**Biofouling leads to reduced shell growth and flesh weight in the cultured mussel *Mytilus galloprovincialis***

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Competitive interactions between cultured mussels and fouling organisms may result in growth and weight reductions in mussels, and compromised aquaculture productivity. Mussel ropes were inoculated with *Ciona intestinalis*, *Ectopleura crocea* or *Styela clava*, and growth parameters of fouled and unfouled *Mytilus galloprovincialis* were compared after two months. Small mussels (=50 mm) fouled by *C. intestinalis* and *E. crocea* were 4.0 and 3.2% shorter in shell length and had 21 and 13% reduced flesh weight, respectively, compared to the controls. Large mussels (=68 mm) fouled by *S. clava*, *C. intestinalis* and *E. crocea* were 4.4, 3.9 and 2.1% shorter than control mussels, respectively, but flesh weights were not significantly reduced. A series of competitive feeding experiments indicated that *S. clava* and *C. intestinalis* did not reduce mussels' food consumption, but that *E. crocea*, through interference competition, did. Fouling by these species at the densities used here reduced mussel growth and flesh weight, likely resulting in economic losses for the industry, and requires consideration when developing biofouling mitigation strategies.

**Keywords:** *Ciona intestinalis*; *Ectopleura crocea*; *Styela clava*; aquaculture; competition; feeding rate

**Introduction**

Biofouling is one of the most significant threats to aquaculture operations through increasing production and management costs and decreasing product value (Fitridge et al. 2012). Ropes, cages, nets and bivalve shells provide hard substrata that are readily colonised by fouling organisms (Khalaman 2001). These communities can contribute a substantial proportion to the total weight of the system (Woods et al. 2012), resulting in increased costs associated with buoyancy and anchoring systems (Claereboudt et al. 1994). Declines in bivalve growth and condition are often attributed to reduced water flow and food availability caused by the clogging of nets and cages by fouling organisms (Lodeiros & Himmelman 1996; Pit & Southgate 2003). For example, giant scallops *Placopecten magellanicus* reared in cleaned nets contained 68% more flesh than those reared in fouled cages (Claereboudt et al. 1994).

In longline mussel aquaculture, fouling organisms often settle and grow on the shells of the mussels as well as on the farm infrastructure (LeBlanc et al. 2003). Biofouling poses several significant problems for mussel culture operations. Firstly, fouling communities are primarily comprised of suspension feeding species that can compete with mussels for food resources (Mook 1981; Daigle & Herbinger 2009; Woods et al. 2012) and reduce oxygen availability (Wallace & Reinsnes 1985). Fouling organisms can also impede the procurement of food by reducing water flow around mussels or interfering with the operational opening of the valves of the mussels (de Sa et al. 2007). Fouling organisms can attain such densities that the byssal threads of the mussels are unable to sustain the combined weight, resulting in mussel detachment and thus, stock loss (Witman & Suchanek 1984). In addition, the periodic removal of fouling organisms increases production costs substantially, reducing farm profitability (Colautti et al. 2006).

Competition for food between mussels and fouling organisms may compromise mussel growth and condition, and thus, aquaculture productivity. Since the primary factor affecting mussel growth is food supply, significant exploitative or interference competition for food resources will influence growth rates (Seed & Suchanek 1992). In New Zealand, biofouling on mussel ropes at two sites in Pelorus Sound was estimated to contribute 13–18% of the total farm clearance rate; such suspension-feeding effects could have a significant impact on carrying capacity (Woods et al. 2012). Although mussels generally have higher clearance rates than most species in fouling communities (Lesser et al. 1992), there are similarities in clearance rates and particle size utilisation between mussels and particular fouling species, such as ascidians (Stuart & Klumpp 1984; Petersen 2007; Daigle & Herbinger 2009). As such, the potential for food competition from fouling species to manifest into growth reductions in mussels is plausible.
Solitary ascidians, such as *Ciona intestinalis* (Linnaeus 1767) and *Styela clava* (Herdman 1881), are common colonists of mussel ropes and may represent significant competitors for food resources (Arakawa 1990; Petersen 2007; Daigle & Herbinger 2009). *C. intestinalis* has successfully invaded coastal areas throughout the world (Lambert & Lambert 1998; Castilla et al. 2005), depressing species richness in epifaunal communities on a local scale and influencing community composition by altering both species presence and abundance (Blum et al. 2007). *C. intestinalis* is highly problematic for mussel aquaculture in the USA (Lesser et al. 1992), New Zealand (Woods et al. 2012) and Canada (Howes et al. 2007), where it increases mussel mortality and decreases growth and condition (Daigle & Herbinger 2009). Similarly, *S. clava* is a common fouling organism in bivalve culture (Lutz-Collins et al. 2009), where its capacity to infest cultivation equipment severely compromises the productivity of mussel (LeBlanc et al. 2007) and oyster (Davis & Davis 2010) farms. In Prince Edward Island, Canada, *S. clava* populations have been steadily declining as *C. intestinalis* populations increase (Ramsay et al. 2008). While the relatively high abundance of *C. intestinalis* may be currently preventing the proliferation of *S. clava* on mussel ropes in Port Phillip Bay (PPB), Australia, *S. clava* remains a significant future threat.

Hydroids too are often a common component of fouling communities. They can contribute significantly to the overall biomass of such communities (Kashin et al. 2000) and are typically ravenous feeders that have comparable ingestion rates to some bivalves (Gilli et al. 1998). Hydroids are often problematic in bivalve culture, fouling the mussel *Perna canaliculus* in New Zealand (Heasman & de Zwart 2004) and reducing the growth of the scallop *Mizuhopecten yessoensis* in Japan (Baba et al. 2007). In PPB, the non-indigenous hydroid *Ectopleura crocea* (Agassiz 1862) has become an increasingly abundant fouling species on mussel ropes over the last five years (Fitridge 2011). Although its deleterious effect on mussel growth and flesh weight has been reported (Fitridge 2011), to the authors’ knowledge, this has not been investigated experimentally.

Most studies investigating the effect of fouling on bivalve productivity use a holistic approach by investigating the entire fouling community, often by studying the effects of a cleaning schedule (Claereboudt et al. 1994; Lodeiros & Himmelman 1996; Taylor et al. 1997; Metri et al. 2002; de Sa et al. 2007). When a single species is considered, incidental observational studies are used rather than controlled experiments (Cropp & Hottle 1992; Daigle & Herbinger 2009). While documenting the effects of whole fouling communities is important, by identifying the species most responsible for any negative effects, management strategies can be tailored towards the removal of these species, and farmers can better determine if removal strategies are cost-effective. This is especially important as removal techniques tend to be ineffective against some species in the fouling community (Piola et al. 2010) and as such, future anti-fouling methods will likely be based on specific action against target organisms (Berntsson & Jonsson 2003; Guenther et al. 2011; Paetzold & Davidson 2011; Cahill et al. 2012).

This study is aimed to determine whether fouling by *C. intestinalis*, *E. crocea* and *S. clava* affects shell growth, flesh weight and the condition of the cultivated mussel *Mytilus galloprovincialis* (Lamarck 1819). In addition, the mechanisms causing any reductions were investigated with competitive feeding experiments in the laboratory.

**Materials and methods**

**Study sites and organisms**

PPB is a large (1930 km²) embayment on the southern coast of Victoria, Australia. Here, a growing aquaculture industry is dominated by the longline cultivation of the Australian blue mussel *M. galloprovincialis* within eight Aquaculture Fisheries Reserves covering roughly 1990 ha. The largest of these are the Pinnacle Channel Aquaculture Fisheries Reserve (1000 ha; 38°16′39″ S, 144°49′19″ E; hereafter PC), the Clifton Springs Aquaculture Fisheries Reserve (318 ha; 38°08′24″ S, 144°33′26″ E), the Grassy Point Aquaculture Fisheries Reserve (252 ha; 38°06′41″ S, 144°41′35″ E) and the Kirk Point – Werribee (KPW) Aquaculture Fisheries Reserve (200 ha; 38°02′28″ S, 144°34′38″ E; hereafter KPW). Collections were done at Workshops Jetty (WJ), Williamstown (37°51′39″ S, 144°54′34″ E; hereafter WJ) and Portarlington Pier (PP) (38°06′44″ S, 144°39′07″ E; hereafter PP).

The solitary ascidian *C. intestinalis* has been recorded from most harbours around the world (Kott 1997) and is presumed to originate from the north-east and north-west Atlantic (Hewitt et al. 2004). It was first recorded in PPB in 1958 (Miller 1966) and has subsequently invaded much of the bay (Keough & Ross 1999). The hydroid *E. crocea* was first recorded in PPB in 1884 as *Tubularia ralphii* (Bale 1884) and has recently emerged as a problematic fouling species for the mussel aquaculture industry in the bay (Fitridge 2011). The solitary ascidian *S. clava* originates from the north-west Pacific and has invaded many coastal areas around the world (Keough & Ross 1999). First recorded from PPB in 1976 from material collected in 1972 (Holmes 1976), it is not currently a major component of the fouling communities associated with mussel aquaculture.

Mussels used in the *S. clava* inoculation experiments were collected from PC. Small mussels were 40.0 ± 1.0 mm (mean ± SE) in length and large mussels were
62.1 ± 0.5 mm in length and had an initial dry flesh weight of 0.49 ± 0.03 g. Initial flesh weights were obtained from 30 mussels from each size class, which were sacrificed prior to experimentation. Flesh weights for these small mussels were not recorded as they were heavily predated by the seastar Coscinasterias muricata during the study, and no analyses were conducted on them. S. clava were collected from WJ, and were 106.8 ± 3.5 mm in length. Small mussels used in the C. intestinalis and E. crocea inoculation experiment were collected from PC and were 50.2 ± 1.0 mm in length and had a dry flesh weight of 0.20 ± 0.02 g, whilst large mussels were collected from PP and were 72.8 ± 1.0 mm in length and had a dry flesh weight of 0.97 ± 0.11 g. C. intestinalis, with an average length of 36.9 ± 1.2 mm, were collected from acetate sheets deployed at PP and E. crocea colonies were collected from mussel ropes within KPW.

For the feeding experiments, mussels were collected from WJ and were 62.8 ± 1.1, 64.8 ± 0.7 and 48.6 ± 0.7 mm in length for the S. clava, C. intestinalis and E. crocea experiments, respectively. S. clava (71.2 ± 5.9 mm) were collected from WJ, C. intestinalis (24.7 ± 1.5 mm) were collected from PP and E. crocea colonies were collected from mussel ropes within KPW.

Biofouling effects on mussel shell growth and flesh weight

Experimental mussel ropes were inoculated with one of the three fouling species to determine whether these organisms had an effect on mussel shell growth or flesh weight. Field experiments were conducted over two months cultivation periods.

S. clava experiments were conducted from December 2011 to February 2012 and the C. intestinalis and E. crocea experiments from February to April 2012. Upon collection, mussels and the fouling organisms were cleaned of epibionts by pulling off any easily removed species by hand, and scraping calcified species off with a paint scraper. Mussels were soaked onto 35 cm rope sections at densities of 25–30 mussels per rope for the small mussel experiments and 20–25 mussels per rope for the large mussel experiments. Five inoculated and five control ropes were used for each mussel size in the S. clava experiment. As the C. intestinalis and E. crocea treatments were run concurrently, a total of 10 inoculated and five control ropes were used for each mussel size. 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 or eight E. crocea colonies (~25 feeding polyps per colony) were used per replicate. Glue was also applied to mussels on the control ropes to act as a procedural control. The level of fouling used was calculated by visually inspecting fouling on mussel ropes in situ and assessing the literature, and scaling the density appropriately to mimic a low-fouling to medium-fouling scenario (see Figure S1) [Supplementary material is available via a multimedia link on the online article webpage]. Experimental rope sections were cable tied to weighted ropes and deployed at PP for two months. Treatment and control rope sections were carefully gardened of any additional fouling by hand every two weeks. Fouling organisms rarely died throughout the study period, but any that did were replaced with new individuals.

Following collection after two months, mussels from each rope section were placed in individual sealable bags and frozen prior to processing. Once thawed, 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 and dissected. The wet and dry weights of the shell and flesh were obtained using an A&D HR-200 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\textsubscript{COOKED MEAT}/ (weight\textsubscript{COOKED MEAT} + weight\textsubscript 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, \((\text{length} \times \text{width} \times \text{depth})^{1/3}\), was used to represent overall mussel size, and along with the commercially important length and flesh weight parameters, was statistically analysed.

Biofouling effects on food consumption of mussels

Laboratory experiments investigating food consumption by mussels and foulers were conducted to determine the amount of phytoplankton consumed and to infer whether exploitative or interference competition was occurring. Mussels and fouling organisms were manually cleaned of epibionts and kept in flow-through aquaria in sand-filtered seawater prior to experimentation. Mussels were soaked onto 15 cm rope sections at a density of six mussels per rope for the S. clava and C. intestinalis experiments, and seven per rope for the E. crocea experiments. These stocking densities were chosen so that during the 2 h experimental period, not all phytoplankton cells were consumed and to reduce variability among replicates. Stacking densities used are notably lower than standard aquaculture production densities, and instead, resemble the densities used in the field experiments. Five replicate runs were conducted for each fouling species, with each run consisting of four treatments: live mussels (mussels-only), the fouling species attached to empty mussel shells (foulers-only), fouler mimics attached to live mussels (mimics) and the fouling species attached to live mussels
(M+F (obs)). Four S. clava, 10 C. intestinalis or four E. crocea colonies were attached to each rope in the foulers-only, and M+F (obs) treatments. The densities of the mussels and foulers closely matched those used in the field experiments, and were thus proportional to low-level to medium-level fouling experienced on commercial mussel ropes. Although similar numbers of C. intestinalis were used, these individuals were smaller than those used in the field experiments. S. clava and C. intestinalis mimics were made from casting resin and a putty mould, and closely matched the size and weight of the live specimens. Previously frozen E. crocea colonies were used as mimics and were matched to live colonies in terms of number of hydranths. The number of mimics attached to mussels in the mimic treatment matched the number of live foulers used in the other treatments. Foulers and mimics were carefully glued onto the shells of randomly chosen mussels with Selleys® Quick Fix™ cyanoacrylate glue. Glue was also applied to the mussels-only treatment in the same manner, to serve as a procedural control.

Experiments were conducted throughout April 2012. Ropes were transferred into 1 μm filtered seawater for 24 h prior to experimentation to allow purging. Ropes were then suspended in individual 451 aquaria with fresh 1 μm filtered seawater, and the mussels and foulers were allowed to acclimate for 1 h and recover from any handling stress. The water temperature remained at 19 °C throughout the experiments. The diatom Chaetoceros muelleri (Lemmmerman 1898) was added to each aquarium at an average initial concentration of 10,652 ± 174 cells ml⁻¹. At this concentration, mussels were not expected to produce pseudofaeces (Riisgard et al. 2011), and final cell concentrations were within the range in which mussel feeding should not be severely compromised (Riisgard et al. 2003). Throughout the experiment, the water was kept homogenous by mixing with two air-stones. Twenty millilitre water samples were taken 5 min after the addition of algae and again every 30 min for 2 h. Cell concentration was determined using a Beckman Coulter Multisizer™ 3 Coulter Counter® with a 100 μm aperture. Cells between 2.6 and 10 μm were counted. The percentage of cells cleared was calculated to standardise for any differences in initial cell concentration.

Control aquaria set up during pilot experiments showed that the cell concentration did not change over the 2 h (t-test; \( p > 0.05 \)) and so, no control tanks were necessary, or included, in the experiment.

**Statistical analysis**

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.

Mixed model factorial ANOVA was used to compare the proportion of cells consumed among treatments. The dependent variable in all tests, the proportion of cells consumed, was log-transformed. Treatment and time were treated as fixed factors. Initially, all treatments were compared, followed by planned comparisons of the mussel-only and mimics treatments. An exploitative effect of fouling on mussel feeding was investigated by using the consumption from the mussels-only and the foulers-only treatments to generate a predicted combined consumption for each run: \( M+F \text{(pred)} = 100 - (100 - \text{mussels-only}) \times (100 - \text{foulers-only})/100 \). These predicted values were then statistically compared to the consumption of the observed fouled treatment (M+F (obs)).

**Results**

**Biofouling effects on mussel shell growth and flesh weight**

Inoculating experimental mussel ropes with C. intestinalis, E. crocea and S. clava significantly reduced growth in both small and large mussels (Figure 1 and 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 2 and 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

![Figure 1. 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 \((\text{length} \times \text{width} \times \text{height})^{1/3}\). 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 and light grey and white bars = the two separate experiments using large mussels. N-values are reported for each experiment above the columns. * = a significant difference in planned comparisons between control and fouled treatments (\( p < 0.05 \)).](image-url)
C. intestinalis and E. crocea, respectively, compared to the control mussels (Figure 3 and Table 1). Mussel condition was significantly lower in small mussels fouled by C. intestinalis, but not in mussels fouled by E. crocea (Figure 4 and Table 1).

In the C. intestinalis and E. crocea experiment, large mussels experienced relatively slower growth rates. Control mussels grew 2.7 ± 0.7 mm in length, whilst mussels inoculated with C. intestinalis grew 0.3 ± 0.4 mm and mussels inoculated with E. crocea grew 1.1 ± 0.6 mm (Figure 2 and 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 ± 0.5 mm, respectively (Figure 2 and 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 3 and Table 1). In addition, the condition of large mussels was not significantly affected by fouling by these three species (Figure 4 and Table 1).

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

| Source of variation | Small                  |             |           | Large                  |             |
|---------------------|------------------------|-------------|-----------|------------------------|-------------|
|                     | d.f. | MS  | F    | P    | d.f. | 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   | 0.07 | 2.83  | 0.127 |
|                     | Residual  | 12  | 0.002 |       |       | 9   | 0.03 |       |       |
|                     | 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   | 0.03 |       |       |
|                     | 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 | 8   | 0.62 |       |       |
|                     | Residual  | 8   | 0.62 |       |       | 8   | 1.83 |       |       |
|                     | Length  | 1   | 23.31 | 12.74 | 0.007 | 8   | 1.83 |       |       |
|                     | Residual  | 8   | 2.13  |       |       | 8   | 0.01 |       |       |
|                     | Flesh weight  | 1   | 0.01 | 1.14  | 0.317 | 1   | 0.01 | 0.38  | 0.404 |
|                     | Residual  | 8   | 0.01 |       |       | 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.

Figure 2. 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 are reported for each experiment above the columns. * = a significant difference in planned comparisons between control and fouled treatments (p < 0.05).
Biofouling effects on food consumption by mussels

Food consumption patterns differed among treatments for all three fouling species (Table 2). *C. intestinalis* cleared 32 ± 3% of all cells after 2 h (Figure 5a). Both the mussels-only and mimics treatments consumed 78 ± 2% of the cells after 2 h (Figure 5a and Table 2). The fouled treatment cleared the most cells, with 85 ± 2% of all cells cleared. This matched the percentage of cells that would have been cleared by the theoretical fouled treatment (Figure 5a and Table 2).

After 2 h, *E. crocea* colonies cleared 5 ± 1% of cells. The mussels-only treatment in these experiments cleared 58 ± 1% of available cells, significantly more than the mimics treatment, which cleared only 46 ± 3% (Figure 5b and Table 2). The fouled treatment cleared 50 ± 3% of cells, which was significantly less than the predicted clearance of 60 ± 1% of cells, based on individual clearance rates (Figure 5b and Table 2).

Similar to the *C. intestinalis* experiments, *S. clava* cleared 30 ± 2% of available cells after 2 h (Figure 5c). The mussels-only and mimics treatments did not significantly differ, and cleared 73 ± 3% and 70 ± 2% of all cells by the end of the experiment, respectively (Figure 5c and Table 2). The fouled treatment again cleared the most cells, clearing 80 ± 4%, which closely matched the predicted clearance of 81 ± 2% of cells (Figure 5c and Table 2).

Discussion

Biofouling effects on mussel shell growth and flesh weight

Inoculating mussel ropes with low to medium densities of *C. intestinalis, E. crocea* and *S. clava* indicated 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 muscle 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. For example, six-month-old mussels fouled by *E. crocea* were 4% shorter and had 23% less flesh than unfouled mussels (Fitridge 2011). In addition, reductions in mussel size and flesh weights were more pronounced in these younger, smaller mussels, compared to larger, 12-month-old mussels. Daigle and Herbinger (2009) observed growth reductions, lower meat yields,
reduced condition and decreased survivorship of mussels on longlines fouled by *C. intestinalis*. Similarly, Osman et al. (1989) found reduced growth in oysters in the presence of sessile fouling organisms, including *C. intestinalis*, and deduced that competition for planktonic food was the most likely cause.

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.

**Biofouling effects on food consumption by mussels**

Feeding experiments were designed to gain an insight into the mechanisms of potential food competition that likely caused decreased shell growth and flesh weights in fouled mussels in the inoculation experiments. The two solitary ascidians, *C. intestinalis* and *S. clava*, had very similar food consumption, and at the level of fouling used in these experiments, mussels consumed more than twice as many cells as the ascidians. The lack of a significant difference between the mussels-only and mimics treatments for both these species suggests little in the way of interference competition, a result bolstered by consumption in the fouled treatment being accurately predicted from individual consumption rates. The similarities between the observed and predicted consumption by the fouled treatment also eliminates any exploitative competition for food. The level of fouling used in these experiments matched the inoculation experiments, and

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**Table 2.** Linear mixed model ANOVA-fixed factor results from feeding experiments comparing the percentage of cells consumed over time for all treatments; the mussels-only treatment (mussels-only) compared to the mussels with fouler mimics treatment (mimics); and the fouled treatment (*M + F (obs)*) compared to the predicted consumption (*M + F (pred)*) calculated from the mussels-only and foulers-only treatments.

| Source of variation | C. intestinalis | E. crocea | S. clava |
|---------------------|----------------|-----------|----------|
|                      | F   | P    | F   | P    | F   | P    |
| All treatments       | d_f |       | 306.9 | 0.000 | 260.9 | 0.000 | 176.4 | 0.000 |
| Treatment           | 3   |       | 306.9 | 0.000 | 349.9 | 0.107 | 181.7 | 0.000 |
| Time                | 3   |       | 152.9 | 0.000 | 1.05  | 0.107 | 181.7 | 0.000 |
| Treatment × time    | 9   | 2.85  | 0.007 | 1.70  | 0.015 | 0.008 |
| Residual            | 64  | 0.02  |       | 0.15  | 0.004 |
| Mussels-only vs mimics | 1   | 0.32  | 0.660 | 23.6  | 0.000 | 1.91  | 0.177 |
| Treatment           | 3   | 131.6 | 0.000 | 146.3 | 0.000 | 97.8  | 0.000 |
| Time                | 3   | 0.15  | 0.639 | 0.18  | 0.968 | 0.18  | 0.907 |
| Treatment × time    | 3   | 0.15  | 0.639 | 0.18  | 0.968 | 0.18  | 0.907 |
| Residual            | 32  | 0.01  |       | 0.02  | 0.02  |       |

Each treatment–species combination consisted of five replicate runs. Analysis was run with log-transformed data. MS<sub>residual</sub> is listed at the bottom of the *p*-values. Boldface values are significant at *p* < 0.05.
thus, did not constitute intense fouling. Exploitative food competition and flow inhibition by *C. intestinalis* has been blamed for reducing mussel growth and condition on heavily fouled mussel ropes (Daigle & Herbinger 2009). There is also evidence of comparable retention efficiencies (Mohlenberg & Riisgard 1978), particle size utilizations (Daigle & Herbinger 2009), clearance and ingestion rates (LeBlanc et al. 2003) and pump characteristics (Petersen 2007) between ascidians and mussels. Despite little evidence of food competition found here, mussel clearance rates are affected by numerous environmental variables *in situ*, and the laboratory conditions used here will not accurately mimic these (Riisgard et al. 2011). As such, exploitative competition between these two taxa in the field remains possible.

The hydroid *E. crocea* cleared relatively few cells compared to the ascidians and mussels, and the presence of *E. crocea* mimics resulted in mussels consuming 11.5% fewer cells. The mussels-only treatment also consumed more cells than the fouled treatment. As such, the predicted consumption, based on individual feeding rates, was well above the observed consumption for the fouled treatment. Interference competition is likely occurring; whereby, *E. crocea* interferes with feeding by the mussels by smothering the valves, reducing water flow and food delivery. Flow resistance of inhalant and exhalent currents in the mussels increases as mussels become smothered, and an increase in back pressure results in reduced pumping rates (Jorgensen et al. 1988). *E. crocea* has a fast growth rate and grows in dense tufts of often several hundred stems arising from a matted hydrorhiza (Watson 1999). These features allow *E. crocea* to quickly overgrow and smother mussels, reducing water flow and food delivery.

In terms of exploitative competition, hydroids may have some selectivity in feeding (Genzano 2005), despite their feeding being regarded as opportunistic, and may be able to exhibit resource partitioning with mussels. Although there is still debate regarding retention efficiencies and particle size utilisation among suspension feeders, in general, *E. crocea* prefers large diatoms and benthic amphipods (Fitridge 2011), while mussels consume a variety of food, from small phytoplankton (Lesser et al. 1992; Ward & Shumway 2004) to large zooplankton (Lehane & Davenport 2006). The small diatom species used in this study (4–8 μm) may explain why no exploitative food competition between the two species was detected, and further investigations into food competition between these species using a variety of food sources, particularly larger diatoms and zooplankton, are needed to determine whether exploitative competition between the two exist, and whether it could be partially responsible for reducing shell growth and flesh weight in fouled mussels.

Phytoplankton biomass is the primary factor influencing feeding behaviour in bivalves (Riisgard et al. 2011), and Lesser et al. (1992) argue that mussels will be superior competitors when food is not limiting. The concentration of the nutrient-rich phytoplankton monoculture used here may have resulted in higher than normal feeding rates in mussels than experienced in the field. In addition, all organisms were starved for 24 h prior to experimentation. This can cause a breakdown of the ascidian mucous net used for feeding, leading to reduced feeding rates and thus, limited competition (Petersen & Riisgard 1992), although mussels also may exhibit reduced filtration rates post starvation (Riisgard et al. 2003).

In natural systems, the spatial separation of suspension feeders can limit food competition between species with comparable filtration rates and particle size utilisation (Stuart & Klumpp 1984). Although fouling communities associated with mussel aquaculture are in close proximity to mussels, resource partitioning among these...
organisms may limit food competition, and thus, ideally, assessments of food competition should take into account particle concentration, type and size (Lesser et al. 1992). Here, an algal monoculture was used to determine the effects of fouling on mussel feeding. While this method has the potential to provide useful insights into these effects, it is by no means an exhaustive investigation into food competition, and no attempt is made to quantify food competition due to the myriad variables affecting feeding in mussels, ascidians and hydroids (Genzano 2005; Petersen 2007; Daigne & Herbringer 2009).

**Industry implications**

The presence of the fouling organisms *C. intestinalis*, *S. clava* and *E. crocea* reduced mussel shell growth and flesh weight, even at low-fouling to medium-fouling levels. The most plausible mechanism driving this effect is food competition. In terms of industry implications, however, the mode by which fouling reduces mussel productivity may be of limited importance.

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 a 5% reduction in meat yield is detrimental to farm productivity (Lance Wiffen, SeaBounty Mussels Pty Ltd, personal communication). Growth reductions of roughly 1 mm 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 fresh water 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.

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