Biofouling in Salmon Aquaculture: the effectiveness of alternative netting materials and coatings in coastal British Columbia

by

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BSc. University of Victoria, 2008

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Abstract

Biofouling in salmon aquaculture is an important issue. The use of copper based antifoulants contributes to marine pollution and managing biofouling on untreated nets incurs a heavy cost on the industry. What is needed is an antifoulant coating that balances the needs of the industry with good environmental practices. This study describes the effectiveness of seven alternative netting treatments and two copper based treatments as compared to an untreated nylon net. Effectiveness was measured in terms of percent net occlusion, percent cover of major fouling groups and biomass. Following eight months immersion, results show that the alternative treatments did not out-perform the untreated nylon control, and that the two copper treatments significantly outperformed the control and all of the alternative treatments tested in this study.

The results demonstrate that the alternative treatments tested in this study were unable to meet the performance standards set by industry, that more research is needed into alternative antifoulant coatings for aquaculture, and that the effectiveness of copper based treatments will continue to be a barrier to the implementation of alternative antifouling treatments.

keywords: biofouling; salmon; copper antifoulant; image analysis

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CHAPTER 1: Introduction

Although global capture fisheries production has remained relatively stable during the past decade, over half of the world's fisheries stocks are estimated to be fully exploited, with another thirty percent in varying states of over-exploitation and depletion (FAO 2010). The decline in capture fisheries and the increase in demand for aquatic food sources from a growing global population are contributing to the growth of the aquaculture industry (FAO 2010). Currently, aquaculture is the fastest growing animal-food-producing sector in the world and for the first time in history the industry is expected to surpass capture fisheries as the main source of food fish (FAO 2010). Of the total global aquaculture production (52.5 million tonnes) the production of Atlantic salmon accounts for roughly 1.36% (1.5 million tonnes). Although aquaculture has been practiced in Asia for over 4000 years, industrialized finfish aquaculture, which is dominated by the culture of Atlantic salmon, is a relatively new activity having become established in the last 40 years (Beveridge 2004; Gibbs 2009) in a few key regions which include: Norway (36% of global farmed salmon production), Chile (28%), Europe (7.4%) and North America (7.4%) (FAO 2010).

In 2010 Canada produced 78,700 tonnes of farmed salmon with an estimated farmgate value of 499.6 million dollars (MOE 2010a). Atlantic salmon are the dominant species (94%; 74, 500 tonnes) followed by Pacific salmon (6%; 4200 tonnes) (MOE 2010a). Salmon production is divided between the East coast Maritime Provinces (mostly New Brunswick and some in Nova Scotia) and British Columbia. On the East coast the industry is generally supported by the public, but in BC the salmon farming industry is a somewhat contentious issue. Most production occurs on the more rural northern half of Vancouver Island where employment is appreciated in the face of dwindling fisheries and declines in the forestry sector. The main salmon farming areas are on the west coast in Clayquot Sound, the area north of Campbell River, and the mainland Sunshine Coast. Opposition can be found anywhere on the coast and it comes in the form of bumper stickers, (e.g. “Farmed Salmon don’t do Drugs”), rallies, and even suing the government (Morton v. British Columbia 2009) . The antagonism about the issues around salmon farming are complex, but they are usually grounded in ideas about protecting wild salmon. What follows is a brief outline of the salmon farming industry and its issues in the province of British Columbia.

Currently, in British Columbia there are 130 salmon farm tenures, roughly 80 of which are in operation at any given time (MOE 2010b). There are thirteen companies operating in the province, with most of the tenures licensed to four major salmon farming companies: Marine Harvest (73 tenures), Mainstream (33 tenures), Grieg Seafood (24 tenures), and Creative Salmon (7 tenures). The majority of the farmed salmon produced in the province is exported to the United States (MOE 2010a).

In British Columbia, the first salmon farms came into operation in the 1970s. The 1980s saw a rapid increase in the number of farms and a shift to predominantly Atlantic salmon culture (Noakes et al. 2000). This rapid expansion led to concerns from a variety of stakeholders which inspired one of the first reports on the salmon farming industry in BC in 1986 (Gillespie 1986). The report referred to the early development of the salmon farming industry as a “gold rush” implying that development had unrestrained growth, high profits, and a general disregard for environmental implications. It outlined a broad set of issues and concerns brought forward by stakeholders. Many of the issues still hold to this day and new issues have since been brought to light, including: threats to salmon enhancement programs (Noakes et al. 2000), market and processing facility competition with the wild caught salmon industry (Gillespie 1986), concerns regarding safe navigation (Gillespie 1986), the unsightly nature of farms negatively affecting tourism (Gillespie 1986) and a whole suite of environmental issues. The environmental issues are both local and global. There has been much debate about the sourcing of fish feed and fish oil which often comes from abroad (Naylor et al. 2000), and concerns regarding predator management protocols (Gillespie 1986). One of the most complex issues is the interaction between farmed and wild fish. There are fears that escaped Atlantic salmon will outcompete or hybridize with wild Pacific salmon (Noakes et al. 2000). There are concerns regarding the threat to the genetic diversity of wild Pacific salmon if farmed Pacific salmon escape (Noakes et al. 2000). There are fears regarding disease and parasite transference between wild and farmed fish (e.g. Hematopoeietic Necrosis virus (IHNv), *Yersinia ruckeri* (the cause of enteric red mouth disease), *Aeromonas salmonicida* (the cause of furunculosis), and *Renibacterium salmoninarum* (the cause of BKD; Noakes et al. 2000), the persistence of therapeutants and antibiotics in the marine environment (Gillespie 1986; Noakes et al. 2000), and last but not least, there are issues with pollution and the accumulation of wastes (both organic and inorganic) on the seafloor and in the water column.

Many of the early unsustainable practices in the industry have been discontinued and producers are continually working towards improving their operational procedures and environmental practices (Stickney and McVey 2002). It is in a company's own self-interest to minimize negative impacts and maintain a healthy growing environment for their fish (Gillespie 1986). Described by Noakes et al. (2000) the industry has improved its practices in many ways: the frequency of escapes has decreased because of better equipment and improved handling, farming Atlantic salmon removes the threat of an interaction between wild Pacific and farmed Pacific salmon, more effective vaccines means they are used in smaller quantities and less often, and improved feeds and feeding practices have reduced the amount food needed to reach market size and also decreases the waste generated by the salmon. Some of the issues that have yet to be resolved include: sustainable feed sources, effective sea lice management, disease transference (in both directions) between farmed and wild fish, the potential introduction of invasive species, and chemical pollution from antifoulants. This project is focused on the latter topic of antifoulant paints.

Salmon are grown inside of large cages made of 25 mm nylon mesh. They rely on currents to exchange dirty, oxygen depleted water inside the cage with clean, oxygen rich water from outside. When marine invertebrates (biofouling) grow on the nets they block the mesh openings which causes a reduction in water exchange resulting in the build-up of wastes and oxygen depletion which are detrimental to the fish. There are three main options for dealing with this issue: net washing, net changing, and antifoulant coatings. In-situ washing of a net is labour intensive, stressful to the fish, and it results in the accumulation of debris below the cages. The pressure washers also damage the nets and compromise the structural integrity of the fibres. An alternative is to change out the fouled net with a clean net, which is still costly, increases the risk of escapes, and stresses the fish. The application of a copper based antifoulant can protect a net for an entire growout under most conditions but can result in unacceptable levels of dissolved copper (Brooks 2000), and company policies often lead to premature net washing which results in the continual need to wash the nets for the rest of the growout cycle.

There is a long history of combating marine biofouling by coating infrastructure with toxic antifoulant paints to deter settling organisms (WHOI 1952). Initially the salmon farming industry coated their nets in antifoulants based on the highly toxic tributyltin (TBT) (Gillespie 1996). Since the global ban on TBT in the 1990s the industry exclusively uses antifoulants based on cuprous oxide. Copper is a naturally occurring element, but in high concentrations it is toxic.

Because of the environmental concerns some companies have chosen not to use them. Creative Salmon does not use antifoulants, and the largest company operating in the province (Marine Harvest) has decided to stop using copper antifoulants at all but a few problematic sites.

Dipping a net in an antifoulant adds anywhere from 25% (Beveridge 2004) to 56% (R. Clarke pers. comm.) to the cost of a net and contributes to marine copper pollution, and net washing (including labour but excluding equipment) can add another 15% (R. Clarke pers. comm.). It shortens the life span of a net and, when done on site, impacts the benthic environment around a farm. Considering that on average there are 12 cages (S. Cross pers. comm.) on each farm in BC, which equates to roughly 1260 salmon cages along the whole coast, then it can be assumed that a substantial portion of a farm's operating budget is spent managing biofouling. Because biofouling management continues to be an economic and environmental issue there is a clear need for viable management practices that balance the needs of the industry with the needs of the environment. In view of this background, this study has two main objectives. The first is based in biology and aims to describe the biofouling community on a salmon farm off of northern Vancouver Island and to contribute to our understanding and future research on this topic. The second is grounded in the needs of industry and will test the effectiveness of several alternative antifoulant coatings on a commercial salmon farm. This was accomplished by first describing the seasonal succession of the biofouling community found at this site. Then for each treatment, the changes in percent net occlusion were quantified starting in winter and continuing through the peak fouling months of summer. The percent net occlusion for the final climax community in September was analysed separately, along with the biofouling community composition and wet weight biomass. All of the treatments were compared, in terms of better or worse performance, to an untreated nylon net. Additionally, two copper treatments were compared to one another (Netrex and Flexgard) as were two Dyneema treatments (Dyneema and Dyneema with Sancure). The results from this project will help industry to make better-informed biofouling management decisions and understand if any of the coatings tested are viable alternatives to an untreated nylon net.

CHAPTER 2: Biofouling

2.1 The Biology of Biofouling

Biofouling is the growth of unwanted organisms on the surfaces of artificial structures immersed in the marine environment (WHOI 1952). Henceforth, the terms ‘biofouling’ and ‘fouling’ are used interchangeably. The fouling community that develops at a site depends on a number of factors which include: substrate type (e.g. soft bottom or rock), geographical location (e.g. latitude), oceanographic characteristics (e.g. current, salinity, temperature, pH), seasonality (e.g. whether a surface was immersed starting in spring or fall) and biotic factors (e.g. competition and predation) (Callow & Callow 2002).

Species are rarely dispersed uniformly in nature (Miller and Ambrose, 1996; Legendre and Fortin, 1989). Instead, patchiness (spatial heterogeneity) is the norm (Miller and Ambrose, 1996) and biofouling is no exception. Biofouling in aquaculture is inherently patchy as well as spatially and temporally specific. The process behind the patterns of aggregation in biofouling communities can be based around the theory of island biogeography (MacArthur and Wilson 1967). An ‘island’ is an area of suitable habitat surrounded by an expanse of unsuitable habitat (MacArthur and Wilson 1967). In the case of aquaculture biofouling, the man-made netting surface is the island, which is otherwise surrounded by open water.

A biofouling community can include all sessile organisms in the area, as well as introduced or invasive species, and some mobile species (Watson and Dürr 2010). However, for the purpose of this study true biofouling organisms are considered to be those organisms which remain attached for the post-settlement stage of their lifecycle (Zongguo et al. 1999). The mobile organisms associated with the complex habitat of a biofouling community (e.g. decapods and nudibranchs) are not included because they tend to be too small or rare to occlude net openings or add substantial weight to a system. They are usually organisms that prey on and forage in the sedentary community thus reducing fouling; and because they are mobile, they are extremely difficult to document accurately since they are able to swim away or hide when there is a major disturbance (e.g. sampling).

The development of a biofouling community occurs as a complex colonization process that is made up of four distinct stages (Figure 1) (Callow and Fletcher 1994; Yebra 2004). Described by Callow and Callow (2002), the first stage of development is a molecular conditioning film of dissolved organic material that accumulates on the newly immersed surface. The second stage is the development of a biofilm that is comprised of bacteria, unicellular algae and cyanobacteria which can form within a few hours. The third stage typically includes diatoms, which quickly reproduce forming a ubiquitous brown-green slime. The biofilm and diatom coatings are referred to as ‘microfouling’. Once this microfouling community is established, macrofoulers arrive in the form of larvae or spores. The early macrofouling community is dominated by fast-growing organisms and is typically followed by slow growing organisms that develop into the final stage which is a dynamic biofouling community (Scheer 1945). As immersion time increases so does the overall complexity of a biofouling community (Railkin 2004) and greater nutrient availability can also lead to increased community complexity (Naranjo et al. 1996). The first stages of bacterial colonization are driven by a mix of biological and physical factors, but as the community moves from diatoms to macrofoulers biological factors (e.g. competition) become more prevalent (Railkin 2004).

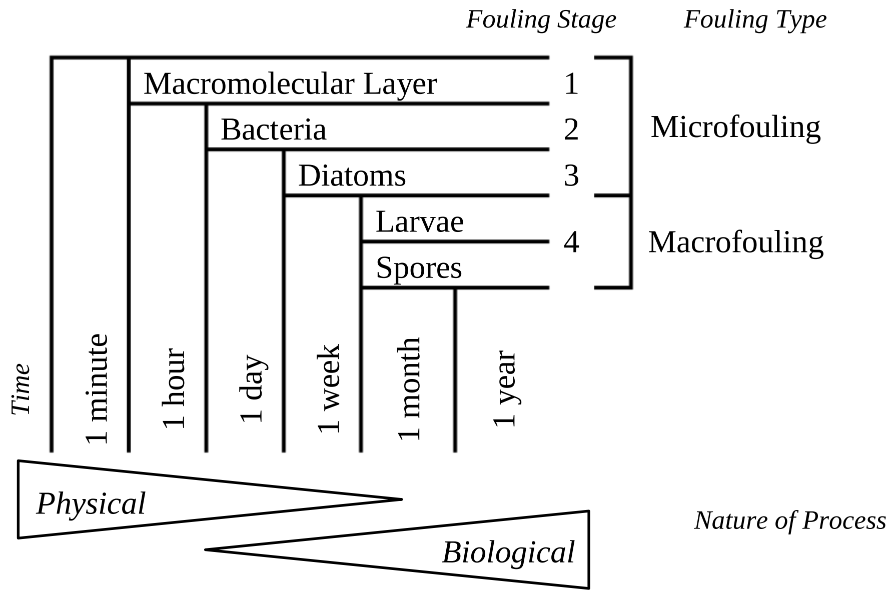

Figure 1: Stages of hard surface biofouling colonization (adapted from Railkin 2004). Microfouling occurs in three general stages and is followed by macrofouling which involves both larvae and spores. Over time the nature of the processes driving colonization change from physical (e.g. surface type) to biological (e.g. competition).

Throughout the world's oceans, over 4000 fouling organisms have been described (summarized in Yebra, 2004). Despite this daunting number of organisms, most aquaculture biofouling can be separated into six macrofouling groups of major concern: algae, hydroids, mussels, barnacles, tube worms, and ascidians (Watson and Dürr 2010). Other groups often found include low growing encrusting organisms like sponges and bryozoans, and members of the Caprellidaea (skeleton shrimp) family. The six broad taxonomic groups include a wide range of organisms with diverse life histories. Both sexual and asexual reproductive strategies are common, with broadcasters (e.g. mussels) being more common than brooders (e.g. barnacles) (Havenhand and Styan 2010). Many organisms (e.g. algae) alternate between sexual haploid and asexual diploid reproduction (Brawley and Johnson 1992), and settlement and re-establishment from fragments is common among encrusting organisms such as sponges (Ayling 1980) and bryozoans (Jackson and Coates 1986). Approaches to larval supply and dispersal also vary. Propagules typically move with cilia, flagella, or muscular contractions; but their movement is limited in comparison to water currents (Chia et al. 1984). For biofouling organisms, establishment is based on the dispersal potential of a species and the distance from source populations (Mcquaid and Miller 2010). Once some individuals establish they become a source for localised dispersal and self-recruitment (Mcquaid and Miller 2010). Fouling organisms also have different strategies, behaviours and cues when settling on a surface and metamorphosing from the planktonic to sessile phase. There are three basic models to describe the way a propagule might encounter a surface: 1) a propagule might settle randomly on whatever surface they are near and post-settlement mortality will remove those that settled in inappropriate locations, 2) a propagule might involuntarily respond to environmental stimuli, and 3) a propagule might actively seek out an appropriate site using behavioural responses to environmental cues (Prendergast 2010). For example, sponge propagules are some of the least discriminating when it comes to settlement, which might be attributed to their generalist nature as adults (Prendergast 2010). Tunicates tend to settle on shaded, downward farcing surfaces likely due to a lack of sedimentation (Prendergast 2010). Barnacles respond to chemical settlement cues from conspecifics (Rodriguez et al. 1993; Kato-Yoshinaga et al. 2000). Molluscs are often more complex with different species responding to everything from substrate colour and roughness, biofilm attraction, and avoidance of adults; some species even have the ability to move after they have settled (Prendergast 2010).

The effects of the accumulation of a biofouling community are felt in all marine industries. Fouling blocks pipes and conduits on everything from ships and desalinization plants, to power stations and refineries (WHOI 1952). These blockages cause operational inefficiencies, equipment failures and increased maintenance requirements (WHOI 1952). In shipping, fouling increases the weight of a vessel and makes it more susceptible to drag forces which results in a reduction of speed and maneuverability and causes an increase in fuel consumption and the need for more frequent dry dock maintenance (WHOI 1952). The fouling of critical navigation equipment such as depth sounders can jeopardize the safety of a vessel (WHOI 1952). Moreover, biofouling has broad ecological ramifications since the biofouling that accumulates on international shipping vessels has been the cause of many invasive species introductions (Davidson et al. 2009).

2.2 Biofouling in Salmon Aquaculture

Similar to shipping, biofouling in the aquaculture industry is an expensive problem (Hodson et al. 1997; Braithwaite and Mcevoy 2005). The traditional methods used to control biofouling at finfish sites are the use of copper-based antifoulant coatings, manually cleaning nets with pressure washers, and drying nets between deployments. Some less frequently used fouling management practices are avoidance-based methods such as cages made of metal mesh (Beveridge 2004), double net systems where one net is left in the water and the other is left on the surface to dry (e.g. Nor-Maer 2012), and fully enclosed rotating cages where half of the cage is out of the water at any given time (Campbell et al. 1982).

When antifoulants are used, fouling does eventually occur but with a delayed start and with a slower rate of accumulation (Braithwaite et et al. 2007; Guenther et al. 2010). Despite being coated, nets with antifoulants are usually still washed. A typical schedule is to wash nets every two weeks in the peak fouling months of summer, every 21 days in spring and fall, and once a month in winter months (R. Clarke, pers. comm.). Washing a treated net causes the antifoulant coating and the organisms that were attached to the nets to accumulate on the seafloor. This disrupts the natural processes that occur in benthic environments (Brooks 2000; Brooks et al. 2002) and changes the bioavailability of the copper antifoulant (Yebra 2004).

In Canada, with the use of current industry standard materials, a salmon net is projected to last ten years, but they often fail break strength testing after less time (K. Onclin, pers. comm.). Currently, the most common alternative to copper treatments is an untreated nylon net. But an untreated net is more susceptible to damage from UV radiation and has to be washed more frequently, both of which contribute to accelerated deterioration of the mesh structure and can reduce the life expectancy of a net by half (K. Onclin, pers. comm.). The increased use of pressure washers also leads to higher risk of premature net failures (K. Onclin, pers. comm.). These factors create a need for more frequent net replacements which has significant economic implications for a salmon farming operation.

The multi-filament netting material that is ubiquitous in the aquaculture industry is an ideal substrate for biofouling organisms because when it is left untreated it is non-toxic, contains many crevices that can accumulate and shelter settling organisms and it has a high surface-area to volume ratio (Hodson and Burke 1994; Hodson et al. 1995; Hodson et al. 1997). Furthermore, the fish contained within the nets can create readily available dissolved nutrients that might otherwise be a limiting factor for the growth of fouling organisms (Cook and Kelly 2007; Corner et al. 2007).

Fouling affects a salmon aquaculture operation in several ways. One of the most visible and direct effects is from the occlusion of net openings (Hodson, et al. 1997; Phillippi et al. 2001; Braithwaite et al. 2007) which occurs when an organism grows across the opening of a net and blocks the transmission of water, and thus oxygen, through a cage. A decrease in net aperture causes a severe reduction in water flow (Ahlgren 1998), resulting in decreased nutrient exchange and a reduction in oxygen supplies (Ahlgren 1998; Beveridge 2004). The recommended minimum amount of dissolved oxygen for cold water fish such as Atlantic salmon is 6 mg per liter (70% saturation), oxygen levels below this stresses the fish making them more susceptible to diseases and infection, and it also slows their metabolism causing them to eat less which results in decreased growth rates and associated economic loss (Beveridge 2004).

The accumulation of biofouling organisms adds substantial weight to system infrastructure (Cheah and Chua 1979; Milne 1979a; Cronin et al. 1999; Braithwaite et al. 2007). In some cases fouling can increase the weight of a net up to 200 times (Milne, 1972 in Beveridge, 2004). This increase in weight and surface area decreases cage buoyancy and increases drag from currents (De Nys and Guenther 2009). Studies show that drag on a fouled net can be 3 to 12.5 times stronger than that of a clean net (Swift et al. 2006; Milne, 1970 in De Nys and Guenther 2009). This increase in drag causes structural fatigue of the nets (Huguenin and Ansuini 1975; Hodson et al. 1997; Hodson et al. 2000) and cage deformation (De Nys and Guenther 2009) which can cause reductions of cage volumes from 45-80% depending on cage size and bottom weights (Aarsnes et al. 1990). Another issue is that biofouling communities can create habitat for harmful diseases and parasites such as net pen liver disease (Andersen et al. 1993), the parasitic nematode *Hysterothylacium aduncum* (González and Gonzalez 1998), the sea louse *Lepeophtheirus salmonis* (Huse et al. 1990; De Nys and Guenther 2009), and amoebic gill disease (Tan et al. 2002; Douglas-Helders et al. 2003). It has also been shown that copper dipped nets can house greater abundance of the organism that causes amoebic gill disease (Douglas-Helders et al. 2003).

From an operational perspective fouled materials not only create more maintenance requirements and system damage, but they are more difficult to work with. The additional weight from fouling organisms makes manual tasks like pulling nets much more difficult or it can mean that heavy equipment needs to be used. Fouled surfaces are also slippery and often covered in sharp, calcareous organisms which decreases worker efficiency and creates a more hazardous work environment.

Biofouling that occurs on aquaculture system infrastructure poses a risk to the cultured stock, thus managing biofouling is of critical importance to the success of an aquaculture operation. Because there are few effective passive management options like antifoulant coatings, manual removal of fouling by washing the nets is still the most reliable management strategy. However, this practice is labour intensive and time consuming which results in more time being spent on system maintenance and less time being spent on animal husbandry.

2.3 Historical Context of Antifoulants

Although the scientific approach to understanding and managing biofouling is relatively new, ancient mariners were well aware of the issues caused by biofouling. The development of antifouling technology is rooted in shipping and the need to protect ship hulls from damage from fouling organisms. In the era of wooden hulled vessels wood boring molluscs were a serious problem since an unprotected ship might arrive at its destination missing a substantial percentage of its hull (WHOI, 1952). The Phoenicians and Carthaginians are thought to have used coatings made of pitch (WHOI, 1952). The ancient Greeks are known to have used tar, wax, and lead sheathing (WHOI, 1952). The writings of Plutarch (45-125 A.D.) mention the need to scrape 'weeds, ooze and filth' from the underside of ships to let them move more easily through the water (WHOI, 1952). From the 13th to the 15th century ships were often coated with pitch, and copper sheathing is known to have been used as early as the 16th century on ship hulls (WHOI 1952). By the late 17th century copper sheathing was recognized as the most effective antifoulant available at that time, and in 1824 the first research into the chemical mechanism behind the antifoulant characteristics of copper sheathing was published (WHOI 1952).

In the late 18th century iron hulls and steam engines were introduced and brought with them a renewed interest in preventing biofouling since the drag it caused dramatically slowed a ship and increased its fuel consumption (WHOI 1952). However, the use of copper sheathing was discontinued due to its dangerous electrolytic corrosion effects on iron hulls (WHOI 1952). As a result, more effort was put into developing coatings and paints that included an antifoulant in their polymer structure (WHOI 1952). These biocidal antifoulant coatings work by creating a toxic boundary layer at the surface of the coating as the component biocide leaches out (Yebra 2004). This boundary layer either deters larvae and spores from settling or outright kills them (WHOI 1952). In the early 20th century biocidal antifoulant paints that contained chemical compounds of lead, arsenic, and mercury became common; these coatings posed such sever environmental and human health risks that they were voluntarily discontinued by the paint industry in the 1960s (Evans et al. 2003). Shortly thereafter, some of the most effective antifoulants created to date were brought to market.

2.4 Environmental Concerns of Antifoulants

Antifoulants based on tributyltin (TBT) were first commercialized in the 1960s. The products quickly gained popularity and by the 1970s semi-enclosed water bodies were showing signs of contamination (Terlizzi et al. 2001). As a result of TBT's high toxicity the International

Maritime Organization (IMO) enacted legislation on a global scale under the International Convention on the Control of Harmful Antifouling Systems on Ships (ICAFS) that prohibits the use of TBT as an antifouling agent (Terlizzi et al. 2001; Cheyne 2004).

The ban on TBT created a revival for copper based antifoulant paints (Terlizzi et al. 2001). These copper based paints are now the most commonly used antifoulant coatings in both shipping and aquaculture (Hodson et al. 1997; Braithwaite et al. 2007). Copper is an essential element and is naturally found in the marine environment but in high concentrations it can be toxic (Voulvoulis et al. 1999). The fate and bioavailability, and thus toxicity, of copper is poorly understood and often controversial (Voulvoulis et al. 1999). However, many studies have shown that high concentrations of copper can have negative effects on aquatic organisms (Nor 1987). Studies using bioassays show that copper sensitivity varies greatly between species, but a generalized model from most sensitive to most tolerant is first microorganisms, followed by invertebrates, then fishes, bivalves, and finally macrophytes (Nor 1987).

High concentrations of copper can inhibit growth (e.g. Debelius et al. 2009a), reduce photosynthesis (e.g Garvey et al.1991), and decrease enzyme activity (e.g. Pinto et al. 2003). Copper can disrupt physiological processes (e.g. Brown et al. 2004), it can induce morphologic changes (e.g. Debelius et al. 2009b), and can cause mortality when levels are too high (e.g. Rai et al.1981).

In Canada, antifoulant coatings are regulated by the Pest Management Regulatory Agency (PMRA). There are five products registered for use as antifoulants in aquaculture, all of which have copper (cuprous oxide: Cu_2O) as the active ingredient (Table 1). Part of the International Maritime Organization’s harmful antifoulant (ICAFS) legislation includes a policy to eventually ban all antifoulants that exhibit harmful effects on the marine environment (Evans et al. 2003). This issue was also raised by the 2007 BC parliamentary report on sustainable aquaculture which suggested that antifoulant coatings be prohibited for use on salmon farms and that the industry should continue research into alternative biofouling management practices (BC Leg. 2007). Thus, there is the potential for the eventual phasing-out of copper based antifoulants in aquaculture (Braithwaite and Mcevoy 2005). The challenge for the implementation of alternative antifoulant coatings is the fact that antifoulants based on cuprous-oxide are exceptionally effective at preventing the accumulation of biofouling organisms. For example, a study by Braithwaite et al. (2007) tested the effectiveness of a common copper based antifoulant used on salmon cages. They found that over a 10 month period the copper treatment significantly reduced the biomass accumulation (1.8 kg m^{-2} as opposed to 4.9 kg m^{-2} on an untreated net), and that the copper treated net was able to significantly reduce net occlusion for at least 150 days during peak fouling months (Braithwaite et al. 2007).

Table 1: Copper based antifoulant coatings that are available in Canada for use in aquaculture (PMRA 2012).

Coating Name	Manufacturer and location
Flexgard XI	Flexabar Aquatech Corporation, USA
Flexgard VI	Flexabar Aquatech Corporation, USA
Netrex AF	Netkem AF, Norway
Solignum	UCP Paints, Canada
Aquanet	Steen-Hansen Maling AS, Norway

2.5 Alternative Treatments

Because of the ecological risks associated with copper antifoulants and the potential for the phasing-out of these products there are incentives to develop and implement alternative antifoulant coatings. When choosing a netting material or treatment there are two other important factors to consider other than antifouling ability, which are surface area and breaking strength. Breaking strength is important because it relates to a material’s ability to resist chafe and breakage. A stronger twine means less risk of escapes as well as less maintenance and repairs. It is important that the breaking strength does not substantially decrease over time.

The surface area of a salmon cage relates to the twine thickness of a material and/or coating. Surface area is important because drag force acting on nets is the main mechanism by which wave and current energy are transferred to net pens (Swift et al. 2006). The mesh size and twine thickness change the total surface area of a net (Hellio & Yebra 2009). Thicker twine and smaller mesh increase the surface area which also equates to an increase in available area for the settlement of fouling organisms (De Nys and Guenther 2009). Consequently, small-mesh cages tend to have higher levels of biofouling than large-mesh cages. Likewise, a thick twine mesh has more surface area than a thinner twine mesh of the same size. Fouling development on netting is also influenced by the three-dimensional structure of the mesh itself. Preferential fouling of mesh intersections has been noted in fouling studies (Hellio & Yebra 2009), therefore, smaller twine and alternative weaves can result in smaller intersections which reduces the surface area available to fouling organisms.

Jacobson and Willingham (2000), state that an ideal antifoulant coating should prevent fouling from hundreds of organisms across the entire range of global climactic and environmental conditions while causing no adverse effects on the marine environment. The authors also describe several key environmental characteristics of an alternative antifoulant, which include: rapid degradation of the compound once released into the marine environment, rapid partitioning and limited bioavailability to non-target organisms, non-hazardous environmental concentrations, minimal toxicity to non-target organisms at concentrations present in the environment, and minimal bioaccumulation (Jacobson and Willingham 2000). Economic considerations for the implementation of a novel coating are that it is possible to apply it over top of pre-existing coatings, it fits within current business models and product application infrastructure (Rittschof 2010), it protects netting from UV and washing damage, and it increases the overall lifespan of a net (K. Onclin, pers. comm.).

In recent years, led mostly by the shipping industry, there has been an increase in the research effort into environmentally sound antifoulants (Braithwaite and Mcevoy 2005). Products loosely grouped as foul-release coatings have shown some potential. They are often silicone (Hodson et al. 2000) or latex (Svane et al. 2006) based and operate on the principle that fouling will occur, but due to low surface adhesion the organisms are easy to remove (Tsibouklis et al. 2000). Other novel approaches are based on changing the characteristics of a surface through the application of coatings that create micro-surface topography (Callow et al. 2002; Ralston and Swain 2009; Fang et al. 2010) or flocking which creates spiky layers that deter settling organisms (Köhler et al. 1999; Phillippi et al. 2001; Micanti 2011). Other directions include biomimecry, since many organisms are naturally able to prevent fouling on their shells, blades or bodies (Ralston and Swain 2009). On smaller systems and in the shellfish industry biological control involving the use of predators or grazers to manage fouling shows promise (Deady 1995; Ahlgren 1998). Other studies have looked at the use of electrical fields (Perez-Roa and Tompkins 2006), and even netting colour (Hodson et al. 2000).

Despite the progress that has been made recently, many of the alternative treatments currently available do not meet the standards set by copper based coatings (Braithwaite and Mcevoy 2005) and the associated performance requirements set by industry. For a company to choose to use an alternative coating it must be worth the effort and cost of applying it, particularly since implementation by industry is currently voluntary.

2.6 Summary

Biofouling in aquaculture is one of the industry's oldest operational and environmental issues. Despite this, much of the information that exists is anecdotal and of limited value (Braithwaite and Mcevoy 2005) or is kept private by commercial growers (Beveridge 2004). Research into biofouling on fish cages first appeared in the literature roughly 30 years ago (e.g. Milne 1979a; Milne 1979b). Authors often noted that there are few quantitative studies of net fouling (e.g. Cronin et al. 1999; Braithwaite and Mcevoy 2005; De Nys and Guenther 2009) and even less information on the economic costs of the management of finfish net fouling (Braithwaite and Mcevoy 2005). While there have been a number of valuable studies on finfish aquaculture biofouling (Svane et al. 2006; Braithwaite et al. 2007; Guenther et al. 2010) there have been few studies done in Canada, and even fewer in the Pacific Northwest. Hall (1962) quantified fouling on salmon cages in New Brunswick. Haegele et al. (1991) considered the value of biofouling organisms as a wild feed source for caged salmon in British Columbia, and Gartner (2010) documented subtidal fouling communities along the B.C. coast. However, due to the study design, the research by Gartner (2010) did not represent the fouling assemblages that are found on vertically suspended aquaculture netting. Considering the scale of the finfish industry, its potential expansion, and the costs biofouling incurs there is a need for more research into viable, environmentally sound biofouling management options.

In view of this background and rationale, this study has two main objectives. The first is based in biology and aims to describe the biofouling community on a salmon farm off of northern Vancouver Island and to contribute to our understanding and future research on this topic. The second is grounded in the needs of industry and will test the effectiveness of several alternative antifoulant coatings on a commercial salmon farm. The results from this project will help industry to make better-informed biofouling management decisions and to understand if any of the coatings tested are viable alternatives to untreated nylon and copper dipped netting.

CHAPTER 3: Materials and Methods

3.1 Test Materials

This study tested the effectiveness of nine net treatments (Netrex, Flexgard, Dyneema, Sancure, ThornD, Solucote, Netpolish, NetCoating, and Tar) at preventing the accumulation of biofouling as compared to an untreated nylon net. The study design had one control, two copper based treatments, and seven alternative treatments (ten treatments in total; Table 2). All mesh was ~25mm square, grow-out mesh.

Table 2: Summary of netting materials and coatings tested in this study. Including the treatment name used throughout this document in both the text and figures. Because there are two treatments with Dyneema netting they are sometimes referred to as ‘untreated Dyneema’ and ‘Dyneema with Sancure.’ The control is untreated nylon.

Treatment	Material	Coating	Colour	Coating Manufacturer
Control	nylon	n/a	white	n/a
Netrex™ [1]	nylon	wax	red	Morenot, Norway
Flexgard® [2]	nylon	paint	red	Flexabar Corp, USA
Dyneema®	Dyneema	n/a	white	n/a
Sancure®	Dyneema	paint	clear	Lubrizol Advanced Materials, USA
ThornD®	nylon[3]	flocking	cream	Micanti, Netherlands
Solucote®	nylon	paint	clear	DSM NeoResins Inc, Netherlands
Netpolish™	nylon	wax	green	Morenot, Norway
NetCoating™	nylon	wax	yellow	Netprotect as, Norway
Tar	nylon	tar	black	Sotranot as, Norway

[1] Netrex AF: 17% cuprous oxide (Cu_2O)

[2] Flexgard VI: 13.6% cuprous oxide (Cu_2O)

[3] Netting manufactured by Micanti, all other nylon nets were manufactured by Badinotti.

3.1.2 Net Materials

Untreated nylon netting was used as the control. Nylon encompasses various synthetic, thermoplastic polymers which are considered to be fairly tough, lightweight and resistant to heat and chemicals (OED 2011). Nylon netting absorbs water and can lose 10-20% of its knot strength when submerged (Badinotti 2011). Nylon netting has a round twine thickness of ~3mm.

It is white in colour. Untreated nylon was chosen as the control because it is the most commonly used alternative to copper treated nets, and is the least expensive, simplest option.

Dyneema is an ultra-high-molecular-weight polyethylene (UHMwPE) fibre created by the Dutch chemical company DSM®. According to the manufacturer (Dyneema 2011) the fibre is made using a gel-spinning process that results in a multifilament fibre that is both strong and supple. The material is 15 times stronger than steel, chemically inert; UV, abrasion, and moisture resistant; and very durable (Dyneema 2011). Nets made with Dyneema do not absorb water, they retain their knot strength, and have limited stretch (Badinotti 2011). The Dyneema net has a somewhat rectangular twine thickness of 2mm wide and 1mm thick.

3.1.3 Coatings

Netrex AF (manufactured by Morenot AS) is a waterborne, wax based, copper antifoulant with 17% cuprous oxide as the active ingredient. It is dark red in colour. It is made with a food-grade micro crystalline wax and, according to the manufacturer (CR Netloft 2011), the coating keeps the netting material supple and prevents UV damage. Netrex adds approximately 1mm to the diameter or the netting fibers. This coating was applied to a nylon net.

Flexgard VI (manufactured by Flexabar-Aquatech Corporation) is a waterborne, paint based, copper antifoulant with 13% cuprous oxide as the active ingredient. It is dark red in colour. The coating stiffens the netting allowing cages to better maintain their shape, it provides UV protection for the netting fibers, and binds and sets knots (Badinotti 2011). Flexgard VI does not alter the initial twine thickness. This coating was applied to a nylon net.

Sancure 1511 (manufactured by Lubrizol Advanced Materials Inc.) is an aromatic, waterborne, urethane polymer. The coating is clear and does not change the net colour. It has a high gloss, is abrasion resistant, and flexible (Lubrizol 2007). Sancure does not alter the initial twine thickness. The coating was applied to a Dyneema net.

ThornD (manufactured by Micanti) is applied by flocking short fibres onto the netting material. It is off-white/cream in colour. The manufacturer claims that this ‘fuzzy‘ surface will deter settling organisms by damaging planktonic cell structure, and by swaying with water movement and dislodging the organisms (Micanti 2011). Nylon netting with a ThornD coating has a round twine thickness of ~5±1mm due to the varying length of the flocking fibres.

Solucote 1003 (manufactured by DSM NeoResins) is a waterborne, polyurethane coating. It is a clear coating. According to the manufacturer the product is a high-performance barrier coating (DSM 2011). Solucote does not alter the initial twine thickness. It was applied to a nylon net.

Netpolish (manufactured by Morenot) is a waterborne, wax based coating. It is light green in colour. It is made from the same food-grade, micro-crystalline wax as the Netrex AF coating, but without cuprous oxide. Its purpose is to seal the fibers thus reducing the available attachment points for biofouling and provides UV protection(CR Netloft 2011). Netpolish adds approximately 1mm to the diameter or the netting fibers. It was applied to a nylon net.

NetCoating (manufactured by Netprotect) is a waterborne, wax based coating. It is oxide yellow in colour. According to the manufacturer this coating prevents damage from UV, improves the strength of a net, helps the cage maintain its shape and makes the net easier to clean (Steen-Hansen 2012). Net-Coating adds approximately 1mm to the diameter of the netting fibers. It was applied to a nylon net.

Tar (manufactured by Sotrenot as) is officially called “Naphtha (petroleum), hydrodesulfurized heavy” (CAS# 64742-82-1). It is naptha-solvent based, and once the product has dried only the bitumen (tar) remains on the net. It is black in colour. The tar coating does not alter the initial twine thickness. This coating was applied to a nylon net.

3.2 Study Site

This experiment was carried out at Shelter Bay, off of the north end of Vancouver Island in British Columbia, Canada. The bay is roughly 30km north-east of Port Hardy, on the mainland of British Columbia (50°57'50.65"N, 127°27'14.63"W; Figure 2). The bay faces North-West (285°) into Queen Charlotte Strait. It is recognized as being a site with heavy wave-action, with waves reaching up to 5m during winter storms. The current runs in a North-East and South-West direction. Overall current speed is roughly 0.2-0.25 knots, making it a relatively low flow site.

The site is operated by Marine Harvest Canada, which is British Columbia’s largest salmon farming company. The 28.3-hectare tenure at Shelter Bay has a relatively flat seabed, with a 40m deep ridge that runs from north to south along the west side of the cages, dropping another 10m on either side. The substrate consists of sand and mud. Over the course of the study the site had seven 120m circumference (38m diameter) polar circle cages (made by Aqualine®) arranged in a double array (Figure 2). Samples were placed in the water in January 2011 and Atlantic salmon (*Salmo salar*) were put into the cages in February 2011.

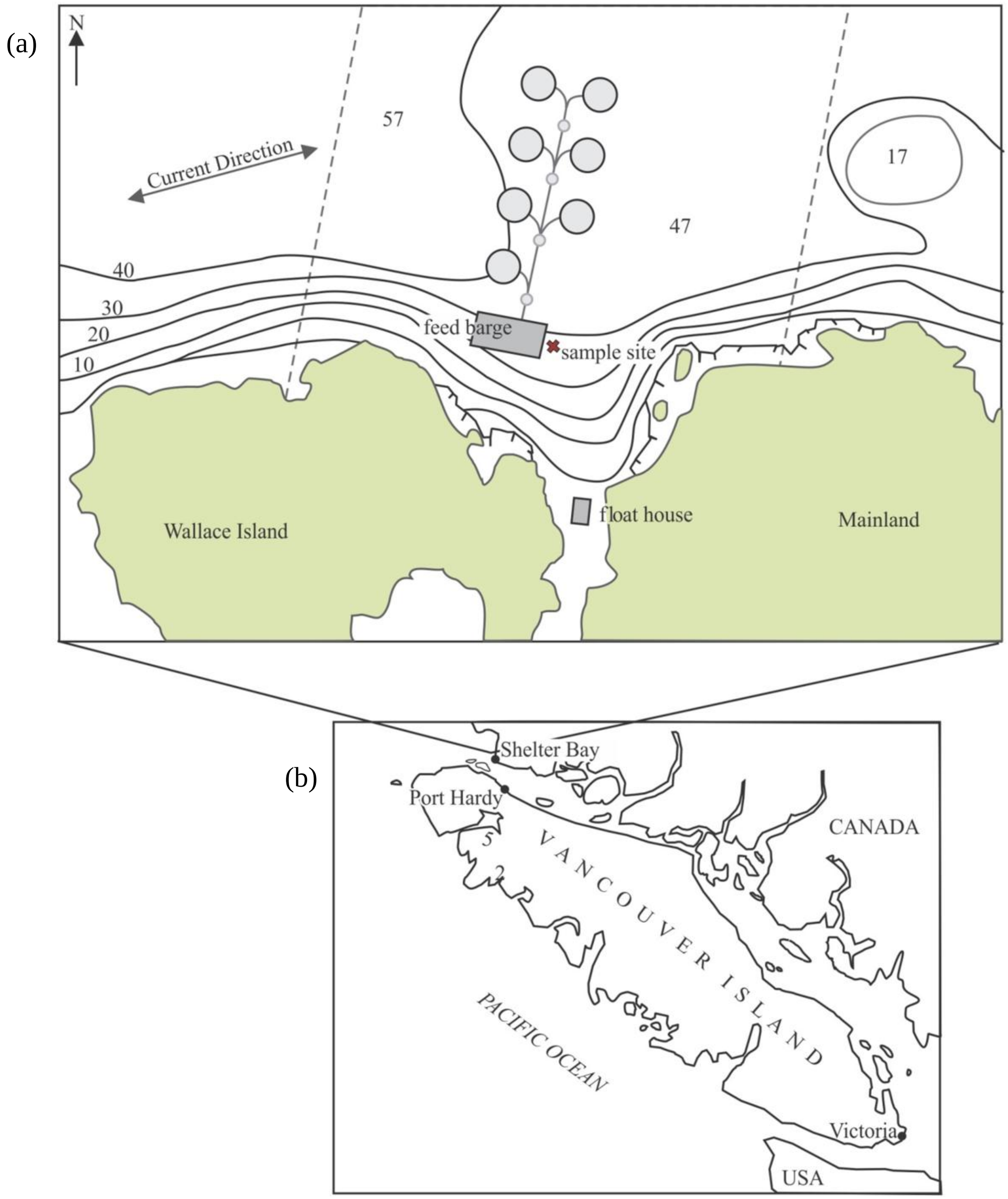

Figure 2: Site map showing (a) the layout of the farm with the sample site located off the East side of the feed barge with the dominant and sub-dominant current running perpendicular to the cage array (depth contours are in meters), and (b) the location of Shelter Bay off of the north end of Vancouver Island.

3.3. Experimental Design

The experiment included each of the ten treatments replicated four times at three depths. Netting was attached with cable ties to 30x30 cm quadrats made of 21 mm PVC pipe (Figure 3b). The PVC quadrats were attached to steel frames, in two rows (Figure 3a). These large frames were hung in sets of two at three depths: 1 m, 5 m and 18 m. The samples that were hung at 1m were below the freshwater lens and protected from surface wave action. 5 m was selected to capture the deeper part of the biologically active surface waters. 18 m represents the lower depth of the salmon cages. Because of the heavy wave action and the lack of locations for suspending samples on a site with polar circles, the frames were hung off of the two anchor chains on the feed barge. This placement resulted in roughly a 10 m distance between the two sets (Figure 3a) of samples which had to be accounted for by a blocking factor in the analysis.

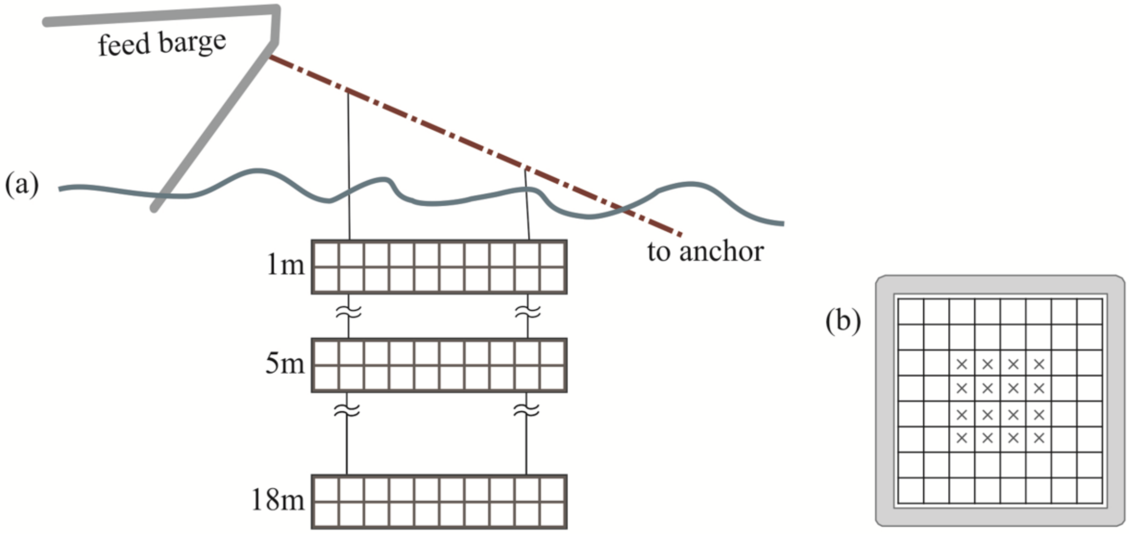

Figure 3: Study design showing (a) the layout of the frames that were hung off of both anchor chains (image not to scale), where every row has all 10 of the treatments (randomized), and (b) the number of meshes (~2.5cm across) included in each quadrat where ‘x’ represents the area of interest/sample area; the two outside rows are ignored to account for edge effects caused by fouling accumulating on the quadrat frames.

Monthly sampling involved pulling each of the steel frames to the surface and placing it in a large container filled with seawater, where samples were photographed with an underwater digital camera (Panasonic Lumix DMC-TS2). A white background was used to maximise the contrast in the images. A frame was used to hold the camera at the same distance from each sample so that all of the photos were at the same scale.

The main indices used to measure the effectiveness of each material in this study are percent net occlusion, percent cover of major biofouling groups, and biomass (wet weight).
To describe the general succession patterns of the biofouling organisms seen over the course of this study a qualitative analysis of the untreated nylon control was considered to be the natural succession sequence. For each month the dominant foulers from all three depths were documented and were combined with a graph showing the mean percent net occlusion over time.

Percent net occlusion (PNO) was determined using modified methods based on work done by Braithwaite et al. (2007). The first step was to determine the average net aperture for each treatment prior to the development of any biofouling. This involved measuring the area of sixteen mesh openings (apertures) at the center of each sample and calculating the average (Figure 4). To determine the area of a mesh aperture it was first manually filled in with a solid colour and the area (in pixels) was determined using the 'measure' tool in ImageJ. This same process was done for each of the 120 samples for seven months of data. The second step was to convert the raw mean aperture for each sample to a percentage using Equation 1. For example, the mean net aperture of an untreated nylon sample in June was 151799 pixels, this value was then divided by the mean clean net aperture for this treatment which is 226134 pixels, and using Equation 1 it was converted to a value of 33%. This number represents an occlusion level of 33% as compared to a clean net with the same treatment which has zero net occlusion.

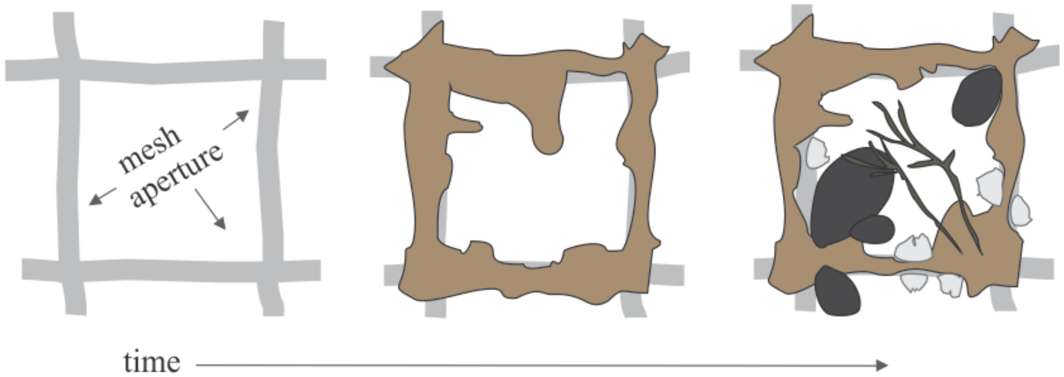

Figure 4: The area of interest of a single mesh aperture showing a generalized representation of how it becomes occluded by fouling organisms over time.

$$\left[1-\left(\frac{\text{PNA time X}}{\text{PNA time 0}}\right)\right]*100$$

Equation 1: Percent Net Occlusion (PNO) at any sample period (time X) is determined by the relationship of a sample to the mean percent net aperture (PNA) prior to the development of biofouling (time 0) (Braithwaite et al. 2007).

Percent cover images were first cropped to include a 4x4 set of mesh at the center of each quadrat (Figure 3b). Sampling was done using Photogrid (Bird 2011), a program specifically designed for sampling percent cover of marine invertebrates from photographs. Percent cover was determined using stratified random sampling (Foster et al. 1991) with an overlay of 100 sample points for each image. The organism below each point was counted. Mobile species were not included. Because of the difficulty in identifying an organism accurately to the species level from photographs this study classified organisms into basic, relevant, functional groups which included: mussels, barnacles, hydroids, tunicates, sabellids, caprellids, crusts, algae and diatom. These groups were chosen based on previous experience, observing the samples, and methods used in other studies (e.g. CRAB 2010). The diatom group consisted of an unknown filamentous diatom that tended to accumulate at the corners of the mesh and formed clumps in and around the filamentous hydroids. Points on clean netting or net apertures were also noted and it is for this reason that this measurement is called ‘Percent Cover’ and not ‘Species Composition’. ‘Other’ was used when a sample point fell on a tag or mobile species.

Biomass was determined at the end of the study as it was sampled destructively. Data were collected by first removing mesh from the frame and leaving it to drip for ten minutes then weighing it (in grams). Because the materials were all different in terms of twine thickness and the weight of the coating, mean wet weight of clean netting (calculated from four samples) was subtracted from each sample to determine the biomass of the biofouling organisms. Data were then extrapolated to represent the wet weight of 1 m^{-2} (rather than 30x30 cm quadrats) in order to make the values more readily comparable to other studies.

3.4. Analysis

Percent Net Occlusion was determined for each month of the study. The data were arcsine transformed to improve normality and the overall fit of the model. The data were skewed because of a high frequency of zeros which was caused by the absence of fouling for the first three months on the 18m samples and the effectiveness of the copper treatments. It is recognized that the arcsine transformation is able to create a nearly normal distribution in this type of situation (Zar 1999). The transformed data were analysed using a marginal mixed model (West et al. 2007), with treatment, depth and block as fixed effects, and time (month) as a repeated measure. Block was treated as fixed instead of random because there were only two levels. Because block was selected arbitrarily, it was only used to account for model variance, and is of no interest in and of itself it was not included in any interaction effects or discussions (Newman et al. 1997). Follow-up pairwise tests were used to compare all treatments to the untreated nylon control, to compare Netrex to Flexgard, and to compare Dyneema to Sancure. The final month of September was analysed separately using a marginal mixed model in order to be able to the describe relationships between all three of the outcome variables used in this study. This model is similar to a 3-factor ANOVA but allowed for the use of the custom contrasts developed in the previous model. The benefit of using a marginalized mixed model for analysing this type of data is that it is able to accommodate heterogeneous variance (West et al. 2007) which were caused by the low values and low variability of the copper treatments as compared to the alternative treatments. The model is also able to account for complex covariance structures between time points. In this case, because of the diatom disturbance event early on in the study, unstructured covariance resulted in the best model fit. Mixed models are recognized as being robust and adaptable making them a powerful tool for the analysis of complex ecological data sets (West et al. 2007). All PNO analysis were done using SPSS v.17 and all error is presented as the mean ±1 standard error.

Percent cover was determined for the final month of September. Data were first standardised by removing the ‘aperture (water)’ and ‘other’ categories and standardising the data by dividing each sample by the total. Data were then square root transformed to approach normality (Clarke 1993). This was followed by the computation of a Bray-Curtis similarity matrix. Data were analysed using permutation based analysis of variance (PERMANOVA) and represented using non-metric multi-dimensional scaling (MDS) in Primer v.6 (Clarke and Gorley 2006). PERMANOVA was used instead of ANOSIM (analysis of similarities) because of its ability to accommodate more complex study designs (including blocking factors) and account for interaction effects (in this case treatment by depth) (Anderson et al. 2008) . The test statistic for PERMANOVA is the pseudo F-ratio, where a large pseudo F-ratio indicates that the samples within the groups (grouped by treatment or depth) differ in terms of community composition.

The significance of the pseudo F-ratio is tested using a permutation test that randomly shuffles the sample labels within and among treatment groups and calculates the pseudo F-ratio for 9999 arbitrary reassignments of the data which is then compared to the pseudo F-ratio of the observed communities and calculates the significance level of the test (Anderson et al. 2008).

Biomass data for the month of September were normally distributed ($D(120) = 1.242$, $p = 0.092$) but had heterogeneous variance ($F(9,110) = 20.0611$, $p < 0.001$). Consequently, the data were analysed using nonparametric methods in Primer v.6. This analysis was chosen so as to be able to assess the interaction effect between treatment and depth and to control for the variability between blocks. Biomass data were converted into a Euclidean distance matrix and analysed using PERMANOVA (Anderson et al. 2008). When data are univariate and converted to a Euclidean distance matrix, the resulting F ratio is the same as a traditional F statistic from an ANOVA (Anderson et al. 2008).

CHAPTER 4: Results

4.1 Succession

Within 24 hours of entering the samples a visible diatom film developed on the netting and continued to grow until April (Figure 5). In May the diatoms had grown long enough to be affected by water currents and shear force and were washed off the netting in an event that is called “sloughing” (Stevenson and Stoermer 1982). Sparse settlement by an unknown filamentous red seaweed was part of the disturbance transition. This was followed by the initial settlement of fast-growing macrofoulers in June (mostly hydroids and caprellids), which continued to develop until September when the more dominant, climax community (barnacles, tunicates, hydroids, sabellids) became established.

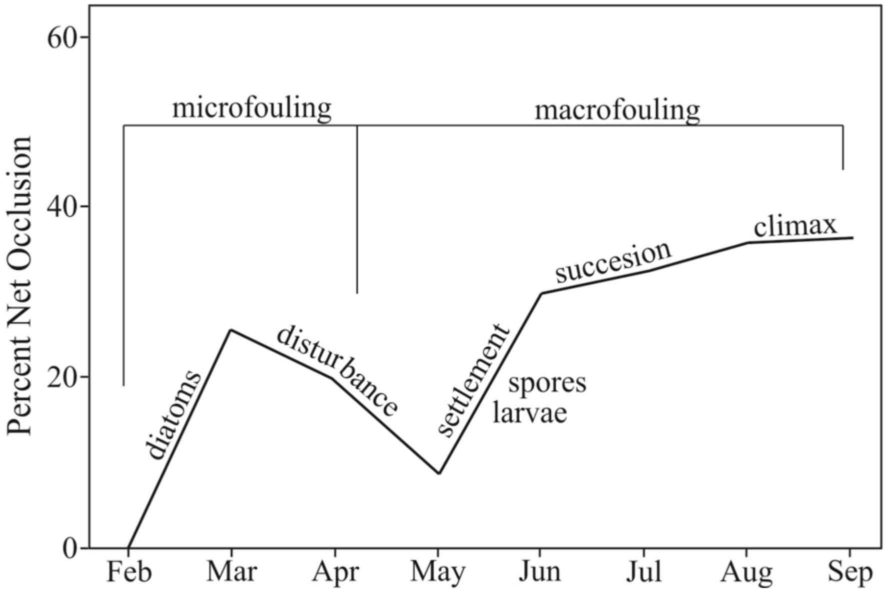

Figure 5: The generalized succession and disturbance pattern seen in this study based on observations of the untreated nylon samples. The line graph represents mean PNO for all depths over eight months.

4.2 Percent Net Occlusion

After a single month of immersion there was a significant increase in percent net occlusion (Figure 6). This was followed by a disturbance event that varied in severity between treatments and depths. Then net occlusion continued to increase until the end of the study.

Analysis of percent net occlusion across all time periods found a significant interaction between treatment and depth ($F(18, 89) = 12.9367$, $p < 0.001$), and both main effects, treatment ($F(9, 89) = 7536.74$, $p < 0.001$) and depth ($F(2, 89) = 102.63$, $p < 0.001$), were found to be significant. Percent net occlusion ranged from 0% to 95%, with a mean for all time periods of 32±0.85% for the alternative treatments (including the control) and 0.61±0.02% for the copper treatments. Each of the three depths (1m, 5m and 18m) were significantly different from the others ($p < 0.001$ for all comparisons) (Figure 7). Overall, the greatest amount of net occlusion occurred at 1m ($\bar{x}$ = 43±1.2% for all treatments), and the lowest PNO occurred at 18m ($\bar{x}$ = 23±1.7% for all treatments).

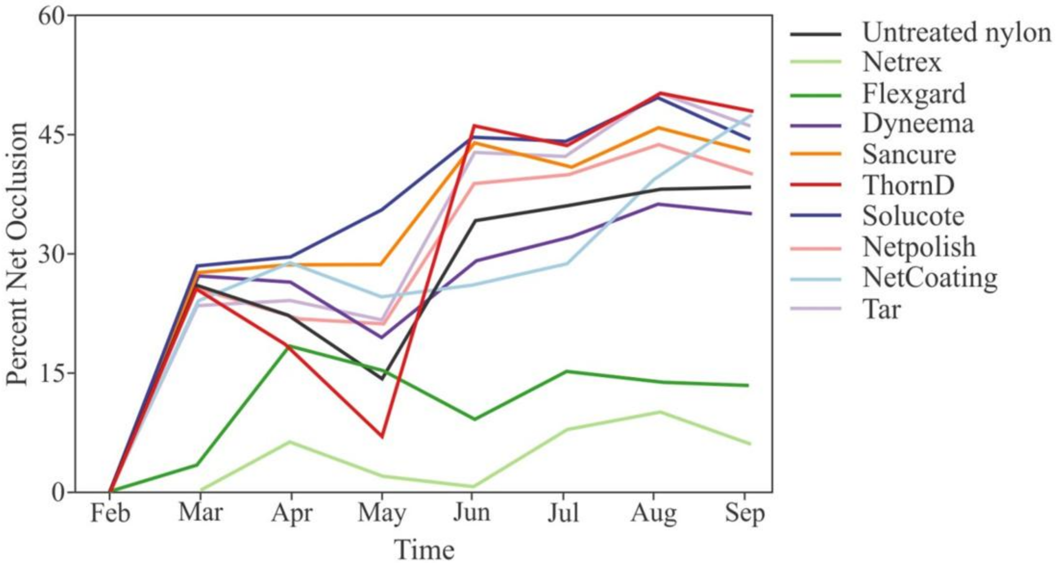

Figure 6: Mean PNO for each treatment over time, showing the patterns of accumulation of disturbance followed by more accumulation (Transformed data).

PNO varied by treatment between depths (Figure 8). All treatments had the highest net occlusion at 1m, and the lowest at 18m. For the control the difference in net occlusion between 1m and 5m was 11%, and the difference between 5m and 18m was 5.6%. Netrex had the least difference between depths (mean difference = 1.6%) and maintained negligible PNO at all three depths. Flexgard had very low PNO at 18m (on par with Netrex) but had higher PNO at 5m and 1m. ThornD had the highest PNO at 18m. Solucote had the highest levels at both 1m and 5m. NetCoating had the greatest difference between any two consecutive depths: 18.5% between 5m and 18m.

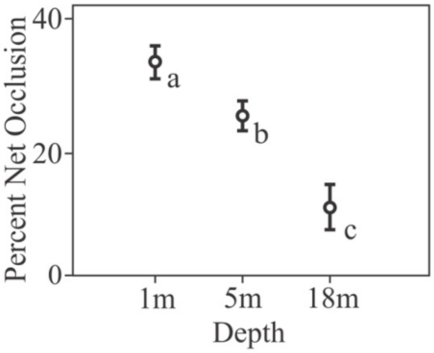

Figure 7: Mean PNO for each depth (Error bars = ±1s.e.; n = 40).

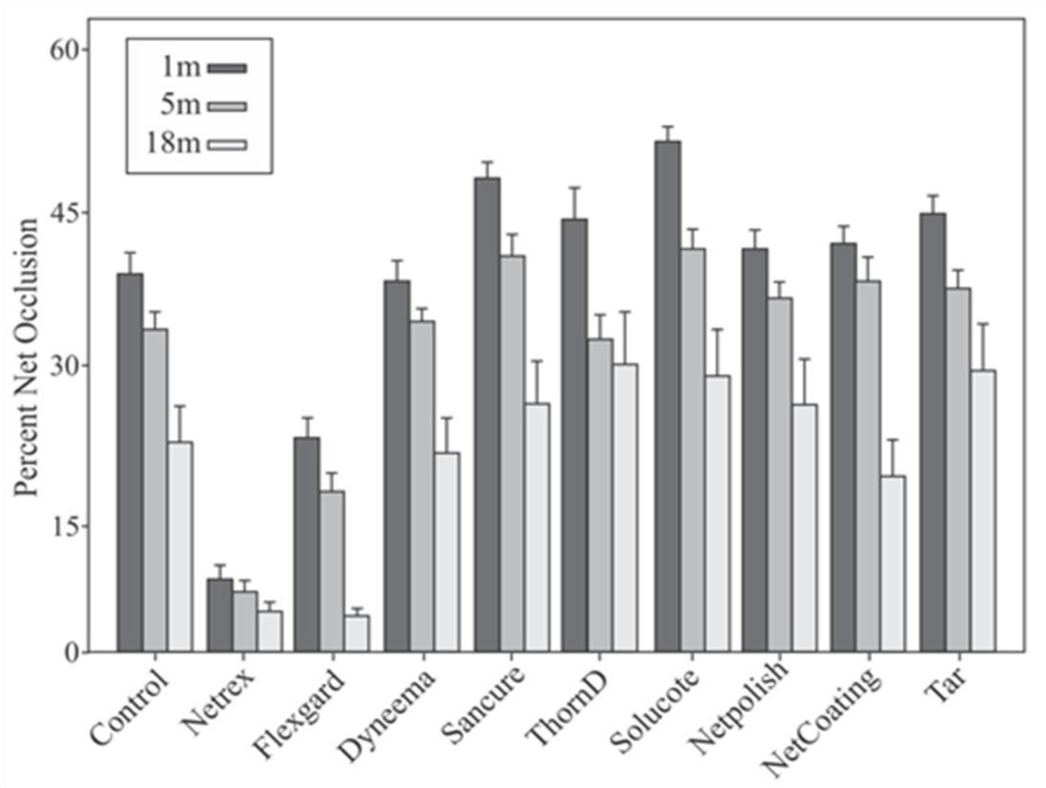

Figure 8: Mean PNO for each treatment for all time periods clustered by depth (Transformed data. Error bars = ±1s.e.; n = 28).

When considering the changes in percent net occlusion over time, between depths, while controlling for block (Figure 9) Solucote ($p < 0.001$) and Sancure ($p = 0.022$) had significantly higher PNO than the control; No other alternative treatments were found to be significantly different from the control. Netrex ($p < 0.001$) and Flexgard ($p < 0.001$) had significantly lower PNO than the untreated nylon control. There was a significant difference between untreated Dyneema and Dyneema with Sancure ($p = 0.025$) with untreated Dyneema performing better than Dyneema with Sancure. There was a significant difference between the two copper treatments ($p < 0.001$) with Netrex performing better in terms of net occlusion than Flexgard. Solucote and Sancure had net occlusion levels that were consistently higher than the untreated nylon control throughout the seven months of this study. The effects of the other alternative treatments were variable; sometimes they were higher, other times lower, but overall maintained statistically non-significantly different PNO levels. The Sancure treatment had higher net occlusion compared to Dyneema at all three depths for the full duration of the study. There was no difference between the two copper treatments at 18m for the duration of the study, but at 5m and 1m, Flexgard had more occlusion than Netrex.

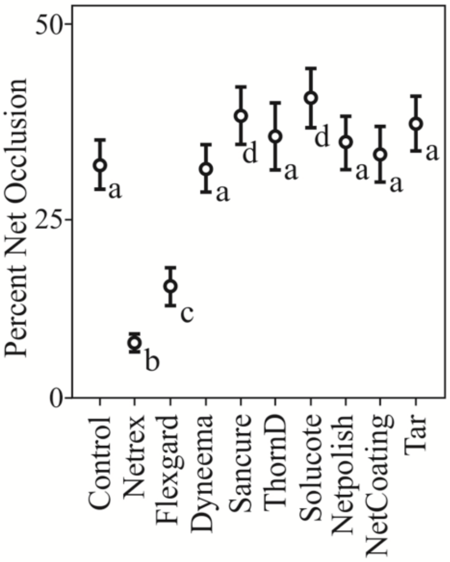

Figure 9: Mean PNO for each treatment averaged across all time periods and depths. (Transformed data. Error bars = ±1s.e.; n = 84).

Analysis of the final month of September found both treatment ($F(9, 89) = 25.147$, $p < 0.001$) and depth ($F(2, 89) = 10.762$, $p < 0.001$) to be significant. There was also significant interaction between treatment and depth ($F(18, 89) = 2.094$, $p < 0.012$). The main driver for the interaction was the NetCoating treatment at 5m (Figure 10). Pairwise comparisons of each treatment to the control found a significant difference with Netrex (p <0.001), and with Flexgard (p <0.001). None of the alternative treatments were found to be significantly different from the untreated nylon control (Figure 11). There was no significant difference between the two copper treatments, and there was no significant difference between Dyneema and Sancure. Comparisons between depths found that overall the 5m depth had significantly less net occlusion than 1m (*p* <0.001) and 18m (*p* <0.001), the other comparisons were found to be non-significant.

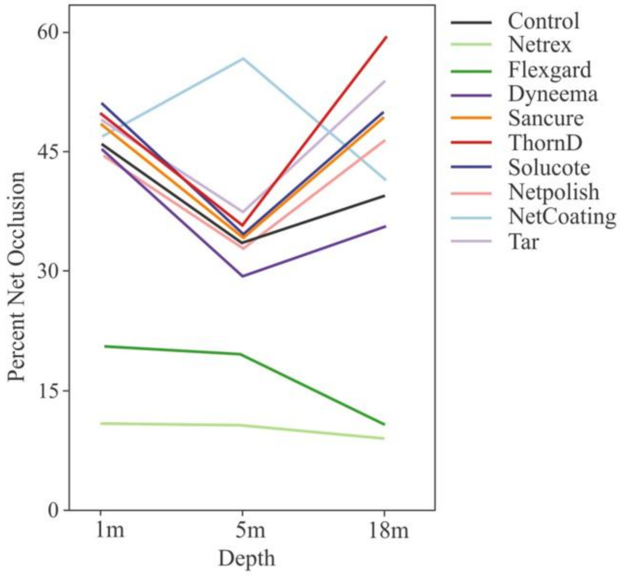

Figure 10: Mean PNO for each treatment by depth for September, showing the significant interaction effect caused by NetCoating at 5m (Transformed data).

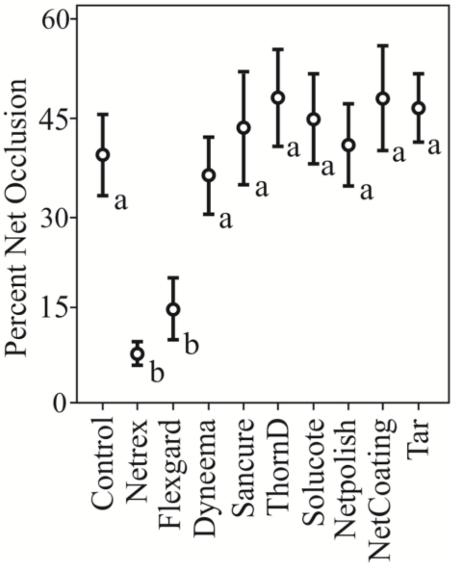

Figure 11: Mean PNO for September for each treatment (Transformed data; Error bars = ±1s.e.; n = 12).

4.3 Percent Cover

The most common occurring percent cover groups were Diatom and Hydroid, followed by Algae and Barnacles. Table 3 shows the dominant taxa from all treatments for each of the major fouling groups in this study. Species documented include: *Mytilus sp*., *Semibalanus cariosus, Plumaria sp*., *Obelia sp*., *Garvei sp*., *Ectopleura sp*., *Chelyosoma sp*., an unknown feather duster worm, *Caprella sp*., *Ulva sp*., and an unknown filamentous red seaweed.

Table 3: Dominant taxa by fouling group.

Group	Genus (and species if known)
Mussel	*Mytilus sp.*
Barnacle	*Semibalanus cariosus (Balanus cariosus)*
Hydroid	*Plumaria sp.*
	Obelia sp.
	Garvei sp.
	Ectopleura sp.
Tunicate	*Chelyosoma sp.*
Sabellid	unknown feather duster worm
Caprellid	*Caprella sp.*
Algae	*Ulva sp.*
	unknown filamentous red seaweed

Analysis of percent cover data showed a significant difference between treatment and depth, and an interaction between treatment and depth (Table 4). All depths were statistically significantly different from one another (*p* <0.001 for all comparisons). Subsequently, pairwise comparisons were done for treatment by depth. 1m percent cover (untransformed data, and excluding copper treatments) was dominated by diatoms (40%), followed by algae (15%) and then hydroid and mussels (both 5%) (Table 5). Pairwise comparisons for each treatment at1m found (table 6) both of the copper treatments (Netrex and Flexgard) to be significantly different from the control due to low levels of fouling organisms. None of the alternative treatments were found to be different from the untreated nylon control (Figure 12a). A significant difference was found between Netrex and Flexgard (*p* = 0.028). There was no significant difference between untreated Dyneema and Sancure.

Table 4: Summary table for PERMANOVA analysis showing significance tests for treatment, depth and treatment*depth.

	df	SS	MS	Pseudo-F	P (perm)
Treatment	9	88361	9817.9	43.26	<0.001
Depth	2	64513	32257.0	142.11	<0.001
Treatment*Depth	18	11635	646.4	2.85	<0.001
Residuals	89	20201	226.98		

At 5m percent cover (untransformed data, excluding copper treatments) was dominated by the Diatom (25%) group, followed by Barnacles (9%), Algae (8%) and Hydroids (7%). Both copper treatments were statistically significantly different from the control due to the dominance of clean net (table 6). The NetCoating treatment was found to be significantly different because of heavy barnacle fouling and an absence of other species. There was no significant difference between the two copper treatments or between untreated Dyneema and Sancure (Figure 12b).

At 18m percent cover (untransformed data, and excluding copper treatments) was dominated by Hydroids (55%), Diatoms (8%) and Sabellids (7%). The two copper treatments were significantly different from the control again due to clean net (Table 6). NetCoating was significantly different due to the presence of barnacles and the absence of sabellids. There was no significant difference between the two copper treatments or between untreated Dyneema and Sancure (Figure 12c).

Table 5: Mean percent cover of major fouling groups (>3% coverage; Untransformed data) for the treatments found to be significantly different at each depth. Untransformed data. Column labels: mus = mussel, barn = barnacle, tuni = tunicate, hyd = hydroid, sabe = sabellid, capr = caprellid, diat = diatom

	algae	mus.	barn.	tuni.	hyd.	sabe.	crust	capr.	diat.	net
1m										
Control	17	3			3		2	5	42	1
Netrex								6		27
Flexgard									5	27
5m										
Control	6		4	3	11				28	
Netrex										28
Flexgard										31
NetCoating	6		56						9	
18m										
Control					48	8			11	7
Netrex					4					27
Flexgard					6					31
NetCoating			12		42				7	10

Table 6: PERMANOVA pairwise comparisons showing significant differences in treatment as compared to the control, separated by depth.

	t	P (perm)
1m		
Netrex	6.323	0.018
Flexgard	5.270	0.026
5m		
Netrex	5.798	0.024
Flexgard	4.992	0.009
NetCoating	3.563	0.012
18m		
Netrex	6.156	0.017
Flexgard	5.808	0.018
NetCoating	2.346	0.019

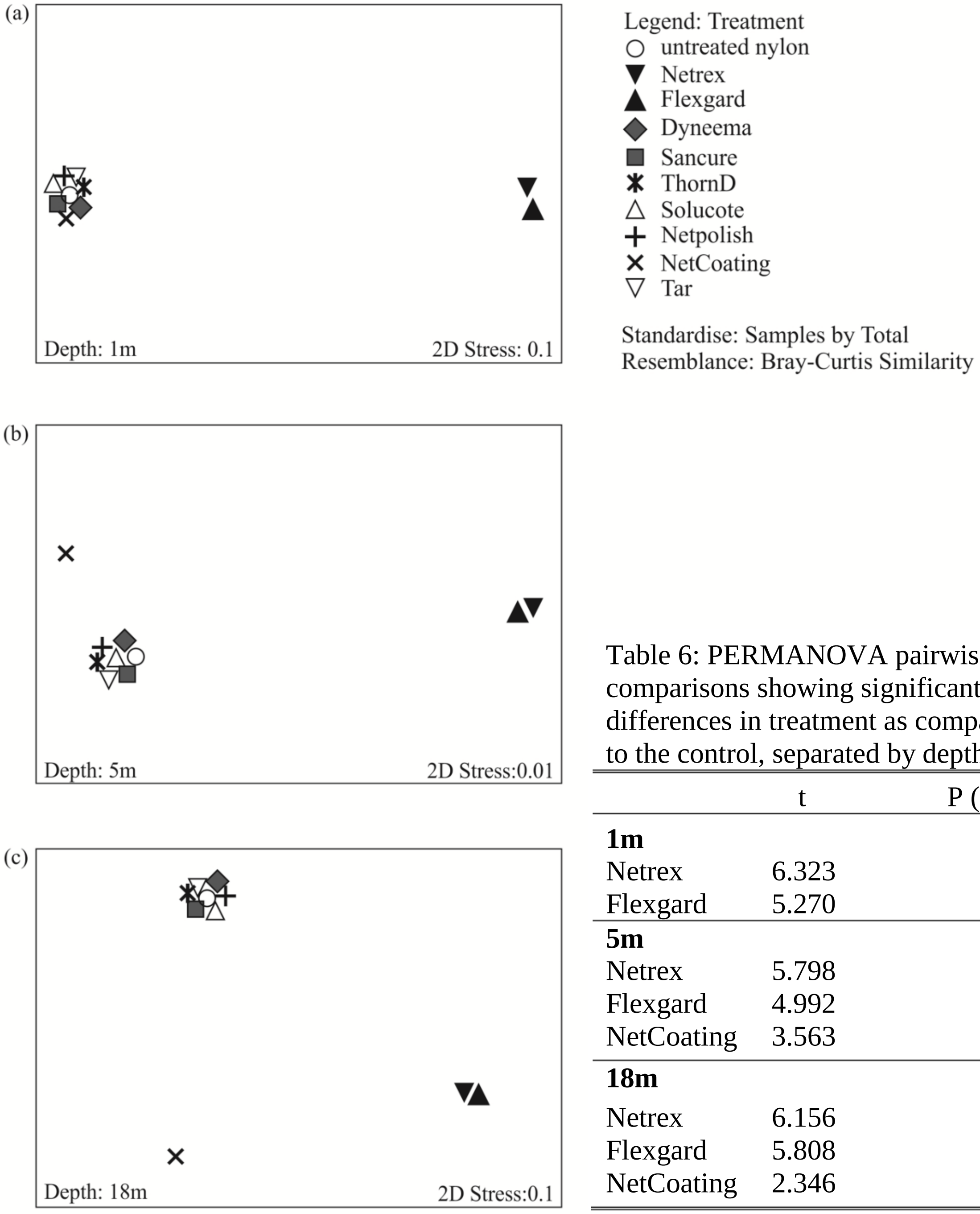

Figure 12: MDS plots for each depth, where symbols closer together are more similar than those further apart. There is strong clustering at 1m within all of the alternative treatments (a). The 5m plot shows the moderate dissimilarity of both copper treatments and NetCoating (b). The 18m plot (c) shows some dispersion within the alternative treatments and the strong dissimilarity between the copper treatments and NetCoating.

4.4 Biomass

Results showed that there was a significant interaction effect between treatment and depth ($F(18, 89) = 16.72$, $p < 0.001$) for the biomass data, as well as a significant effect for treatment ($F(9, 89) = 56.48$, $p < 0.001$). Depth was found to be non-significant ($F(2, 89) = 0.05$, $p = 0.96$). Mean biomass for the alternative treatments at all depths was 657±77g/m^{-2}, with a maximum 2312g/m^{-2}and a minimum of 212g/m^{-2}. The copper treatments had a mean of 13.85± 4.04 g/m^{-2}, and a maximum of 72g/m^{-2}. The interaction effect between treatment and depth was mainly driven by the NetCoating treatment at 5m (Figure 13). Follow-up pairwise tests (Figure 14) comparing all treatments to the untreated nylon control ($\bar{x} = 603.33 \pm 61$ g/m^{-2}) showed Netrex ($\bar{x} = 6.6 \pm 3.1$g/m^{-2}, $p < 0.001$), Flexgard ($\bar{x} = 21 \pm 6.9$g/m^{-2}, $p < 0.001$), Dyneema ($\bar{x} = 485.66 \pm 53$g/m^{-2}, $p = 0.096$), ThornD ($\bar{x} = 1020.00 \pm 88$g/m^{-2}, $p < 0.001$), and NetCoating ($\bar{x} = 1021.66 \pm 226$g/m^{-2}, $p < 0.001$) to be significantly different from the control. There was a significant difference between Netrex and Flexgard ($p = 0.003$) and between untreated Dyneema and Dyneema with Sancure ($p = 0.044$).

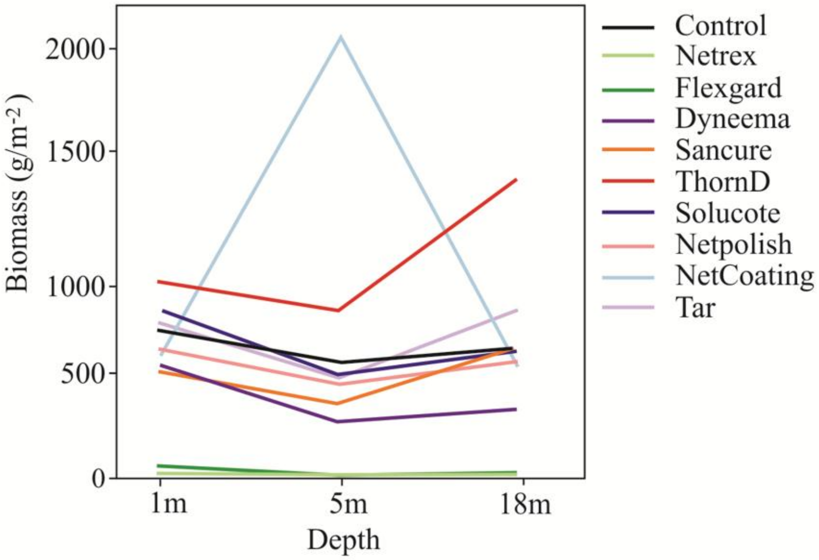

Figure 13: Mean Biomass for each treatment showing the strong depth interaction with NetCoating at 5m.

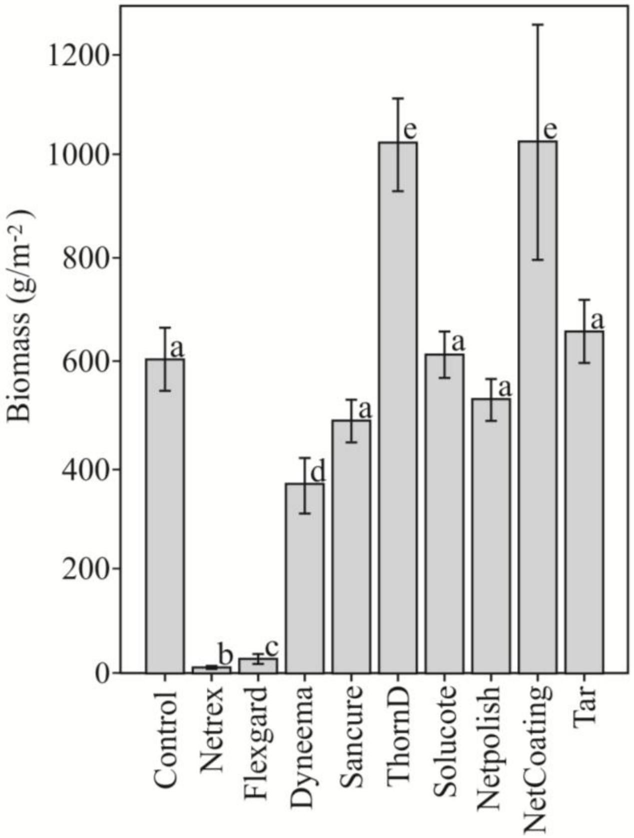

Figure 14: Biomass for each treatment showing significantly different treatments (Error bars = ±1s.e.; n = 12).

CHAPTER 5: Discussion

Results from this study demonstrated that none of the alternative treatments tested were able to outperform untreated nylon to a level that would make it cost effective to use the alternative. It was also shown that antifoulants based on cuprous oxide are very effective at preventing the accumulation of biofouling. Because of the diversity of organisms and complexity of ecological processes the development of an effective, alternative antifoulant coating is extremely challenging.

Throughout this discussion the term "operationally effective" is used. This term refers to the idea that if a treatment is effectively preventing the accumulation of biofouling organisms then the net is clean enough to not warrant any management actions. Conversely, if a net is not effective then net occlusion reaches critical levels and some sort of washing or maintenance is required. Generally, the protocol on a farm is "if it's dirty, wash it," irrespective of the time of year or the type of fouling present.

5.1 Percent Net Occlusion

Percent Net Occlusion is an important measure for aquaculture biofouling studies because of its effect on water exchange. Over time some degree of fouling, and associated percent net occlusion, accumulated on all of the treatments. The levels of net occlusion seen in this study are similar to those seen in other studies. A study on net occlusion on 50-70cm mesh tuna cages in Australia found 80.6% coverage of fouling organisms after five months immersion (Svane et al. 2006). A study in the Shetland islands in Scotland found 80-100% net occlusion on untreated half-mesh (25mm) nets after approximately eight months immersion time (Braithwaite et al. 2007). Net occlusion rates up to 62% were documented on 25mm mesh salmon cages in

Norway (Guenther et al. 2010). However, because of the variability in study design and the temporal and spatial specificity of biofouling, direct comparison of net occlusion rates between studies is difficult.

5.2 Percent Cover

Percent cover is a common measure in biofouling studies (Phillippi et al. 2001; Svane et al. 2006; Guenther et al. 2010) and is often used in biological studies (e.g. Butler & Connolly 1999; Underwood & Chapman 1996; Addison et al. 2008). Because the data collection methods in this study were based on broad taxonomic groups, rare and unique species were not accounted for, which is reasonable from an operational standpoint because industry is not interested in the species with low frequency of occurrence since they do not cause significant issues on a farm. The dominant fouling organisms that occurred are considered to be highly cosmopolitan taxa (e.g. *Mytilus sp*. (Koehn 1991); *Semibalanus cariosus* (Noda 2004)). It was noted that there was far less mussels than in other years, likely due to lower seawater temperatures which affects spawning (R. Clarke, pers. comm.).

Because the patterns and differences seen in the end results were so clear, with none of the alternatives performing better than the control, percent cover was not determined for the entire course of the study. The two treatments that were significantly different from the control were dissimilar because they had high frequencies of organisms that are of particular concern to farmers (barnacles and sabellids). Good performance in terms of percent cover can be seen with the two copper treatments. Both treatments were very effective at preventing the accumulation of fouling organisms to a point where at the end of the study the samples were nearly devoid of any fouling, with the exception of operationally insignificant levels of fouling from hydroids at 18m which are known to develop resistance to copper (Piola et al. 2009) and at 1m either diatoms on Netrex or caprellids on Flexgard.

5.3 Biomass

Having low biomass results in better buoyancy and reduces the stress from drag on a system. The biomass wet weights in this study, with a maximum of 2312g/m^{-2} and a mean for the all of the alternative treatments at all depths of 657±77g/m^{-2}, are within the range of values found by other researchers. For example 4900g/m^{-2} was found on untreated nylon 25mm half-mesh after 10 months immersion at 3m in the UK (Braithwaite et al. 2007). 2200g/m^{-2} was documented in on 80mm mesh in southern Australia (Cronin et al. 1999), and 7800g/m^{-2} was noted on 20mm mesh in Tasmania (Hodson et al. 2000). As with percent net occlusion, comparisons between studies is challenging due to different infrastructure components, cultured species and latitude. When extrapolated to a commercial sized salmon net the application of an antifoulant can decrease the weight on a system by several tonnes (Cronin et al. 1999; Braithwaite et al. 2007). If the mean biomass found in this study (657g/m^{-2}) is extrapolated to the vertical surface area of a cage (area = 2160m^{-2} for a cylinder cage that is 120m circumference and 18m deep) then the mean total biomass on only the vertical surfaces of a single cage would be 1419.12kg, with a maximum of 4993.92kg.

5.4 Multiple Measures

Because of the different characteristics of biofouling organisms and the unique spatial patterns of biofouling community development it is important to use multiple measures to quantify the extent of the impact biofouling has on aquaculture system infrastructure. The choice of methods also relates to the questions being asked. For example, biomass is critical to understanding the impact of fouling on drag forces, whereas percent net occlusion is important for water exchange and fish health. An issue raised by Braithwaite et al. (2007) is that although a sample may have low biomass, the associated PNO might be very high. For example, netting near the surface may have a high occurrence of filamentous green algae that could severely occlude the net openings, but would have a low biomass. Alternatively, a deeper sample that is predominantly fouled by ascidians might have a very high wet weight, but the associated PNO could be low. It is also possible to have both high net occlusion and high biomass.

More research is needed into the relationship between the type of fouling and the effect it has on net occlusion and water exchange. This is because all foulers are not created equal and each biofouling group or particular species causes problems in a different way. For example, large kelps with buoyant pneumatocists can accumulate to a point where they can pull anchor ropes nearly to the surface; whereas, low-growing algae can cause severe net occlusion and a reduction in water flow. Different types of organisms have different types of net occlusion. Porous net occlusion from *Caprella sp*. will alter water flow differently from deformable hydroids or hard shellfish (Swift et al. 2006).

A major operational consideration on a salmon farm is the effort it takes to remove an organism because it relates directly to the number of passes with a pressure washer that are required to clean a net. For example kelps are much easier to wash off when they are young and the holdfasts have not yet fully encircled several mesh squares. Although they both have very different attachment strategies, barnacles with their glue and mussels with byssal threads are some of the most difficult organisms to wash off once they have set. Contrary to this, diatom fouling still lets water move through the mesh and is very easy to clean. Similarly, hydroids are often easy to wash off, but they are sometimes able to regenerate very quickly if the entire organism is not fully removed (Guenther et al. 2010). The ability to regenerate causes similar issues as those organisms which are able to re-settle once they have been suspended in the water column because they are able to become established and dominant far more quickly than an organism that arrives as a spore or larvae. Because of these complexities there is a need for more research into the relationship between the type of species causing the net occlusion, the reduction in water flow into a cage and the best management strategy.

The relationships between the results for the different indices used in this study are complex (Table 7). The copper treatments outperformed the control in all cases, but some of the alternative treatments fared better or worse than the control for only one or two of the indices, and in some cases only at certain depths.

Table 7: Significant results for indices used in this study (PNO over time, PNO for the final month of September, percent cover, and biomass), where 'X' marks a significant result for the associated index. Including treatment, depth and the treatment*depth interaction. Super-script represents, when relevant, the depths where the significance was found and whether it was higher (↑) or lower (↓) than the control.

	PNO time	PNO Sept	% Cover	Biomass
Treatment	X	X	X	X
Depth	X$^{1:5:18}$	X$^{5: 1,18}$	X$^{1m:5m:18m}$	
Treatment*Depth	X	X	X	X
Netrex	X$^{\downarrow}$	X$^{\downarrow}$	X$^{\downarrow\ 1m:5m:18m}$	X$^{\downarrow}$
Flexgard	X$^{\downarrow}$	X$^{\downarrow}$	X$^{\downarrow\ 1m:5m:18m}$	X$^{\downarrow}$
Dyneema				X$^{\downarrow}$
Sancure	X$^{\uparrow}$			
ThornD			X$^{\uparrow\ 5m}$	X$^{\uparrow}$
Solucote	X$^{\uparrow}$			
Netpolish				
NetCoating			X$^{\uparrow\ 5m:18m}$	X$^{\uparrow}$
Tar				

5.4.1 Time

Change in PNO demonstrated that time plays an important role in the development of a biofouling community. Both the time of year in which a net is put in the water and the amount of time it remains there will affect the composition of a biofouling community. If the levels that occurred in September were seen on a cage it would immediately be washed. It is also likely that early season storm events would have caused major disturbances in the biofouling community and initiated complex stochastic successional patterns (e.g. Braithwaite et al. 2007). The disturbance event between April and May was caused by the removal of diatoms which is a naturally occurring event (Railkin 2004). Filamentous diatoms naturally slough off of a surface when they grow too long. However, in this case, the sampling effort may have made the disturbance more dramatic since the frames were pulled to the surface in order to sample which subjected the samples to stronger shear forces.

5.4.2 Depth

Depth played a significant role in this study. The effect was documented in PNO over time, PNO for the final month of September and differences in community composition. However, there was no difference in biomass between depths; therefore, despite each depth having significantly different community composition and rates of net occlusion, the overall weight of the different taxa was similar at each depth.

5.4.3 Treatment by Depth Interaction

The interaction between treatment and depth was significant when considering PNO over time, PNO for September, percent cover, and biomass. This implies that over time there are different patterns of accumulation and disturbance of PNO for different treatments at different depths. The interaction between treatment and depth for biomass was mainly caused by a single treatment at a particular depth: NetCoating at 5m which experienced heavy fouling from barnacles that was not seen on any other samples. However, the influence was not significant enough to alter the mean; therefore, there was no significant difference from the control in terms of biomass for this treatment.

5.4.4 Treatment

Treatment was found to be a significant effect for all indices used in this study. It was particularly strong because of the effectiveness of the copper treatments when compared to the control. Surprisingly, the untreated nylon performed relatively well considering some of the alternative treatments performed worse in terms of PNO over time and biomass.

Overall, the alternative treatments did not out-perform the untreated nylon control, and in some cases the alternative treatment performed worse. Netpolish and Tar were both found to not be significantly different from the control when considering all of the indices used in this study. Dyneema, Sancure, ThornD, Solucote and NetCoating were all found to be significantly different in one or more of the indices used in this study. Dyneema had significantly less biomass, but was otherwise not different from the control. Sancure had significantly more biomass and PNO over time, but it did not have significantly different percent cover or PNO in September. ThornD had significantly different percent cover at 5m due to the absence of barnacles and the presence of sabellids which resulted in significantly higher biomass. Solucote maintained high PNO over the entire course of the study, but by September the other treatments had caught up and it was found to not be different from the control. NetCoating had significantly different percent cover at 5m and 18m which resulted in significantly higher overall biomass.

Sancure and Solucote were both found to have the highest PNO over time. Both coatings did not dramatically change the surface of the netting in terms of texture because the treatments go on thin; or colour since both coatings are clear. Sancure is an adhesive and protective coating that is sometimes used by one of the local net lofts to bind and set knots on a loose net. Solucote is a high-performance barrier coating for specialty textiles (essentially a waterproofer). Both coatings changed the surface texture in a way that quickly and continuously supported the growth of biofouling organisms.

Netpolish and NetCoating are both wax based coatings used by companies that specifically manufacture antifoulants. They are both used to protect a cage from UV damage. Netpolish was not found to be different from the control in any of the indices; however, at 5m NetCoating experienced a dramatic barnacle set that was not observed on any other treatment. A qualitative review of both treatments percent cover over time showed that Netpolish had very similar community composition to the control. NetCoating, on the other hand, maintained Diatom fouling into July, which was much later into the study than the other treatments. Then it experienced a disturbance event and at 1m and 18m the community composition quickly lined up with the control so that by September it was not found to be different from the control, but at 5m barnacle spores were able to quickly capitalize on the free space that became available.

5.4.5 Dyneema and Sancure

When considering all of the indices used in this study there was a significant difference between the two Dyneema treatments (untreated Dyneema and Dyneema with Sancure) (Table

8). Untreated Dyneema had significantly less PNO over time and was found to have significantly less biomass than Dyneema with Sancure. This implies that the Sancure treatment alters the surface of the Dyneema net in a way that does not alter the type of organisms present, or the final net occlusion but does increase biomass and net occlusion over time.

Table 8: Significant results comparing the two copper treatments and the two Dyneema treatments for indices used in this study (PNO over time, PNO for the final month of September, percent cover, and biomass), where ‘X’ marks a significant result and super-script represents the depths where the significance was found.

	PNO time	PNO Sept	% Cover	Biomass
Netrex: Flexgard	X		X ^{1m}	X
Dyneema: Sancure	X			X

5.4.6. *Netrex and Flexgard*

When considering all of the indices used in this study the two copper treatments (Netrex and Flexgard) significantly outperformed the untreated nylon control and all of the alternative treatments tested in this study (Table 8). The cuprous oxide based treatments were not completely immune to biofouling since they did experienced minor fouling from diatoms and some hydroids which was documented in both percent cover and net occlusion analysis. However, the levels were not significant enough to warrant any management actions. Of the two copper treatments, the wax based Netrex treatment had fewer fouling organisms and was thus able to maintain a lower PNO throughout the study. In September there was no significant difference between Netrex and Flexgard in terms of PNO. However, there was a measurable difference in percent cover and the associated community composition between the two copper treatments which resulted in a statistically significant difference but not operationally significant difference of 14.32 ±7.6g/m-2 in biomass between the two treatments. This was caused by Flexgard having some operationally non-significant diatom fouling at 1m.

5.6 Challenges in Aquaculture Field Research

The common experimental design used to test antifoulants is to use small quadrats of netting (e.g. Hodson et al. 2000; Braithwaite et al. 2007). This is because commercial salmon farms are massive and it is rarely possible to create a full scale, sufficiently replicated study. When studies use full-sized cages psuedoreplication is common (e.g. Hodson et al. 1995) because of the cost of the infrastructure and the sampling effort required. When an antifouling study is done on a fully-operational salmon farm with cages as replicates regular farm maintenance causes psuedoreplication because the cages do not get washed at the same time (e.g. Guenther et al. 2010). Compounding this is the spatial variability on a farm. Because a farm covers such a large area (e.g. at Shelter Bay the distance between the cages nearshore and the outer cages is ~150m) each cage is subjected to different environmental conditions (e.g. upwelling, dominant vs. sub-dominant current direction, oceanographic variability, shading from the cages themselves). Because of this, smaller scale quadrat studies have more control over some of the confounding variables and tend to be more realistic in terms of logistics. The issue is that quadrats are not exposed to the same effects as a full sized cages, which include: nutrient levels, particulates, faeces, and hydrodynamic forces (Svane et al. 2006), currents caused by the fish swimming in circles in the cages, and the potential for the cultured stock to feed on the biofouling (Moring and Moring 1975). Like a laboratory study, this creates issues in terms of using a small scale study represent a larger scale phenomenon. In this study, the quadrat samples were meant to represent a full-sized salmon cage. The most obvious difference between the quadrats and the cages was a lack of kelp on the quadrats. This may have been caused by pulling the quadrats to the surface for sampling could have dislodged the kelp spores from the net; kelp did settle on the steel frames which showed that algae spores were present but were mostly unable to grow on the quadrats. Another difference is that the movement of the barge from wave action was different from the movement of the 120m circumference polar circles. Despite the movement of the barge, the anchor chains turned out to be a good place to hang the frames because the knots were over a meter above the water line making it easier to retrieve the samples and tie them back on in rough weather.

As discussed earlier, biofouling is site and time specific, therefore the effectiveness of a treatment will vary between years and between sites. This variability can be caused by something as simple as the improper application of the coating, or complex issues like the fact that there are some organisms that are naturally tolerant of cuprous oxide and some are be able to develop tolerances with exposure (Piola et al. 2009), as well as annual variation in biotic and abiotic factors. Other major concerns facing the aquaculture industry and antifouling studies are the effects of climate change, which may alter species distributions (Walther et al. 2002) resulting in a change to the dominant fouling community, and the introduction of invasive species, which in some cases the invasive species can cause worse fouling than the native species (e.g. the hydroid *Ectopleura larynx* (Guenther et al. 2010)).

5.5 Additional Variables

A factor that would have improved this study and increased the relevance of the tests for industry is tensile strength. Most salmon cages are made out of Raschel knitted netting (Beveridge 2004). The preferred pattern is the super-knot pattern, where the mesh intersection is reinforced (Moe et al. 2007). There are ISO standards (ISO 2002b) for determining the breaking load and elongation of knotless mesh (Moe et al. 2007); these standards have specified temperature and humidity levels for dry samples, but not for wet samples. The test involves putting a single square of mesh between two hooks and pulling on it until it breaks and measuring the pull (kN) on a load cell (Moe et al. 2007). Summarized by Beveridge (2004), a needs to meet a certain set of performance criteria in terms of material type and tensile strength. It needs to be slightly denser than water to facilitate hanging, but not so dense as to make it difficult to work with or affect the floatation on a system; the netting should resist tears from floating objects and predators; be able to support some fish biomass when the nets are lifted for harvesting; it should resist deformation from currents; it should endure stretching without breaking; and netting needs resist abrasion. All these factors make tensile strength extremely important. Having strong nets means that the fish are kept safely inside the cages, thus a reduction in tensile strength is a threat to the structural integrity of a cage and increases the rick of escapes. Nylon netting loses strength simply by getting wet (Badinotti 2011) and it has been found that the application of some alternative, wax-based antifoulant coatings can decrease tensile strength by 13% and cause a 11% reduction in mesh size on 25mm half mesh (Moe et al. 2007). Over time, fouling organisms can cause damage to the netting fibers and reduce the break strength of the material (C. Edwards unpublished data). Because of the importance of tensile strength it would have been valuable to include measures of it prior to immersing the samples, and at the end of the study.

Another valuable addition to this type of study would be to test the effects of pressure washing on the different materials. If the assumption is that fouling will still occur, but at lower levels, then the nets still need to be washed. But, if a coating is removed from the netting after the first wash then it is of little value to a farm operation because they are then paying the full cost of dipping a net and the full cost of washing a net. If a coating were able slow the decline in breaking strength of a net and resist removal from a pressure washer then it is valuable to a farm irrespective of its antifoulant properties.

5.7 Better Management Practices

Because effective alternative antifoulant treatments are currently unavailable better management practices need to be implemented. If a site continues to use copper treated nets then it should be done with the least impact on the marine environment. A treated net should be left unwashed for as long as possible. Washing it prematurely means that the highest concentrations of copper from the coating will accumulate in the benthic environment below the cages. When the coating itself is washed off of a net, the active antifoulant ingredient remains imbedded in the wax or paint coatings and leaches out below and around the cages. A better option is to let the copper slowly leach out of the paint or wax so that the marine environment has more time to assimilate it into non-bioavailable forms. Capitalizing on the effectiveness of treated nets means that the most value is obtained for the price of dipping a net since prematurely washing a copper dipped net not only increases the time spent washing, but also increases the number of times a net has to be dipped. A net should be dipped or washed, not both.

Despite the effectiveness of copper treated aquaculture nets there is a trend to move away from the use of these products. This shift is caused by an increasing awareness of the impact these products have on organisms in the marine environment. The use of environmentally sound alternatives reduces the salmon farming industry’s contribution to marine copper pollution. This improvement in environmental sustainability improves public perception of the industry and improves consumer perception of the product on the shelves. Thus, there are several reasons to stop using copper based antifoulants, but as it stands the ideal antifoulant described in the introductory chapter does not yet exist. Factors that could improve biofouling management might involve including the identification of major fouling plankton in the routine plankton sampling which could help to inform choices on when and when not to wash. For example, if fouling is present but not at a critical level then not washing a net when there is a lot of mussel plankton in the area might reduce the severity of the settlement because organisms already fouling the net will be able to spatially outcompete the mussels. A camera on a drop line could be used to determine the severity and depth of biofouling because visual checks are limited to the area that can be seen from the surface. And better educational material could be included in standard operating procedures so that farm workers can make better informed decisions regarding biofouling management. Although a blanket policy regarding seasonality and net washing schedules means that there is never any doubt about when a net should be washed (e.g. summer months wash every two weeks) it also means that sometimes nets are getting washed more often than they need to be.

Currently, the main environmentally sound alternative is an untreated nylon net, which is unable to withstand the abuse from repeated pressure washings, and exposure to UV thus what is needed is either a coating that provides protection to nylon or a different net material. An ideal coating would be able to withstand pressure washing without chipping or cracking, and provide UV protection; fouling would still occur but it could be washed off without damaging the net. An ideal net material would be made of a fiber that is strong, resistant to UV damage, and does not breakdown from repeated pressure washing. Ideally, the strength of the fibre would mean that the twine could be thinner which would let more water pass through the net, reduce drag from currents, and have less surface are for foulers to settle on. Also, a net made of a high-strength fibre could be anchored more tightly which makes it harder for predators to get at the fish and helps the net keep its shape in currents. Whether an alternative coating or alternative net material is used it should be accepted that fouling will still occur, but the cage would be able to withstand the abuse from biofouling management and would contribute to an increase in the overall lifespan of a net.

CHAPTER 6: Conclusion

As the commercial scale, intensive finfish farming industry continues to develop so will the need for viable solutions for operational issues and environmental concerns. Having effective biofouling management practices is critical to the overall economic performance of a finfish site; however, there are other ways to look at the incentives to implement alternative antifoulant coatings. Financially, it is expensive to dip a net in antifoulant paint and biofouling eventually accumulates on a treated net which will then need to be washed. This means that in some cases a company is paying for both the cost of the antifoulant coating and the cost of net washing. A shift away from toxic coatings is beneficial to the marine environment because it reduces chemical pollution, but it also helps improve the public perception of the industry' environmental policies (Braithwaite and Mcevoy 2005) and it can help to improve consumer perceptions of farmed salmon (Hodson and Burke 1994; Hodson et al. 1997). Because there is potential for copper based antifoulants to eventually be banned it is a good idea to have viable alternatives already available.

Although biofouling is an extremely important operational issue on a salmon farm, there are other factors to consider when choosing a netting material or coating, especially considering the current lack of effective alternative treatments. These factors include: surface area and drag; breaking strength (initial and decrease over time); initial cost, as well as the cost of net washing, maintenance, and disposal; and finally, the total cost of all these factors over the life time of a net. For future finfish aquaculture biofouling studies, the inclusion of tests on the breaking strength of the treatments and the effect of net occlusion on water exchange would make for a study that accounts for the variables that are important to both science and industry. A direction for future research is to increase our understanding of the relationship between the type and severity of fouling, and the changes in water exchange into and out of a cage.

The dominant theme found throughout this research project is that copper treated nets are extremely effective at preventing the accumulation of biofouling organisms when considering all three of the indices used in this study (species composition, biomass and percent net occlusion). This effectiveness will continue to be a barrier to the development and implementation of alternative antifouling treatments. This study also demonstrates that the alternative treatments tested were unable to meet the performance standards set by industry, and that more research is needed into effective, alternative antifoulant coatings for aquaculture operations.

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Appendix A: SPSS v.17 Syntax

Arcsine Transformation:

```
COMPUTE PNOArsin=ARSIN(SQRT(PNO/100)).
```

Marginalized Linear Mixed Model: Percent Net Occlusion over Time:

```
MIXED PNOArsin BY Treatment Depth Block Month
/FIXED=Treatment Depth Block Treatment*Depth | SSTYPE(3)
/METHOD=REML
/PRINT=R
/REPEATED=Month | SUBJECT(SampleID) COVTYPE(UN)
/EMMEANS=TABLES(Treatment) COMPARE (Treatment REFCAT('O')
ADJ(BONFERRONI)
/EMMEANS=TABLES(Depth) COMPARE ADJ(BONFERRONI)
/TEST = 'compare copper P Q' ALL 0 0 1 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1/3 1/3 1/3 -1/3
-1/3 -1/3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
/TEST = 'compare dyneema R S' ALL 0 0 0 0 1 -1 0 0 0 0 0 0 0 0 0 0 0 0 0  0 0 0 0 0 0
1/3 1/3 1/3 -1/3 -1/3 -1/3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0.
```

Marginalized Linear Mixed Model: Percent Net Occlusion for September:

```
MIXED PNOArsin BY Treatment Depth Block
/CRITERIA=CIN(95) MXITER(100) MXSTEP(5) SCORING(1)
SINGULAR(0.000000000001) HCONVERGE(0,
ABSOLUTE) LCONVERGE(0, ABSOLUTE) PCONVERGE(0.000001, ABSOLUTE)
/FIXED= Treatment Depth Block Treatment*Depth | SSTYPE(3)
/METHOD=REML
/EMMEANS=TABLES(Treatment) COMPARE (Treatment) REFCAT('O')
ADJ(BONFERRONI)
/EMMEANS=TABLES(Depth) COMPARE ADJ(BONFERRONI)
/TEST = 'compare copper P Q' ALL 0 0 1 -1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1/3 1/3 1/3 -1/3
-1/3 -1/3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
/TEST = 'compare dyneema R S' ALL 0 0 0 0 1 -1 0 0 0 0 0 0 0 0 0 0 0 0 0  0 0 0 0 0 0
1/3 1/3 1/3 -1/3 -1/3 -1/3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0.
```