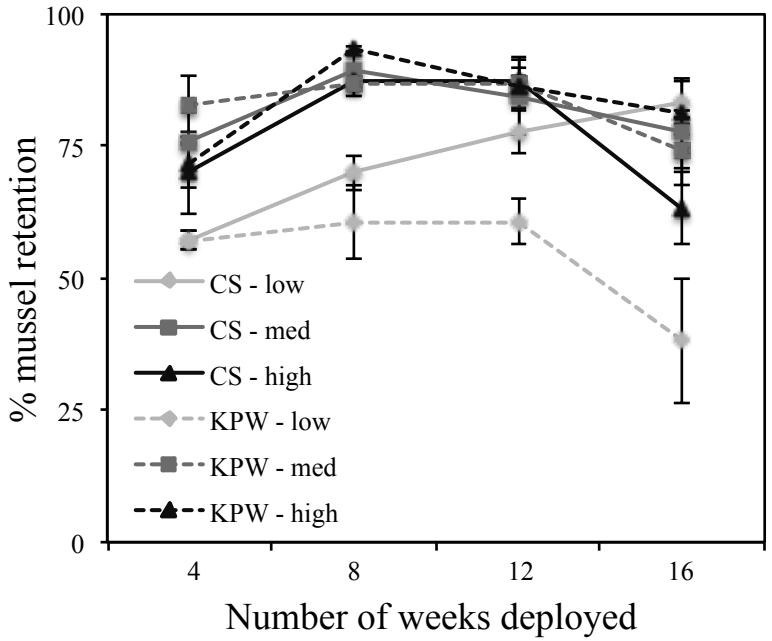


Figure 16: Mean percent mussel retention (± standard error) on ropes stocked at either low (200 mussels/m), medium (400 m/m) or high (800 m/m) densities deployed at Clifton Springs (CS) and Kirk Point Werribee (KPW) for 4, 8, 12 or 16 weeks.

Table 5: Results of a three-way ANOVA comparing the effects of site (Clifton Springs or Kirk Point), density treatment (low: 200 mussels/m; med: 400 m/m; or high: 800 m/m) and time (4, 8, 12, or 16 weeks deployment) on mussel retention. All factors were treated as fixed.

Source	df	MS	*F*	p
Site	1	301.761	3.810	0.057
Treatment	2	2325.888	29.370	<0.001
Time	3	752.094	9.497	<0.001
Site*Treatment	2	830.729	10.490	<0.001
Site*Time	3	146.220	1.846	0.151
Treatment*Time	6	108.269	1.367	0.247
Site*Treatment*Time	6	293.829	3.710	0.004
Error	48	79.194		

Note: Untransformed data

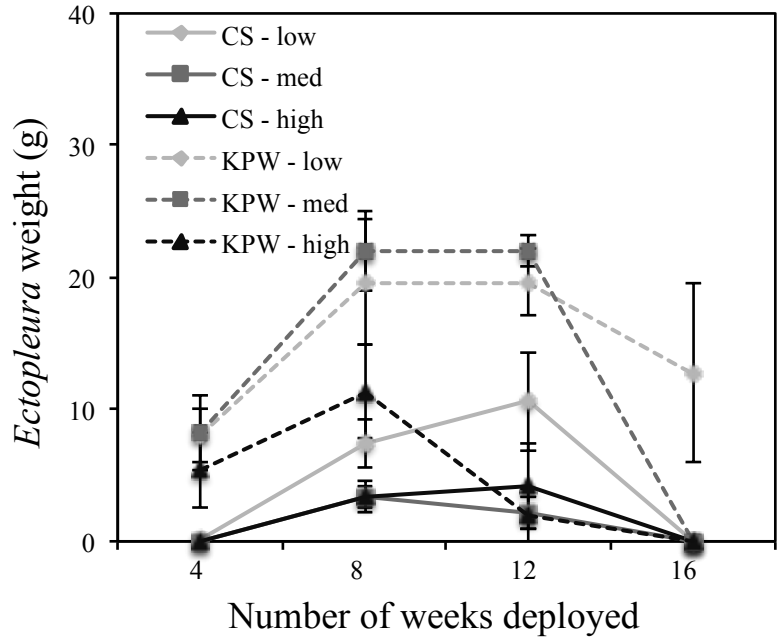


Figure 17: Mean *Ectopleura crocea* weight (± standard error) accumulated on ropes stocked at either low (200 mussels/m), medium (400 m/m) or high (800 m/m) densities deployed at Clifton Springs (CS) and Kirk Point Werribee (KPW) for 4, 8, 12 or 16 weeks.

Table 6: Results of a three-way ANOVA comparing the effects of site (Clifton Springs or Kirk Point), density treatment (low: 200 mussels/m; med: 400 m/m; or high: 800 m/m) and time (4, 8, 12, or 16 weeks deployment) on the quantity of *Ectopleura crocea* on ropes. All factors were treated as fixed.

Source	df	MS	*F*	p
Site	1	7.797	20.471	<0.001
Treatment	2	2.764	7.257	0.002
Time	3	9.714	25.503	<0.001
Site*Treatment	2	0.225	0.592	0.557
Site*Time	3	3.740	9.818	<0.001
Treatment*Time	6	0.262	0.687	0.661
Site*Treatment*Time	6	0.568	1.492	0.201
Error	48	0.381		

Note: Cube root transformed data

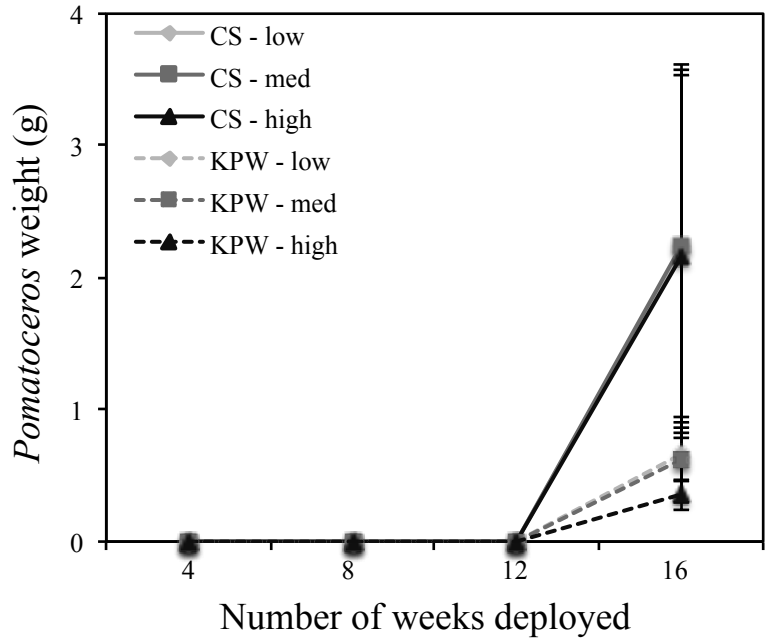


Figure 18: Mean *Pomatoceros taeniata* weight (± standard error) accumulated on ropes stocked at either low (200 mussels/m), medium (400 m/m) or high (800 m/m) densities deployed at Clifton Springs (CS) and Kirk Point Werribee (KPW) for 4, 8, 12 or 16 weeks.

Table 7: Results of a three-way ANOVA comparing the effects of site (Clifton Springs or Kirk Point), density treatment (low: 200 mussels/m; med: 400 m/m; or high: 800 m/m) and time (4, 8, 12, or 16 weeks deployment) on the quantity of *Pomatoceros taeniata* on ropes. All factors were treated as fixed.

Source	df	MS	*F*	p
Site	1	12.404	4.300	0.060
Treatment	2	0.064	0.022	0.978
Site*Treatment	2	0.025	0.009	0.991
Error	12	2.885		

Note: Log transformed data. Only last time period when worms were present

Depth

Five different taxa accumulated on the plates during the experiment. Overall fouling was approximately 40% less at 10 m depth compared to 5 m ($F_{1, 13} = 12.1$, $p<0.001$; Figure 19). The problematic fouling hydroid *E. crocea* covered more than 20% of the plates but was completely absent at 10 m. Of the other species, *E. georgiana* was more abundant at 10 m ($F_{1, 13} = 5.50$, $p = 0.04$) and *A. aureoradiata* more abundant at 5 m ($F_{1, 13} = 9.00$, $p = 0.01$).

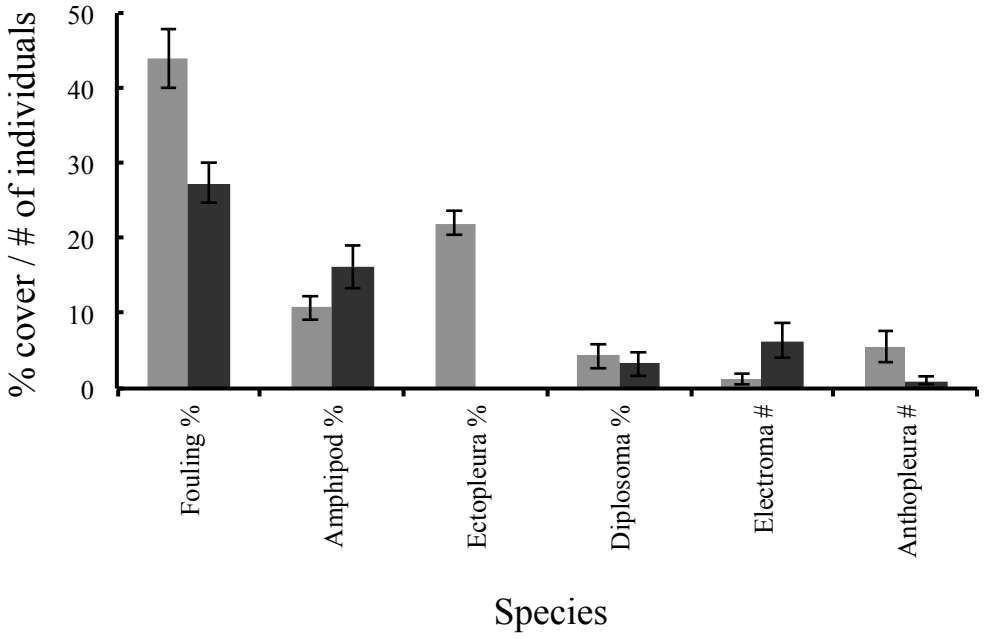


Figure 19: Mean percent cover (± standard error) of total biofouling, amphipod tubes, *Electroma georgiana, Ectopleura crocea, Diplosoma listerianum* and *Anthopleura aureoradiata* at shallow (5 m; light grey) and deep (10 m; dark grey) zones of the water column.

Biofouling patterns

A diverse fouling assemblage of 16 species belonging to seven phyla covered the plates within the three mussel aquaculture zones from July 2011 to April 2013. The six most abundant species were tubiculous amphipods, *D. listerianum*, *E. crocea*, *C. intestinalis*, *P. taeniata* and *M. galloprovincialis*.

Tube-dwelling Corophiidae amphipods were common colonists at all farms throughout the study period (Figure 20). Pinnace Channel typically exhibited higher cover of amphipod tubes compared to Clifton Springs and Kirk Point, and peaked at just below 70% cover for the September 2012 collection period. In general, cover was greatest during the cooler times of the year.

Cover of the ascidian *D. listerianum* was highly variable, with continual changes in the farm that exhibited the highest cover of this ascidian (Figure 20). Although a common constituent of the communities developing on plates, commonly covering >30% of the available space, no discernable temporal or spatial patterns were evident, and each of the farms recorded the heaviest fouling by this species on at least one survey. *D. listerianum* also recruited over much of the year.

E. crocea exhibited no distinct settlement period (Figure 20). Cover was highest at Kirk Point throughout most of the study and peaked during the April 2011 period at 75%. Besides the September 2011 and February 2013 periods, *E. crocea* colonies were absent on plates from Pinnace Channel. Cover during late 2011 and early 2012 appeared to be greater than during late 2012 and early 2013.

C. intestinalis was completely absent at PC throughout the study period and was only recorded at Kirk Point during the February period in 2012 when all but one plate was lost. During this period at Clifton Springs, *C. intestinalis* was present in high numbers during both 2012 and 2013 (58–60% cover; Figure 20). Lower numbers were also present on plates from Clifton Springs during the June period (10% cover).

Similarly, cover of *P. taeniata* varied through time, and peaked during spring (September/October) of both years (Figure 20). These worms were common at Clifton Springs during both 2012 and 2013, only common at Kirk Point in 2012, and consistently rare at Pinnace Channel.

Mussel spat recruitment was temporally and spatially restricted in Port Phillip Bay, with Clifton Springs clearly exhibiting highest recruitment during the December period (Figure 20). Here, cover was highest in 2011 (29%) compared to Kirk Point (1%) and Pinnace Channel (3%), and also in 2012 (30%) compared to Kirk Point (5%) and Pinnace Channel (2%).

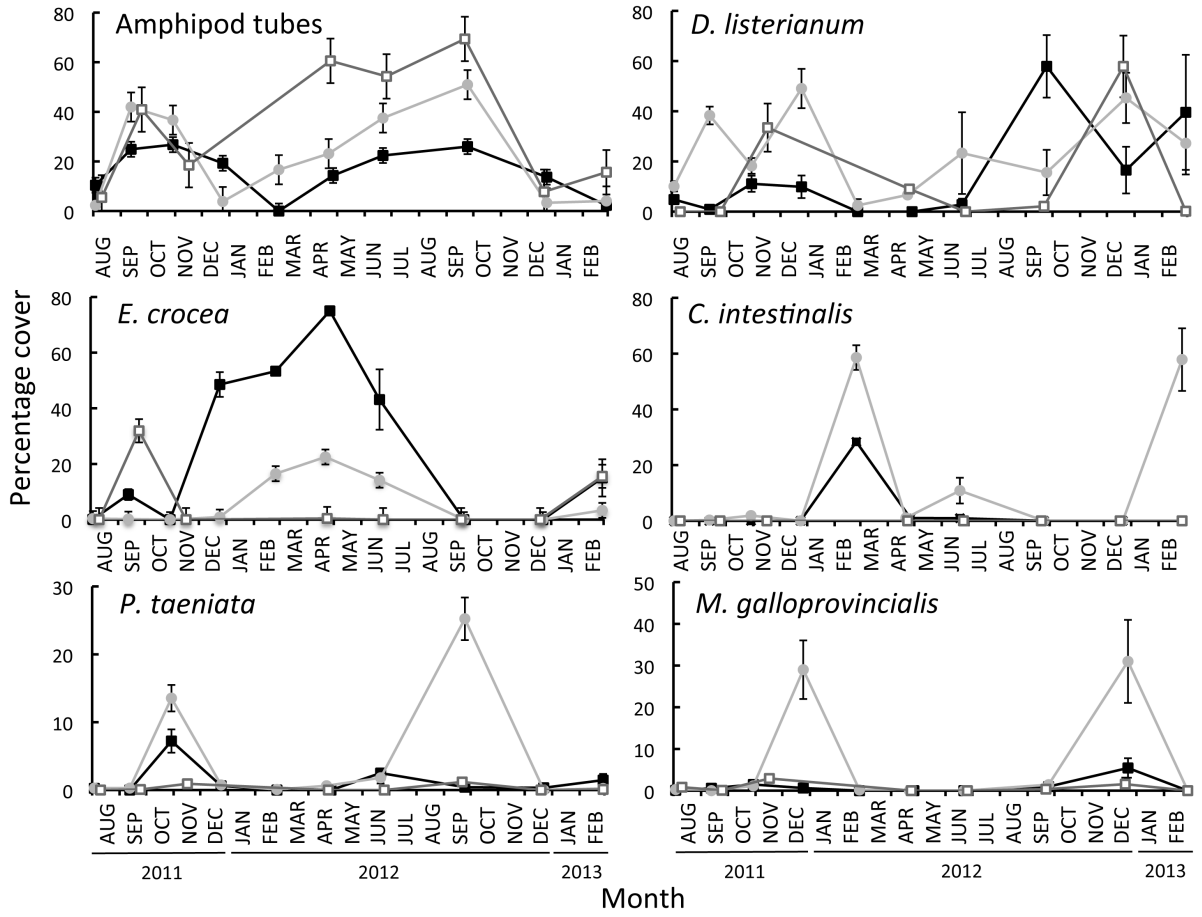


Figure 20: Mean percentage cover (± standard error) of the six most abundant species at Clifton Springs (light grey circles), Kirk Point (black squares) and Pinnace Channel (dark grey open squares). The middle point between plate deployment and collection was used to assign collection periods into months. Note the varying scales of the y-axes.

Objective 3: Test the effectiveness of existing and new biofouling treatment methods to develop cost-efficient, implementable on-farm treatments.

Individual treatments

The results of treatments are discussed individually for each species, to allow direct comparisons between all treatment methods for each species to be made. In all cases, short-term exposure to regular tap freshwater was ineffective at causing mortality of the tested fouling organisms (besides *C. intestinalis* at 30 s duration) and also had no deleterious effects on shellfish survival. It shall therefore not be discussed further.

E. crocea

All treatments were very successful against *E. crocea* causing 100% mortality with the exception of a 10 s immersion in ambient 2% citric acid which caused only 40% mortality (Figure 21).

C. intestinalis

Immersion in 40 ^{o}C seawater for 10 or 30 s induced some mortality (approx. 66%), but a longer exposure to this temperature for 60 s, or temperatures of 50 ^{o}C and higher for any length of time caused 100% mortality (Figure 22). 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. *C. intestinalis* was unaffected by hydrated lime concentrations of 4%, but 6% resulted in 50% mortality whether exposed to air pre- and post-treatment or not.

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 (Figure 23). At ambient temperature, 2 and 5% acetic acid killed roughly half of the ascidians. 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 ascidians. A similar pattern was observed for 5% acetic acid at 40 °C. Near 100% mortality was observed for 50 °C treatments regardless of duration or acetic acid concentration. Like acetic acid, under ambient temperatures, citric acid killed roughly 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. *S. clava* was not affected by 4% hydrated lime unless there was a pre- and post-treatment air drying. Only during 60 s dips with 30 s pre- and post-treatment air drying was some mortality observed. A solution of 6% hydrated lime resulted in >50% mortality for all treatment combinations tested.

M. galloprovincialis (30 mm)

Temperatures of 40 °C for 10, 30 or 60 s, and 50 °C for 10 s showed little to no mortality of mussels (Figure 24). Any duration above 10 s for 50 and any length of time at 60 °C resulted in 100% mortality. The only significant mortality in the acetic acid treatments occurred at 50 °C, with slightly higher mortality at the higher acid concentration. Results from the citric acid trials suggest, similarly 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 little 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%.

M. galloprovincialis (60 mm)

Adult 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 (Figure 25). Acetic acid only killed mussel at 50 °C. Citric acid appeared to have some impacts, often inducing 20-40% mortality when exposure time was 30 s or higher. Hydrated lime treatments (4 and 6% for 60 s, and for 30 s air, 60 s dip, 30 s air) did not result in any mussel mortality.

O. angasi (15 mm)

Heated treatments only induced oyster mortality at 50 and 60 °C; 100% mortality occurred at both these temperatures following a 30 or 60 s immersion, while a 10 s dip only killed 40% of oysters for the 60 °C treatment (Figure 26). Acetic acid was only effective at 50 °C regardless of concentration. Similarly, citric acid was by far most effective at 50 °C, with 100% mortality at this temperature for 30 s regardless of concentration.

O. angasi (50 mm)

Very similar results were found for 15 and 50 mm *O. angasi.* 50 and 60 °C for 30 s or longer killed all oysters (Figure 27). Only 50 °C for 30 s resulted in 100% mortality for the acetic acid treatments. Similarly, total mortality was only found for 50 °C for 30 s for all citric acid treatments.

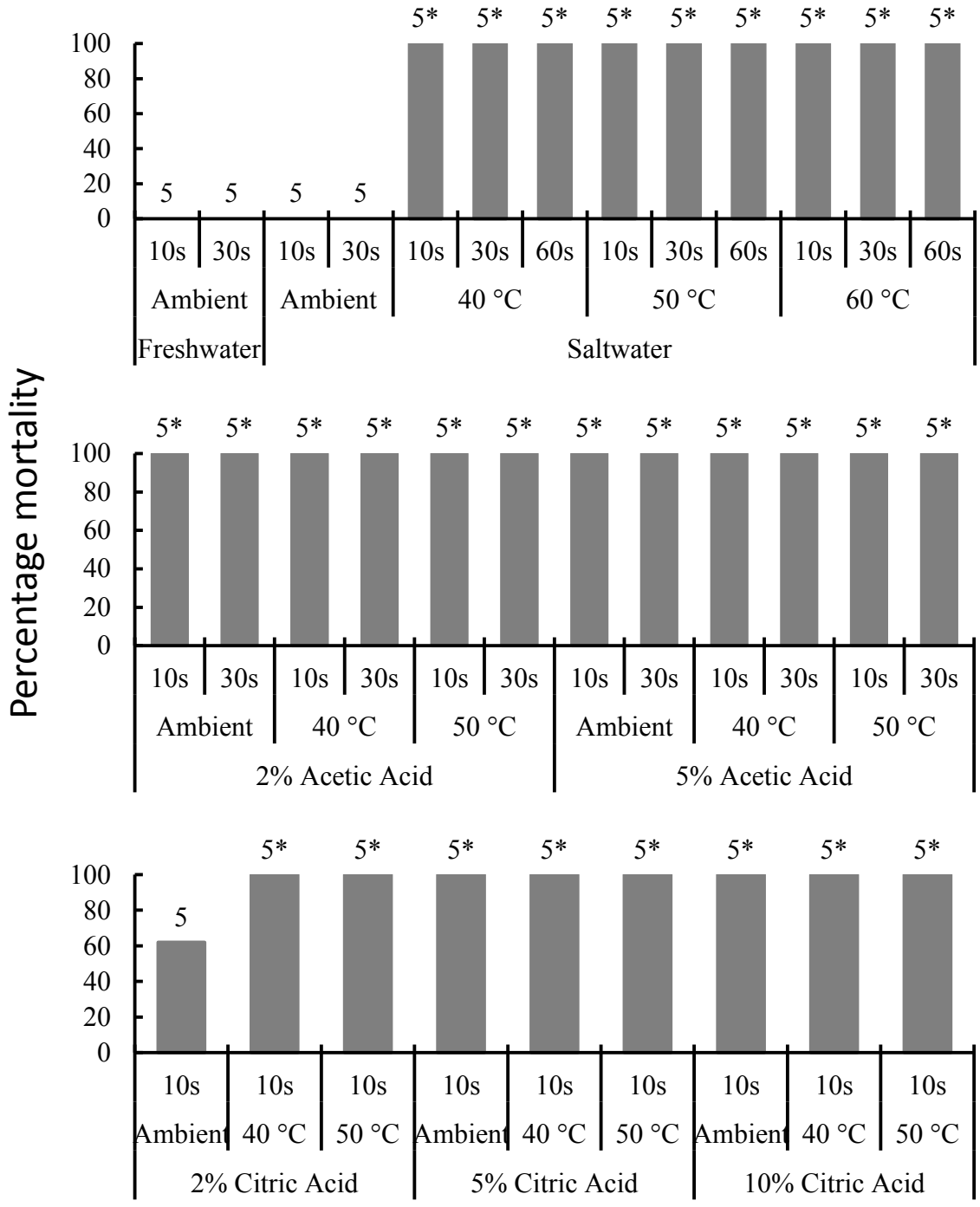


Figure 21. Percentage mortality of *Ectopleura crocea* when exposed to freshwater, heated saltwater, acetic acid solutions and citric acid solutions. N-values represented by numbers above the bars. Asterisks represent significant difference based on Fisher's tests comparing treatment to control.

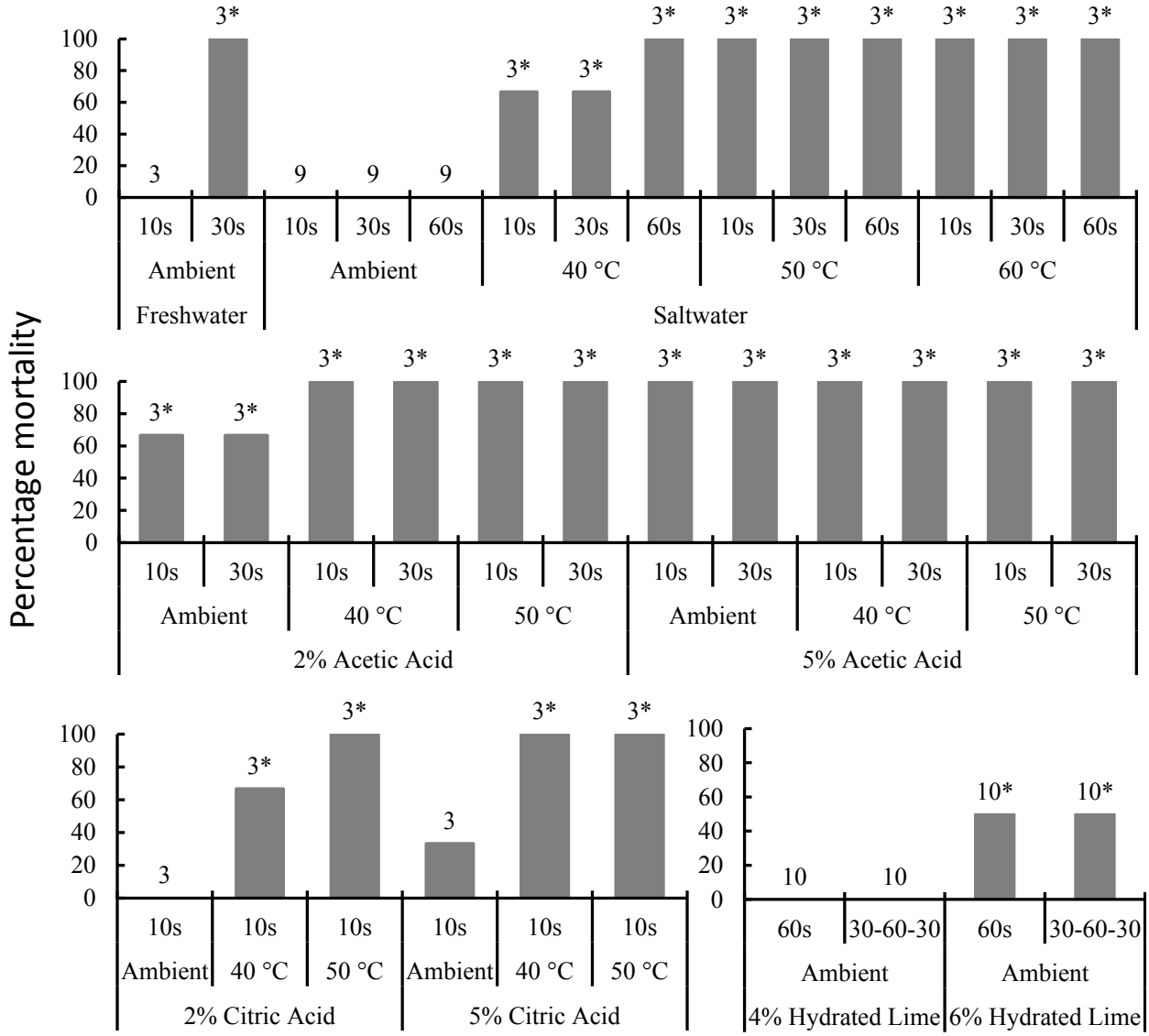


Figure 22. Percentage mortality of *Ciona intestinalis* when exposed to freshwater, heated saltwater, acetic acid solutions, citric acid solutions and hydrated lime solutions. N-values represented by numbers above the bars. Asterisks represent significant difference based on Fisher's tests comparing treatment to control.

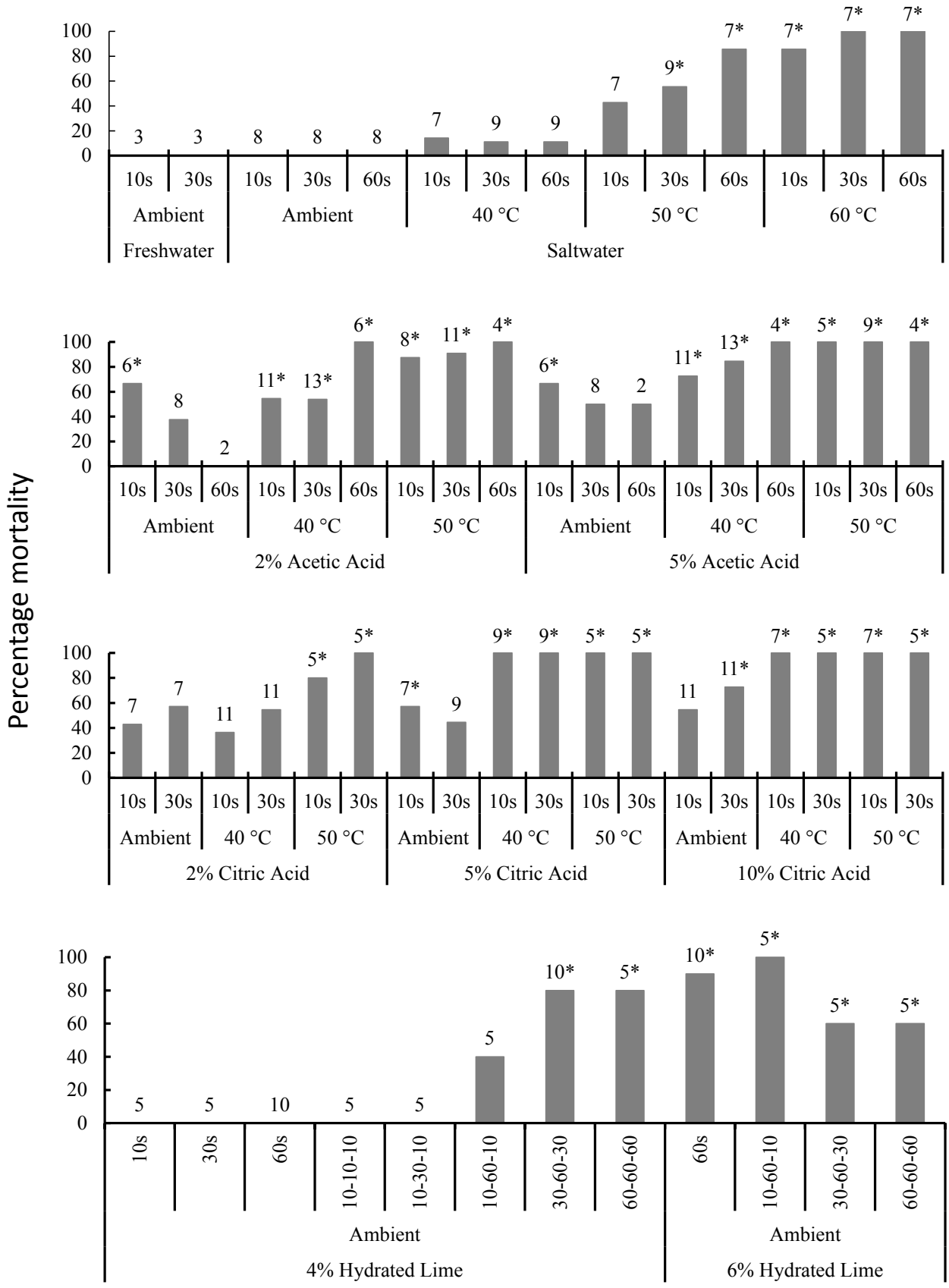


Figure 23. Percentage mortality of *Styela clava* when exposed to freshwater, heated saltwater, acetic acid solutions, citric acid solutions and hydrated lime solutions. N-values represented by numbers above the bars. Asterisks represent significant difference based on Fisher's tests comparing treatment to control.

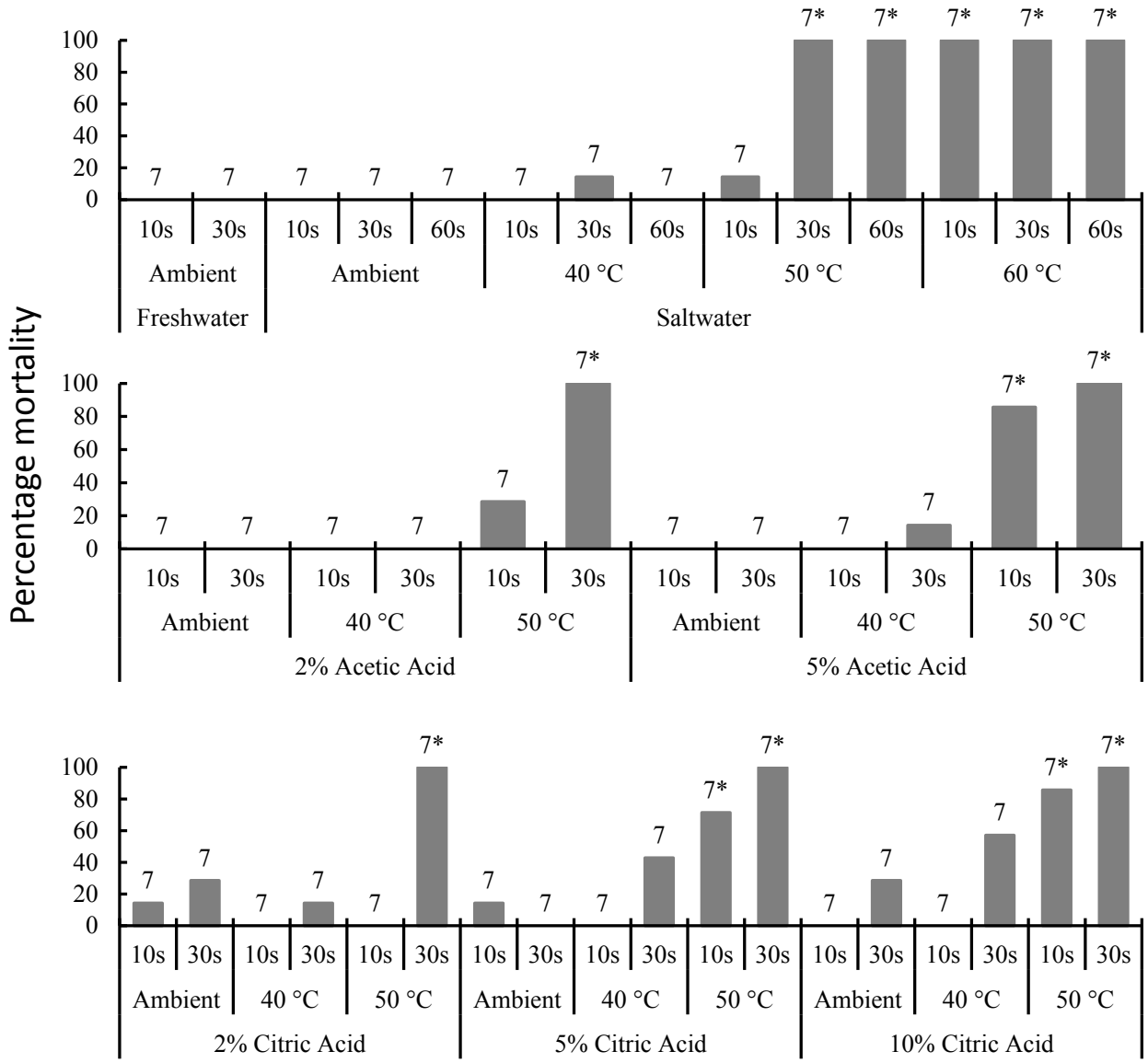


Figure 24. Percentage mortality of 30mm *Mytilus galloprovincialis* when exposed to freshwater, heated saltwater, acetic acid solutions and citric acid solutions. N-values represented by numbers above the bars. Asterisks represent significant difference based on Fisher's tests comparing treatment to control.

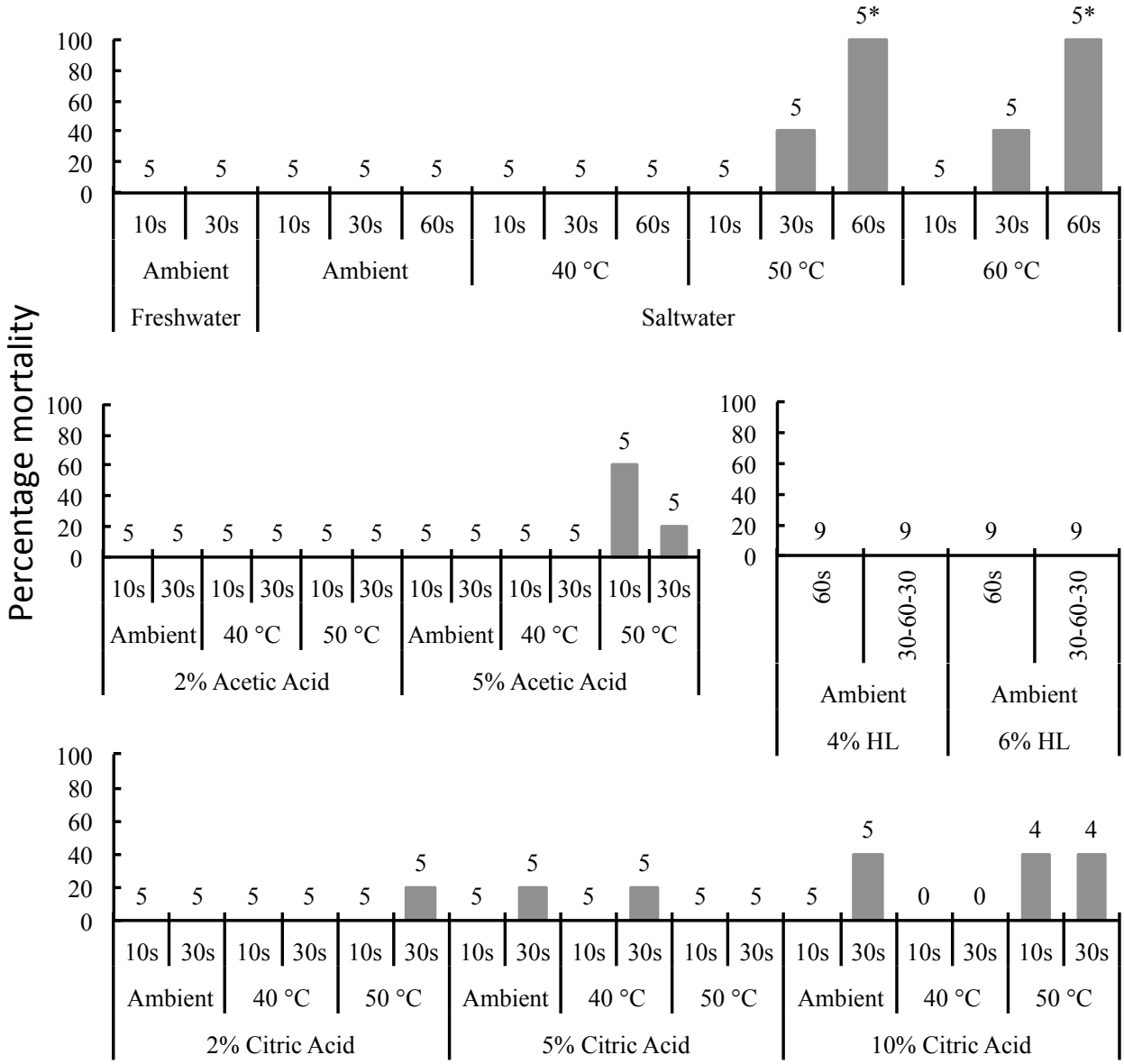


Figure 25. Percentage mortality of 60mm *Mytilus galloprovincialis* when exposed to freshwater, heated saltwater, acetic acid solutions, hydrated lime and citric acid solutions. N-values represented by numbers above the bars. Asterisks represent significant difference based on Fisher's tests comparing treatment to control.

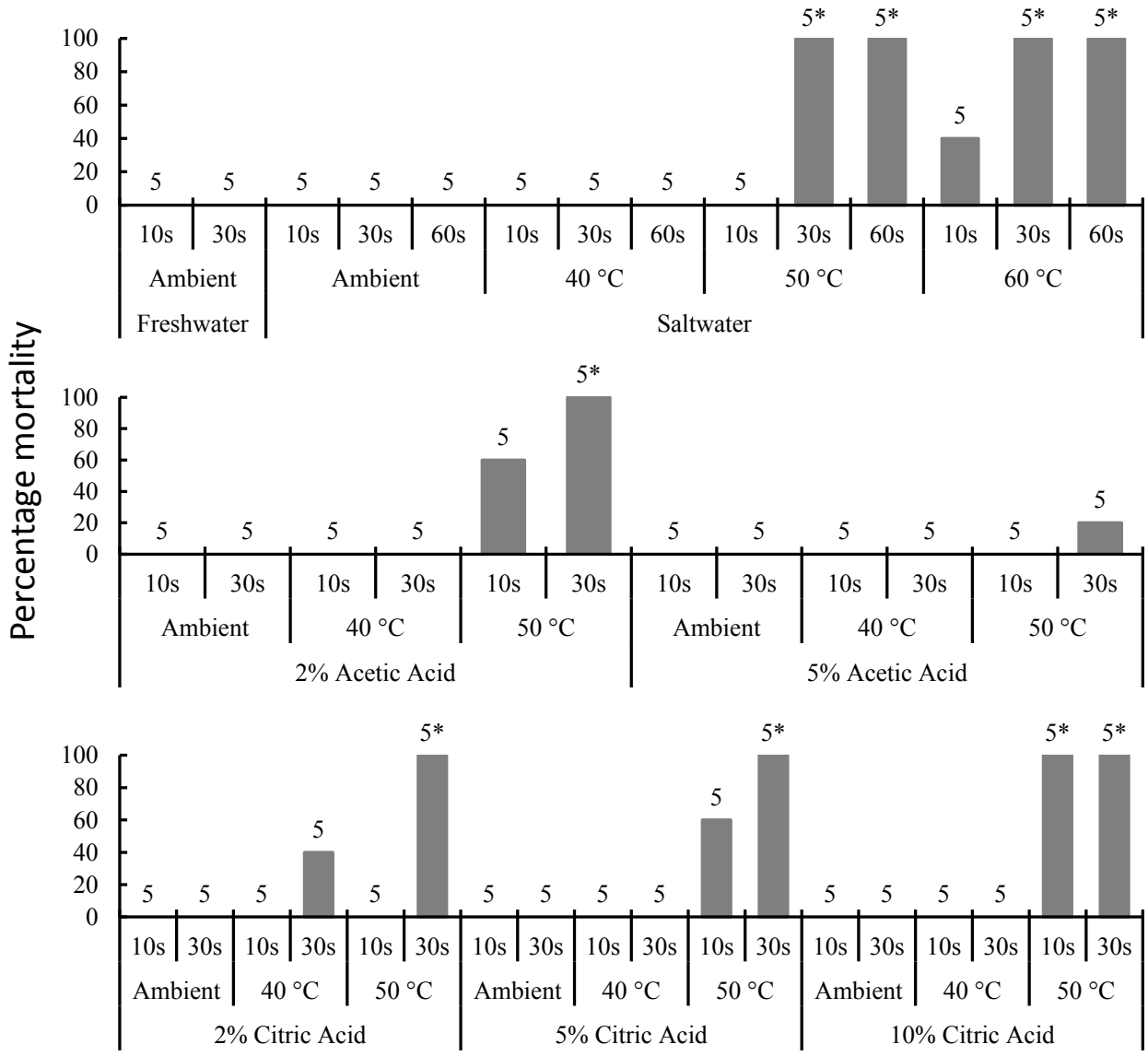


Figure 26. Percentage mortality of 15mm *Ostrea angasi* when exposed to freshwater, heated saltwater, acetic acid solutions and citric acid solutions. N-values represented by numbers above the bars. Asterisks represent significant difference based on Fisher's tests comparing treatment to control.

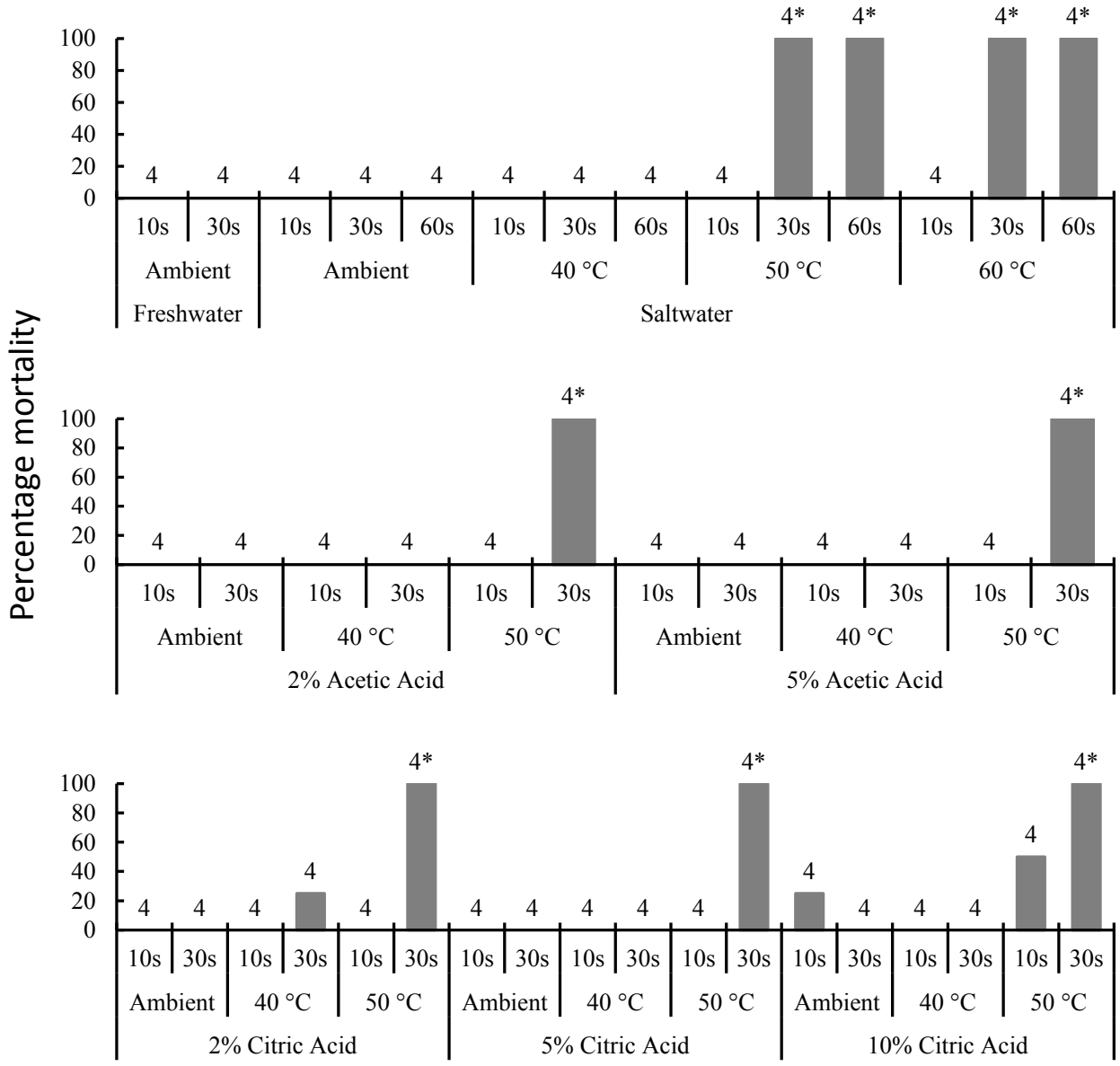


Figure 27. Percentage mortality of 50mm *Ostrea angasi* when exposed to freshwater, heated saltwater, acetic acid solutions and citric acid solutions. N-values represented by numbers above the bars. Asterisks represent significant difference based on Fisher's tests comparing treatment to control.

On-shore treatment trials

Mussel survival

All of the on-shore treatment trials brought about some degree of *M. galloprovincialis* mortality (Figure 28). Exposure to 40 ^{o}C seawater for 60 s, and a 30 s immersion in a 2% acetic acid solution heated to 40 ^{o}C brought about low levels of mortality, comparable to control ropes (approximately 3%; Table 8). The greatest treatment effects were seen in ropes exposed to a 30 s immersion in a 5% acetic acid solution, where on average more than 10 % of mussels died. The 6% hydrated lime treatment, combined with air exposure, resulted in approximately 5% mortality (Figure 28).

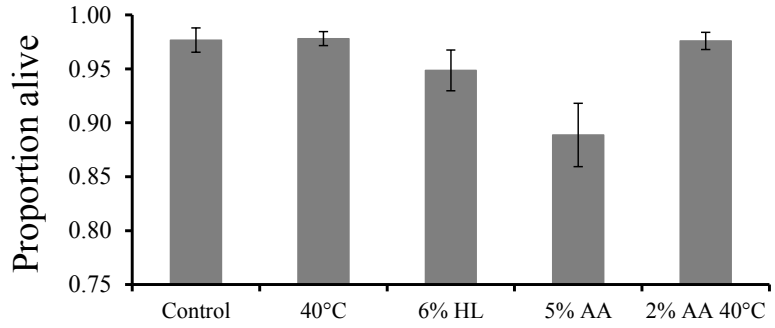


Figure 28. Proportion of *Mytilus galloprovincialis* surviving exposure to selected treatments during on-shore trials. 40 °C = 60 s immersion in 40 °C seawater; 6% HL = air dried for 30 s, dipped for 60 s in a 6% hydrated lime solution, and air dried for a further 30 s; 5% AA = 30 s immersion in a 5% acetic acid solution; 2% AA 40 °C = 30 s immersion in a 2% acetic acid solution heated to 40 °C.

Biofouling survival

Fouling on the rope sections consisted of colonial tunicates (*D. listerianum),* hydroids *(A. operculata),* juvenile mussels (*M. galloprovincialis*), little wing pearl shells *(Electroma georgiana)*, coral worms (*P taeniata)*, juvenile Northern pacific seastars *(A amurensis)*, sponges (*Sycon* sp.), and polychaete worms.

All treatments affected the survival of the colonial tunicate *D. listerianum* (Figure 29), an ubiquitous species that although doesn't appear to currently impact production in Port Phillip Bay, it does elsewhere (Gittenberger 2009). Acetic acid was particularly effective, with both the heated and unheated options completely eliminating the tunicate (Table 8). The hydrated lime treatment reduced biomass by two-thirds, however this was not significant ($F_{1, 20} = 1.30$, $p = 0.27$). Exposure to 40°C seawater reduced biomass by approximately 15%. None of the treatments significantly affected the biomass of the hydroid *A. operculata* (Figure 29; Table 8).

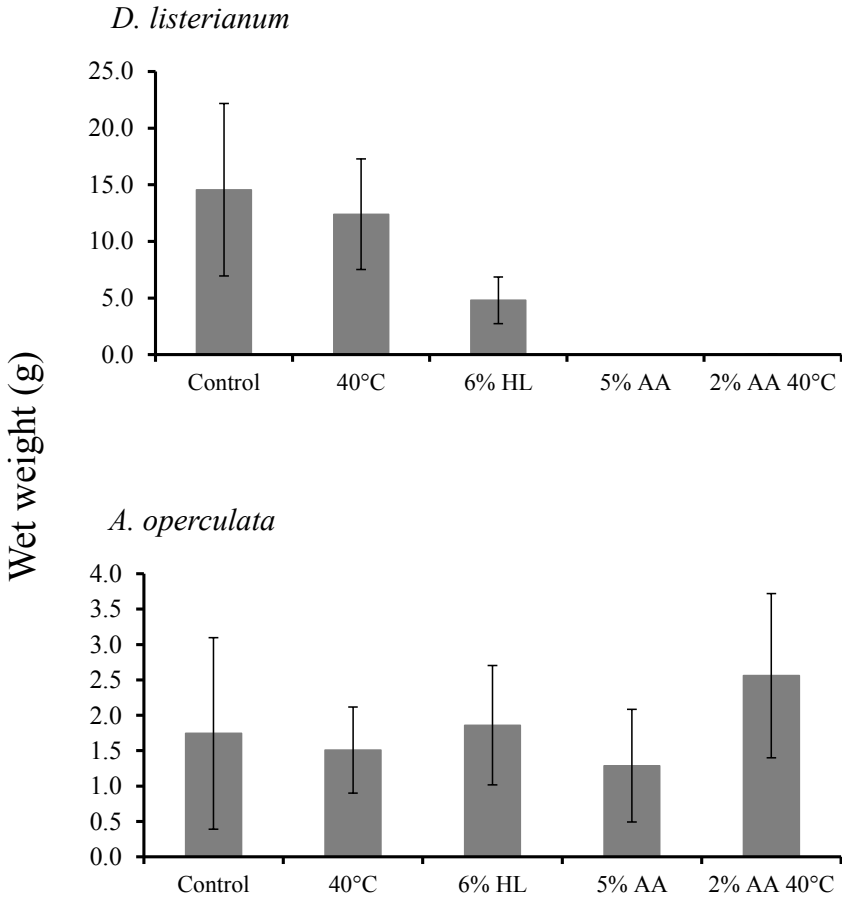


Figure 29. Wet weight of live *Diplosoma listerianum* and *Amphisbetia operculata* on rope sections after exposure to selected treatments during on-shore trials. 40 °C = 60 s immersion in 40 °C seawater; 6% HL = air dried for 30 s, dipped for 60 s in a 6% hydrated lime solution, and air dried for a further 30 s; 5% AA = 30 s immersion in a 5% acetic acid solution; 2% AA 40 °C = 30 s immersion in a 2% acetic acid solution heated to 40 °C.

Table 8. Results of *a priori* tests comparing treatment to control ropes for the proportion of adult mussels alive, and the quantity (g) of alive *Diplosoma listerianum* and *Amphisbetia operculata*. 40 °C = 60 s immersion in 40 °C seawater; 6% HL = air dried for 30 s, dipped for 60 s in a 6% hydrated lime solution, and air dried for a further 30 s; 5% AA = 30 s immersion in a 5% acetic acid solution; 2% AA 40 °C = 30 s immersion in a 2% acetic acid solution heated to 40 °C.

Source of variation	df	Propn mussels alive*		*Diplosoma***		*Amphisbetia***	
		F	p	*F*	p	*F*	p
Control v 40°C	1	0.003	0.995	0.003	0.955	0.063	0.804
Error	20		0.009		0.743		0.602
Control v 6%HL	1	1.483	0.238	1.303	0.267	0.093	0.763
Error	20		0.009		0.743		0.602
Control v 5%AA	1	13.855	**0.001**	15.996	**0.001**	0.042	0.839
Error	20		0.009		0.743		0.602
Control v 2%AA40°C	1	0.002	0.969	15.514	**0.001**	0.372	0.549
Error	20		0.009		0.743		0.602

Note: *Exponential transformation, **Log transformation; MS error is listed below p-values

Hydrated lime and 40 °C seawater both resulted in approximately 50% mortality of juvenile *M. galloprovincialis* fouling the mussel ropes (Figure 30; Table 9). Exposure to a 5% acetic acid solution was also reasonably effective, bringing about 40% mortality. The tube worm *P. taeniata* responded little to the treatments, with each resulting in only 10 - 20% mortality (Figure 30; Table 9). Heating seawater to 40 °C resulted in mortality at the upper end of the scale. Hydrated lime was the most effective treatment against the little wing pearl shell *E. georgiana* (approx. 55% mortality), followed by 2% acetic acid heated to 40 °C (52%) and a 5% acetic acid solution (48%). Heated seawater alone was ineffective, resulting in the same level of mortality observed in control ropes (Figure 30; Table 9).

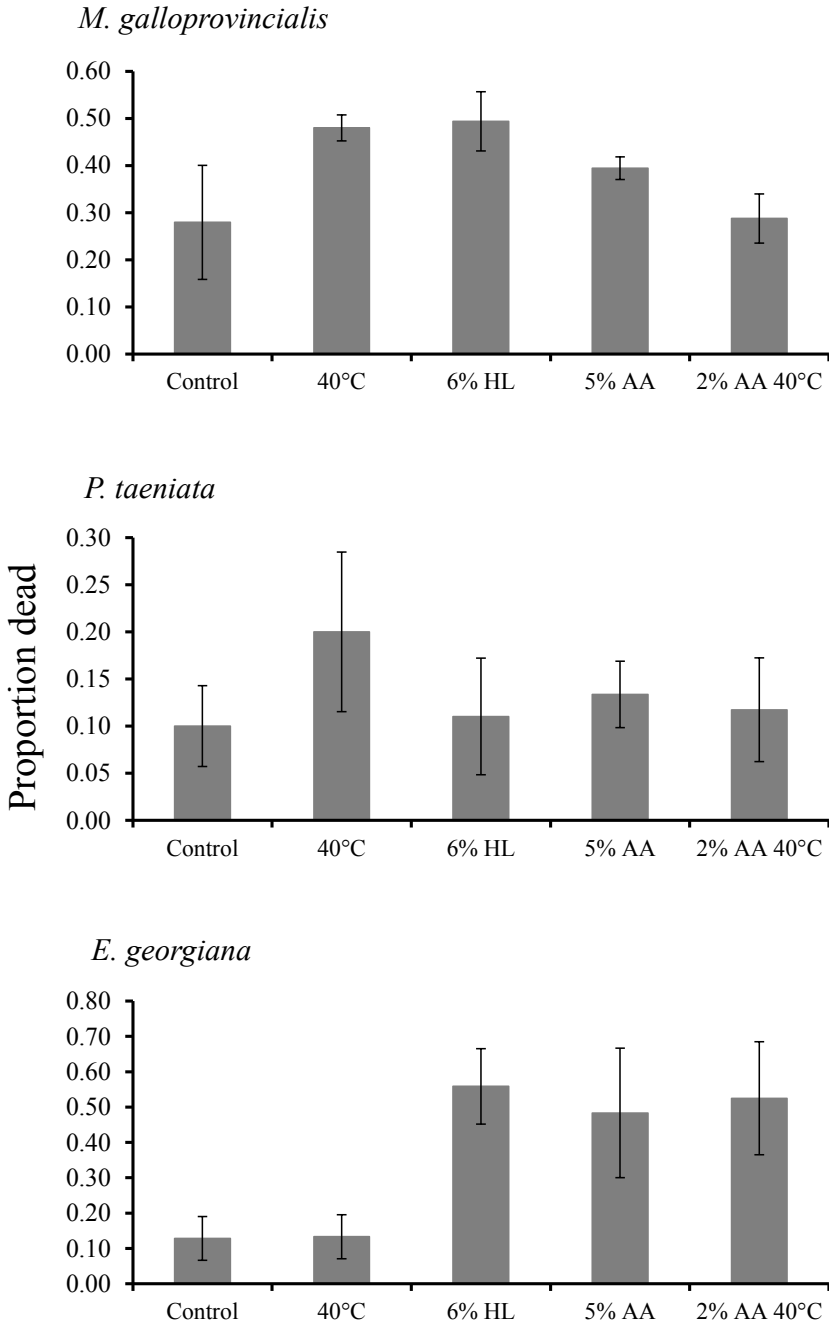


Figure 30. Proportion of *Mytilus galloprovincialis*, *Pomatoceros taeniata* and *Electroma georgiana* suffering mortality from exposure to selected treatments on rope sections during on-shore trials. 40 °C = 60 s immersion in 40 °C seawater; 6% HL = air dried for 30 s, dipped for 60 s in a 6% hydrated lime solution, and air dried for a further 30 s; 5% AA = 30 s immersion in a 5% acetic acid solution; 2% AA 40 °C = 30 s immersion in a 2% acetic acid solution heated to 40 °C.

Table 9. Results of *a priori* tests comparing treatment to control ropes for the proportion of mussel spat, *Pomatoceros taeniata* and *Electroma georgiana* dead. 40 °C = 60 s immersion in 40°C seawater; 6% HL = air dried for 30 s, dipped for 60 s in a 6% hydrated lime solution, and air dried for a further 30 s; 5% AA = 30 s immersion in a 5% acetic acid solution; 2% AA 40 °C = 30 s immersion in a 2% acetic acid solution heated to 40 °C.

Source of variation		Propn spat dead		Propn *Pomato.* dead		Propn *Electroma* dead	
	df	*F*	p	*F*	p	*F*	p
Control v 40°C	1	4.45	**0.048**	1.461	0.241	0.001	0.979
Error	20		0.023		0.017		0.078
Control v 6%HL	1	5.075	**0.036**	0.015	0.903	5.896	**0.025**
Error	20		0.023		0.017		0.078
Control v 5%AA	1	1.464	0.24	0.166	0.688	4.013	0.059
Error	20		0.023		0.017		0.078
Control v 2%AA40°C	1	0.008	0.931	0.044	0.836	5.011	**0.037**
Error	20		0.023		0.017		0.078

Note: MS error is listed below p-values

The seastar *A. amurensis* and the sponge *Sycon* sp. were completely eliminated from ropes exposed to the hydrated lime and acetic acid treatments (Figure 31). Heating seawater to 40 °C had no significant impact on seastar presence, but reduced the number of sponges by two thirds (Table 10). Hydrated lime and acetic acid also eliminated polychaete worms on the rope sections, although there were some small levels of survival when 2% acetic acid was heated to 40 °C. Heat alone was not a successful treatment to bring about mortality in polychaetes (Figure 31).

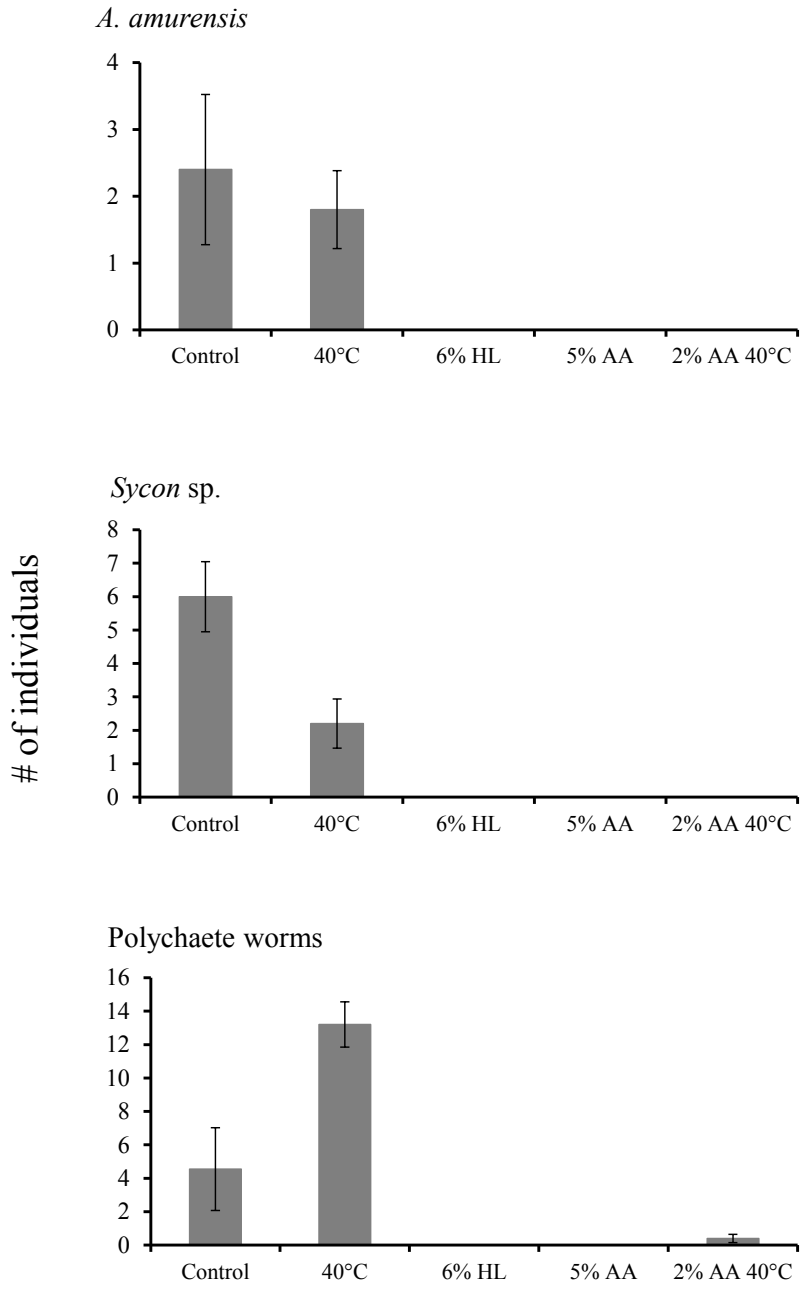


Figure 31. Number of living individuals of *Asterias amurensis*, *Sycon* sp. and polychaete worms on rope sections after exposure to selected treatments during on-shore trials. 40 °C = 60 s immersion in 40 °C seawater; 6% HL = air dried for 30 s, dipped for 60 s in a 6% hydrated lime solution, and air dried for a further 30 s; 5% AA = 30 s immersion in a 5% acetic acid solution; 2% AA 40 °C = 30 s immersion in a 2% acetic acid solution heated to 40 °C.

Table 10. Results of *a priori* tests comparing treatment to control ropes for the number of *Asterias amurensis*, *Sycon* sp. and polychaete worms alive. 40 °C = 60 s immersion in 40 °C seawater; 6% HL = air dried for 30 s, dipped for 60 s in a 6% hydrated lime solution, and air dried for a further 30 s; 5% AA = 30 s immersion in a 5% acetic acid solution; 2% AA 40 °C = 30 s immersion in a 2% acetic acid solution heated to 40 °C.

Source of variation		*Asterias*		*Sycon*		Polychaete	
	df	*F*	p	*F*	p	*F*	p
Control v 40°C	1	0.562	0.462	22.012	**<0.001**	20.856	**<0.001**
Error	20		1.6		1.640		8.06
Control v 6%HL	1	9.000	**0.007**	54.878	**<0.001**	142.047	**<0.001**
Error	20		1.6		1.640		8.06
Control v 5%AA	1	9.000	**0.007**	54.878	**<0.001**	142.047	**<0.001**
Error	20		1.6		1.640		8.06
Control v 2%AA40°C	1	9.000	**0.007**	54.878	**<0.001**	136.787	**<0.001**
Error	20		1.6		1.640		8.06

Note: MS error is listed below p-values

Discussion

Measure the effects of key biofouling species on mussel spat survival and grow-out.

Inoculating mussel ropes with low to medium densities of *C. intestinalis*, *E. crocea* and *S. clava* determined that fouling by these species reduced shell growth and flesh weight in the cultured mussel *M. galloprovincialis*. In two months, fouled mussels grew 1.6–3.0 mm less than unfouled mussels; such growth reductions will increase the time until mussels reach marketable size. Reductions in flesh weight, and thus edible product, are also of importance to the industry. Fouling by the three species reduced mussel flesh weight to varying degrees, with decreases in flesh weight ranging from 8 to 21%. Flesh weight and condition were only significantly reduced in the small mussels, potentially because the same quantity of fouling organisms were used in small and large treatments, resulting in higher levels of fouling relative to mussel mass in the small treatments. In addition, higher filtration rates and superior competition for resources in larger mussels (Thompson & Bayne 1974; Riisgard & Randlov 1981) may have resulted in less pronounced reductions in flesh weights and thus, condition in larger mussels. The lack of decreased condition in most mussels suggests that any decrease in meat yield is likely directly attributable to smaller overall mussel size. The manipulative experiments here confirm several observational/mensurative studies investigating fouling on mussel lines (de Sa et al. 2007; Daigle & Herbinger 2009; Fitridge 2011; Fitridge & Keough 2013).

Physiological trade-offs occur when organisms are under stress, whereby energy expenditure is allocated away from growth and reproduction, and instead, put towards physiological defences that increase survival (Stearns 1992). Reduced growth and a lower gonadosomatic index have been observed in mussels that experienced reduced food availability and longer aerial exposure (Petes et al. 2008). These stressed mussels allocated energy away from shell, somatic and gonadal growth, and instead, likely invested in costly physiological defences (Petes et al. 2008). In addition, mussels exposed to low food availability are unable to maintain ripe gametes, have lower fecundity and produce smaller eggs (Bayne et al. 1978). Exploitative or interference food competition between mussels and the three fouling species tested in this study may have led to decreased food consumption, resulting in the reallocation of energy away from growth and reproductive output, more energy used for physiological defences aimed at increasing survival (and thus, future reproduction) and adult mussels with unripe gametes that produce fewer and smaller eggs. These outcomes, coupled with lower overall energy obtained to use for additional processes, would manifest into mussels with reduced flesh weights and smaller morphometric parameters compared to unfouled mussels with a potentially higher food intake. The results presented here demonstrate the effect of the fouling species on mussel shell growth and flesh weights at a specific density of fouling. Changes in the level of fouling would likely alter the magnitude of shell growth and flesh weight reductions, and strong negative correlations between *C. intestinalis* density and marketable mussel product have been found (Daigle & Herbinger 2009). As the density of foulers represented approximately low-level to medium-level fouling, the detrimental effects these species have in mussel aquaculture are likely more severe than found here, although whether the magnitude of the effects are directly proportional to the level of fouling is unknown.

Fouling by *E. crocea* in the commercial culture of *M. galloprovincialis* may be significant at several stages in the production cycle, not only deleteriously affecting growing mussels, but consuming critical supplies of wild mussel larvae as prey. *E. crocea* colonies captured an assortment of prey items. Although it is difficult to assess with certainty the actual 'availability' of planktonic organisms to *E. crocea* as prey items, all of the taxa identified in this study are known prey of tubulariid hydroids (Gili et al. 1996; Genzano 2005). The most common items were various crustaceans, diatoms and bivalves. Pelagic cnidarians are known to consume bivalve larvae (Purcell et al. 1991) and the ingestion of bivalves is also reported in benthic cnidarians. For example, Coma et al. (1999) report that bivalve larvae contributed 14% to the diet of the benthic coral reef hydrozoan *Nemalecium lighti*, and bivalves also featured in the diet of *Eudendrium racemosum* at certain times of the year, representing almost 20% of its diet in summer (Barange 1988). In this study, mussel larvae were the third most common prey item in Port Phillip Bay, confirming that *E. crocea* is a predator of mussel larvae. Mussel larvae approaching the settlement stage actively 'test out' any substratum they encounter as a suitable settlement site. Plantigrade larvae (those settling out from the plankton) were consumed more readily than trochophore or veliger larvae (younger, smaller larvae which are still considered planktonic), regardless of density. The plantigrades were observed to land on the hydroid stolons or tentacles, and were then either passed directly to the mouth of *E. crocea* or plucked from

surrounding stolons using its tentacles. The confounding design of this experiment is noted, but the difference in behaviour of different mussel larval stages leads to acceptance that this result is an effect of mussel stage.

A. amurensis typically prefer small mussels of approximately 20-30 mm and therefore, in commercial mussel culture, juvenile mussels are at most risk of predation by *A. amurensis*. However, mussel lines in Port Phillip Bay do not reach the substrate and juvenile seastars would likely drop off grow-out lines prior to becoming large enough to prey on mussels. Mussel spat would thus likely be the only mussel stage in danger of predation by these seastars for commercial farmers within the bay.

Test farm management methods that will discourage and/or avoid biofouling episodes.

A multitude of factors have various effects on mussel larval settlement and post-mortality (see Brenner & Buck 2010). Surface area and structure are two primary factors influencing the success of spat collector ropes (Walter & Liebezeit 2003; Filgueira et al. 2007). Increased surface area reduces competition for space and food, and increased structural complexity may provide mussel spat with greater protection from predators (Walters & Wethey 1996). Here, ropes with greater complexity and surface area achieved superior spat settlement, and preferential settlement (Lutz & Kennish 1992) and higher adhesive strength (Brenner & Buck 2010) on filamentous substrata has been identified in mussels previously. 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). When evaluating the spat collecting capabilities of various rope types an interesting aspect to investigate is the attachment location of the spat. Clumps of spat attached to each other are disadvantageous as more are lost during harvesting (Lekang et al. 2003). This was, however, beyond the scope of this experiment.

Unsurprisingly, ropes that yielded the highest spat were also the most heavily fouled, likely due to the same physicochemical properties that made the ropes more efficient collectors. Despite the low level of fouling on the currently used black spat ropes, switching to aqualoop or super christmas tree would increase spat collections by 3-5 times, with less than a 2-fold increase in biofouling biomass. However, ascertaining the actual quantitative benefits of this strategy requires additional experimentation.

Dropping lines to deeper water may be a beneficial management tool during periods of heavy fouling, particularly by *E. crocea*. However, this method may concurrently result in increased settlement of other fouling species such as the bivalve *E. georgiana*. The effect of depth needs to be examined at other mussel farms around the bay, throughout the year and at a variety of depths in order to ascertain the best depths to place mussels at different times of the year. In addition, information on mussel growth at different depths is crucial to ensure maximum production is not overly compromised whilst trying to reduce biofouling, as food availability may change with depth.

Stocking mussel lines too sparsely may encourage less fouling but farmers are at risk of losing stock, since low stocking density resulted in low mussel retention. A stocking density of 400 mussels/m here produced optimum mussel retention and a moderate amount of fouling accumulation, in particular by the problematic *E. crocea*. Information on the effect of stocking density on mussel growth would be necessary in choosing the optimum stocking density, and this information has been documented for mussel industries elsewhere (Lauzon-Guay et al. 2005; Cubillo et al. 2012), where density was either not related or negatively related to growth rate. These studies found variable results dependent on site and season, and as such similar experiments looking at long-term effects of initial density within Port Phillip Bay would be required to make any tangible recommendations.

During the documentation of spatial and temporal fouling patterns, some form of biofouling was present on all plates collected throughout the study period, indicating that complete avoidance of biofouling would be impossible. However, there were clear differences in the composition of fouling communities among farms and throughout the year, potentially providing an opportunity for farmers to reduce the impacts of particularly detrimental foulers. Similarly, conspicuous yearly peaks in the settlement of mussel spat at a single location provide opportunities to maximise each stage of mussel production through strategically planned husbandry practices.

Mussel production occurs across an approximately 18-month cycle, from initial spat settlement to the harvest of adult mussels. At several points in the production cycle, mussels are stripped and re-socked for growout and can be transported among farms. Within Port Phillip Bay, translocations of mussels among farms are frequent events, mainly to optimise mussel growth, and farmers have already decided that increased productivity at Pinnace Channel is sufficient to justify transport costs. Currently, however, there is no synchronicity between the timing of translocations and the occurrence of biofouling. By informing the timing of these husbandry practices with knowledge of the spatial and temporal patterns of spat settlement and the settlement of damaging biofouling species, the extent and cost of biofouling could be reduced. For example, typically in Port Phillip Bay, ropes are stripped three months after spat settlement, and spat are re-socked at appropriate densities. Using settlement information obtained during the farming cycle to avoid biofouling, farm production strategies could ensure that spat collector ropes were deployed at Clifton Springs in early November in an attempt to avoid the settlement of *P. taeniata*, although impacts by this worm to spat or spat collection are likely minimal. Then, during January, spat could be stripped and moved to Pinnace Channel for growout. In moving stock away from Clifton Springs, the February peak in *C. intestinalis* is avoided, as well as the gradual build up of *E. crocea* throughout the year. Moving more susceptible stock such as small mussels away from fouling by the notoriously detrimental species *C. intestinalis* and *E. crocea* is highly recommended, as at even modest densities these foulers reduce mussel growth (Fitridge & Keough 2013; Sievers et al. 2013).

C. intestinalis presents an interesting case. In this study, it was absent at Pinnace Channel, a pattern consistent with its distribution in relatively calm water in Victoria (Keough & Ross 1999). It also shows considerable year-to-year variation in abundance at individual sites habitats (Keough 1983; Svane 1983), so that even sites at which *C. intestinalis* is abundant may experience years when it is not a problem. This kind of variation has been documented in Port Phillip Bay (Johnston & Keough 2003). The movement of stock to Pinnace Channel would reliably have allowed this species to be avoided, but it is also possible that the cost of fouling at Clifton Springs (and possibly Kirk Point) could vary from year to year. Monitoring of *C. intestinalis* settlement in early summer could allow determination of the risk posed by this species and allow decisions to be made about translocation or the timing of re-socking.

Suggesting husbandry practices based on *E. crocea* patterns from the data is difficult due to the seemingly unpredictable nature of its settlement. Although Kirk Point often had the highest cover of *E. crocea*, a longer data-set is needed to find out if there are consistent yearly trends or a point in time when accurate future predictions could be made. In general, Pinnace Channel exhibited low fouling by all undesirable species, likely due to its relative geographical isolation and the different environmental conditions experienced at this farm due to it being more reflective of the oceanic southern parts of the bay. As such it is an optimal growout location if food availability and thus mussel growth rates are comparable to other sites. Measuring and comparing growth rates at different farms is important, as higher fouling may be associated with better conditions. Moving stock to areas with low fouling may also mean moving stock to areas with reduced food levels, and any effect on mussel growth may be negligible.

If stock translocation is not feasible or logistically impractical, farmers can still take advantage of distinct temporal peaks in the settlement of particular species if such peaks occur at similar times year to year. For example, grading and re-socking practices, during which fouling is removed, could be postponed until, or initially scheduled to occur after these peaks in settlement. In the present example, such a strategy could potentially be used at Clifton Springs; grading and re-socking of stock, depending on the frequency required, could be done after the heavy settlement of *C. intestinalis* and *P. taeniata* during the February and September/October periods, respectively. This would not only remove newly settled foulers, which are likely to be more easily removed, but also lead to cleaned mussels being returned to the water at a time when the recruitment of these species is low.

Beyond the strategies outlined above, knowledge of fouling patterns will provide insight into what the next dominant fouling species will be, when it will settle and where it will be most problematic. Management and removal practices – such as those investigated here – can then be tailored ahead of time to suit the needs of each farm. The ability to focus these practices more precisely will increase their efficacy and reduce overall production costs, especially since future antifouling methods will likely focus on specific action against target organisms in localised regions (Berntsson & Jonsson 2003; Guenther et al. 2011; Paetzold & Davidson 2011; Cahill et al. 2012).

Test the effectiveness of existing and new biofouling treatment methods to develop cost-efficient, implementable on-farm treatments.

E. crocea was extremely susceptible to the treatments tested. In terms of cost, time and efficacy, the recommended treatments to use against fouling by this hydroid would be a 10 s dip in one of: 40 ^{o}C seawater, an ambient 2% acetic acid solution or an ambient 5% citric acid solution. *C. intestinalis* was similarly susceptible to the treatments, with 10 s immersions in one of: a 40 ^{o}C 2% acetic acid solution, an ambient 5% acetic acid solution or a 40 ^{o}C 5% citric acid solution the best options. *S. clava* was more resilient, and if present in large numbers on mussel lines will require more harsh treatments. Those that achieved 100% mortality were a 40 ^{o}C 2% acetic acid solution for 60 s and a 40 ^{o}C 5% citric acid solution for 10 s. All these recommended treatment options when tested against small and large mussels and oysters resulted in no mortality. When temperature of 50 ^{o}C and higher were used some mussel and oyster mortality was observed, and as such these temperatures are not advised, especially when coupled with the significant cots of heating water to such a degree.

Although immersing fouled shellfish in freshwater is a simple, cheap and environmentally friendly technique, with few detrimental effects on the cultured shellfish (Denny 2008), like others, we found it to be ineffective at killing most of the fouling species tested here (Carver et al. 2003; Denny 2008). The treatment durations used here were very short however, as this is most feasible for farmers. Despite this, freshwater baths have been used to treat incursions of fouling organisms, based on the principle that fouling organisms are more sensitive to treatment. For example, immersion of Akoya pearl oysters in freshwater effectively controls polychaete infestations without inducing oyster mortality (Velayudhan 1983). However, most freshwater treatments require exposure times of minutes to days depending on the life stage of the target organism (eg. algal plantlets vs gametophytes; Forrest & Blakemore 2006). Exposures to brine solutions are similarly inconsistent, with the possibility of rapid mortality of some algal species through osmotic stress (Sharp et al. 2006), but poor success against some tunicates (Carver et al. 2003).

Heat treatments have been used to successfully combat problematic biofouling in many marine industries (eg. Perepelizin & Boltovskoy 2011), and is appealing due to its benign environmental effects and ease of application (Rajagopal et al. 1995). The tubeworm *P. taeniata* was resilient to heated seawater, and calcareous taxa such as these worms and barnacles typically show higher resistance to heated treatments compared to softer bodied taxa (Blakemore & Forrest 2007). Similar to the results here, a range of common algal and invertebrate fouling organisms were negatively affected by heat treatments (Forrest & Blakemore 2006; Blakemore & Forrest 2007). Importantly, this technique can lead to some shellfish mortality (Carver et al. 2003), as found here when temperatures reached 50 ^{o}C.

Both spray and immersion techniques have been implemented extensively using acidic and alkaline chemicals. Acetic acid has been used as a herbicide against aquatic (Spencer & Ksander 1995) and terrestrial plants (Young 2004), with seemingly no environmental side-effects. In mussel culture, low concentrations of acetic acid are particularly successful against soft-bodied tunicates and algae (LeBlanc et al. 2007; Denny 2008; Piola et al. 2010), regardless of the method of application. However, some mussel mortality may be experienced (Carver et al. 2003; LeBlanc et al. 2007) and the application of acetic acid also affects non-target organisms and may hamper naturally occurring biocontrol (Paetzold et al. 2008). Other acids used less commonly but with some success on tunicates are silicic, formic and citric acid (Denny 2008). The most common alkaline substance in use is lime, and treatments have been conducted using both quicklime (calcium oxide) and hydrated lime (calcium hydroxide). Hydrated lime is used in pond aquaculture and has a 'low' environmental impact (Boyd 1999). Lime is considered effective against tunicates (Carver et al. 2003; Denny 2008), but less successful against other fouling species (Piola et al. 2010).

Develop integrated biofouling control strategies and producing a handbook for farmers.

This objective has been the focal point of the work in this project: to combine all of the biofouling strategies into a simple handbook which farmers can use as a reference point to enable them to make decisions regarding biofouling control, depending on the level of fouling and their own personal farming capabilities (see Appendix).

Conclusion

Biofouling clearly impacts the mussel industry in Port Phillip Bay through a variety of ways. The presence of the fouling organisms *C. intestinalis*, *S. clava* and *E. crocea* reduced mussel shell growth and flesh weight, even at low- to medium-fouling levels. The most plausible mechanism driving this effect is food competition, but it is consequences, rather than mode of action, that matters to industry implications. *E. crocea* may also affect the settlement and recruitment of mussel larvae through predation. Although some authors suggest that removal of fouling may not be cost effective as mussels may be lost in the cleaning process and the cost of fouling removal may be more than the reduced profits caused by fouling (de Sa et al. 2007), the recommendation from this study is that mussel growers should consider methods to reduce fouling. The impact of fouling on mussel condition is an important consideration for farmers in the market place. For example, >7% of shell fouling by tubeworms can produce mussels considered less than 'A' quality, costing the Scottish industry £300,000 – 500,000 per annum (550,000 - 915,000 AUD; Campbell & Kelly 2002). In Port Phillip Bay, a 5% difference in meat yield would be considered significant to production (Lance Wiffen, SeaBounty Pty Ltd., personal communication) and could affect consumer perception of product quality. Reduced shell growth and flesh weight could represent significant impacts to production over time through loss of revenue, as does a decline in the availability of wild mussel larvae for culture associated with hydroid predation on mussel larvae.

A variety of treatment options were found to be effective against key fouling species that were concurrently not harmful to mussels. However, prevention is better than treatment in most cases and manipulating farm management strategies is a relatively easy way to achieve this. Different rope types used to collect spat or to grow mussels accrue different types and quantities of biofouling. Choosing the best rope for the job will thus reduce the impacts of fouling on production. Similarly, as shown here, manipulating initial mussel densities and the depth of ropes can have substantial influences on fouling and farm efficiency.

In addition to manipulating husbandry practices, monitoring biofouling provides another means of avoiding biofouling that is simple and inexpensive. The information gained can be used to modify and focus husbandry and management strategies to reduce the quantity and impact of biofouling within aquaculture. For these strategies to be optimised, accurate long-term data on fouling should be collected from all the farms in an area. Husbandry could respond to fouling if there is predictable seasonality in settlement. Clearly, this requires a relatively high degree of certainty about spatial and temporal recruitment patterns. However, in a practical sense, if husbandry was highly adaptive, low-level monitoring could give short-term warnings when the risk of fouling by particular species might be high. Monitoring of biofouling at appropriate spatial and temporal scales is likely to deliver benefits that outweigh the costs for many aquaculture industries where biofouling affects efficient production.

As all biofouling communities vary temporally and spatially, growers should monitor settlement at individual sites throughout the growing season and be cautious about using sites that experience consistently high levels of fouling when on growing juvenile mussels or when trying to capture wild mussels on collector ropes. These sites and the associated fouling may be detrimental to the condition of young stock or affect the ability of wild mussel larvae to successfully settle out from the plankton and recruit, but are less damaging if the mussels utilising that site are close to harvestable age. However, some caution should still be taken if the fouling occurs on harvestable mussels, as although the effects on condition may be negligible, heavy fouling may lead to increased drag on mussel lines and result in lost stock, as the weight of the fouling pulls mussels from the culture lines. This is commonly experienced as a result of biofouling in mussel culture (Heasman & de Zwart 2004; Ramsay et al. 2008) and is also seen in wild mussel populations (Inglis 1994; O'Connor et al. 2006).

The end result of much of this research, the biofouling handbook, is a portable, easy to use guide for farmers to identify fouling species, and determine if treatment is necessary and if so which treatment is best suited to their situation.

Implications

This research defines the key fouling species affecting the mussel culture industry and makes recommended strategies for their avoidance and management.

Outcomes of this research will benefit the following end users:

1. Farm managers

2. State mussel growing agencies

3. Regulatory agencies

4. Other aquaculture industries

5. Other scientists involved in related aquaculture biofouling research

Management / Industry

Reference data on patterns of fouling and the key species within different aquaculture zones at key times in the mussel production cycle are available to industry that will facilitate the ability to avoid fouling episodes.

Production of a handbook featuring the key fouling species likely to be encountered by farmers, a 'decision tree' to guide farmers in decisions pertaining to the control of biofouling species at key times in the production cycle, and outlining step-by-step methods to avoid, control and treat these key fouling species.

Other aquaculture industries

The information on fouling species gathered during this research will be of equal importance to other related industries such as oyster, scallop and finfish aquaculture, all of which experience biofouling.

Other researchers

Reference data has already been published in peer reviewed literature, whilst further manuscripts are in preparation. These publications will provide a basis for further research into investigating biofouling in the mussel culture industry.

Recommendations

Industry implications

Mussels are often sold domestically as live produce via wholesale markets, or directly to restaurants and consumers by the farmers (Weston et al. 2001). Shell length and flesh weight are important indicators of mussel quality and value, and reductions in meat yield are detrimental to farm productivity. Growth reductions of roughly 1mm per month and flesh weight reductions of 8–21%, as found here, will increase the time until harvesting, reduce the quantity of edible product and result in economic losses. The three fouling species studied here are common foulers within mussel aquaculture around the world, and likely have significant impacts on farm productivity in these regions.

Variations in the level of fouling, and the composition of the fouling community, will affect the extent to which mussel productivity is reduced. Effects will also likely differ across temporal scales and in different locations due to the effects of food availability and temperature on feeding. Biofouling removal techniques, such as grading and resocking practices or treatment baths, coupled with the calculated placement of mussel ropes to attempt to avoid periods of heavy fouling, especially when dealing with smaller mussels, would be advised when settlement of these three species is high.

Further development

- Implementation of a biofouling monitoring programme to better understand local fouling patterns and processes. If possible, environmental variables such as temperature and salinity, among others, should be concurrently recorded to investigate the influence of these variables on fouling patterns. In addition, studying the hydrodynamics of aquaculture areas may provide insights into connectivity among farms, and help explain fouling patterns and thus aid in reducing fouling. If monitoring is conducted within regions of intensive aquaculture, these data could be continually uploaded to an online database, as already occurs in many shellfish aquaculture industries for the detection of harmful phytoplankton (Trainer et al. 2003). The management of biofouling could then be enhanced if, for example, there are yearly trends where a specific fouling species becomes abundant at one farm and successively becomes abundant at others in a distinct sequence.

- Further investigation of depth as a tool to mitigate biofouling, and the effects of depth on mussel growth and condition. These studies should be completed at a range of temporal and spatial scales.

- Further exploration of density as a tool against biofouling and the long term effects of density on mussel retention, growth and condition.

- Long term studies of rope type on fouling success throughout the production cycle and fouling seasons.

- Further experimentation on intermediate temperatures for heat treatments (such as 42, 44, 46 and 48 ^{o}C).

- Creation of a detailed cost-benefit analysis of various treatment options looking at the cost of heating water and purchase of acids *vs* the benefits gained by reducing biofouling based on mussel growth reductions caused by fouling.

Extension and Adoption

Management / Industry

The outcomes of the project will mainly be disseminated to industry through the distribution of the biofouling flip guide.

The research will be further communicated through an industry workshop to Australian mussel growers and managers to coincide with a 2014 meeting of the Australian Mussel Industry Association.

Other researchers

Reference data has already been published in peer reviewed literature, whilst further manuscripts are in preparation. The data will be presented at conferences such as Australasian Aquaculture 2014.

Project coverage

The project was reported on in 2011 in the September/October issue of the University of Melbourne newspaper, *Voice*. The monthly publication is distributed on the University's campuses and published as a supplement to the Fairfax media newspaper *The Age* on the second Monday of each month. It is also available on line and by free email or hard copy subscription.

Published on *VOICE* (http://voice.unimelb.edu.au)

On the quest for marine pests

Volume 7 Number 9 September 12 - October 9 2011 [1]

Developing methods to prevent and treat outbreaks of troublesome marine pests in the Victorian mussel industry is the goal of a new project led by the University of Melbourne. Charlotte Crawford reports.

Dr Isla Fitridge's research assists the seafood industry's battle against "biofouling".

University of Melbourne researcher, Dr Isla Fitridge, a postdoctoral Fellow with the Department of Zoology, says fresh, plump blue mussels are a favourite of many Australian seafood lovers.

"But what many consumers may not realise is they very likely had to fight for survival in the preceding months against 'biofouling' – greedy, smothering marine pests," she says.

"In the case of blue mussels, biofouling can be quite the epitome of 'foul play': it can smother the shells, preventing the mussels from feeding effectively, or compete with them for food, reducing their size and quality.

"Aquaculture, or the farming of aquatic organisms such as fish and shellfish for human consumption, uses a variety of ropes, floats and nets. Biofouling not only negatively affects the condition of this farm infrastructure but can also threaten the seafood being cultured.

"The extra costs associated with cleaning biofouling from ropes, floats and mussels, as well as the direct effects on mussel condition, incur great economic losses for mussel growers."

While the three-year project will focus on mussel farms in Port Phillip Bay in Victoria, the prevention and treatment strategies will have broad value across the national industry. The project will determine the distribution and development of biofouling species in Port Phillip Bay mussel farms and measure the effects of key species on mussel survival. New treatment methods will be developed that are hoped to be cost-effective, easy to implement by the growers and have no impact on the surrounding environment.

"Ultimately, by reducing the problem of biofouling, the project aims to help the industry to provide even more high quality, healthy mussels to Australian consumers," Ms Fitridge says.

The $300,000 project is supported by funding from the Fisheries Research and Development Corporation (FRDC) on behalf of the Australian Government.

Project materials developed

Scientific papers

Fitridge, I., Dempster, T., Guenther, J., de Nys, R. (2012). The impact and control of biofouling in marine aquaculture: a review. Biofouling: The Journal of Bioadhesion and Biofilm Research, 28:7, 649-669

Sievers, M., Fitridge, I., Dempster, T. and Keough, M. J. (2013) Biofouling leads to reduced shell growth and flesh weight in the cultured mussel *Mytilus galloprovincialis*. Biofouling, 29(1): 97-107.

Sievers, M., Dempster, T. Fitridge, I., and 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(2): 203-212.

Conferences & Presentations

Fitridge, I. (2011) Foul friends or foe? The impact of invasive hydroids in commercial mussel culture. Proceedings of the 6th International Conference on Marine Bioinvasions, Barcelona, Spain, 23–25 August 2011.

Sievers, M**.,** Fitridge, I. (2012). Foul play: Are mussels being out-muscled? Proceedings of the joint Australian Marine Sciences Association Inc. and New Zealand Marine Sciences Society Conference "Marine Extremes – and Everything in Between", Hobart, Australia, 1–5 July 2012.

Fitridge, I., Keough, M.J. (2012). Foul friends or foe? The impact of hydroid biofouling in commercial mussel culture. Proceedings of the Australasian Aquaculture Conference, Melbourne, Australia, 1-4 May 2012.

Fitridge, I., Sievers, M. (2013). The impact and control of invasive species in Australian mussel culture. Proceedings of the 7th International Conference on Marine Bioinvasions, Vancouver, Canada, 20–22 August 2013.

Fitridge, I. (2013). In a pickle: developing methods to control biofouling in aquaculture. Proceedings of the ANZPAC workshop on biofouling management for sustainable shipping, Melbourne, Australia, 5-9 May 2013.

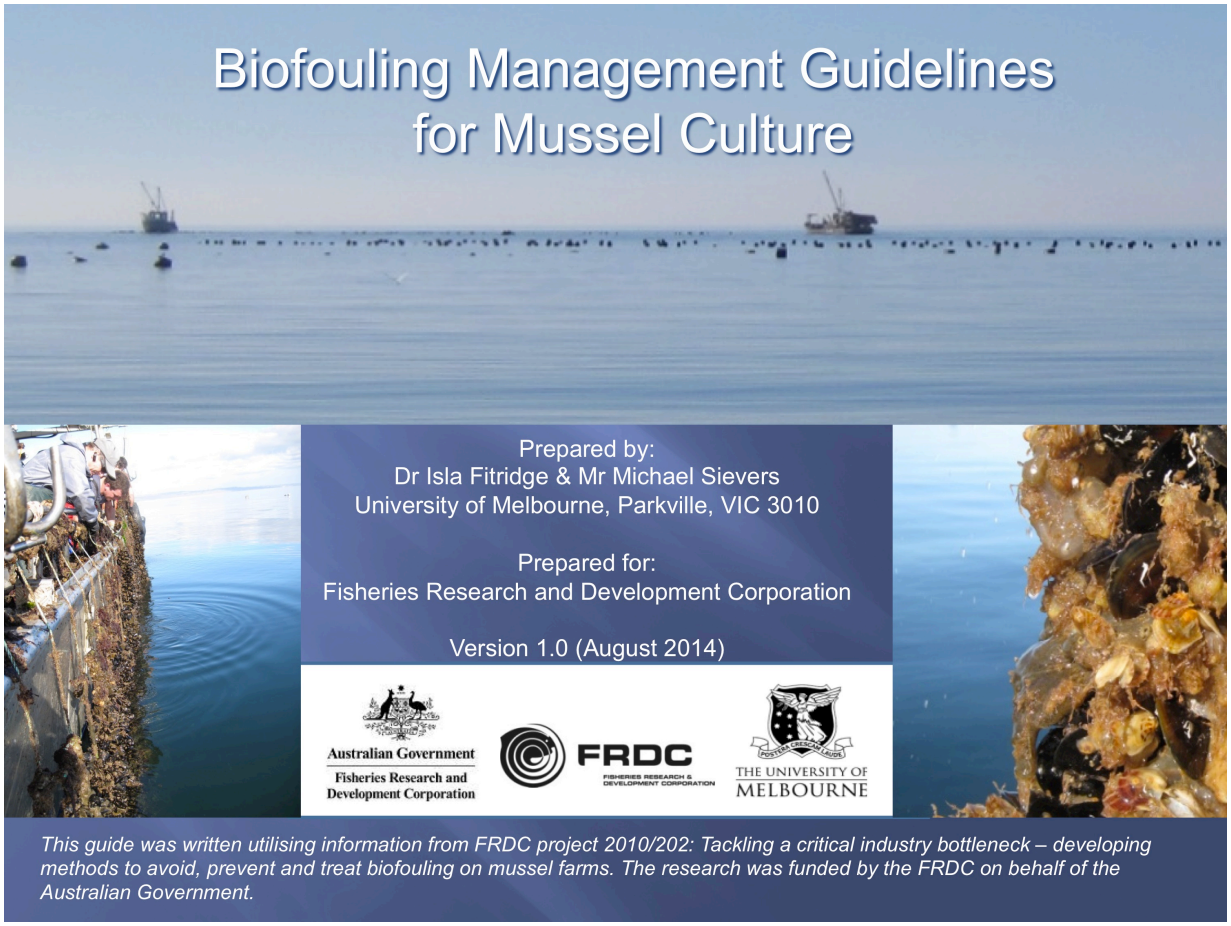
Handbook for farmers

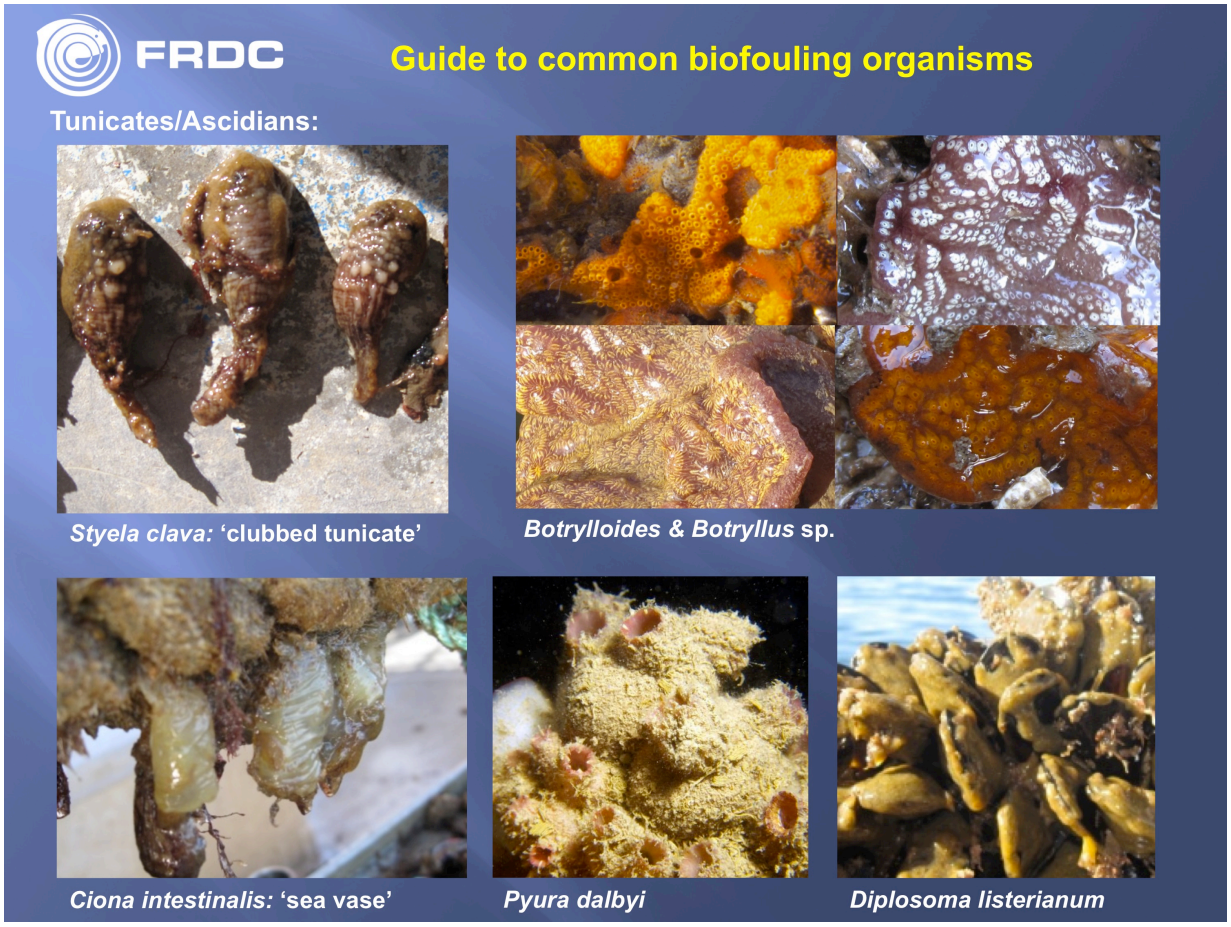
Aspects of the project have been brought together in a handbook outlining the species to be aware of, and methods to deal with outbreaks, in order to develop the most effective biofouling strategies for farmers. The handbook is intended to be produced as a simple to use 'flip guide', allowing easy identification of key fouling organisms and outlining a range of options for treatment or management. Printed on waterproof card, it is intended to be an 'on board' tool for farmers; a handy reference during all stages of the production cycle. The handbook features key fouling species likely to be encountered by farmers, a 'decision tree' to guide farmers in decisions pertaining to the control of biofouling species at key times in the production cycle, and outlining step-by-step methods to avoid, control and treat these key fouling species.

Study trip to Canadian mussel farms

The fostering of industry ties with other researchers is key to further understanding patterns and behaviours of fouling species. Prince Edward Island, in Eastern Canada's Gulf of St Lawrence, is a hotspot for mussel fouling. The PEI mussel industry is, with the support of various local and federal government funding agencies and academia, leading the development of methods to mitigate the impact of non-indigenous tunicates on shellfish operations. Dr Isla Fitridge travelled to Canada to report on the Canadian biofouling experience.

Handbook for farmers





Guide to common biofouling organisms

FRDC

Worms:

Pomatoceros taeniata: 'Coral/White worm'

Barnacles:

Amphibalanus variegatus

Elminius modestus

Seastars:

Asterias amurensis: 'Northern Pacific seastar'

Coscinasterias muricata: '11-armed seastar'

Anemones:

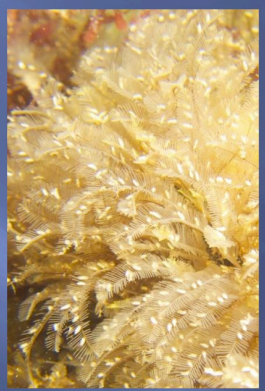
Anthopleura aureoradiata

FRDC

Guide to common biofouling organisms

Hydroids:

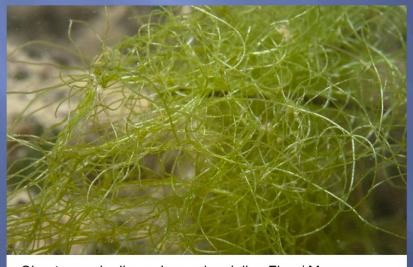
Ectopleura crocea: 'Pink mouthed hydroid'



Aglaophenia sp.

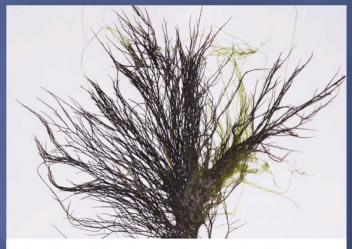
Obelia dichotoma: 'Winter hydroid'

Seaweeds:



Chaetomorpha linum. Image by: Julian Finn / Museum Victoria, Rights/Licence: CC BY (Attribution)

Chaetomorpha sp.



Gracilaria chilensis, pressing by J. Pocklington. Image by: Blair Patullo / Museum Victoria Rights/Licence: CC BY (Attribution)

Gracilaria chilensis

Undaria pinnatifida: 'Japanese kelp'

Monitor, manage or treat?

In order to decide wether monitoring, management or treatment is required, it is important to ask the following questions:

1) Characteristics of biofouling: What type of fouling is it?

2) Risk assessment: What stage of production is affected?
What are the risks of action *versus* no action?

	Low priority	→	High priority
Number of lines affected	few	many	all
Density of biofouling	<10%	10- 40%	>40%
Degree of fouling establishment	Starting to establish (juveniles)	Somewhat established	Well established (adults)
Potential impact	Lowest	Medium	Highest
Action	**Monitor**	**Manage**	**Treat**

Monitor, manage or treat?

Monitor

Monitoring is as simple as **being aware** of the 'usual' flora and fauna of your culture area and keeping **written records** of **what** biofouling is prevalent, **when** it appears and **where** it becomes a nuisance. This information can be critical in allowing you to be prepared for future outbreaks.

If the biofouling is **new or unusual** or particularly heavy, **notify the relevant authority** immediately and collect a sample. It may be an introduced marine pest. Introduced species are opportunistic and can often be highly problematic as biofouling.

Monitor, manage or treat?

Manage

Ensure ropes are stocked at an **appropriate density** to avoid biofouling episodes. A density of **400 mussels per metre** will provide an optimum balance of good mussel retention and reduced fouling intensity.

Drop stock to depths of greater than 5 metres in anticipation of fouling periods; fouling can be up to 40% less at depths of 10 metres.

Plan **re-socking** for periods immediately **after settlement** of problem fouling species. The re-socking process will dislodge newly settled fouling.

Keep floats, vessels and other infrastructure **clean**; these act as hosts for biofouling, re-introducing problem organisms to culture areas and potentially **spreading pests** between them.

Monitor, manage or treat?

Treatment option 1 (T1):

Seawater + 5% glacial acetic acid	
Protect	Flammable: avoid sources of ignition (flame/spark) Acetic acid can irritate skin, eyes and the respiratory system. Wear Nitrile gloves and eye protection when handling, and avoid inhalation of vapours. Store in a well ventilated, bounded, secure storage area, away from direct sunlight.
Prepare	Dissolve acetic acid in seawater in a large tank to a concentration of 5% (50ml of glacial acetic acid per litre of seawater) in a well ventilated area. Ensure the solution is well mixed.
Procedure	1. Hoist fouled line from water 2. Dip line in solution for 30 seconds 3. Remove line from solution. 4. Return to water

For acetic acid MSDS see: http://www.sciencelab.com/msds.php?msdsId=9922769

Monitor, manage or treat?

Treatment option 2 (T2):

Seawater + 6% hydrated lime (calcium hydroxide)	
Protect	Hydrated lime can irritate skin, eyes and the respiratory system. Wear gloves and eye protection when handling, and avoid inhalation of dust. Store in a cool protected place away from moisture, strong oxidants or acids and to minimize dust emissions. Storage in steel or concrete bins and silos, or plastic lined bags, is appropriate.
Prepare	Dissolve hydrated lime in seawater in a large tank to a concentration of 6% (60g of hydrated lime (~99% calcium hydroxide) per litre of seawater) in a well ventilated area. Ensure the solution is well mixed.
Procedure	1. Hoist fouled line from water 2. Air dry for 30 seconds 3. Dip line in solution for 60 seconds 4. Remove line from solution 5. Air dry for 30 seconds 6. Return to water

For calcium hydroxide MSDS see: http://www.sciencelab.com/msds.php?msdsId=9927122

Monitor, manage or treat?

Treatment option 3 (T3):

40°C seawater	
Protect	Heated liquids and steam can burn. Wear gloves and eye protection when handling.
Prepare	Heat seawater to 40°C in a large tank
Procedure	1. Hoist fouled line from water 2. Dip line in solution for 60 seconds 3. Remove line from solution. 4. Return to water

Monitor, manage or treat?

Treatment option 4 (T4):

40°C **seawater + 2% acetic acid**	
Protect	Flammable: avoid sources of ignition (flame/spark) Acetic acid can irritate skin, eyes and the respiratory system. Wear Nitrile gloves and eye protection when handling, and avoid inhalation of vapours. Store in a well ventilated, bounded, secure storage area, away from direct sunlight.
Prepare	Dissolve acetic acid in seawater (heated to 40°C) in a large tank to a concentration of 2% (20ml of acetic acid per litre of seawater) in a well ventilated area. Ensure the solution is well mixed.
Procedure	1. Hoist fouled line from water 2. Dip line in solution for 30 seconds 3. Remove line from solution. 4. Return to water

For acetic acid MSDS see: http://www.sciencelab.com/msds.php?msdsId=9922769

Monitor, manage or treat?

Treatment option 5 (T5):

40°C **seawater + 5% citric acid**	
Protect	Flammable: avoid sources of ignition (flame/spark) Citric acid can irritate skin, eyes and the respiratory system. Wear Nitrile gloves and eye protection when handling, and avoid inhalation of vapours. Store in a well ventilated, bounded, secure storage area, away from direct sunlight.
Prepare	Dissolve citric acid in seawater (heated to 40°C) in a large tank to a concentration of 5% (50g of 99.5% anhydrous citric acid per litre of seawater) in a well ventilated area. Ensure the solution is well mixed.
Procedure	1. Hoist fouled line from water 2. Dip line in solution for 10 seconds 3. Remove line from solution. 4. Return to water

For citric acid MSDS see: http://www.sciencelab.com/msds.php?msdsId=9923494

‘Quick pick’ treatment decision guide

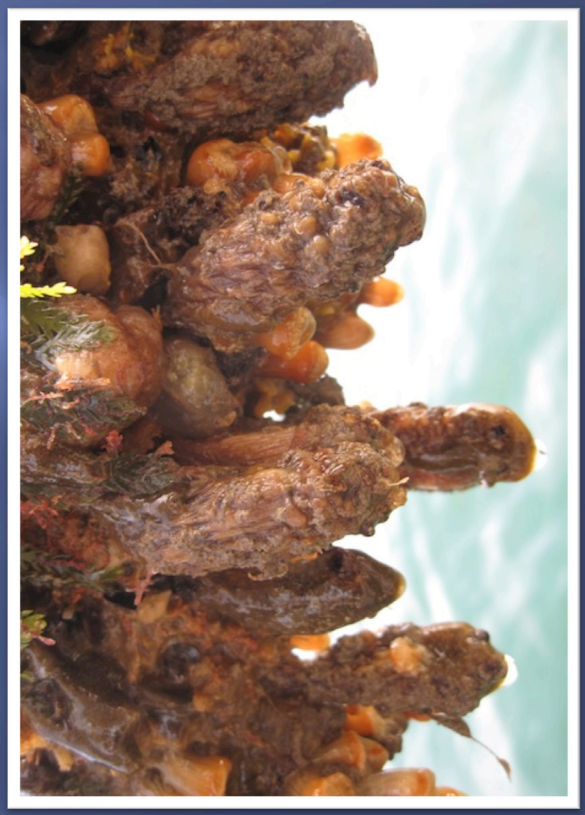
FRDC

Fouling species	T1	T2	T3	T4	T5
	Seawater + 5% Acetic acid	**Seawater + 6% hydrated lime**	**40°C Seawater**	**40°C seawater + 2% acetic acid**	**40°C seawater + 2% citric acid**
Styela clava & possibly other tough leathery tunicates	50% mortality	0% mortality	10% mortality	50% mortality	100% mortality
Ciona intenstinalis & possibly other soft tunicates, anemones	100% mortality	50% mortality	100% mortality	100% mortality	100% mortality
Ectopleura crocea & possibly other hydroids, seaweeds	100% mortality	As yet untested	100% mortality	100% mortality	100% mortality
Impacts on harvestable ***Mytilus galloprovincialis***	0-8% mortality*	0-3% mortality	0% mortality	0% mortality	0% mortality

* Impacts more substantial for smaller mussels

FRDC

Clubbed Tunicate: *Styela clava*



Clubbed Tunicate: *Styela clava*

Key features

- Brown, stalked, tough and leathery club-shaped
- Solitary individuals grow up to 16 cm long
- Often thickly covered with other marine fuzz-like fouling

Impacts

- Forms dense groups that compete with mussels for space and food leading to reduced mussel growth and condition
- Additional weight caused by extensive fouling can pull stock from lines
- Increased operational costs
- Decreased yields

Recommended treatment

- With access to heat: T5
- Without access to heat: T1 (if mussels >60mm or fouling severe)

Sea Vase: *Ciona intestinalis*

Sea Vase: *Ciona intestinalis*

FRDC

Key features

•Smooth, fragile and gelatinous

•Transparent with a yellow hue

•Solitary individuals grow up to 20 cm long

Impacts

•Competes with mussels for space and food leading to reduced mussel growth and condition

•Additional weight caused by extensive fouling can pull stock from lines

•Increased operational costs

•Decreased yields

Recommended treatment

•With access to heat: T3

•Without access to heat: T1 or T2 (if mussels >60mm or fouling severe)

FRDC

Pink Mouthed Hydroid: *Ectopleura crocea*

Pink Mouthed Hydroid: *Ectopleura crocea*

Key features

•Distinctive tufts of straight, tubular stems topped with large flower-like heads

•Often highly matted towards the base, trapping sediment

•Yellowish coloured stems up to 12 cm long, pink heads with long tentacles

Impacts

•Competes with mussels for space and food leading to reduced mussel growth and condition

•Preys on mussel spat

•Increased operational costs

•Decreased yields

Recommended treatment

•With access to heat: T3

•Without access to heat: T1 (if mussels >60mm or fouling severe)

Northern Pacific Seastar: *Asterias amurensis*

Juvenile

Adult

Key features

•5 arms (although occasionally <5)
•Yellow with distinctive purple markings
•Only juveniles are likely to be found on mussel lines

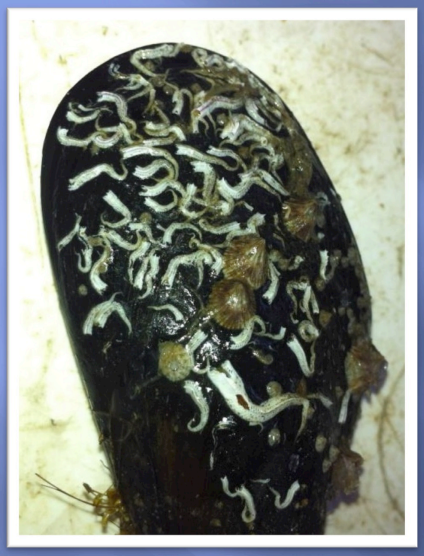
Impacts

•Active predator of mussels
•Juveniles (<5 cm) only capable of consuming mussels <1 cm
•Adults prefer mussels >2 – 3 cm
•May reduce spat settlement and survival
•Subsequent decrease in yields

Recommended treatment

•Pilot studies on juvenile seastars suggest:
With access to heat: T4
Without access to heat: T1

Coral/White worm: *Pomatoceros taeniata*



Key features
•Hard calcareous white ridged tube
•Readily colonise hard surfaces including mussel shells
•Older individuals may have purple stripes along ridges
•Grows to 3 cm

Impacts
•Grow on mussel shells reducing aesthetics, product sale value and thus profits
•Heavily fouled mussels are often discarded
•Difficult to remove calcified tube

Recommended treatment
•None of the treatments here are effective against white worm. FRDC project 2011/241 recommends dipping in seawater heated to 45-50^{o}C for 45-50 seconds. However, in our study, 50^{o}C treatments for 30 & 60 seconds resulted in high mussel mortality. We recommend caution when using these high temperatures against white worm.

Summary

Monitoring biofouling
•Simple and ineffective
•Provides valuable information that may enable the avoidance of fouling and a pre-emptive treatment regime to be put in place

Alter husbandry practices
•Optimising stocking densities, rope types, line depth, cleaning schedules, etc will reduce the need for fouling treatment

Treatments
•Acquiring equipment to allow the use heated treatments is highly recommended, as this eliminates or reduces the need for chemicals, and treatments with heat suggested here result in no mussel mortality.

Contacts

The National Introduced Marine Pest Information System (NIMPIS):
http://data.daff.gov.au/marinepests/

THE NATIONAL SYSTEM FOR THE PREVENTION AND MANAGEMENT OF MARINE PEST INCURSIONS

Australian Pesticides and Veterinary Medicines Authority (APVMP):
http://apvma.gov.au/

Australian Government
Australian Pesticides and Veterinary Medicines Authority

For advice on the use of chemicals in aquaculture:
http://business.qld.gov.au/industry/fisheries/aquaculture/using-chemicals-in-aquaculture

Australian Mussel Industry Association:
http://www.australianmussels.com.au/

AUSTRALIAN MUSSEL INDUSTRY ASSOCIATION

Report on study trip to Canadian mussel farms

Dr Isla Fitridge, University of Melbourne

Aims

1. To meet with key personnel at the Department of Fisheries and Oceans Canada (Chris Mills, Fisheries & Aquaculture Officer), the PEI Department of Fisheries, Aquaculture and Rural Development (Aaron Ramsay, Brian Gillis, Kim Gills), and the University of PEI (Jonathon Hill), to discuss the Canadian approach.

2. To undertake site visits of affected mussel and oyster leases and view *in situ* control strategies directly.

Introduction

Prince Edward Island's aquaculture industry annually produces more than 22,500 MT of shellfish and finfish (Figure 1). In terms of shellfish specifically, the Island industry is the largest producer of oysters in Eastern Canada and the largest producer of blue mussels (*Mytilus edulis*) in North America (over 20,000 MT, valued at €20.5 million). The PEI mussel aquaculture industry has been developed as an economically and environmentally sustainable business since its conception in 1980. There are in excess of 100 growers farming across 295 leases, which collectively span 10,000 acres of production area. Mussels are harvested year round, reaching market size (60 mm) after 12 – 18 months. PEI has a strict leasing policy, with a designated Lease Management Board responsible for the development of the industry, co-managed by the Department of Fisheries and Oceans Canada, the PEI Department of Fisheries, Aquaculture and Rural Development, and the PEI aquaculture industry. An extensive mussel monitoring program has been designed to monitor mussel larvae, potential toxic algae, water quality, meat yields, predators and fouling organisms. The industry has collaboratively developed extensive Environmental Codes of Practice, including such areas as introductions or transfer of live shellfish, predator control, waste management and biofouling control.

Figure 1. Mussel and oyster leases at Savage Harbour, growing part of the 22,500 MT of shellfish produced around the shores of PEI.

The mussel industry in PEI has been detrimentally impacted by tunicate biofouling since 1998. There are four species in question, all of which are non-indigenous to the PEI region: two colonial species, the golden star (*Botryllus schlosseri*) and violet (*Botrylloides violaceous*) tunicates; and two solitary species, the vase (*Ciona intestinalis*; Figure 2) and clubbed (*Styela clava*) tunicates. In order to protect the sustainability and productivity of the PEI mussel aquaculture industry, efforts at controlling the spread of these tunicates and development of mitigation measures to reduce their impact have been made.

Education – industry and other maritime end users

The PEI experience exemplifies that industry education is a critical part of biofouling mitigation and control – if the industry realises the impact upon itself, then growers police themselves very stringently. In order to lessen the incidences of species transfers, an awareness campaign was set up to target different maritime users who share the same water space as the mussel growers, including recreational boaters and commercial oyster fisherman. The campaign consists of a series of information boards erected at boat ramps throughout the Island, to inform other maritime users which pest species are known to be present in that area, and those which are not (Figure 2). It provides images of each organism for clear identification, and information regarding the importance of washing down gear after leaving one bay and entering another in order to lessen the spread of these organisms. The campaign has proven to be an important tool in raising awareness about marine pests and combating unwanted species transfers. The violet and golden tunicates are unfortunately found in every bay, but the vase and clubbed tunicates are currently contained to specific areas and their transfer needs to be closely policed.

Figure 2. Signs are erected at all boat ramps alerting boat users to the problematic species present and absent from that area (left); the Vase Tunicate extensively fouls mussel lines in Savage Harbour, PEI (right).

Containment

In order to manage the spread of the four tunicates to non-infested waters, an Introduction & Transfers Committee has been formed chaired by the Department of Fisheries and Oceans (DFO). It consists of approximately 30 members: a representative from every production area and representatives of the PEI Aquaculture Alliance and the PEI Shellfish Association. The IT code is a national guideline on managing transfers of product within, or into, PEI from tunicate infested waters, and covers genetics, disease and habitat impacts of transfers. If a grower finds evidence of a known pest or new species in their production area, they contact the DFO who go out to confirm the presence *in situ*. This then leads to a survey of the entire area, to determine how broad the infestation is. A notice is then sent out to the Introduction and Transfers Committee and a broader search is conducted to determine where the infestation is located in the bay, and how widely established it is. The Committee then decides whether to restrict the Bay or not. Even if only partly affected, the industry will normally call the whole bay as infested. This approach is taken because the drawing of arbitrary lines across a bay and designating part of the bay as clear and part as infested will require annual monitoring to support such a decision. From a management perspective, if a pest species is present in a bay then it is considered to be affecting the whole bay. All waters within PEI infested by any of these tunicates are designated as 'restricted waters', and the application of the licensing and transfer protocols has been effective in reducing the spread of tunicates to non-infested areas. The application of this 'like to like' policy, whereby transfers of mussels can only occur between bays with similar tunicate profiles, is supported by industry and has, for example, restricted the vase tunicate to the northern and south eastern states of PEI.

Mitigation

The PEI industry has tested a variety of innovative methods to enable mussel growers to manage their crops effectively against tunicates, including ultrasound, chlorine, light, UV, lime, acetic acid and high pressure washing. All treatments show a great deal of variability and practicality, highlighting the need for treatments to be cost effective and user friendly. This research has been possible through the support of several funding bodies including the Atlantic Canada Opportunities Agency (ACOA), the Aquaculture Collaborative Research and Development Program of Fisheries and Oceans Canada (ACRDP), the Aquaculture Innovation and Market Access Program of Fisheries and Oceans Canada (AIMAP) and the Aquaculture and Fisheries Research Initiative of the Government of PEI (AFRI). Equipment has been developed and improved upon by industry within these sources of funding to best suit their own individual farm practice.

The typical treatment for minor fouling, and on fouling of juvenile mussels, is a 4% by volume hydrated lime slurry, applied using a hose. Mussel socks are raised out of the water for approximately 20 seconds, receive the spray, and air dried for approximately 45 seconds before being returned to the ocean. Alternatively, the lime slurry is applied through a series of nozzles, which spray both sides of the sock evenly. A lime recovery system has been developed into this technique, allowing the lime solution to be recirculated, thereby making this process quite cost effective. One standard bag of lime can treat approximately 300 2.5m socks. However, there are some problems associated with lime use. The lime has to be fresh, as if it becomes exposed to air it loses its potency as the PH gets neutralised. In addition, there is no way to tell if the concentration is maintained and therefore continues to be efficient over time. A second treatment, particularly for the vase tunicate, is high pressure water spraying (approximately 400 – 600 psi; Figure 4). This treatment is included in normal maintenance practices, and as spraying strengthens the attachment of mussel stock, meaning there is no need to double sock, the costs are mitigated. Spraying is carried out every 4 weeks, and is very effective when the vase tunicate is approaching its reproductive peak at approximately 4cm in length. The treatment ruptures the vase tunicate, which in turn is thought to inhibit further recruitment of larval stages for 2 – 3 weeks afterwards.

Figure 3. High pressure spraying of mussel socks in Savage Harbour

Conclusions / Recommendations

The collaborative management approach taken by the PEI industry, with the support of various local and federal government funding agencies and academia, has led to the development of site specific physical and chemical mitigation methods, enabling the future sustainable development of the PEI mussel industry. It is an approach which is highly effective. It is recommended that the Australian industry, government and academia similarly work together to further understand the complexities of biofouling patterns and develop and implement strategies to avoid, prevent and treat outbreaks. The amalgamation of scientific research and industry know-how could enable Australian production to grow shrewdly and sustainably.

Appendices

List of researchers and project staff:

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Footnotes:

Pomatoceros taeniata has been recently reclassified as *Spirobranchus taeniata*
Mytilus galloprovincialis has been recently reclassified as *Mytilus planulatus*

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