Co-culturing green sea urchins, *Strongylocentrotus droebachiensis*, with blue mussels, *Mytilus edulis*, to control biofouling at an integrated multi-trophic aquaculture site

by

Andrea Bartsch
B.Sc., University of Victoria, 2007

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

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in the Department of Geography

Supervisory Committee

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Prevention and removal of biofouling from nets and product is a huge expense in the aquaculture industry. Of the many technologies that slow the accumulation of biofouling, copper-based coatings are used most commonly as they are a relatively inexpensive and effective option. However, they can leach into the marine environment and have potentially harmful impacts on marine life. In previous studies, sea urchins have shown potential as a non-toxic alternative to control fouling. In this field study, five different stocking densities (*i.e.* 0, 30, 60, 90, 120 urchins net^{-1} or 0, 2.46, 4.91, 7.37, 9.82 urchins m^{-2}) of green sea urchins, *Strongylocentrotus droebachiensis,* were randomly placed in 30 mussel predator exclusions nets (with six replicates per density treatment) in order to test the effect of urchin density on biofouling intensity and urchin/mussel growth. Mussel predator exclusion nets were chosen to house the urchins since they are necessary to protect mussels from diving ducks and sea otters on the west coast of Vancouver Island, British Columbia, Canada. The urchins provide a means of controlling biofouling as well an additional marketable crop to offset predator net expenses. After 174 days, the percent net occlusion, mussel growth, and urchin growth were quantified. Nets with urchins were significantly less fouled than those without urchins. Fouling on nets with higher stocking densities of urchins (90 and 120 urchins net^{-1}) was significantly less than that on nets with the lowest stocking density (30 urchins net^{-1}). Fouling was no longer significantly reduced at densities >60 urchins net^{-1} or 4.91 urchins m^{-2}. While fouling was significantly reduced in the presence of urchins, it was not completely eliminated as they were only able to access the inside surface of the nets. There was no significant difference in mussel growth at the different urchin stocking densities, but urchin somatic growth and gonad growth did decline with increasing urchin stocking density. Mussels and sea urchins can be successfully co-cultured with no food inputs, but there is a trade-off between biofouling control and urchin growth.

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Chapter 1 : Introduction

1.1 Sustainable Aquaculture

The finfish aquaculture industry – especially in British Columbia (BC), Canada – has been criticized by the public and environmental non-government organizations for the perceived negative environmental impacts of the industry. These include production of fish waste (*i.e.* uneaten food and feces), escapes and negative interactions with wild species, spread of disease and parasites to wild populations, use of hormones and chemotherapeutants, marine mammal entanglement, copper-based antifouling paints, noise pollution, and esthetic degradation of the shoreline. The discharge of fish waste through open net-pens and copper-based antifouling paints are two such environmental impacts that will be considered in this thesis.

Both the aquaculture industry and the research community have responded to these perceived environmental impacts and have developed a variety of strategies to potentially reduce the environmental impacts of finfish mono-culture. Some examples of these strategies include: land-based systems (Colt et al., 2008; Tal et al., 2009), closed containment systems (Fullbirth et al., 2009; Cahill et al., 2010), production of lower trophic level species (*e.g.* tilapia), and integrated multi-trophic aquaculture (IMTA).

1.2 Integrated Multi-trophic Aquaculture (IMTA) and Sustainable Ecological Aquaculture (SEA)

IMTA is the integration of fed species (*e.g.* finfish, shrimp) with organic extractive species (filter feeders [*e.g.* mussels, scallops, oysters, cockles] and detritivores [*e.g.* sea urchins, sea cucumbers]) and inorganic extractive species (*e.g.* kelp, Nori) within the same oceanographic area, usually a single farm tenure (Chopin et al., 2007). The potential benefits of this aquaculture practice are removal of excess nutrients in the water (by the extractive species), economic diversification, and greater social acceptability. IMTA economically diversifies the aquaculture business because the extractive species are also additional marketable seafood products (Whitmarsh et al., 2006; Neori, 2008; Bunting and Shpigel, 2009), which means the business no longer needs to rely on only one species. This has the potential to make companies more economically resilient since seafood markets fluctuate on an annual and seasonal basis (FAO, 2011). Therefore, growing multiple species reduces economic pressures when one species does not perform well in a given year.

Research on IMTA is taking place globally (*e.g.* Chile, China, Israel, South Africa, and the United Kingdom). In Chile, Israel, and South Africa, abalone and seaweeds have been cultured together (Shpigel et al., 1996; Bolton et al., 2006; Buschmann et al., 2007) while in the United Kingdom, researchers have integrated oysters, sea urchins, and seaweeds into salmon farms (Kelly et al., 2007; Rodger et al., 2007). There is also a large nation-wide research effort on IMTA in Canada. In the Bay of Fundy, on the east coast of Canada, extractive species such as mussels, sea urchins, polychaete worms, and kelps have been integrated around and under polar-circle cages which contain Atlantic salmon (*Salmo salar*). Meanwhile, in Kyuquot Sound (Figure 1.1), on the west coast of Canada, extractive species have been integrated around and under steel-frame net pens which contain sablefish (*Anoplopoma fimbria*).

In regards to waste removal, one of the goals of IMTA research is to determine how effective various species are at removing dissolved inorganics or particulate organics from the water column and how to grow them in ways that maximize their waste removal efficiency. The Kyuquot Sound facility (Figure 1.1), where my research was conducted, is also a sustainable ecological aquaculture (SEAfarm) site, which is an IMTA site, but also incorporates the additional principles of using native or established species where possible, sustainable energy sources (eg. wind power), and organic farming principles. This includes not using chemicals or antibiotics. Finding an alternative for copper-based antifoulant is especially important for SEAfarm aquaculture sites.

1.2.1 Mussels in IMTA and SEAfarms

The blue mussel, *Mytilus edulis*, was the organic extractive species used for this research project, but is also one of the dominant fouling species at many aquaculture sites. *Mytilus edulis* can filter fish waste (feces and excess feed) from the water column (Reid et al., 2010) and it has elevated growth rates when cultured adjacent to fish pens compared to controls grown away from fish pens (Stirling and Okumus, 1995; Peharda et al., 2007; Sara et al., 2009). In addition to being a promising species from an IMTA standpoint, *M. edulis* is also a widely consumed seafood product with established local, national, and international markets.

Sea otters (*Enhydra lutris*) have been reintroduced to the northwest coast of Vancouver Island (Watson et al., 1997) and there is a large population of them in Kyuquot Sound. The sea otters, as well as surf scoters (*Melanitta perspicillata*), predate on exposed mussels (Wursig and Gailey, 2002; Dionne et al., 2006), which is why the bivalve had not been grown at the Kyuquot SEAFoods site prior to being introduced to the farm for this research. In order to grow mussels at this site, they needed to be protected in predator-exclusion nets. These nets, however, would be an additional equipment expense for the farm and an additional surface on which biofouling could develop. Building individual nets for each mussel line would not be economically feasible when compared to the profits that could be made by selling the mussels. The alternative, a skirted net around the whole mussel grow-out system, would not exclude ducks because the top of the system is open (unlike traditional shellfish rafts). For individual predator nets to be economically feasible, an additional species would also need to be produced in the nets to further off-set the cost of building them. The green sea urchin, *Strongylocentrotus droebachiensis,* was a promising candidate based on the reasons outlined in section 1.2.2 below.

1.2.2 Sea Urchins in IMTA and SEAfarms

Sea urchins have received some attention as a potential aquaculture organism because of the lucrative market in Japan for sea urchin gonads or uni. In Canada, aquaculture-related research has focused on the green sea urchin, *S. droebachiensis,* and there is a growing understanding of how to effectively culture the species *(*Pearce et al., 2002a,b,c, 2004; Robinson et al., 2002; Daggett et al., 2005, 2006, 2010)*. Stronglyocentrotus droebachiensis* is one of the economically valuable sea urchin species on the west coast of Canada, praised for its sweet tasting uni (D. Macey, D&D Pacific Fisheries Ltd., personal communication). It should be noted, however, that Canadian sea urchin exports to Japan have declined in recent years (FAO, 2011). This decline is due to: 1) decreased demand in Japan as younger generations are consuming less seafood and 2) increased exports from Chile and Russia (Sonu, 2003; D. Macey, D&D Pacific Fisheries Ltd., personal communication).

In addition to being economically valuable, sea urchins have shown some promise as a biological method of biofouling control. Lodeiros and Garcia (2004) found that *Lytechinus variegatus* significantly reduced fouling on both pearl nets and scallops compared to controls without urchins, but reported that *Echinometra lucunter* was not as effective at controlling fouling. In addition, Ross et al. (2004) found that the sea urchins *Echinus esculentus* and *Psammechinus miliaris* also significantly reduced fouling on pearl nets and reported that *P. miliaris* was associated with increased scallop growth rate (Ross et al., 2004).

In BC, *S. droebachiensis* shows potential as a biological-fouling control species. Edwards and Cross (2008) tested the fouling control potential of green urchins in a small-scale experiment and found that the species was capable of controlling fouling on nylon nets when held at higher stocking densities. *Strongylocentrotus droebachiensis* has also been observed recruiting into shellfish containment structures and reducing fouling in B.C. (C. Day, Taylor Shellfish, personal communication). However, Switzer et al. (2011) did not find *S. droebachiensis* to be effective at controlling tunicate fouling on oyster shells, although they admitted that urchins were placed with oysters that already had well developed fouling communities and that urchins may be more effective at fouling control if added to the system before fouling becomes developed. The economic value of *S. droebachiensis* and its biofouling control potential makes it an ideal candidate to integrate into the mussel predator nets.

1.3 Biofouling in Aquaculture

Biofouling is the natural recruitment and attachment of aquatic species onto any surface. These species recruit more slowly onto some surfaces (*e.g.* smooth metal) than others, but any surface will become fouled over time. Biofouling is a problem for any sea-based industry and aquaculture is no exception. The cost of preventing and removing biofouling from surfaces is a significant expense in the aquaculture industry in terms of labour and resources (Durr and Watson, 2010). Nylon nets are a widely-used material in aquaculture and are especially prone to fouling. The large surface area and heterogeneous surface structure of these nets make them an ideal place for fouling organisms to attach. Despite their susceptibility to fouling, these nets are still used in the industry due to their low cost, light weight, high strength, and flexibility.

Measures must be taken to prevent and remove fouling because it increases net drag (Swift et al., 2006), decreases water flow (Gansel et al., 2009; Madin et al., 2010), and over-weights structures, which can lead to net breakage and species escapes or to decreased water quality within nets. In addition, for shellfish farmers, biofouling increases the processing time and reduces the market value of their products (Durr and Watson, 2010).

Treating the nets with a copper-based coating is the most commonly used method in Canada to prevent biofouling. This method does slow the recruitment of fouling organisms onto Nylon nets (Braithwaite et al., 2007), but the nets still require periodic cleaning. There is evidence that the copper slowly leaches from these nets into the surrounding water, which has potential negative environmental repercussions (Andersson and Kautsky, 1996; Hall and Anderson, 1999; Katranitsas et al., 2003; Braithwaite and McEvoy, 2005; Kullman et al., 2007). A variety of non-toxic methods of biofouling prevention have been utilised with varying success. Non-toxic alternatives that have shown some promise include acetic acid (Piola et al., 2010), biofilms (Qian et al., 2007), conductive coatings (Huang et al., 2010), and heat treatment (Rajagopal et al., 2005). Biological alternatives are also being considered for combating fouling; herbivorous fish (Kvenseth, 1996), crabs (Hidu et al., 1981; Enright, 1993), sea cucumbers (Ahlgren, 1998), shrimp (Dumont et al., 2009), and sea urchins

(Lodeiros and Garcia, 2004; Ross et al., 2004; Edwards and Cross, 2008; Switzer et al., 2011) have all been trialed for biological fouling prevention or removal, with varying degrees of success. There is no method of fouling prevention that is 100 percent effective. Therefore, cleaning nets is still an expense to aquaculture sites, even if preventative methods are also used. Power washing (underwater or on land) is the most commonly used method to remove fouling.

1.4 Study Site Description

The research described in Chapters 2 and 3 of this thesis was completed in Kyuquot Sound at the commercial Surprise Island SEAfarm and IMTA facility (50° 02’ 47.39” N, 127° 17’ 49.28” W) (Figure 1.1). Flow at the site is dominated by a prevalent counter clockwise current. The prevailing current moves downstream from the fish pens to the shellfish nets and kelp lines, which means that the waste from the fish should be carried to the extractive species (data are unavailable to confirm this). There is significant freshwater input into the bay in the winter and spring. The average depth at this site is 28 m and the temperature range is from 7 to 16°C in the upper 10 m of the water column.

During the time frame of the present research (May 2009 to October 2010), the fed species on this farm was sablefish (*Anoplopoma fimbria*), which was given a commercial feed pellet. Downstream from the sablefish were Japanese and weathervane hybrid scallops (*Patinopectin yessoensis* X *P. caurinus*), blue mussels (*Mytilus edulis*), green sea urchins (*S.droebachiensis*), and kelp (*Saccharina latissima*). There were also sea cucumbers (*Parastichopus californicus)* below the sablefish net pens. The site was also licensed to farm Pacific oysters (*Crassostrea gigas*) and basket cockles (*Clinocardium nuttallii*), but these species were not being grown at the time of the research. Nutrients were only added to the system at the highest trophic level (sablefish) and all of the other species consumed the waste products of the finfish and other naturally occurring nutrients, with no additional feed requirements. The mussels and sea urchins were introduced to the site for the purpose of this research.

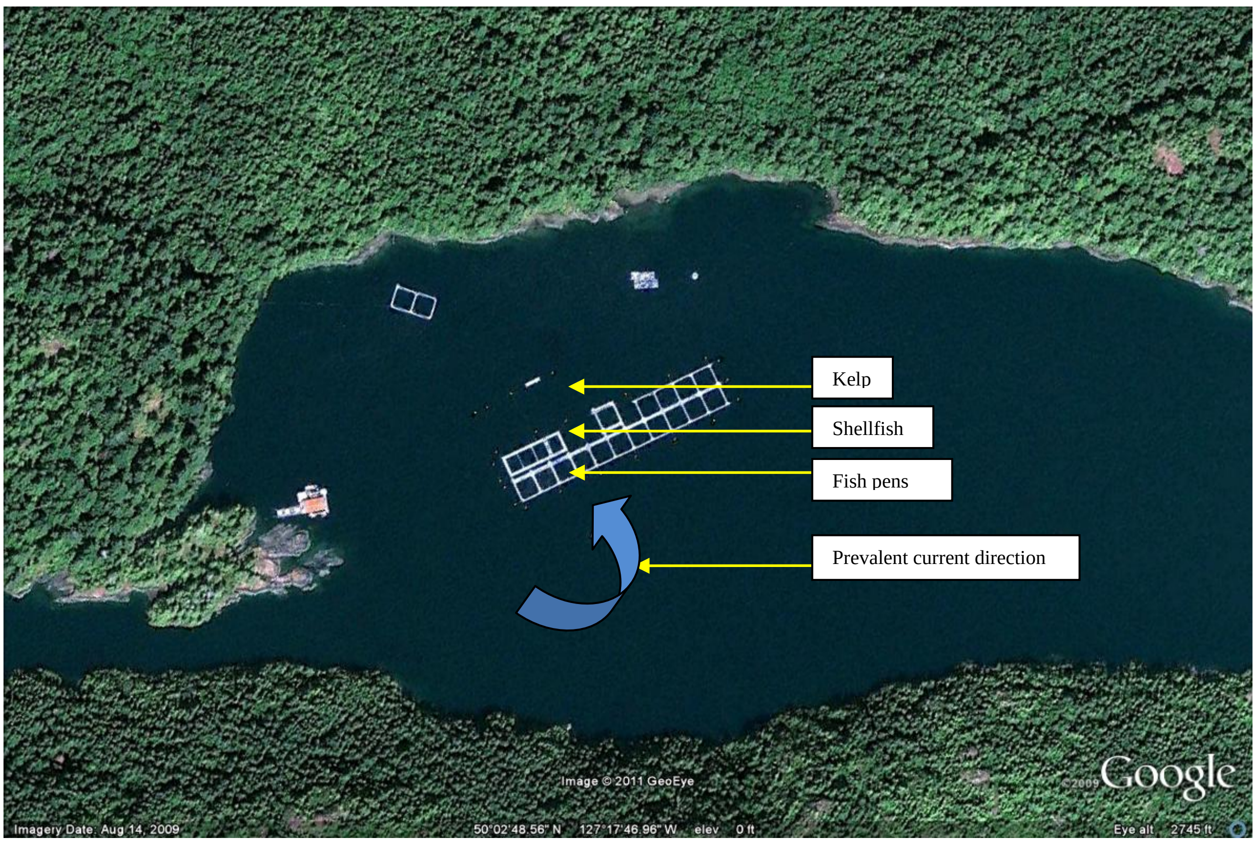

Figure 1.1 Study location at Kyuquot SEAfood Ltd., Surprise Island SEAfarm, Kyuquot Sound, British Columbia, Canada (Google Earth, 2011)

1.5 Research Objectives and Hypotheses

1) Can *S. droebachiensis* effectively control fouling on large surface area nets with a wide range in depth?

H_{01}: There will be no statistically significant difference in biofouling accumulation on mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{A1}: There will be a statistically significant difference in biofouling accumulation on mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{02}: There will be no statistically significant difference in biofouling accumulation on mussel predator nets at different depths (1, 2.5, 4 m).

H_{A2}: There will be a statistically significant difference in biofouling accumulation on mussel predator nets at different depths (1, 2.5, 4 m).

2) Does urchin stocking density and/or depth influence the growth of *S. droebachiensis* and *M. edulis?*

H_{03}: There will be no statistically significant difference in mussel growth (length, whole wet weight, wet meat weight, meat yield, dry meat weight, and ash-free dry meat weight) in predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{A3}: There will be a statistically significant difference in mussel growth (length, whole wet weight, wet meat weight, meat yield, dry meat weight, and ash-free dry meat weight) in predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{04}: There will be no statistically significant difference in mussel growth (length, whole wet weight, wet meat weight, meat yield, dry meat weight, and ash-free dry meat weight) in predator nets at different depths (1, 2.5, 4 m).

H_{A4}: There will be a statistically significant difference in mussel growth (length, whole wet weight, wet meat weight, meat yield, dry meat weight, and ash-free dry meat weight) in predator nets at different depths (1, 2.5, 4 m).

H_{05}: There will be no statistically significant difference in urchin growth (test diameter, whole wet weight, gonad weight, and gonad yield) in mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{A5}: There will be a statistically significant difference in urchin growth (test diameter, whole wet weight, gonad weight, and gonad yield) in mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{06}: There will be no statistically significant difference in urchin gonad quality in mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{A6}: There will be a statistically significant difference in urchin gonad quality in mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

1.6 Research Limitations

Some of the shortcomings of this research project include: no particulate organic matter (POM) measurements, no sampling in the middle of the experiment, and no controls for biofouling or urchin somatic/gonadal growth away from the farm. POM data would have been useful to help explain spatial trends in mussel growth and biofouling across depths and at increasing distances from the fish pens as well as to confirm the dispersion pathways of nutrients from the fish pens, but it was not feasible during this experiment. Mussels and urchins were only measured at the beginning of the experiment and at the end, after 174 days. It would have been interesting to examine the growth trends at least once between the beginning and end of the experiment, but additional sampling periods were ruled out because the urchins were difficult to access once in the nets. The additional handling may have caused stress and therefore changes in growth rates to the organisms, and it may have led to unintentional removal of biofouling. Finally, having control nets as well as urchins grown away from the farm would have enhanced the study. This would have allowed for comparing urchin somatic and gonadal growth between individuals exposed to the additional nutrients from the fish pens and those with no additional nutrient inputs. Similarly, it would have been interesting to compare biofouling accumulation with additional nutrient inputs from the farm to a reference site.

1.7 Thesis Structure

This thesis is divided into four chapters, which are able to stand alone as separate documents. Chapter 1 (Introduction) provides all of the necessary background information for the thesis and explains the rationale, objectives, and hypotheses of the research. Chapters 2 and 3 are results chapters and were prepared as separate manuscripts, which will be submitted for peer-reviewed publication. Chapter 2 examines the potential of sea urchins as a biological method of biofouling control and, more specifically, provides biofouling control results for the green sea urchin, *S. droebachiensis,* reared on mussel predator nets. Chapter 3 examines the growth of the green sea urchin, *S. droebachiensis,* with the blue mussel, *Mytilus edulis*, in co-culture. Finally, Chapter 4 (Conclusion) summarizes the key findings of the thesis, places them into context with previous research, and provides future research directions.

Chapter 2 : Optimal stocking density of green sea urchins, *Strongylocentrotus droebachiensis*, for controlling biofouling accumulation on mussel predator nets

Abstract

Prevention and removal of biofouling from nets and product is a huge expense in the aquaculture industry. Of the many technologies that slow the accumulation of biofouling, copper-based coatings are used most commonly as they are a relatively inexpensive and effective option. However, they can leach into the marine environment and have potentially harmful impacts on marine life. In previous studies, sea urchins have shown potential to be a non-toxic alternative to control fouling. In this field study, five different stocking densities (*i.e.* 0, 30, 60, 90, 120 urchins net^{-1} or 0, 2.46, 4.91, 7.37, 9.82 urchins m^{-2}) of green sea urchins, *Strongylocentrotus droebachiensis,* were randomly placed in 30 mussel predator exclusion nets (with six replicates per density treatment) to test the effect of urchin density on biofouling intensity. After 174 days, nets with urchins were significantly less fouled than those without urchins. Fouling on nets with higher stocking densities of urchins (90 and 120 urchins net^{-1}) was significantly less than that on nets with the lowest stocking density (30 urchins net^{-1}) as well as on the control nets with no urchins. Fouling was no longer significantly reduced at densities >60 urchins net^{-1} or 4.91 urchins m^{-2}. While fouling was significantly reduced in the presence of urchins, it was not completely eliminated as urchins were only able to access the inside surface of the nets, allowing fouling organisms to attach to the outside surface. *Strongylocentrotus*

droebachiensis does effectively slow the accumulation of fouling organisms, but does not eliminate the need for periodic manual net cleaning.

2.1 Introduction

Biofouling is an expensive problem in the aquaculture industry. Fouling increases net drag (Swift et al., 2006), decreases water flow (Gansel et al., 2009; Madin et al., 2010), and over-weights structures, which can lead to net breakage and species escapes or to decreased water quality within nets. There is also the cost of antifouling technologies and labour to manually remove fouling. In addition, for shellfish farmers, biofouling increases the processing time and reduces the market value of their products (Durr and Watson, 2010). Overall, the prevention and removal of fouling represent a large portion of farm operating costs. Aquaculture is even thought to increase the presence of fouling since some fouling species utilize the waste products of cultured species (Lojen et al., 2005).

The traditional method used to prevent net fouling is through the application of copper-based coatings, which reduce net fouling in comparison to untreated nets (Braithwaite et al., 2007). However, these coatings leach into the marine environment over time and may have adverse effects on marine life (Andersson and Kautsky, 1996; Hall and Anderson, 1999; Katranitsas et al., 2003; Braithwaite and McEvoy, 2005; Hollows et al., 2007; Kullman et al., 2007). Non-toxic alternatives that have shown some promise include acetic acid (Piola et al., 2010), biofilms (Qian et al., 2007), conductive coatings (Huang et al., 2010), and heat treatment (Rajagopal et al., 2005). Biological alternatives are also being considered for combating fouling; herbivorous fish (Kvenseth, 1996), crabs (Hidu et al., 1981; Enright, 1993), sea cucumbers (Ahlgren, 1998), shrimp (Dumont et al., 2009), and sea urchins (Lodeiros and Garcia, 2004; Ross et al., 2004; Edwards and Cross, 2008; Switzer et al., 2011) have all been utilised for biological fouling prevention or removal, with varying degrees of success.

One of these biological alternatives, which has shown promise, is the sea urchin. Lodeiros and Garcia (2004) found that *Lytechinus variegatus* significantly reduced fouling on both pearl nets and scallops compared to controls without urchins, but reported that while *Echinometra lucunter* did reduce biofouling, it was not as effective as *L. variegatus*. In addition, Ross et al. (2004) found that the sea urchins *Echinus esculentus* and *Psammechinus miliaris* also significantly reduced fouling on pearl nets and reported that *P. miliaris* was associated with increased scallop growth rates (Ross et al., 2004).

In British Columbia (BC), Canada, the green sea urchin, *Strongylocentrotus droebachiensis,* shows potential as a biological-fouling control species. Edwards and Cross (2008) tested the fouling control potential of *S. droebachiensis* in a small-scale experiment and found that the species was capable of controlling fouling on nylon nets when held at higher stocking densities. *Strongylocentrotus droebachiensis* has also been observed recruiting into shellfish containment structures and reducing fouling in oyster culture in BC (C. Day, Taylor Shellfish, personal communication). However, Switzer et al. (2011) did not find *S. droebachiensis* to be effective at controlling tunicate fouling on oyster shells, although they admitted that urchins were placed with oysters that already had well developed fouling communities and that urchins may be more effective at fouling control if added to the system before fouling becomes developed.

There has been an established *S. droebachiensis* fishery in BC since 1987 (FAO, 2011) and much research has been done on how to effectively culture them (Pearce et al., 2002a,b,c, 2004; Robinson et al., 2002; Daggett et al., 2005, 2006, 2010). The green sea urchin is also being used in research as an integrated multi-trophic aquaculture (IMTA) species on both the west and east coasts of Canada. The principle of IMTA is to integrate extractive species into fish farms that can utilize the waste of other organisms. Also, organisms that can be sold as a seafood product are favoured. All of these factors make *S. droebachiensis* an ideal candidate to control biofouling at an IMTA site as well as other aquaculture facilities.

The objectives of this study were to determine: 1) if *S. droebachiensis* can effectively control fouling on large surface area nets with a wide range in depth and 2) how many urchins are required per unit of surface area to keep fouling at low levels. The null and alternative hypotheses are listed below.

H_{01}: There will be no statistically significant difference in biofouling accumulation on mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{A1}: There will be a statistically significant difference in biofouling accumulation on mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{02}: There will be no statistically significant difference in biofouling accumulation on mussel predator nets at different depths (1, 2.5, 4 m).

H_{A2}: There will be a statistically significant difference in biofouling accumulation on mussel predator nets at different depths (1, 2.5, 4 m).

2.2 Methods

2.2.1 Study Site

This study took place at Kyuquot SEAfoods Ltd., which is a Sustainable Ecological Aquaculture farm (SEAfarm) and IMTA facility in Kyuquot Sound on northwestern Vancouver Island, British Columbia, Canada (50° 02' 47.39" N, 127° 17' 49.28" W) (Figure 2.1). The farm is located in a sheltered bay with a prevailing counterclockwise tidal current and an average depth of 28 m. At the time of this experiment, the farm was growing sablefish (*Anoplopoma fimbria*), Japanese and weathervane hybrid scallops (*Patinopectin yessoensis* X *P. caurinus*), blue mussels (*Mytilus edulis*), California sea cucumbers (*Parastichopus californicus*), green sea urchins (*Strongylocentrotus droebachiensis*), and kelp (*Saccharina latissima*). The shellfish and kelp species were grown downstream of the residual tidal current from the fish pens.

Controlling fouling is a major operating expense at this farm because fish pens, scallop lantern nets, mussel nets, and shellfish all require fouling removal. The urchins used in this experiment were collected intertidally by hand from Little Espinosa Inlet, Nootka Sound (49° 57’ 33.15” N, 127° 17’ 49.28” W) and were, on average, 41.77 mm (n=1,800, SE=0.13) in diameter at the beginning of the experiment.

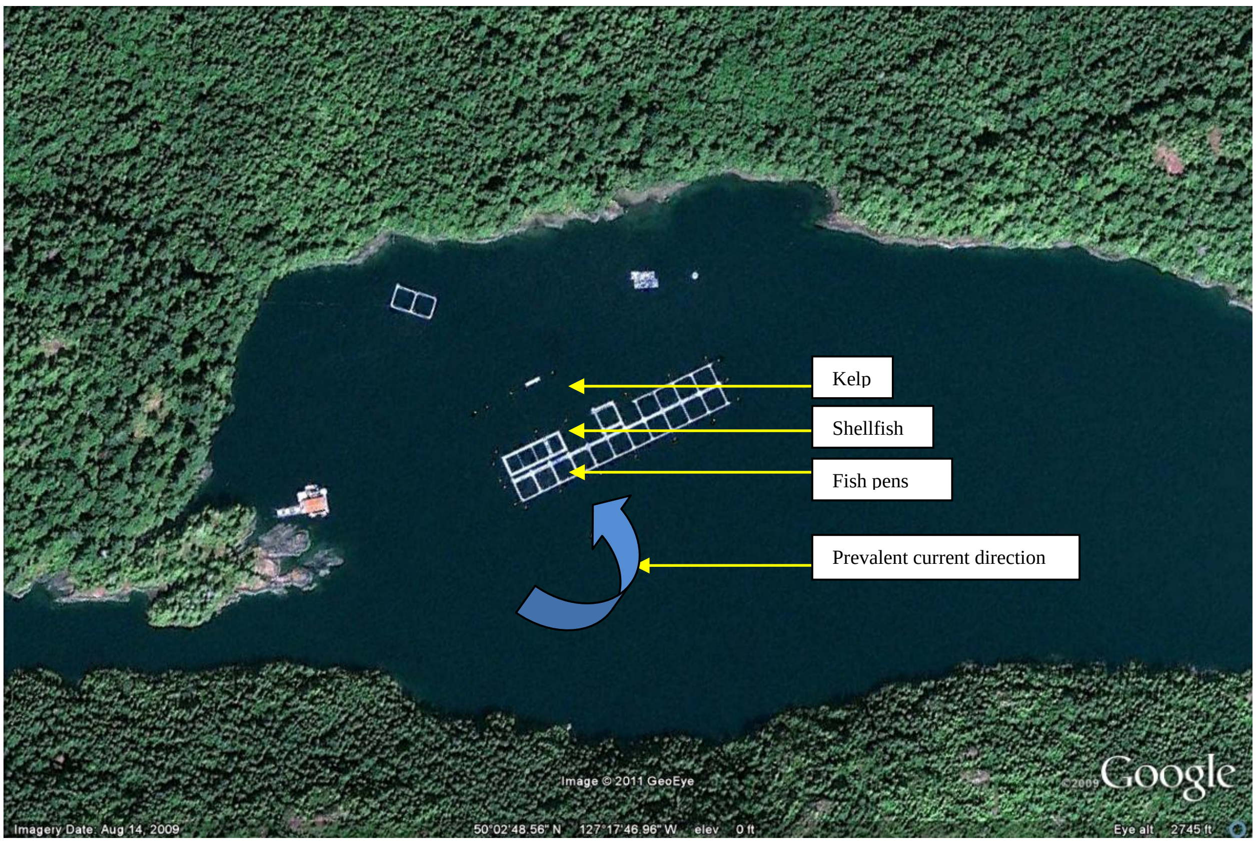

Figure 2.1 Study location at Kyuquot SEAfoods Ltd., Surprise Island SEAfarm, Kyuquot Sound, British Columbia, Canada (Google Maps, 2011).

2.2.2 Experimental Design

The mussel nets were placed on the prevailing downstream current from the stocked sablefish pens. Each of the 30 closed-bottom, cylindrical nylon nets was

5 m long and 0.75 m in diameter (surface area = 12.2 m^2) with a mesh size of 12.7 x 12.7 mm. The nets were suspended from the surface (0.25 m between each net) with one mussel sock suspended vertically in the centre of each net, extending from approximately 0.25 to 4.75 m depth. The nets were stocked with five different densities of urchins (0, 30, 60, 90, 120 urchins net^{-1} or 0, 2.46, 4.91, 7.37, 9.82 urchins m^{-2}) and there were six replicates of each stocking density in a completely randomized blocked design to account for distance from the fish pens. The first nets in block A were 8.73 m from the fish pens and the last nets in block F were 3 m from the kelp lines. Blocks A-F were 8.73, 10.68, 12.55, 14.48, 16.31 and 18.08 m from the fish pens, respectively (measured to the centre of each block). The urchin stocking densities evaluated were based on a review of urchin literature that included a stocking density of urchins, an indication of net surface area, and a qualifying or quantifying statement on net cleanliness or urchin survival (Lodeiros and Garcia, 2004; Ross et al., 2004; Cook and Kelly, 2007, 2009; Edwards and Cross, 2008). Not all of these studies were investigating fouling control directly.

The nets and mussel socks were deployed on October 8, 2009. The urchins were added to the nets on April 19, 2010. Biofouling was quantified on October 10 and 11, 2010 after 174 days of urchin deployment. There was no additional food provided to the mussels or urchins during the study. The general health of both organisms was monitored during the experimental period using a Seaviewer, Sea-Drop 950 underwater camera.

2.2.3 Sampling Methods

Biofouling was quantified at the end of the experiment by taking underwater photographs of the fouled nets in front of a contrasting blue background with a Panasonic DMC-TZ5 camera (with a rigid wire frame attached to the Panasonic DMW-MCTZ5 underwater housing to ensure the images were taken from the same distance). Each net was photographed at three depths (1, 2.5, 4 m) with one photograph taken at each depth for each replicate net (random sampling). A photograph was also taken of an unfouled net using the same technique. The images were processed using GIMP 2© to refine the scale of the image and ImageJ© to calculate the percent net aperture (PNA) of the nets in pixels. For this experiment, PNA was the size of the net opening for a single, randomly chosen square of mesh and was calculated by drawing a polygon over the unfouled portion of the square and measuring the area of the polygon. The percent net occlusion (PNO) of the nets was calculated by quantifying the PNA of a clean net compared to the fouled net using the following modified equation from Braithwaite et al. (2007):

$$PNO = 1 - (PNA_{day\ x} / PNA_{clean\ net}) \times 100$$

2.2.4 Statistical Analysis

Statistical analyses were completed in R using a 3-way analysis of variance model (ANOVA). Urchin stocking density and depth were included as fixed independent variables in the model and block (distance from fish pens) was included as a random variable. The interaction between block and the other independent variables was not included in the model (*i.e.* model 2 design). These interactions with block were unlikely due to the small spatial scale of the experiment and this assumption was confirmed by interaction plots. Tukey's HSD test (p<0.05) was used to make post-hoc comparisons among the density and depth treatments.

An arcsine transformation was used to give the proportion data a normal distribution, rather than a binomial distribution. A variety of plotting methods were used to test the assumptions of the ANOVA model such as residuals plots. In addition, the Shapiro-Wilk test was used to test for normally distributed residuals (W=0.982, p=0.239) and the Bartlett test was used to test for homogeneous variance of the residuals (χ^2=2.37, p=0.667 for urchin stocking density; χ^2=5.09, p=0.078 for depth). All of the assumptions of the ANOVA model were met.

2.3 Results

2.3.1 Urchin Stocking Density

The 3-way ANOVA results revealed that the effects of density and depth were both significant, but there was no significant interaction between them (Table 2.1). All of the nets in treatments with urchins were significantly less fouled than the control nets without urchins (p=0.027 for 30 urchin net^{-1} and p<0.001 for 60, 90, and 120 urchins net^{-1}). Similarly, the nets with 90 and 120 urchins net^{-1} were significantly less fouled than those with 30 urchins net^{-1} (p<0.001 for both comparisons) (Figure 2.2A). There were no statistically significant differences in net occlusion between nets with 30 and 60 urchins net^{-1} and those with 60, 90, and 120 urchins net^{-1}. Nets without urchins were 40% more occluded than the those with 90 or 120 urchins net^{-1} with an average decrease in net fouling of 8% with each increasing stocking density treatment (with the exception of 90 to 120 urchins net^{-1}) (Figure 2.2A).

2.3.2 Depth

There was no significant difference in fouling between 2.5 and 4 m depths, but nets were significantly more fouled at 1 m depth compared to the lower two depths (p=0.008 and p<0.001 respectively) (Figure 2.2B). Overall, the urchins cleaned the nets relatively equally at all depths, which indicated that the urchins were spaced out around the entire inner surface of the nets. This was confirmed by video monitoring during the experiment. The fouling species assemblages were different depending on depth. *Mytilus edulis* and *M. trossulus* were prevalent near the surface, while demosponges, ascidians, and encrusting bryozoans were more common at deeper depths. Hydroids were common along the entire depth of the nets.

Table 2.1 ANOVA table for percent net occlusion

Variable	df	*F*	*p*
Density	4	19.49	<0.001
Depth	2	8.99	<0.001
Block	5	0.44	0.818
Density*Depth	8	1.54	0.160
Error	70		

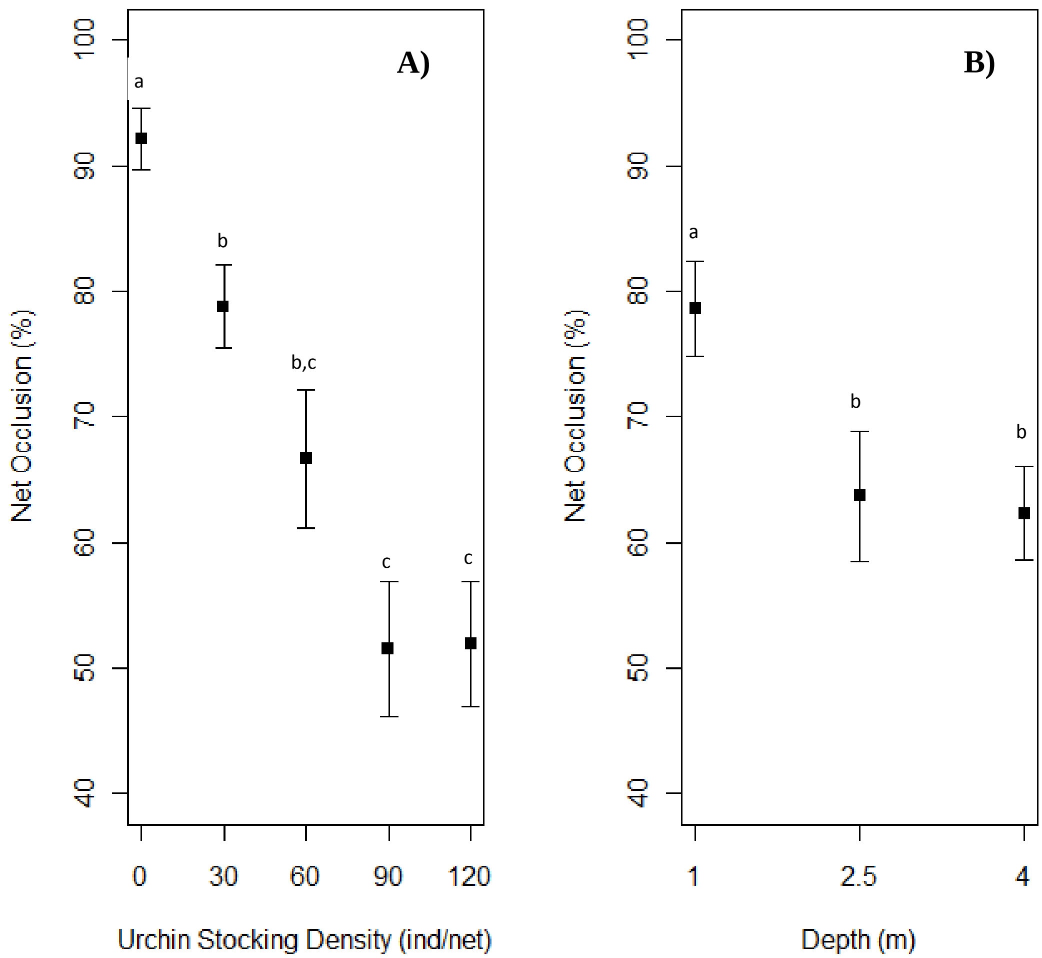

Figure 2.2 Percent net occlusion of mussel predator nets with (A) different stocking densities of urchins (0, 30, 60, 90, 120 urchins net^{-1}) and (B) at three different sampling depths (1, 2.5, 4 m). Different letters above means denote treatments within figures that are significantly different (Tukey's test, *p*<0.05). n=6. Error bars represent standard error.

2.3.3 Survival and Escapes

There were no mortalities during this experiment and few escapes. There were 19 nets in which no urchins escaped, 9 in which less ≤6.6% of the individuals escaped, and 2 nets in which 10.0% and 12.2% of the urchins escaped. It is assumed that these urchins escaped rather than died because urchins were observed attaching to the outside of the nets and no dead urchins were observed in the nets during monitoring. On average, the urchins grew 0.86 cm in test diameter over the 174-day study.

2.4 Discussion

The results of this study indicate that *S. droebachiensis* can effectively reduce fouling on mussel predator nets at a pilot scale. To date, urchins have only been evaluated for biological fouling control in pearl nets or other small shellfish containment units (Loderios and Garcia, 2004; Ross et al., 2004; Switzer et al., 2011). In this study, urchins had free range to graze in 5-m long nets and spaced themselves out relatively evenly over the entire inner surface of the nets.

While significant differences in biofouling control were not detected among the three highest stocking density treatments, all the treatments with urchins were significantly less fouled than the control without urchins. Also, the two highest stocking density treatments (90 and 120 urchins net^{-1}) were significantly less fouled than the lowest stocking density treatment (30 urchins net^{-1}). It is likely that the differences between 30 and 60, as well as 60 and 90 urchins net^{-1}, would have been statistically significant if more replicates had been established. However, there appeared to be no trend in the difference in fouling between the two highest density treatments (90 and 120 urchins net^{-1}), therefore 90 urchins net^{-1} or 7.37 urchins m^{-2} was the maximum stocking density required to control biofouling in this experiment.

While urchins did remove biofouling, none of the nets were completely unfouled at the end of the trial. For example, some of the nets accumulated mussel sets on their outside surfaces. Urchins will graze on small juvenile mussels when they first settle on the nets, however, they only had access to the inner net surface, which allowed mussels and other organisms to settle and survive on the outside of the nets. This did not matter when the fouling organisms were small, but as they grew outward they began to occlude the net openings, at which point they were too large for the urchins to graze. The cleanest nets in the trial were still approximately 30% occluded. In order to better understand the ability of sea urchins to prevent biofouling, a method is needed to quantify fouling on the inner *vs.* outer surface of the nets.

While not 100% effective on their own, *S. droebachiensis* could still act as a replacement for copper-based coatings. These coatings reduce the amount of fouling on nets and make them easier to clean (Braithwaite et al., 2007), but nets still require manual cleaning periodically. Urchins also reduce the amount of fouling on nets and make them easier to clean by only allowing one side of the nets to foul. Nets with high stocking densities of urchins (90 urchins net^{-1}) were only 51.5% occluded compared to 92.1% occluded with no urchins (control) in the 6 month experiment. However, nets treated with copper-based antifoulant were only 98.7% occluded compared to control nets, which were 3.1% occluded in a 10 month experiment by Braithwaite et al. (2007).

While sea urchins may not be as effective at controlling fouling as copper coatings, they are much more environmentally benign. The urchins in this experiment were not given any additional feed inputs, which means that they performed an environmental service (biofouling removal) without additional organic inputs into the local environment. Also, unlike copper-based net treatments, the urchins do not add deleterious substances into the environment [see Braithwaite and McEvoy (2005) for a summary of toxic antifouling paints and materials].

Of the many experimental alternatives to copper-based treatments, urchins are a relatively low maintenance option. They were able to effectively clean both the horizontal bottom of the nets as well as the large vertical surface area. There are other species which have shown potential for controlling biofouling. Herbivorous fish have been shown to effectively reduce both biofouling and sealice on fish (Kvenseth, 1996), but Deady et al. (1995) found that they are prone to escaping. Crabs (Hidu et al., 1981; Enright, 1993), sea cucumbers (Ahlgren, 1998) and shrimp (Dumont et al., 2009) have also been cited as effective alternatives to control biofouling. However, none of these species have been tested in substantially vertical environments such as the nets in this study.

An advantage to biological methods of biofouling control, including the urchin, is that the nets do not need to be removed from the water. Acetic acid (Piola et al., 2010), freshwater (Forest and Blakemore, 2006), and heat treatment (Rajagopal et al., 2005) have been suggested as environmentally benign methods to control fouling, but all of these methods require that the nets be brought to the surface. There is also research on conductive coatings (Huang et al., 2010) and biofilms (Qian et al., 2007) to control fouling on aquaculture nets, but both of these methods are very technical and not well developed.

Urchins can provide an additional seafood product to aquaculture businesses, rather than only being an expense to control biofouling. The increased cost of required manual net cleaning can be offset from the profits made by selling sea urchin gonads. There were no mortalities in any of the treatments over the experimental period and relatively few escapes out of the open-top nets, indicating that urchins can be kept successfully in these environments. The urchins grew an average of 0.86 cm in test diameter over this six month experiment, which equates to a growth rate of 1.4 mm month^{-1}. Growth rate of adult urchins is not well documented, but there has been a wide range of juvenile *S. droebachiensis* growth rates reported in the literature. Juvenile green urchin growth ranges from 0.2 to 1.1 mm month^{-1} in the field (Swan, 1958; Miller and Mann, 1973; Lang and Mann, 1976; Himmelman et al., 1983; Himmelman, 1986; Raymond and Scheibling, 1987; Meidel and Scheibling, 1998) and from 0 to 1.8 mm month^{-1} in the laboratory (Raymond and Scheibling, 1987; Daggett et al., 2005). Daggett et al. (2005) reported a maximum growth rate of 3.0 mm month^{-1} in the laboratory under ideal feeding and temperature conditions. Based on these reported growth rates, the urchins in this experiment had a relatively high growth rate, especially considering their diet was limited to biofouling. They may have been exposed to supplemental food, however, in the form of sablefish feces or uneaten feed. Cook and Kelly (2007) reported that urchins grew faster when reared on salmon farms than when grown at a distance from the farms.

There is more incentive for farmers to use *S. droebachiensis* to keep their nets clean if they can also sell them as a high-value product.

Despite the advantages of using sea urchins to control biofouling, there are some challenges. Commercial-scale quantities of hatchery-produced sea urchins are not currently available in North America so sourcing large numbers of individuals could be problematic. However, sea urchins are relatively easy to spawn and culture in the larval phase so hatcheries could be readily established if the urchin became commonly used in aquaculture. For the purpose of this experiment, urchins were collected from the wild, but this is not recommended for commercial-scale operations because removing wild urchins would reduce the overall perceived environmental benefits of the biofouling control technique by reducing wild populations of sea urchins.

2.5 Conclusions

Strongylocentrotus droebachiensis significantly reduced the amount of fouling on mussel predator nets when compared to controls without urchins. While *S. droebachiensis* may not be as effective at keeping the nets clean as copper-based coatings (based on a 10 month experiment by Braithwaite et al., 2007), it is a much more environmentally benign method. Also, because there is an already established market for *S. droebachiensis*, there is an economic incentive for farmers to incorporate green sea urchins into their farms.

Chapter 3 : Co-culture of green sea urchins, *Strongylocentrotus droebachiensis*, and blue mussels, *Mytilus edulis*, at an IMTA facility

Abstract

Mussels are heavily predated on by sea otters on northwestern Vancouver Island, which means they need to be cultured in predator-exclusion nets. These nets are expensive to build and can become heavily fouled over time. The green sea urchin (*Strongylocentrotus droebachiensis)* was introduced into blue mussel (*Mytilus edulis*) predator exclusion nets at an integrated multi-trophic aquaculture (IMTA) facility as a means of controlling biofouling and providing an additional marketable crop to offset predator net expenses. The objective of this study was to measure the performance of *S.droebachiensis* at different stocking densities (0, 30, 60, 90, 120 urchins net^{-1} or 0, 2.46, 4.91, 7.37, 9.82 urchins m^{-2}) and to determine whether or not the presence of sea urchins impacted *M. edulis* growth. The mussels and urchins were co-cultured for 174 days and then size and weight parameters were measured to quantify growth of both *M. edulis* and *S. droebachiensis.* Urchin growth did decline at increasing urchin stocking densities, there was only significant difference in mussel growth at different urchin stocking densities at 1 m depth. Mussels and sea urchins can be successfully co-cultured with no food inputs, but urchin growth may be significantly reduced at high stocking densities of urchins as there is less biofouling available for the animals to feed on.

3.1 Introduction

Integrating additional seafood species into finfish aquaculture has potential ecological and economic benefits. Both shellfish and kelp species (organic and inorganic extractive species) have been shown to remove some of the excess nutrients from the water column around finfish farms (Troell et al., 2003; Reid et al., 2010) and detritivores have been reported to consume solid finfish and shellfish waste (Tsutsumi et al., 2005; Cook and Kelly, 2007; Paltzat et al., 2008). The various species being integrated into fish farms also have the potential to reduce the environmental impact of finfish aquaculture, increase farm profits as additional seafood products, and improve public perception of the industry (Whitmarsh et al., 2006; Neori, 2008; Bunting and Shpigel, 2009). Integrated multi-trophic aquaculture (IMTA) has the potential to make a highly criticized industry more ecologically sustainable, economically resilient, and socially acceptable.

Mussels are able to filter fish feces and excess fish feed from the water column (Reid et al., 2010) and have shown elevated growth rates when grown adjacent to salmon pens in comparison to controls grown away from the pens (Stirling and Okumus, 1995; Peharda et al., 2007; Sara et al., 2009). On the east coast of Canada, Cooke Aquaculture Ltd. has successfully integrated commercial-scale mussel long line rafts alongside salmon net pens. On northwestern Vancouver Island [British Columbia (BC), Canada], however, mussels cannot be grown as an extractive species without some form of predator protection as there are large populations of sea otters (*Enhydra lutris*) and surf scoters (*Melanitta perspicillata*) in the area, which both predate heavily on exposed mussels.

Sea urchins could also be environmentally and economically important when integrated into any type of marine aquaculture facility. Some echinoid species are able to significantly reduce biofouling accumulation on aquaculture nets (Lodeiros and Garcia, 2004; Ross et al., 2004; Chapter 2) and consume fish waste (feces and excess feed) (Cook and Kelly, 2007). There is also an established market in BC for the export of green sea urchins, *Strongylocentrotus droebachiensis*, and red sea urchins, *S. franciscanus,* to Japan (FAO, 2011).

The rationales for co-culturing mussels and green sea urchins in this study were: 1) provide an additional marketable product (sea urchins) that would generate additional revenue for the farm and 2) utilize an organism (sea urchins) that would feed on the biofouling accumulating on the predator nets and, thus, simultaneously clean the nets to allow higher water flow to the mussels. An increase in water flow to the mussels could lead to an increase in food availability and growth rate. It was expected that the upper limit of urchin stocking density would be constrained by urchin growth (less fouling and therefore less food availability to the urchins), while the lower limit of urchin stocking density would be constrained by mussel growth (more fouling leading to decreased water flow and decreased food availability to the mussels). *Mytilus edulis* and *S. droebachiensis* were chosen for this study because they are both established species in BC and have had seafood markets in BC for over 20 years. The objectives of this study were to measure how urchin stocking density and/or depth influence the growth of both *S. droebachiensis* and *M. edulis*. The null and alternative hypotheses are listed below.

H_{01}: There will be no statistically significant difference in mussel growth (length, whole wet weight, wet meat weight, meat yield, dry meat weight, and ash-free dry meat weight) in predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{A1}: There will be a statistically significant difference in mussel growth (length, whole wet weight, wet meat weight, meat yield, dry meat weight and ash-free dry meat weight) in predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{02}: There will be no statistically significant difference in mussel growth (length, whole wet weight, wet meat weight, meat yield, dry meat weight, and ash-free dry meat weight) in predator nets at different depths (1, 2.5, 4 m).

H_{A2}: There will be a statistically significant difference in mussel growth (length, whole wet weight, wet meat weight, meat yield, dry meat weight, and ash-free dry meat weight) in predator nets at different depths (1, 2.5, 4 m).

H_{03}: There will be no statistically significant difference in urchin growth (test diameter, whole wet weight, gonad weight, and gonad yield) in mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{A3}: There will be a statistically significant difference in urchin growth (test diameter, whole wet, gonad weight, and gonad yield) in mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{04}: There will be no statistically significant difference in urchin gonad quality in mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

H_{A4}: There will be a statistically significant difference in urchin gonad quality in mussel predator nets with different stocking densities of sea urchins (0, 30, 60, 90, 120 urchins net^{-1}).

3.2 Methods

3.*2.1 Location and Experimental Design*

The study took place in Kyuquot Sound on northwestern Vancouver Island, BC, Canada at a commercial Sustainable Ecological Aquaculture farm (SEAfarm) and IMTA facility (50° 02' 47.39" N, 127° 17' 49.28" W) (Figure 3.1). The farm is located in a sheltered bay with a prevailing counter-clockwise tidal current and an average depth of 28 m. The mussel nets were placed in the prevailing downstream current from the stocked sablefish pens. Each of the 30 closed-bottom, cylindrical nylon nets was 5 m long and 0.75 m in diameter (surface area = 12.2 m^2) with a mesh size of 12.7 x 12.7 mm. The nets were suspended from the surface, 0.25 m apart, with one mussel sock suspended in each net from approximately 0.25 to 4.75 m depth.

The nets were stocked with five different densities of urchins (0, 30, 60, 90, 120 urchins net^{-1} or 0, 2.46, 4.91, 7.37, 9.82 urchins m^{-2}) and there were six replicates of each stocking density in a completely randomized block design to account for varying distance (8.79, 10.68, 12.55, 14.48, 16.31 and 18.08 m) from the fish pens (Figure 3.2). These stocking densities were based on a review of urchin literature that included a stocking density of urchins, an indication of net surface area, and a qualifying or quantifying statement on net cleanliness or urchin survival (Lodeiros and Garcia, 2004; Ross et al., 2004; Cook and Kelly, 2007, 2009; Edwards and Cross, 2008).

The nets and mussel socks were deployed on October 8, 2009 and the urchins were added to the nets on April 19, 2010 after collection from the reference site at Little Espinosa Inlet (49° 57’ 33.15” N, 127° 17’ 49.28” W) (Figure 3.1). The urchins and mussels were measured and collected for laboratory analysis on October 10 and 11, 2010 after 174 days. There was no additional food provided to the mussels or urchins during the study and the nets were monitored for overall health of both organisms during the experimental period using a Seaviewer, Sea-Drop 950 underwater camera.

Figure 3.1 Research site located at the Surprise Island SEAfarm owned by Kyuquot SEAfoods Ltd. in Kyuquot Sound, British Columbia, Canada and the reference site at Little Espinosa Inlet, Nootka Sound.

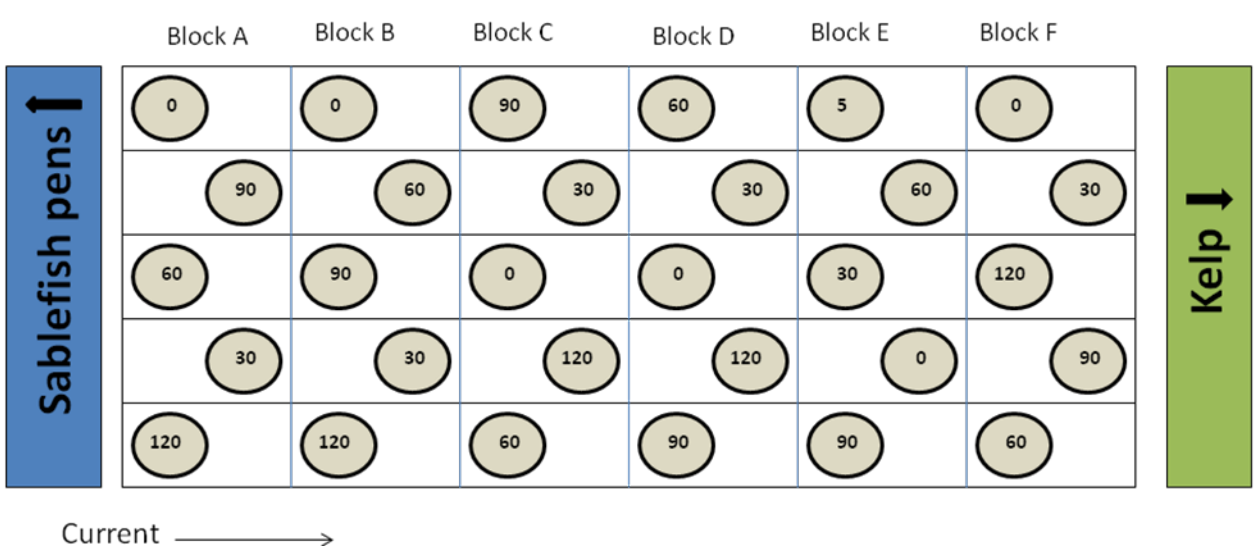

Figure 3.2 Experimental design of mussel predator exclusion nets stocked with sea urchins at densities of 0, 30, 60, 90, or 120 urchins net^{-1}. The first nets in block A were 8.73 m from the fish pens and the last nets in block F were 3.00 m

from the kelp lines. Blocks A-F were 8.73, 10.68, 12.55, 14.48, 16.31 and 18.08 m from the fish pens, respectively (measured to the centre of each block) and the nets were 0.25 m apart.

3.2.2 Mussels

On day 0 of the experiment (day 192 of mussel deployment), mussel shell length was measured using vernier calipers (accuracy 0.1 mm) to ensure there was no effect of block (distance from fish) and to record trends in growth at different depths (average shell length across all depths was 38.9 mm (n=900, SE=0.3)). Ten randomly-sampled mussels were measured at each of 1, 2.5, and 4 m depth on each of the 30 mussel socks. Their distance from the fish cage (*i.e.* block effect) had no significant effect on mussel size (ANOVA, DF=5;880, F=0.39, p=0.858). However, depth had a statistically significant impact on mussel length (ANOVA, DF=2;880, F=19.61, p<0.001). Mussels grown at 1 m depth were significantly smaller than those grown at 2.5 and 4 m depth (Tukey test, p<0.001 for both comparisons). There was no significant difference in mussel length between 2.5 and 4 m depths.

On day 174, ten mussels were randomly selected from each of 1, 2.5, and 4 m depths on each of the 30 mussel socks. These mussels were immediately frozen for later laboratory analysis. After the mussels were thawed at room temperature and cleaned, their shell lengths were measured with vernier calipers (accuracy 0.01 mm). Whole wet weight and meat wet weight were measured and meat index was calculated as in Chatterji et al. (1984):

meat index = (meat wet weight / whole wet weight) x 100

The meat was then dried at 60°C for 48 hours to constant weight and weighed again. Finally, three samples from each batch of 10 mussels were randomly selected to combust at 550°C for 3 hours in order to remove the organic content. The remaining inorganic weight was subtracted from the meat dry weight to calculate the meat ash-free dry weight (organic content of the mussel meat). The same individuals were used to measure length, total wet weight, wet meat weight, meat yield, dry meat weight, and ash-free dry meat weight.

3.2.3 Urchins

On day 0 of the experiment, the test diameters of all urchins in each replicate net were measured using vernier calipers (accuracy 0.1 mm). The average test diameter was 41.8 mm (n=1800, SE=0.1). On day 174, the test diameters of all urchins in each replicate net were measured again and the average test diameter was 50.3 mm (n=1757, SE=0.1). Then, six randomly-chosen urchins from each net were transported to the laboratory for further analysis, which took place within three days of the urchins being removed from the nets. The urchins were kept alive in seawater tanks during this period. In addition, four urchins were collected from a reference site (a nearby inlet where the urchins were originally collected, Figure 3.1) for laboratory analysis. There were no urchin mortalities during transport.

The live urchins were weighed accurately to 0.01 g. The tests were then carefully opened and the gonads were removed with forceps. The gonads were blotted with a paper towel to remove excess moisture and weighed. Gonad yield was calculated for each individual using the equation in Pearce et al. (2002a):

gonad yield = (towel-blotted gonad wet weight / whole wet weight) x 100

Gonad quality was assessed by rating the colour, brightness, firmness, and texture [based on the methods of Pearce et al. (2002a). These four parameters were given a rating from 1 to 4 (1=best, 4=worst). The rating for all four parameters were added together to produce an overall quality score out of 16 for each urchin. The same individuals were used to measure whole wet weight, gonad weight, gonad yield, and gonad quality.

3.2.4 Statistical Analysis

Statistical analyses were completed in R using 3-way analysis of variance models (ANOVA) for the mussel parameters measured. Urchin stocking density and depth were included as fixed independent variables in the model and block (distance from fish pens) was included as a random variable. 2-way ANOVA models (density and block) were used to test the urchin growth parameters. Depth was not included as an independent variable (urchins had free range to move around in the nets). The interaction between block and the other independent variable(s) were not included in the models (*i.e.* model 2 design).

These interactions with block were unlikely due to the small spatial scale of the experiment and this assumption was confirmed by interaction plots. Tukey's HSD test (p<0.05) was used to make post-hoc comparisons among the density and depth treatments.

A square-root transformation was used on the mussel ash-free dry weight data in order to meet the assumption of normally distributed residuals. A log transformation was used on the urchin diameter, urchin whole wet wet, and urchin wet gonad weight data in order to meet the assumption of normally distributed residuals. A variety of plotting methods were used to test the assumptions of the ANOVA models, such as residuals plot and interactions plots. In addition, the Shapiro-Wilk test was used to test for normally distributed residuals and the Bartlett test was used to test for homogeneous variance of the residuals. All the assumptions of the ANOVA model were met.

3.3 Results

3.3.1 Mussels

Table 3.1 ANOVA tables for mussel length, total wet weight, meat wet weight, meat yield, meat dry weight, and meat ash-free dry weight after 174 days in predator nets stocked with different densities of sea urchins.

Mussel Length					Total Mussel Wet Weight			
Variable	**df**	***F***	***P***		**Variable**	**df**	***F***	***P***
Density	4	2.85	0.023		Density	4	2.59	0.035
Depth	2	353.91	<0.001		Depth	2	309.94	<0.001
Block	5	6.83	<0.001		Block	5	6.53	<0.001
Density*Depth	8	5.54	<0.001		Density*Depth	8	4.98	<0.001
Error	848				Error	848		
Mussel Meat Wet Weight					Mussel Meat Yield			
Variable	**df**	***F***	***P***		**Variable**	**df**	***F***	***P***
Density	4	1.20	0.308		Density	4	1.53	0.191

Depth	2	290.87	<0.001		Depth	2	46.77	<0.001
Block	5	6.17	<0.001		Block	5	1.69	0.020
Density*Depth	8	4.89	<0.001		Density*Depth	8	1.61	0.119
Error	848				Error	848		
Mussel Meat Dry Weight					Mussel Ash-Free Dry Weight			
Variable	**DF**	***F***	***P***		**Variable**	**DF**	***F***	***P***
Density	4	4.41	0.002		Density	4	1.09	0.363
Depth	2	293.33	<0.001		Depth	2	79.30	<0.001
Block	5	7.50	<0.001		Block	5	1.89	0.097
Density*Depth	8	4.83	<0.001		Density*Depth	8	2.45	0.015
Error	848				Error	237		

3.3.1.1 Mussel Length

Density,, depth, and their interaction all significantly influenced mussel length (Table 3.1). At 1 m depth, mussels were significantly shorter in nets with 60 urchins net $^{-1}$ than those held at 0, 90, and 120 urchins net $^{-1}$ (*p*=0.012, 0.003 and 0.002 respectively), but there was no significant difference in mussel length between nets with 30 and 60 urchins net $^{-1}$ (Figure 3.3A). Mussel length in nets with 30 urchins net $^{-1}$ was also significantly shorter than in nets with 120 urchins net $^{-1}$ (*p*=0.040). There were no statistically significant pair-wise differences in mussel length among urchin stocking densities at 2.5 or 4 m depth.

Mussels were significantly longer at 2.5 and 4 m than at 1 m depth (*p*<0.001) at all urchin stocking densities (Figure 3.3A). Mussel length was significantly longer at 4 m than at 2.5 m depth at stocking densities of 30 and 60 urchins net^{-1} (*p*=0.0274 and p<0.001 respectively), but there were no other significant differences between these two depths at any of the other stocking densities.

3.3.1.2 Total Mussel Wet Weight

The results of the 3-way ANOVA showed that density, depth, and their interaction all significantly influenced total mussel wet weight (Table 3.1). At 1 m depth total mussel wet weight was significantly lower in nets with 60 urchins net $^{-1}$ than in those with 0, 90, and 120 urchins net $^{-1}$ (p=0.040, p<0.001 and p<0.001 respectively), but there was no significant difference in total mussel wet weight between nets with 30 and 60 urchins net $^{-1}$ (Figure 3.3B). Total mussel wet weight in nets with 30 urchins net $^{-1}$ was also significantly lower than in nets with 90 and 120 urchins net $^{-1}$ (p=0.010 and p<0.001 respectively). There were no significant differences among urchin densities in total mussel wet weight at 2.5 m, but at 4 m depth mussels were significantly heavier in the 60 urchin net^{-1} treatment than in the 120 urchin net^{-1} (p=0.005).

Total mussel wet weight was significantly heavier at 2.5 and 4 m than at 1 m depth (p<0.001) at all urchin stocking densities (Figure 3.3B). Total mussel wet weight was significantly higher at 4 m than at 2.5 m depth at stocking densities of 30 and 60 urchins net^{-1} (p=0.0244 and p<0.001 respectively), but there were no other significant differences between these two depths at any of the other stocking densities.

3.3.1.3 Mussel Meat Wet Weight

The results of the 3-way ANOVA showed that mussel meat wet weight was significantly influenced by depth and the interaction between density and depth, but not by density (Table 3.1). At 1 m depth, mussel meat wet weight was significantly lower in nets with 30 and 60 urchins net $^{-1}$ than in those with 90 and

120 urchins net $^{-1}$ (p<0.001 for both comparisons), but there was no significant difference in meat wet weight between nets with 0, 30 and 60 urchins net $^{-1}$ (Figure 3.3C). Mussel meat wet weight in nets with 30 urchins net $^{-1}$ was also significantly lower than in nets with 90 and 120 urchins net $^{-1}$ (p=0.009 and p<0.001 respectively). Mussel meat wet weight was also significantly lower in nets with 0 urchins net^{-1} than in nets with 120 urchins net^{-1} (p=0.052). There were no significant differences in mussel meat wet weight among densities at 2.5 or 4 m depths.

Mussel meat wet weight was significantly heavier at 2.5 and 4 m than at 1 m depth (p<0.001) at all urchin stocking densities (Figure 3.3C). Mussel meat wet weight was significantly heavier at 4 m than at 2.5 m depth at stocking densities of 30 and 60 urchins net^{-1} (p=0.0225 and p<0.001 respectively), but there were no other significant differences between these two depths at any of the other stocking densities.

3.3.1.4 Mussel Meat Yield

The results of the 3-way ANOVA showed that mussel meat yield was significantly influenced only by depth (Table 3.1). There were no significant differences in mussel meat yield at different stocking densities of urchins (Figure 3.3D). However, mussels grown at 2.5 and 4 m depth had significantly higher meat yields than those grown at 1 m (p<0.001 for both comparisons), but there was no significant difference in mussel meat yield between 2.5 and 4 m (Figure 3.3D).

3.3.1.5 Mussel Meat Dry Weight

The results of the 3-way ANOVA showed that density, depth, and their interaction all significantly influenced mussel meat dry weight (Table 3.1). At 1 m depth, mussel meat dry weight was significantly lower in nets with 30 and 60 urchins net $^{-1}$ than in those with 90 and 120 urchins net $^{-1}$ (p<0.001 for all comparisons), but there was no significant difference in mussel meat dry weight between nets with 0, 30, and 60 urchins net $^{-1}$ (Figure 3.3E). There were no statistically significant differences in meat dry weight among urchin densities at 2.5 or 4 m.

Mussel meat dry weight was significantly greater at 2.5 and 4 m than at 1 m depth (p<0.001) at all urchin stocking densities. Mussel meat dry weight was significantly greater at 4 m than at 2.5 m depth at a stocking density of 60 urchins net^{-1} (p=0.009), but there were no other significant differences between these two depths at any of the other stocking densities (Figure 3.3E).

3.3.1.6 Mussel Meat Ash-Free Dry Weight

The results of the 3-way ANOVA showed that mussel meat ash-free dry weight was significantly influenced by depth and the interaction between density and depth, but not by density (Table 3.1). At 1 m depth, mussel meat ash-free dry weight was significantly lower in nets with 30 and 60 urchins net $^{-1}$ than in nets with 120 urchins net $^{-1}$ (p=0.026 and p=0.009 respectively), but there were no other significant pair-wise differences among stocking densities (Figure 3.3F).

Mussel meat ash-free dry weight was significantly higher at 2.5 and 4 m than at 1 m depth (p<0.001) at all urchin stocking densities. There were no significant differences in mussel meat ash-free dry weight between 2.5 and 4 m depths at any of the urchin stocking densities (Figure 3.3F).

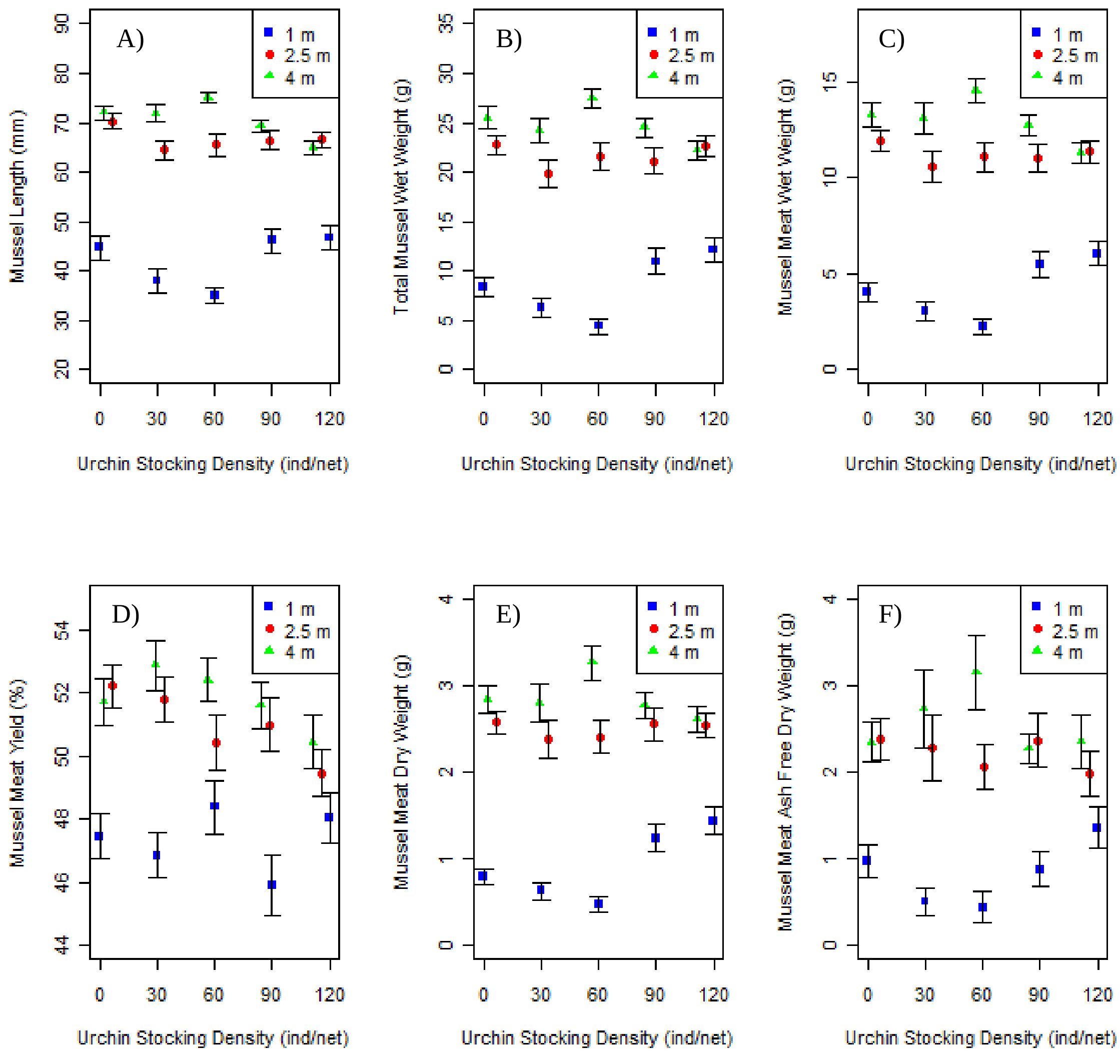

Figure 3.3 Mussel (A) length, (B) total wet weight, (C) wet meat weight, (D) meat yield, (E) dry meat weight, and (F) ash-free dry meat weight after 174 days in predator nets stocked with different densities of sea urchins. n=60. Error bars represent standard error.

3.3.2 Urchins

Urchin stocking density had a significant effect on all urchin variables measured except urchin gonad quality (Table 3.2).

Table 3.2 ANOVA tables for urchin test diameter, whole wet, gonad weight, gonad yield, and gonad quality after 174 days of feeding on biofouling in mussel predator nets.

Urchin Test Diameter					Urchin Whole Wet Weight			
Variable	**df**	***F***	***P***		**Variable**	**df**	***F***	***P***
Density	4	39.06	<0.001		Density	4	5.19	<0.001
Block	5	2.67	0.021		Block	5	0.27	0.926
Error	1748				Error	137		
Urchin Gonad Weight					Urchin Gonad Yield			
Variable	**df**	***F***	***P***		**Variable**	**df**	***F***	***P***
Density	4	55.68	<0.001		Density	4	48.7	<0.001
Block	5	0.42	0.834		Block	5	0.40	0.846
Error	137				Error	137		
Urchin Gonad Quality								
Variable	**df**	***F***	***P***					
Density	4	1.95	0.105					
Block	5	2.61	0.027					
Error	137							

Urchin somatic growth (measured by test diameter) decreased with increasing urchin stocking density with all pair-wise comparisons among densities being significant (p<0.001 for all post hoc comparisons), with the exception of 60 and 90 urchins net^{-1} (Figure 3.4).

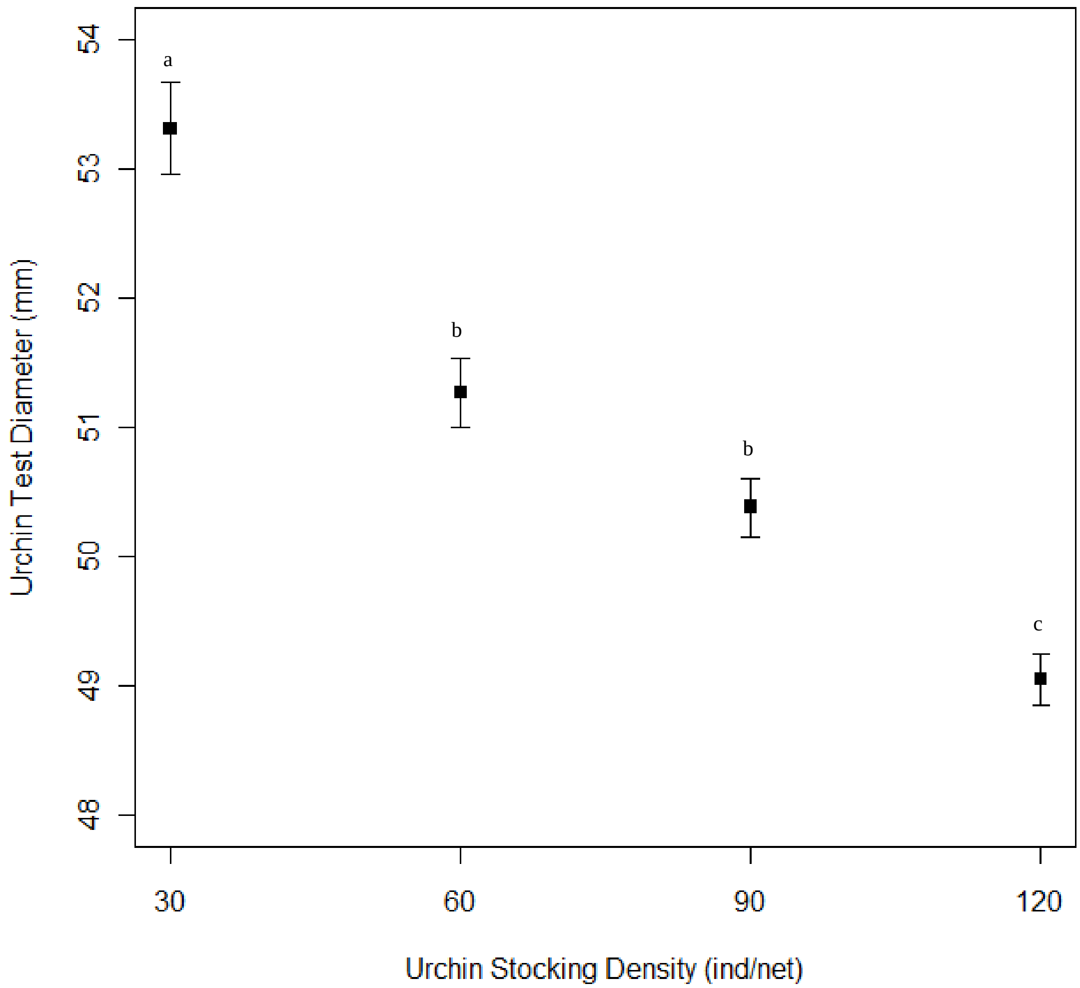

Figure 3.4 Urchin test diameter after 174 days of feeding on biofouling in mussel predator nets at stocking densities of 0, 30, 60, 90, and 120 urchins net^{-1}. Different letters above means denote significantly different treatments (Tukey test, *p*<0.05). n=1,757. Error bars represent standard error.

Urchin whole wet weight was significantly lower at 120 urchins net^{-1} than at 30 urchins net^{-1} with no other significant pair-wise comparisons among the densities (Figure 3.5A) (p<0.001). Urchin gonad weight was significantly higher in nets with 30 urchins net^{-1} than at the reference site (p<0.032) and in nets with 60, 90, and 120 urchins net^{-1} ($p<0.001$ for all comparisons) (Figure 3.5B). There was no difference in gonad weight between the reference site, nets with 60 urchins net^{-1}, and nets with 90 urchins net^{-1}, but they all had significantly heavier gonads than in nets with 120 urchins net^{-1} ($p=0.032$, $p<0.001$ and $p<0.001$ respectively) (Figure 3.5B).

There was no significant difference in gonad yield between farm-raised urchins and the urchins from the reference site for stocking densities of 60, 90 and 120 urchins net^{-1}, but urchins grown at low density on the farm (30 urchins net^{-1}) had significantly higher gonad yield than urchins at the reference site ($p<0.001$) (Figure 3.5C). There were no statistically significant pair-wise differences in gonad quality among the urchins grown at different stocking densities or between farm-raised urchins and urchins from the reference site (Figure 3.5D).

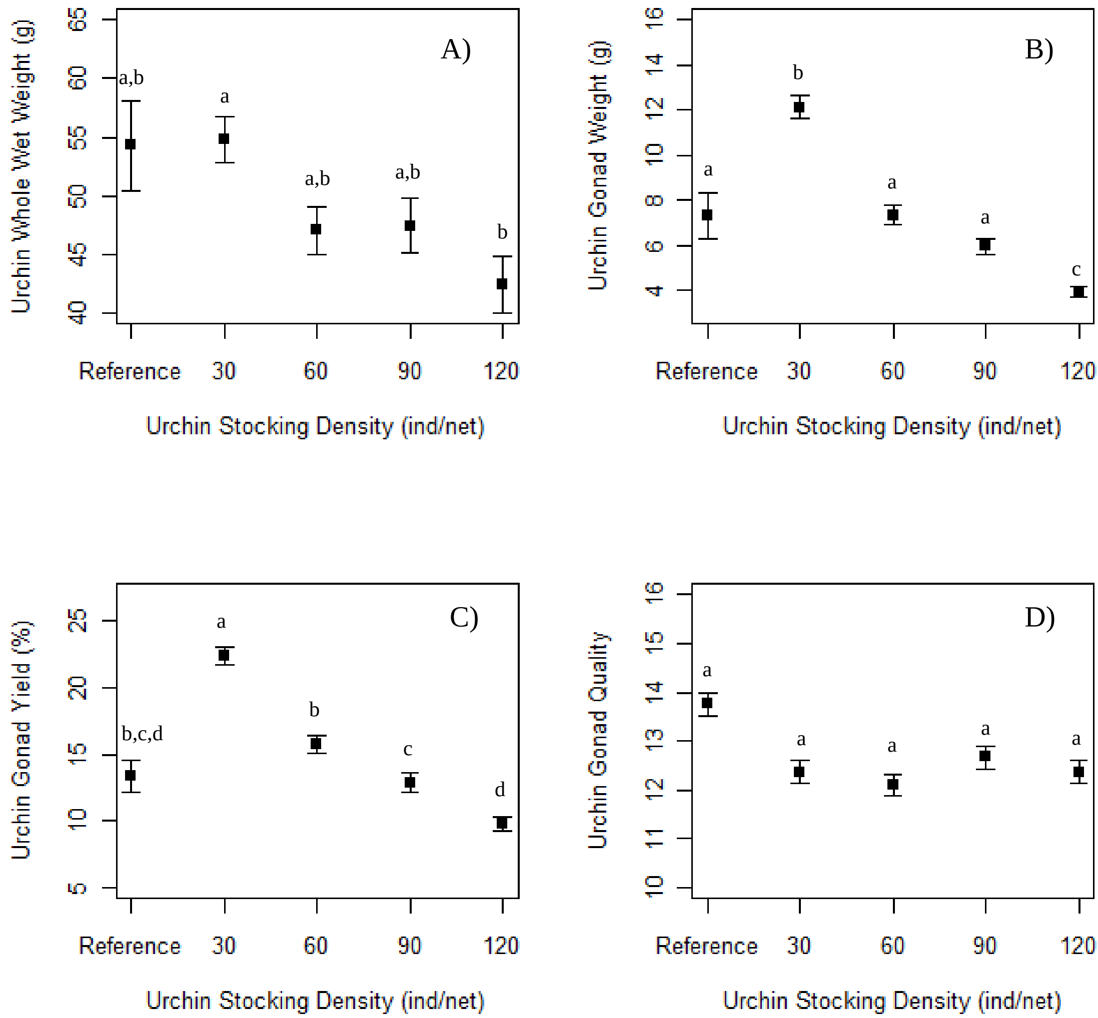

Figure 3.5 Urchin (A) whole wet weight, (B) gonad weight, (C) gonad yield, and (D) gonad quality after 174 days of feeding on biofouling in mussel predator nets at stocking densities of 0, 30, 60, 90, and 120 urchins net^{-1}. Different letters above means denote significantly different treatments (Tukey test, p<0.05). n=36. Error bars represent standard error.

3.4 Discussion

3.4.1 Interpretation

The effect of urchin density on all mussel growth parameters (except meat yield) was significantly affected by the interaction with depth; there were few significant pair-wise differences among urchin densities at 2.5 and 4 m depth, but many at 1 m depth. At 1 m depth, mussel growth (*i.e.* length, wet total weight, wet meat weight, and dry meat weight) was significantly inhibited at the lower urchin stocking density of 60 urchins net^{-1} than at 90 and 120 urchins net^{-1} with no significant difference between 0, 30, and 60 urchins net^{-1} for wet and dry meat weight. Therefore, at 1m depth, higher urchins stocking (90 and 120 urchins net^{-1}) densities did improve mussel growth compared to lower urchin stocking densities (60 urchins net^{-1}). Biofouling was reduced in nets with 90 and 120 urchins compared to nets with lower stocking densities of urchins or no urchins, which may have resulted in more food availability to the mussels through the large net apertures (Chapter 2). It is also possible that the urchins were thinning the mussel sock at 1m by consuming small mussel, which may have resulted in increased mussel growth. Sea urchins were shown to feed on small mussels by Cook and Kelly (2009) and on mussel meat (Meidel and Scheibling, 1999).

For mussel length and total wet weight at 1 m, mussels grown in nets with 0 urchins net^{-1} were also significantly larger than in nets with 60 urchins net^{-1}. The urchins may have inhibited mussel growth by grazing on the small mussels or their physical presence may have interrupted mussel feeding. It is likely that the mussels closed their valves and ceased feeding when urchins were present on the mussel socks. Throughout the experiment, urchins were observed attached to the mussel socks in the upper 1.5 m of the water column. This was also evident when the mussel socks were removed from the water at the end of the experiment. At higher stocking densities, the increased food availability (from cleaner nets) or thinning effects may have outweighed the effect of feeding interruption by urchin grazing. Regardless of these trends, mussels grown at 1 m depth were undersized and could not be sold as a seafood product at the time of harvest.

There was little difference in mussel growth among densities at 2.5 or 4 m depth. There was no trend in mussel growth because all of the mussels at these depths reached maximum size (approximately 70mm in length. *M. edulis* does not grow to be larger than this. It is possible that a trend in mussel growth would have been observed with an interim sampling period.

The mussels grew larger at greater depths, *i.e.* 2.5 and 4 m, than near the surface. Conversely, Karayucel and Karayucel (2000) found no difference in shell length of *M.edulis* grown at 2 and 6 m, whereas Fuentes et al. (2000) found that *M. galloprovincialis* grew longer at 2.5 m than at 7.5 m depth. While the mussels in this study grew to a greater size at deeper depths during mussel and urchin co-culture, it is unlikely that urchin stocking density had any influence on the increased mussel growth at 2.5 and 4 m because this trend in mussel growth was already evident before the urchins were added to the nets. The reduced growth rates at 1 m may have been a result of the large freshwater inputs at this site during the winter and spring from creek run-off and precipitation, which creates a freshwater layer in the upper water column.

Urchin diameter, whole wet weight, gonad wet weight, and gonad yield all declined at increasing urchin stocking densities. This is most likely a result of competition for food because biofouling, and possibly some fish feces and uneaten fish feed, was the only food resource available to the urchins. Siikavuoplo et al. (2007) also found decreased gonad yield as well as increased mortality at increasing stocking densities of *S. droebachiensis*, albeit with substantially higher stocking densities in a contained system.

There were no significant differences in gonad quality among stocking densities or between the farmed urchins and urchins from the reference site. The method used to quantify gonad quality, however, was highly subjective. In addition, taste and smell were not considered and these are extremely important measures of gonad quality. These parameters were not measured as there were no professional urchin processors available to grade the urchins at this time. Finally, gonad wet weight decreased with increased stocking densities and gonad size is also an important measure of gonad quality. Based on the gonad weight difference alone (the only discernable difference), gonad quality decreased with increased stocking density of urchins and did not differ from the reference site at high farm densities.

Interestingly, at low density, the farm-raised urchins had significantly higher gonad wet weights and gonad yields than urchins from the reference site. At higher stocking densities, gonad wet weight of cultured urchins was either similar to (60 and 90 urchins net^{-1}) or significantly less than (120 urchins net^{-1}) wild urchins at the reference site. Based on these results, the low-density, farmed urchins would have a higher economic value than the wild urchins or the high-density farmed urchins.

3.4.2 Application for IMTA

Both the mussels and urchins performed well when co-cultured in this experiment. The mussels grown at 2.5 and 4 m depth reached maximum market size in only 12 months and the urchins increased their test diameter by an average of 20% over 6 months (by 27.5% at 30 urchins net^{-1}). The average urchin growth rate in test diameter was 1.4 mm month^{-1}. The farm-raised urchins also had increased gonad yield at low densities (*i.e*. 30 urchins net^{-1}) compared to the wild-caught urchins. All urchins were healthy, with bright green, erect spines, at the end of the experiment, even the individuals grown at high densities. The nets also completely prevented predation on mussels by surf scoters, sea otters, and other predators. This experiment shows that mussels and urchins can be successfully co-cultured in an IMTA system.

In order to enhance urchin growth at higher stocking densities, additional prepared or natural feeds [see Pearce 2002a,b,c and Robinson et al. (2010) for urchin feed requirements] could be added to the nets in order to remove the affect of competition on urchin growth. However, this would be an additional expense to the farm and may not be economical considering the expense of building the predator nets. The predator nets were costly (approximately $2,000 to build 30 nets when using a donated, recycled salmon net) and time consuming (approximately 180 hours) to build, which needs to be taken under consideration when implementing this method of co-culturing mussels and urchins. The numbers are approximate and are based on untrained student labour.

The other economic consideration is the current value of sea urchins. Currently, wild caught green sea urchins are purchased by processing/exporting companies for CAD 2.20 to CAD 6.60 kg^{-1} in BC (D. Macey, D&D Pacific Fisheries Ltd., personal communication). The quantity and market value of green sea urchins exported from BC has declined since it peaked in 1992 (FAO, 2011). The decline in value is due to: 1) decreased demand in Japan as younger generations are consuming less seafood and 2) increased exports from Chile and Russia (Sonu, 2003; D. Macey, D&D Pacific Fisheries Ltd., personal communication). Both the Chilean and Russian fisheries are able to sell their products at lower value than in BC because of lower labour costs, however the urchin fishery in Chile has already started to decline due to poor management and the Russian fishery is harvesting urchins at an unsustainable rate (D. Macey, D&D Pacific Fisheries Ltd., personal communication). This could lead to an increase in the value of *S. droebachiensis* in the future. With the recovery in the export market, *S. droebachiensis* could be an additional high-value product grown at IMTA facilities. Careful consideration of price fluctuations and the cost of production will ultimately determine the viability of this form of co-culture at a commercial scale.

3.4.3 Future Directions

The sea urchin is a species of great interest for IMTA because of its economic potential and lack of feed preferences. While there was no significant trend in mussel growth at different stocking densities of sea urchins (at 2.5 and 4 m depth), perhaps there would be a positive trend in shellfish growth during an interim sampling or if urchins were co-cultured with other shellfish or finfish species, at IMTA farms. The red sea urchin, *S. franciscanus*, also has an established market as a seafood product in BC. This species has not yet been evaluated for biofouling control or growth and gonad production at an IMTA facility.

Finally, the urchins used in this study were taken collected from the wild. This was necessary because there were no hatchery-reared sea urchins available. In order for mussel and sea urchins to be commercially co-cultured under the principles of IMTA, the urchins would need to be hatchery-reared to avoid depleting wild populations.

3.5 Conclusions

The predator exclusion nets did successfully protect *M. edulis* from predation and the addition of *S. droebachiensis* did not negatively impact *M. edulis* growth. Being able to grow the two seafood products in the nets has the potential to make the used of predator exclusion nets more economically viable. The limiting factor when co-culturing *M. edulis* and *S. droebachiensis* was competition for food at higher stocking densities of *S. droebachiensis,* which could be avoided with additional feed inputs. The co-culture of *M. edulis* and *S. droebachiensis* is biologically feasible and, with a recovery in export price of *S. droebachiensis,* could also be economically appealing for IMTA and other aquaculture facilities.

Chapter 4 : Conclusions

4.1 Introduction

The aquaculture industry around the world has been criticized by the public and environmental non-government organizations for the perceived negative environmental impacts of the industry. Salmon farming around Vancouver Island, British Columbia (BC), Canada is particularly targeted by the media and activist groups, which has led to a tarnished public image for the entire finfish aquaculture industry in this region. Some of the perceived negative environmental impacts include production of fish waste (*i.e.* uneaten food, feces), escapes and negative interactions with wild species, spread of disease and parasites to wild populations, use of hormones and chemotherapeutants, marine mammal entanglement, copper-based antifouling paints, noise pollution, and esthetic degradation of the shoreline.

One strategy, which aims to reduce some of the environmental impacts of aquaculture, is integrated multi-trophic aquaculture (IMTA). IMTA is the integration of fed species (*e.g.* finfish, shrimp) with organic extractive species (filter feeders [*e.g.* mussels, scallops, oysters, cockles] and detritivores [*e.g.* sea urchins, sea cucumbers]) and inorganic extractive species (*e.g.* kelp, Nori) within the same oceanographic area, usually a single farm tenure (Chopin et al., 2007). The benefits of this aquaculture practice are removal of excess nutrients in the water (by the extractive species), economic diversification, and greater social acceptability. IMTA economically diversifies the aquaculture business because the extractive species are also additional marketable seafood products

(Whitmarsh et al., 2006; Neori, 2008; Bunting and Shpigel, 2009), which means the business no longer needs rely on only one species.

The blue mussel, *Mytilus edulis*, has been shown to remove fish feces and fish feed from the water column (Reid et al., 2010). However, on the west coast of Vancouver Island, exposed mussel socks are predated on by sea otters and surf scoters (Wursig and Gailey, 2002; Dionne et al., 2006). In this study we created predator exclusion nets for the mussels and stocked the inside of the closed-bottom nets with sea urchins in order to potentially off-set the cost of building the nets. Urchins are a seafood delicacy in Japan and there is an established market for the local green sea urchin, *Stronglyocentrotus droebachiensis*. Both of these species meet the criteria of both IMTA and Sustainable Ecological Aquaculture SEAfarm products. There is also some evidence that sea urchins can reduce biofouling on aquaculture nets (Lodeiros and Garcia, 2004; Ross et al., 2004; Edwards and Cross, 2008).

In this study, mussels and sea urchins were co-cultured in the predator exclusion nets at different stocking densities of sea urchins. After 174 days, the growth of the mussels and urchins were quantified. Net biofouling was also quantified.

4.2 Summary of Findings

1) Can S. droebachiensis effectively control fouling on large surface area nets with a wide range in depth?

The results of this study indicate that *S. droebachiensis* can effectively reduce fouling on mussel predator nets on a commercial scale. Significant differences were not detected among the three highest stocking density treatments, but all the treatments with urchins were significantly less fouled than the control without urchins. Also, the two highest stocking density treatments (90 and 120 urchins net^{-1}) were significantly less fouled than the lowest stocking density treatment (30 urchins net^{-1}). The difference in fouling between the two highest density treatments was not significant , therefore 90 urchins net^{-1} or 7.37 urchins m^{-2} was the maximum stocking density required to control biofouling for this experiment.

Nets with high stocking densities of urchins (90 urchins net^{-1}) were only 51.5% occluded compared to 92.1% occluded with no urchins (control) in the 6 month experiment. However, nets treated with copper-based antifoulant were only 98.7% occluded compared to control nets, which were 3.1% occluded in a 10 month experiment by Braithwaite et al. (2007). The urchins were not as effective as copper-based antifouling because they were not able to control fouling on the outside surface of the nets.

While sea urchins are not as effective at controlling fouling as the copper coatings, they are much more environmentally benign. The urchins in this experiment were not given any additional feed inputs, which means that they performed an environmental service (biofouling removal) without additional waste inputs into the local environment. Also, unlike copper-based net treatments, the urchins do not add deleterious substances into the environment [see Braithwaite and McEvoy (2005) for a summary of toxic antifouling paints and materials].

2) Does urchin stocking density and/or depth influence the growth of S. droebachiensis and M. edulis?

The only significant differences in mussel growth at different densities of urchins were at 1 m depth. Mussel growth (*i.e.* length, wet total weight, wet meat weight, and dry meat weight) was significantly higher at higher urchin stocking densities of 90 and 120 urchins net^{-1} than at 60 urchins net^{-1} with no significant difference between 0, 30, and 60 urchins net^{-1} for wet and dry meat weight. At high urchin densities the cleaner nets may have led to the increased trend in mussel growth. For mussel length and total wet weight at 1 m, mussels grown in nets with 60 urchins net^{-1} were also smaller than in nets with 0 urchins net^{-1}. The physical presence of the urchins may have interrupted mussel feeding (throughout the experiment, urchins were observed attached to the mussel socks in the upper 1.5 m of the water column). Sea urchins were shown to feed on small mussels by Cook and Kelly (2009) and on mussel meat (Meidel and Scheibling, 1999). It is possible that the urchins inhibitied feeding at low stocking densities until the increased net aperature (cleaner nets) at higher densities outweighed urchins interference with mussel feeding. There was little difference in mussel growth among densities at 2.5 or 4 m depth. This was likely because the urchins were not observed attaching to the mussel socks at the deeper depths. There is no indication that grazing on biofouling by urchins had any impact on mussel growth at any depth examined.

The mussels grew larger at deeper depths, 2.5 and 4 m, than near the surface, however it is unlikely that urchin stocking density had any influence on the increased mussel growth at 2.5 and 4 m because this trend was already evident before the urchins were added to the nets.

While a trend in mussel growth at 2.5 and 4 m depth was not detected at different urchin stocking densities, an interim sampling may have detected some of these trends before the mussels reached maximum size at the end of the experiment. Urchin diameter, whole wet weight, gonad wet weight, and gonad yield all declined with increasing urchin stocking density. This is most likely a result of competition for food because biofouling, and possibly some fish feces and uneaten fish food, was the only food resource available to the urchins.

There were no significant differences in gonad quality(based on colour, brightness, firmness and texture only) among stocking densities or between the farmed urchins and wild urchins from the reference site based on the quality parameters used. Based on the gonad weight difference alone (the only discernable difference), gonad quality decreased with increased stocking density of urchins.

Interestingly, at low density, the farm-raised urchins had significantly higher gonad wet weights and gonad yields than urchins from the reference site. At higher stocking densities, gonad wet weight of cultured urchins was either similar to (60 and 90 urchins net^{-1}) or significantly less than (120 urchins net^{-1})

wild urchins at the reference. Based on these results, the low-density, farmed urchins would have a higher economic return than the wild urchins or the high-density farmed urchins.

3) Overall Trends

In summary, increasing stocking densities of urchins significantly decrease biofouling, but this trend is not statistically significant at stocking densities higher than 60 urchins net^{-1} (or 4.91 urchins m^{-1} of net). Urchin somatic and gonadal growth decline at increasing stocking densities of urchins while there was only an impact of urchin stocking density on mussel growth detected at 1m. Therefore, there is a trade off between biofouling control by urchins and urchin growth.

4.3 Research Contributions

This research showed that *S. droebachiensis* significantly reduced biofouling on aquaculture nets. This supports the findings of Lodeiros and Garcia (2004) and Ross et al. (2004) who reported the sea urchins *Lytechinus variegatus* and *Psammechinus miliaris* to be effective at controlling biofouling. The present study builds on the work of Lodeiros and Garcia (2004) and Ross et al. (2004) by showing that urchin growth may decline with increasing stocking densities and that urchins are able to clean nets with a large vertical surface area. The finding that urchin growth declines with increasing stocking density is important because any aquaculture businesses wishing to utilize urchins as a method of biofouling control and an additional seafood product, need to consider this trade-off before integrating urchins in their site. Also, urchins did promote increased mussel growth in the top of the water column at low density. This supports the findings of Ross et al. (2004) who showed that increased scallop shell growth was associated with the urchin, *P. miliaris*. Finally, this research confirms that *S. droebachiensis* can effectively control biofouling on a vertical surface over a wide range of depths and will disperse themselves evenly around the entire surface area of the nets (based on monitoring with an underwater video camera). This finding suggests that the green urchins is a practical method to control biofouling because most aquaculture nets have a large vertical component.

This research also adds to the understanding of how to effectively integrate different species into IMTA or SEAfarm sites. The method allowed for mussels to be grown in predator exclusion nets, which is important for areas with large populations of diving ducks and sea otters. The study also demonstrates an effective way to integrate sea urchins into IMTA or SEAfarm sites.

Finally, this research provides further information to the body of literature on biological fouling control (Hidu et al., 1981; Enright, 1993; Kvenseth, 1996; Ahlgren, 1998; Lodeiros and Garcia, 2004; Ross et al., 2004; Dumont et al., 2009). This study is another example of how naturally-occurring species can be utilized to slow the accumulation of biofouling on aquaculture nets. This research also adds to a broader body of literature of non-toxic alternatives to copper-based antifouling net treatments (Hidu et al., 1981; Enright, 1993; Kvenseth, 1996; Ahlgren, 1998; Lodeiros and Garcia, 2004; Ross et al., 2004; Rajagopal et al., 2005; Qian et al., 2007; Dumont et al., 2009; Huang et al., 2010; Piola et al., 2010). These non-toxic alternatives, including the green sea urchin, have the potential to help reduce some of the environmental impacts of aquaculture while improving the public perception of the industry.

4.4 Future Research Directions

In this study, the mussels grown at 2.5 and 4 m depth reached maximum size. An interim sampling period for mussel growth would clarify trends in mussel growth at different stocking densities of sea urchins.

The green sea urchin was able to effectively reduce biofouling in this study, but urchin growth rate did decline at increasing stocking densities. In order to use sea urchins as a method of biofouling control at a commercial scale in BC, more work needs to be done on different size classes of urchins; alternative urchin species; biofouling trends over time, seasonally and at different depths; urchin performance on non-IMTA sites; and gonad quality when taste and smell are included as parameters.

The green sea urchins used in this study were small adults at the beginning of the experiment. It is important to understand how juvenile urchins perform in a similar experiment. It would be more practical for growers to be able to use the mussel predator-exclusion nets as grow-out nets for urchins, rather than stocking them with adult urchins. However, their small size may necessitate a nursery stage prior to stocking in predator-exclusion nets.

In addition to different size classes, alternative species need to be tested. The red sea urchin, *Stronglyocentrotus franciscanus*, and the purple sea urchin, *S. purpuratus*, are also native species in B.C. These species have not yet been tested as a method of biological fouling control and may perform better than the green urchins. There is already an established export market for red urchins (FAO, 2011) and some interest in creating a fishery for purple urchins (Workman, 1999), which make them appealing candidates for integration into aquaculture sites.

In regards to the green sea urchin, biofouling, urchin growth and mussel growth were only measured at the end of the experiment. In order to develop a better understanding of biofouling prevention by sea urchins, these measures should be taken regularly over time and at least seasonally. Also, there was an effect of urchin stocking density on mussel growth at 1m. More work could be done in order to better understand this pattern, although these mussels were undersized and therefore may not warrant further investigation.

Urchin growth and ability to control biofouling also needs to be investigated at non-IMTA sites. Integrating urchins in shellfish farms, with no fish waste inputs, may result in lower growth rates of urchins and different patterns of biofouling reduction. It is possible that the urchins would require additional feed inputs at non-IMTA sites.

At the IMTA site, urchin gonad quality needs to be assessed further using gonad taste and smell as additional parameters. These are both important parameters that are used by urchin processors and heavily influence their value in the seafood market. These parameters were not included in this experiment because it is a skill that takes training to develop and there are few people in BC that are able to accurately assess these qualities.

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