# Evaluation of the properties and effectiveness of antifouling coatings in the coastal Newfoundland marine environment


By

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Abstract

Biofouling, the accumulation of organisms on submerged aquatic surfaces, can be detrimental to the aquaculture industry. In the Atlantic Canadian provinces, excluding Newfoundland and Labrador (NL), fouling by aquatic invasive tunicate species has had large economical impacts on the mussel aquaculture industry. In NL, invasive tunicates have not necessitated management, control, or removal at mussel aquaculture sites, but prevention is an important management tool.

Antifouling coatings can prevent the movement of native and invasive species from a support harbour to a site. In a field study, different antifouling coatings, with different antifouling properties, (chemical and mechanical) painted on wooden panels, were submerged in coastal outports to determine the effectiveness of the coatings in the NL marine environment. A laboratory study was also conducted to determine if micro-surface topography of a coating differed amongst coatings.

Coatings which contained biocides, such as Econea®, and copper and zinc, prevented biofouling accumulation for a period up to 12 months, post-deployment. Among all sites, Micron CF (Econea®) had minimal biofouling accumulation after the first year (less than 5%) and by the end of the trial had less than 60% of the area settled by fouling organisms. Foul release coatings (e.g., Hullspeed 3000) were not effective in this application. Scanning electron microscopy images showed varied textures, in structure and volume, between the different types of antifouling properties, which may promote or prevent initial settlement. All properties together are important to the efficacy of an antifouling technology.

In the aquaculture industry, where vessels are not moving great distances between the docks and the farms and where operations often require vessels to move slowly to complete various work on the farm sites, the use of ecofriendly biocide antifouling coatings (e.g.,Micron CF) would be the best defense against the movement and spread of fouling organisms and aquatic invasive species.

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# List of Abbreviations

A- Argentia

AC- Arnold's Cove

AIS- Aquatic invasive species

Be- Belleoram

Bu- Burin

DFLR- Department of Fisheries and Land Resources

FRC- Foul release coating

FT- Foxtrap

GLM- General linear model

GLMM- Generalized linear mixed model

IMO- International Maritime Organization

IUPAC- International Union of Pure and Applied Chemistry

LB- Little Bay

MEPC- Marine Environment Protection Committee

MUN- Memorial University of Newfoundland

NL- Newfoundland and Labrador

PE- Prince Edward Island

RNYC- Royal Newfoundland Yacht Club

SEM- Scanning electron microscopy

SPC- Self polishing co-polymer

TBT- Tributyltin

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# Co-Authorship Statement

The work described in the present thesis was conducted by Ashley Bungay with guidance from Dr. Cynthia McKenzie, Cyr Couturier, and Dr. Erika Merschrod. Ashley Bungay was responsible for all field and laboratory data collection. Statistical analysis for each chapter was conducted by Ashley Bungay. All Chapters were written by Ashley Bungay with intellectual and editorial input by Dr. Cynthia McKenzie, Cyr Couturier, and Dr. Erika Merschrod.

Potential manuscripts in primary literature resulting in work for the following Chapter 2 will be co-authored by Bungay, A.N., McKenzie, C.H., Matheson, K.

Potential manuscripts in primary literature resulting in work for the following Chapter 3 will be co-authored by Bungay, A.N., McKenzie, C.H., Couturier, C., Merschrod S., E.F.

# Chapter 1: Introduction

## 1.1 Introduction

Biofouling is the unwanted growth of organisms on submerged surfaces (Blöcher et al., 2013). It not only occurs on artificial structures (e.g., docks and vessels) that are immersed but also on natural substrates such as seaweeds and rocks. Biofouling can have a negative effect on many industries such as the shipping industry, as biofouling increases the weight and drag of the vessel, which in turn increases the fuel and maintenance costs, since more fuel is consumed to maintain the same speed (Perez et al., 2015; Zabin et al., 2018). The International Maritime Organization (IMO) recognizes shipping as a major threat to the world's oceans, as aquatic invasive species have been introduced to new environments by ships (IMO., 2020). Many marine species can be carried in a ships' ballast or on a ships' hull, some of these species, such as invasive tunicates, may survive and establish a population in a new environment (IMO., 2020). The IMO has been at the forefront of the effort to address the concern of species transfer through shipping along with many other groups, including governments, economic sectors, non-government organizations and international treaty organizations (IMO., 2020).

In 2006, the issue of the transfer of invasive species was formally brought to IMO's attention and in 2007 the IMO's Marine Environment Protection Committee (MEPC) agreed to the development of biofouling related guidelines, which were adopted in 2011 by MEPC for ships (IMO., 2020). The objective of these guidelines was to provide guidance to various stakeholders, including antifouling paint manufacturers (MEPC., 2011). In 2012, MEPC developed guidelines for recreational vessels as well, being similar those for ships (MEPC., 2012). Both sets of guidelines have sections which focus on antifouling systems which constitute coatings, paints, surface treatments or devices that are used on a ship to control or prevent the attachment of unwanted organisms (IMO., 2020). According to the MEPC guidelines, when choosing an antifouling system for a ship, different factors such as time between dry-docking, ship speed, operating profile, ship construction and legal requirements for the sale and use of the system should be considered (MEPC., 2011). For recreational crafts, factors to be considered are very similar: periods between hauling or drying out, speed and usage of craft, and material of hull and where to apply the antifouling system (MEPC., 2012). As well as having guidelines for the use of antifouling systems, the IMO publishes regulations related to antifouling systems. The legal requirement of the coating is important due to restrictions on biocide, chemical causing adverse effect on an organism, used in antifouling systems. These biocide compounds leach into the sea, killing marine organisms attached to the hull of a ship (IMO., 2020). Developed in the 1960s, tributyltin (TBT) was one of the most effecting antifouling coatings but was proven to cause deformation in oysters and sex change in whelks (IMO., 2020). In 1989 the IMO recognized the harmful effects of organotin (organic tin) compounds and in 1990 IMO's MEPC adopted a resolution, recommending that Governments adopt measures to eliminate TBT containing antifouling coatings (IMO., 2020). In 1999 the MEPC was called to develop an instrument to address the harmful effect of these coatings (IMO., 2020). This resulted in a global prohibition of organotin compounds on ships in 2001 and a complete ban in 2008 (IMO., 2020).

Biofouling can have negative effects on industries other than shipping, such as the aquaculture industry. Fouling organisms can constrict net openings used in finfish aquaculture, reducing the flow of water through the cage and may reduce the amount of light which is available to the fish (Bazes et al., 2006). The reduction in the amount of water flow can restrict oxygen and nutrient exchange and waste removal within the cage system (Braithwaite et al., 2007). Biofouling organisms which attach to the cage and netting can create habitats that are suitable for harmful diseases and parasites which can affect the health of the fish (Edwards et al., 2015; Tan et al., 2002).

Biofouling can also impact the infrastructure of a farm site. Increases in mesh occlusion, due to biofouling covering the mesh of the nets, increases the drag forces on the nets and fouled nets may have 12.5 times the current-induced forces of that of a clean net. This can severely deform a cage which, in turn, decreases the volume in the cage subsequently increasing stocking densities to levels which might cause stress (Fitridge et al., 2012; Sterling et al., 2016).

In shellfish aquaculture, biofouling can cause problems in different ways such as physical damage (e.g., shell damage) to the cultured organism, competition with cultured species for food, oxygen and space, and additional costs of cleaning and replacement of fouled equipment (Fitridge et al., 2012; Sievers et al., 2017). Physical damage to the shell can reduce growth and therefore lengthen the production cycle since the species will invest energy into shell regeneration rather than muscle tissue (Fitridge et al., 2012). Tunicates, and several other biofoulers, colonize the artificial substrates created by mussel line suspension, and, since they are filter feeders, they compete for food and oxygen, which can restrict the amount that is available to the culture species (Cahill et al., 2012; Paetzold et al., 2012). The increase in the weight on the infrastructure can increase operational costs due to the requirement for more floatation, but the additional weight in mussel culture may cause the stocks to drop from the rope, in suspension culture (Arens et al., 2011a; Fitridge et al., 2012,). Therefore, it is important to protect cultured shellfish from the impacts of biofouling.

Invasive species increase biofouling risk. Invasive tunicate species have had devastating effects on the shellfish aquaculture industry in the Maritime Provinces of Canada. In Prince Edward Island (PE) there are four reported invasive or exotic tunicates: *Styella clava, Ciona intestinalis, Botrylloides violaceus* and *Botryllus schlosseri*. Ramsay et al. (2008) reported that *C. intestinalis* colonized the PE Brudenell estuary in epidemic proportions. PE has a large blue mussel (*Mytilus edulis*) aquaculture industry for which these invasive species create issues. Cost for the control of fouling by tunicates in PE is estimated at approximately $5M annually (Cordell et al., 2013). Since its discovery in Nova Scotia, *C. intestinalis* has been devastating to the Nova Scotia mussel industry as well as other provinces (Daigle &

Herbinger., 2009). Therefore, it is important to protect the bivalve industry from invasive tunicate species. Native species can also create issues for industry. Algae species can increase weight and drag on aquaculture infrastructure, making it important that biofouling is properly managed (Woods et al., 2012).

*B. schlosseri* was first detected on a recreational boat in Argentia (A), Placentia Bay, NL in 2006, and has since spread to various areas in Placentia Bay, NL. In 2011 it was subsequently detected in Foxtrap (FT), Conception Bay, NL (Figure 1.1.1, McKenzie et al., 2016a). *B. violaceus* was first detected on a dock in Belleoram (Be), Fortune Bay, NL in 2007 and has since been confirmed as being present in Foxtrap (FT), Conception Bay. *C. intestinalis* was first discovered in Little Bay (LB), Placentia Bay, NL, on the wharf structure, in 2012 (Sargent et al., 2013).

Mitigation, such as vessel and wharf cleaning, has taken place for all three invasive tunicate species in NL. Since these species have been detected by DFO, preventing the return settlement and the spread of these species has been an important aspect of the mitigation process, one which can be achieved with the use of antifouling technologies (McKenzie et al., 2016b, personal comm. McKenzie). In 2019 the Newfoundland shellfish aquaculture industry was valued at $14.8M (DFLR., 2019). Since these aquatic invasive species have not yet invaded NL shellfish farming sites, the industry currently does not have to control, clean, or remove invasive tunicate fouling, which could be devastating to the farmers. NL.

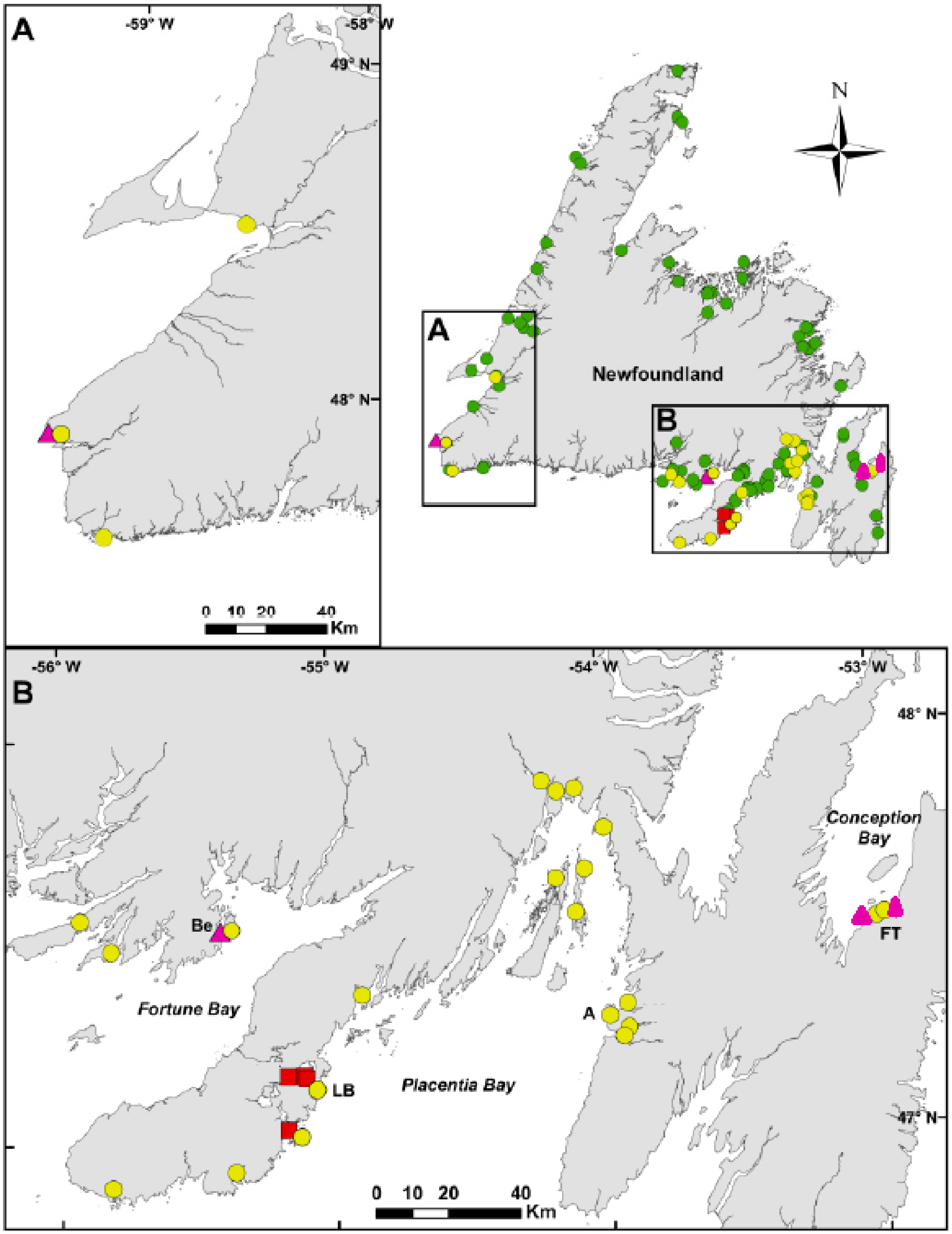


Figure 1.1.1: Invasive tunicate distribution in Newfoundland at the beginning of this study, showing the locations of the first discovery of invasive tunicates in NL. Absence of tunicates (green circles), *Botryllus schlosseri* (yellow circles), Argentia (A), Foxtrap (FT), *Botrylloides violaceus* (purple triangles), Belleoram (Be), *Ciona intestinalis* (red squares) Little Bay (LB). (Modified from McKenzie et al., 2016a).

There are many different methods or treatments to mitigate biofouling in shellfish and aquaculture, most of which involve the removal of fouling after it has established. Fouled equipment used in aquaculture can be submerged in baths which may contain acetic acid, hydrogen peroxide or fresh water to kill the biofouling organisms on the shellfish stocks (Rolheiser et al., 2012). Pressure washing kills and/or removes fouling organisms from shellfish and other substrates (Arens et al., 2011a, b). Exposing the fouling to air may also be used to eliminate the already present fouling community from a submerged surface (Hillock & Costello., 2013). In-situ cleaners are also used to remove fouling organisms from netting on finfish pens (Hodson et al., 1997; personal observation). Introducing other species, which feed on the fouling, into the culture system is another way to remove biofouling from the system (Lodeiros & García., 2004). Lodeiros & García. (2004) studied the use of adding sea urchins to limit the growth of algae fouling on nets used for bivalve culture. Zeinert et al. (2021) found that crab could effectively remove biofouling on a fish net pen enclosure in the Caribbean. Periwinkles have also been shown to control algae fouling on trays for oyster culture (Cigarría et al., 1998; Enright et al., 1983). All these methods focus on the removal of biofouling once the community has already established, instead of preventing the settlement of these organisms on the substrate.

There are some antifouling methods which concentrate on preventing the attachment and growth of organisms on substrates. Edwards et al. (2015) studied the use of different coatings and netting materials. The different antifouling materials that were used include nylon fiber (non-treated netting), Dyneema™ (polyethylene fiber), Netpolish™ (waterborne wax coating), Aquacoating™ (waterborne wax coating), ThronD™ (netting made of short fibers), and Netrex™ (copper-based wax coating). The study determined that the copper-based coating, containing 17% cuprous oxide, was the most effective at preventing biofouling, due to the presence of biocide. Mert et al. (2014) studied the use of Econea® as an antifoulant and determined that Econea® was better at preventing the settlement of hard foulers such as barnacles and worms than soft fouling organisms, but a conditioning film still formed.

For certain species of diatoms (*Thalassiosira pseudonana)* and polychaetes (*Hydroides elegans)* zinc pyrithione, a common biocide in antifouling coatings, was found to be more toxic than copper but for the amphipod (*Elamopus rapax)* zinc pyrithione and copper had the same effect on the organisms (Bao et al., 2008). Not all biocides will be effective against all biofouling organisms.

**1.2 Background**

Biofouling is often described as occurring in four steps as discussed by Martín-Rodríguez et al. (2015) and Kerr and Cowling. (2003). The first step is the adsorption of organic particles on the surface of the substrate, which develops the conditioning film that constitutes the molecular fouling and promotes the next step, primary colonizers. Primary colonizers are pioneer motile bacteria and benthic diatoms; these microorganisms form complex multispecies biofilms which in turn promotes step three, macroalgal zoospores which then settle on the substrate. Finally, the last step in the formation of biofouling is the settlement of invertebrate larvae which forms the complex macroscopic fouling community seen on many fouled substrates (Kerr and Cowling., 2003; Martín-Rodríguez et al., 2015;). Even though biofouling is described in this way, after the conditioning film is formed organisms can adhere to the surface simultaneously (Blöcher et al., 2013). The prevention of fouling occurs at the conditioning film stage; if a conditioning film can be interrupted or altered then fouling organisms will not find the substrate to be a suitable habitat.

The conditioning film is an important aspect of biofouling, starting the growth, but if a substrate does not have the right parameters to promote settlement, attachment can be prevented. Rosenhahna. (2008) provided a diagram which was modified by Hellio and Yebra. (2009) that helps explain how biofouling attaches to a surface. There are four main categories which are used: chemistry, mechanical, structure, and polarity (Figure 1.2.1). According to the diagram chemistry parameters which affect attachment include hydration, wettability, conformation, and bioactivity.  Biocides (chemicals toxic to fouling organisms, used in coatings) can deter organisms from choosing that surface.

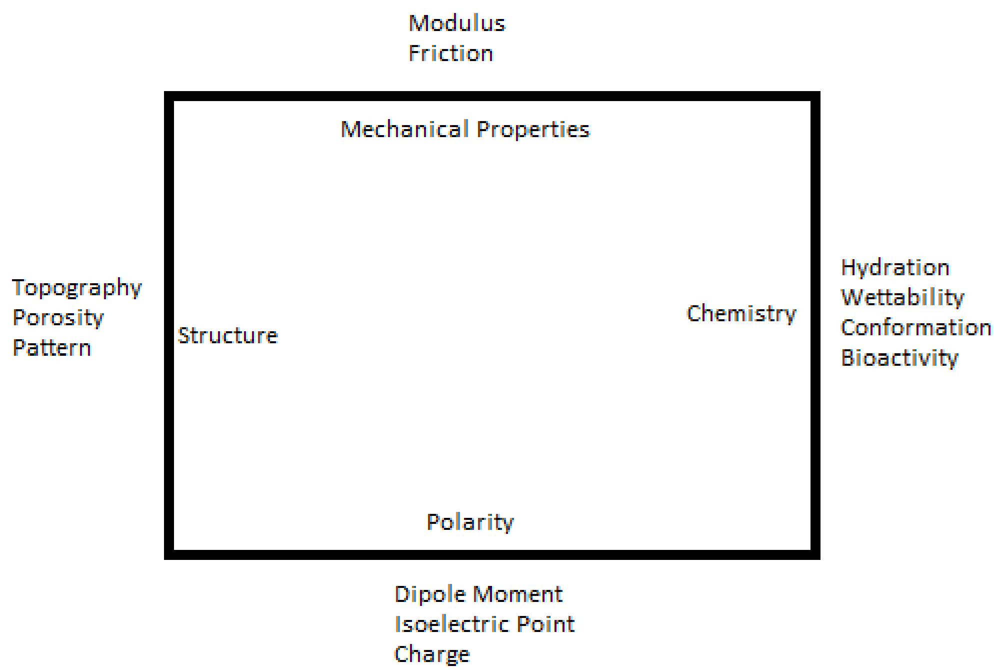


Figure 1.2.1: Examples of properties which can influence the settlement of biofouling organisms (modified from Hellio and Yebra., 2009 and Rosenhahna., 2008).

Mechanical properties include modulus (the relationship between tensile and compression forces) and friction. A lack of friction can make it hard for an organism to stay attached to a substrate. For structure, three parameters are represented: topography of the surface, porosity of the surface and the pattern or periodicity of the surface. Some surfaces may have pores, or patterns which will support a nanoscale interaction between the surface of an antifouling coating and the adhesive chemicals and/or mechanisms used by a biofouling organism. Surface structure of the coating may have important properties which impact its effectiveness. For example, if there are cavities in the microstructure of the coating, then organisms may have the opportunity to settle (Berntsson et al., 2000; Larsson et al., 2010; Stafslien et al., 2015).

Polarity is also an important characteristic of a surface, it includes a dipole moment, isoelectric point, and charge, which can influence how the adhesive chemicals and surface chemical interact (Vladkova., 2008). For the purpose of this study polarity was not studied. This study focused more generally on all biofouling organisms, where polarity would have been for specific species-substrate interactions.

All these properties are important when mitigating or preventing the growth and spread of fouling organisms. Antifouling coatings, which rely on these properties, can be used to prevent biofouling from accumulating on many different surfaces. Not all antifouling coatings are made equal; there are coatings which contain biocides and coatings that use physical properties to prevent fouling attachment.

The most common types of coatings (paints) used in antifouling applications have chemical properties with self-polishing copolymer (SPC) with research shifting focus to a combination of chemical/mechanical properties with foul release coatings (FRC) (Buskens et al., 2013; Yang et al., 2010). SPC paints contain biocides, which differ in the type and amount depending on the paint. The biocide is released through a reaction of water with the coating polymer which is often a methacrylate polymer (Ciriminna et al., 2015). The release of chemicals from the surface can signal to an organism that this surface is toxic (Yebra., 2004). Some biocides which can be included in these coatings (and are being investigated in this study) are copper, in the form of cuprous oxide ($Cu_2O$) and cuprous thiocyanate ($CuSCN$), and zinc pyrithione (Figure 1.1.3), also called zinc omadine. The International Union of Pure and Applied Chemistry (IUPAC) name for zinc pyrithione is 2-pyridinethiol-1-oxide zinc salt. Econea®

(Figure 1.1.4) is often called tralopyril and has the IUPAC name 2-(p-chlorophenyl)-3-cyano-4-trifluoromethyl pyrrole. Econea® is an organic biocide which breaks down into simpler more environmentally friendly compounds (compared to metals) in water (Mert et al., 2014).

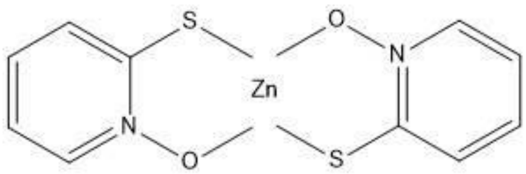


Figure 1.2.2: Chemical structure of zinc pyrithione, 2-pyridinethiol-1-oxide zinc salt.

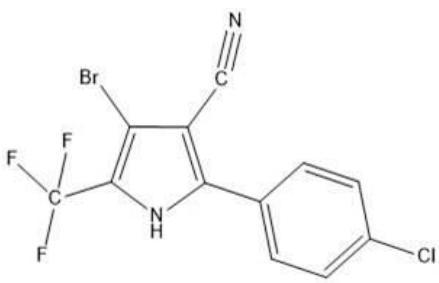


Figure 1.2.3: Chemical structure of Econea®, 2-(p-chlorophenyl)-3-cyano-4-trifluoromethyl pyrrole.

FRC works to reduce the attachment strength of biofouling organisms to the substrate, such that an organism will be dislodged with increased force acting upon it (Dafforn et al., 2011). There are two types of FRC, one which contains silicones and one which contains fluoropolymers (Lejars et al., 2012). Silicone based paints are made with polymers which contain silicon-oxygen backbones and organic side chains. The side groups along the silicon-oxygen back bone are often methyl groups, which provide non-stick properties which prevents or weakens attachments (Dürr &Thomason., 2010). Fluoropolymers are polymers which have fluorinated groups and have a relatively high modulus which encourages adhesive bond fractures through peeling (Dürr &Thomason., 2010).

A study by Stafslien et al. (2015) using fouling release coatings consisting of a mixture of polytrifluoropropylmethylsiloxane ($CF_3$-PDMS) and 2-[methoxy(polyethyleneoxy)propyl]-trimethoxysiloxane (TMS-PEG), found that for the bacterial species *Cellulophaga lytica* and *Halomonas pacifica,* 99% and 100% physical removal was obtained, respectively, with high amounts of TMS-PEG and $CF_3$-PDMS. Also, the lowest barnacle adhesion strength occurs with the highest TMS-PEG and $CF_3$-PDMA contents (Stafslien et al., 2015).

The surface structure properties (e.g., texture) of the antifouling coatings are another important aspect which can have an impact of the effectiveness of the coating. Surface properties of a coating are based on nanotechnology, which is science at the scale of atoms and molecules and deals with objects and material that can be made, controlled, or manipulated at a nanometre scale (Hellio and Yebra., 2009). Organisms that foul surfaces do so through the secretion of adhesive polymers, and these interactions are determined within a few nanometres of the surface (Smith., 2006).

**1.3 Purpose of Proposed Research**

The purpose of this research was to compare the effectiveness of different antifouling coatings, applied to wood, in the coastal NL marine environment over a seventeen-month period. There are many different types of coatings, and each one works differently to prevent the settlement of biofouling species. There are coatings that rely on chemical properties, such as biocides, to prevent the settlement of organisms, while other coatings rely on mechanical properties to reduce the attachment strength of organisms on a surface. Common amongst all coatings is the importance of the microstructure of the surface. In this thesis, chemical biocides, mechanical properties, and the surface structure properties of these coatings were studied to determine their relative effectiveness in the prevention of biofouling on wooden substrate surfaces typically found at aquaculture sites and support harbours, such as wooden docks and support vessels. This study also has implications for biofouling prevention on other structures, equipment and gear utilized at aquaculture locations.

The prevention of biofouling from aquaculture vessels, cages and other equipment will benefit the industry in ways such reduced production costs due to less laborious cleaning of nets, ropes, floats, and other infrastructure, from decreasing the amount of fouling, specifically by invasive species, being transported to the sites. Less cleaning will in turn result in less stress to the animals which will prevent chronic stress related mortalities (Østevik et al., 2021). Less competition for food and physical space, with invasive and native fouling organisms, will also benefit the shellfish industry.

### 1.4 Study Objectives and Hypothesis

The overall goal of this study was to compare various coating types for chemical, mechanical, and surface properties which affect the settlement of biofouling organism. The objectives were: 1) to compare the chemical (biocide) and mechanical (non-biocide) properties of antifouling coatings applied to wood in field trials and 2) investigate how microscopic surface structure topography of the coatings affects the ability of a coating to prevent biofouling. It was hypothesized that coatings containing biocides would be more effective in preventing antifouling than coatings without the use of chemical biocides, as the coatings containing biocides will deter more organisms from settling on the surface. Coatings with higher biocide content should also prevent fouling more than coatings with lower biocide content. It was also hypothesized that coatings with more texture or pores would have more settlement, as there is more opportunity for the nanostructure of the surface and the adhesive chemicals of fouling species to interact on a microscale.

# Chapter 2: The effectiveness of chemical (biocide) and mechanical (non-biocide) coatings to prevent biofouling in the coastal Newfoundland environment

## 2.1 Introduction

Biofouling is the unwanted growth of aquatic organisms on the surface of submerged aquatic substrates. Biofouling not only occurs on manmade structures that are immersed (e.g., docks) but also on natural substrates such as seaweeds and rocks (Maréchal and Hellio., 2009). This growth can be devastating for industries which rely on the marine environment, such as the aquaculture industry.

In open ocean finfish culture, the fish are contained in cages with netting, which provides a large amount of substrate suitable for fouling organisms. Fouling organisms can constrict net openings, reducing the flow of water through the cage and may reduce the amount of light which is available to the fish (Bazes et al., 2006). The reduction in the amount of water flow can restrict the amount of dissolved oxygen available, in addition to nutrient exchange and waste removal within the cage system (Braithwaite et al., 2007). Biofouling organisms which attach to the cage and netting can create habitats that are suitable for harmful diseases and parasites which can affect the health of the fish (Edwards et al., 2015; Tan et al., 2002).

Biofouling can also impact the infrastructure of farm sites as well as have negative effects on the stocks. Increases in mesh occlusion, due to biofouling covering up the mesh of the nets, increases the drag forces on the nets and fouled nets may have 12.5 times the current-induced forces of that of a clean net. This can severely deform a cage which, in turn, decreases the volume in the cage therefore increasing stocking densities to levels which might cause stress (Fitridge et al., 2012; Sterling et al., 2016).

In shellfish aquaculture, biofouling can cause problems in different ways such as physical damage to the organism, competition for food, oxygen, and space, increasing the mass of the longlines in mussel culture, and increasing the cost for cleaning equipment and shellfish for market (Davidson et al., 2017; Fitridge et al., 2012; Seivers et al., 2017). Physical damage to the shell can reduce growth and therefore lengthen the production cycle as the species will invest energy into shell regeneration rather than tissue (Fitridge et al., 2012). Tunicates colonize the artificial substrates created by mussel line suspensions, and since they are filter feeders they compete for food and oxygen, which can restrict the amount that is available to the culture species (Cahill et al., 2012; Paetzold et al., 2012). The increase in the weight of the infrastructure can increase operational costs due to the need for more floatation and more frequent cleaning, but the additional weight in mussel culture may cause the stocks to drop from the rope, in suspension culture (Arens et al., 2011a, Fitridge et al., 2012). Therefore, it is important to protect aquaculture from the impacts of biofouling.

Some of the most devastating biofouling organisms to the aquaculture industry are tunicate species. Some species of tunicates are native to NL marine waters, but there are also three species of invasive tunicates: Golden Star (*Botryllus schlosseri*), Violet (*Botrylloides violaceus*) and Vase (*Ciona intestinalis*). In other Atlantic provinces, such as PE, fouling by tunicate species does cause issues in the shellfish aquaculture industry. The first documented case of Vase tunicate (*Ciona intestinalis*) in Canada was in Nova Scotia, it was reported on mussel farm in Lunenburg, it has since become devastating to mussel aquaculture in other Atlantic provinces (Carver et al., 2006; Daigle & Herbinger., 2009). In PE there are four reported invasive or exotic tunicates, clubbed tunicate (*Styella clava*), Vase tunicate, Golden Star tunicate and Violet tunicate. Ramsay et al. (2008) reported that *C. intestinalis* colonized the Brudenell estuary in epidemic proportions. PE has a large mussel aquaculture industry in which these invasive species create issues, as previously discussed. It was estimated that the costs, associated with controlling fouling by tunicates in PE, was $5M annually (Cordell et al., 2013). In 2019, the Department of Fisheries and Land Resources (DFLR), Government of NL reported that the aquaculture shellfish industry was valued at $14.8M, this is without the control of invasive species (DFLR., 2019). A control cost of $5M or more could be economically devastating to NL shellfish growers. Therefore, it is important that preventative measures be taken in NL to prevent the spread of these species to aquaculture sites, reducing the cost required to control and mitigate tunicates if they were to become a problem for the industry.

Biofouling is often described as occurring in four steps. The first step is the adsorption of organic particles on the surface of the substrate, which develops the conditioning film that constitutes the molecular fouling and promotes the next step, primary colonizers. Primary colonizers are pioneer motile bacteria and benthic diatoms, these microorganisms form complex multispecies biofilms which in turn promotes step three, macroalgal zoospores which then settle on the substrate. Finally, the last step in the formation of biofouling is the settlement of invertebrate larvae which forms the complex macroscopic fouling community seen on many fouled substrates (Kerr and Cowling., 2003; Martín-Rodríguez et al., 2015). Even though biofouling is described this way, after the conditioning film is formed, the organisms can adhere to the surface simultaneously (Blöcher et al., 2013). When preventing biofouling the surface of a substrate is altered such that one or more steps involved in the development of biofouling cannot occur, such as a surface being too toxic for an invertebrate organism to grow.

By understanding the mechanisms of biofouling settlement, surfaces can be manipulated to prevent it. There are different surface parameters which can be changed to deter the settlement and growth of organisms on a surface (Rosenhahn., 2008). If the chemical makeup of a surface is too toxic, it will not support growth or will kill an organism, which then may not settle. If the mechanical properties of a substrate are manipulated, an organism may find it hard to stay attached to a surface due to a decrease in friction or roughness (Chase et al., 2016). Antifouling coatings can be used to change the chemical and mechanical properties of a substrate to prevent biofouling.

The most common type of biocide containing coatings are self-polishing copolymer (SPC), where biocides are released through a reaction of water with the coating polymer which is often a methacrylate polymer (Ciriminna et al., 2015) There are different biocides available, and the amount of biocide in a coating is often variable. Copper is the most commonly used biocide in antifouling coatings today, but before its ban in 2008, tributyltin (TBT) was a very common biocide (Bao et al., 2014; Magin et al., 2010). It was reported that TBT coatings had adverse effects on several mollusc species, such as inhibiting reproduction in Pacific oysters (IMO., 2020; Ytreberg et al., 2015). Zinc biocides are becoming more common as well are environmentally friendly biocides, which are often organic compounds that degrade quickly in the water (Mert et al., 2014).

Another type of coating used to prevent fouling is foul release coating (FRC). FRC works to reduce the attachment strength of biofouling such that it will slough off as hydrodynamic forces increase (Dafforn et al., 2011). These types of coatings are commonly made with hydrophobic siloxane-based materials, decreasing the attachment strength of fouling organisms (Stafslien et al., 2015). Ideally the attachment strength of the organism to the substrate would be low enough that the fouling would be released once the vessel is moving (Stafslien et al.,2015). Newer graphene-based nanomaterials are being examined as FRC for ship hulls (Selim et al., 2022).

In the following study, a variety of coatings were tested on wooden panels to determine their ability to prevent biofouling in NL marine coastal waters. SPC coatings containing copper, zinc and eco-friendly biocides were obtained as well as different FRCs. Since there are aquatic invasive species in NL, which also have negative effects on the aquaculture industry in other parts of Canada, the focus of this study was to determine if there was a coating which would prevent the spread of these species to aquaculture locations in NL.

## 2.2 Methodology

Twelve antifouling coatings were chosen for their preventative properties, different biocides, and physical properties. Table 2.1.1 provides a list of the different coatings that were employed in this study and the antifouling properties and components being investigated.

Table 2.2.1: Antifouling coatings compared and their antifouling properties, percentage of biocide by weight.

| **Antifouling Coating** | **Biocide or Non-biocide** | **Antifouling Property/Component** |
|---|---|---|
| Interlux BottomKote | Biocide | Cuprous oxide (25-50%) |
| Interlux Epoxycop | Biocide | Cuprous oxide (25-50%) |
| Interlux Micron CSC | Biocide | Cuprous oxide (25-50%) |
| Interlux Tri-Lux II | Biocide | Cuprous thiocynate (10-25%) |
| Interlux Micron CSC | Biocide | Cuprous oxide (25-50%) |
| Interlux Micron CF | Biocide | Econea®/ Zinc Pyrithione (1-10%) |
| ePaint ZO | Biocide | Zinc Pyrithione (1-5%) |
| ePaint Ecominder | Biocide | Zinc Pyrithione (1-5%) |
| Interlux Brightside | Non-biocide | Polyurethane-non antifouling |
| Matchless Super Marine | Non-biocide | Enamel- non antifouling |
| Hullspeed 3000 series | Non-biocide | Silicone fouling release |
| ePaint EP21 | Non-Biocide | Silicone fouling release |
| Control (no coating) | Non-Biocide | Wooden control |
| Control (no coating) | Non-Biocide | Wooden control |

The twelve coatings were compared to an untreated plywood control. Each coating was applied to a small wooden panel, 12.5cm x 12.5cm. The panels were hand painted using a paint brush. Two coats were applied to the front and back and the sides of each panel. Each coat was left to dry completely for twenty-four hours before the second coat was applied. All coatings, except for, Matchless Super Marine and Hullspeed 3000 series, were applied as recommended by the manufacturers without any additional treatment to the wooden panel. Hullspeed needed to be mixed with an activating agent before it was applied and was mixed according to the manufacturer's specification. Super Marine required that the wooden panel be sanded to ensure its adherence to the wood.

The same type of wood was used support panel, and all small, coated panels were attached to this support panel. Support panels were all treated with the same type of coating (Micron CSC Shark White). These support panels were coated by hand using a roller due to their larger surface area. For each support panel, there were fourteen different coating panels attached, one of each of the twelve different antifouling coatings and two control (untreated wood) panels. These panels were distributed randomly (Figure 2.2.1) on the support panel using a randomly generated numbering system. Each small panel was attached to the support panel using two plastic zip ties. Each support panel was given a different colored tag to distinguish it from the other support panels. Five units were deployed and randomly spaced, on wharves and floating docks, at each site. Bricks were attached to the bottom of each block to keep it submerged.

Figure 2.2.1: Twelve antifouling coatings that were applied to wooden panels and placed on larger wooden panels for deployment in Arnold's Cove, Placentia Bay, after being submerged for 3 months.

Four deployment sites were chosen based on the documented recruitment of invasive tunicate species and on previous research by McKenzie et al. (2016a, b). The sites, shown in Figure 2.2.2, were the Royal Newfoundland Yacht Club (RNYC), Manuels, Conception Bay, NL; Arnold's Cove (AC), Placentia Bay, NL; Little Bay (LB), Placentia Bay, NL; and Burin (Bu), Placentia Bay, NL. As mentioned above, in Newfoundland only three species of invasive tunicates have been discovered, some of these species overlap between the chosen deployment sites.

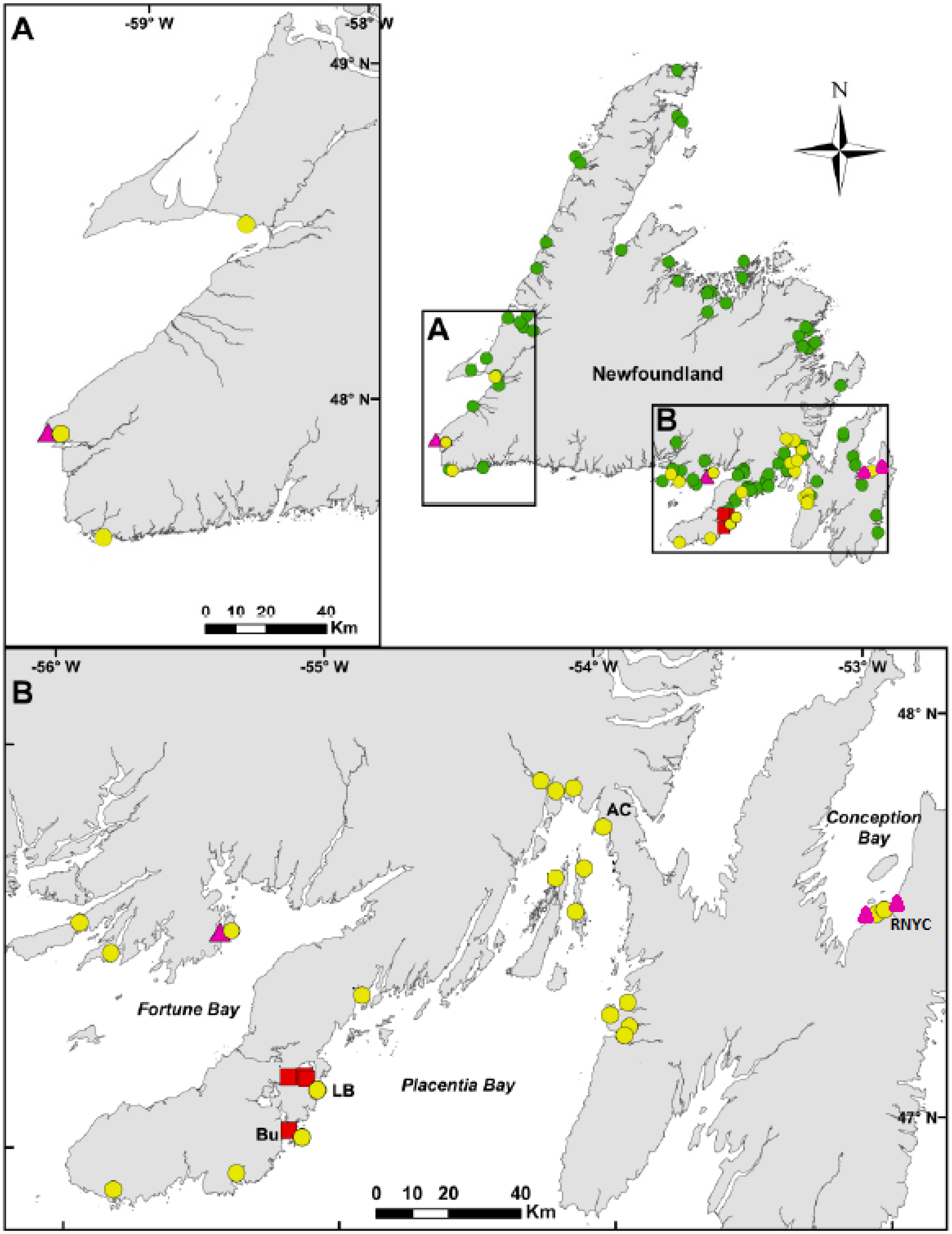


Figure 2.2.2: Studies sites, Royal Newfoundland Yacht Club (RNYC), Manuels, NL; Arnold's Cove (AC), NL; Little Bay (LB), NL; Burin (Bu), NL (Modified from McKenzie et al., 2016a).

To obtain the percent coverage of biofouling growth for each of the panels, photos were taken of the panels nine times during the field experiment. Photography of the coatings occurred monthly at each deployment site in the 2016 season and bi-monthly in the 2017 season. All units were left to overwinter (not removed from the water) at all sites between the two field seasons. Individual panel photographs were taken using a Nikon DS300, using a polarizing lens. Photos were also taken using a Nikon coolpix underwater camera with the aid of a diver; these photos were used to calculate the percent coverage of biofouling, since it gave a more accurate representation of how the organisms were growing on the panels, compared to when the panels were removed from the water. A GoPro was also used to take a video of the panels via diver; the diver would take a slow video of the panels stopping for a short period of 3-5 seconds in front of each before moving to the next coating; screen shots of these videos were used to calculate the coverage on the panel, if the other underwater photos were not of sufficient quality, owing to too much fresh water or other visual disturbances ( e.g. suspended particles).

The underwater photos were used to estimate percent coverage by macrofouling organisms, as described below. The images were processed Using GIMP 2.8.18 to give the image a flat perspective so that the coverage could be estimated using Image J (ver. 1.5i). The fouling was estimated manually in Image J by circling the fouling and using the calculated area against the total area to obtain a proportion estimate of biofouling on the individual panel, as in Equation 2.1.1:

Equation 2.2.1: Equation used to calculate the coverage of biofouling on each panel:

$$\text{Coverage}=\frac{\sum x}{\text{Total Area}}$$

where x is the area of the individual fouling species

The estimation of percent biofouling coverage included both native and non-native species as well as mobile (sea stars) and non-mobile species (tunicates). Coverage by aquatic invasive species (AIS) was also calculated as a total and by individual invasive species. Fouling by AIS was difficult to calculate after a period, since, in August of both years, algae started to grow on the panels (there were AIS under the blades of algae that could not be estimated from a photo). Therefore, even though there may have been AIS on a panel, a percent coverage could not be estimated. A such the presence or absence of AIS was recorded from divers' records and was used to complete statistical analyses.

Statistics were performed to determine if there was a significant change in the amount of biofouling coverage present on wooden panels painted with different coatings, at different times of the year and deployment sites. There were fourteen different coatings sampled over nine sampling dates and four different deployment sites. To determine if the relationship between percent coverage and coating type differed with sampling date, and the relationship between site differed with coating type, a two-part model with a binomial GLMM and GLM statistical model was used. The model which fit the biofouling data was a hurdle model (a two-part model). This model looked at site independently of the other variables. A binominal was used on presence/ absence (of biofouling organisms) data with respect to the different sites where the panels were deployed. At each site the number of successes (defined as any amount of growth on the panel for the purpose of this model) out of a total number of trials (N=14 panels) on each block within the sites was used to evaluate if there were differences between the sites (Equation 2.2.2 below). Then the individual successes were evaluated as their proportions with respect to coating type and time/month (Equation 2.2.3 below), since site was evaluated in this first part of the hurdle model.

Equation 2.2.2: Binomial model used to determine differences in percent biofouling coverage between deployment sites:

$$\frac{\text{Success}}{\text{Trials}} = e^{\eta} + \epsilon(\text{Binomial})$$

where $\eta=\beta_0+\beta_{site}$ X Site

Equation 2.2.3: Linear model use to determine relationships between time and coating type:

$$Coverage=\beta_0+\beta_{coating} \text{ X Coating}+\beta_{time} \text{ X time}+\beta_{paint*time} \text{ X Paint X Time}$$

**2.3 Results**

For the purpose of this study, biofouling included any organisms that were interacting with the surface of the experimental panels at the time the photo was taken of the surface (Figure 2.3.1). Using Image J (ver. 1.5i), a percent coverage was calculated. Golden Star tunicate was present and observed on individual panels in three of the sites, while Vase tunicate was only present and observed at two sites. A third species of tunicate, Violet tunicate, also present in Newfoundland, and does grow at the RNYC and was observed growing on rope, and kelp growing on the docks on the other side of the yacht club.

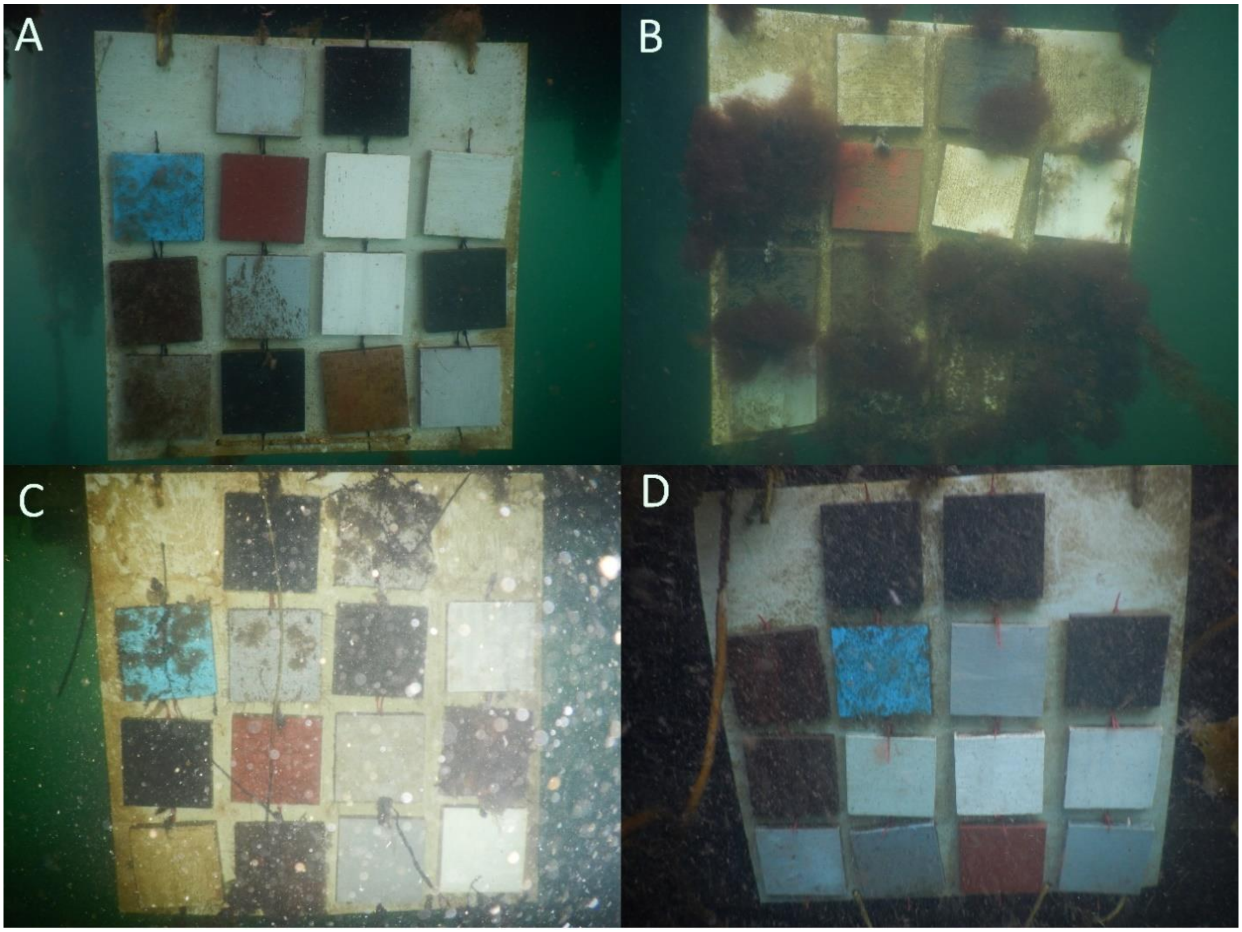


Figure 2.3.1: Surface of panels photographed under water after months of deployment, showing the attachment and growth of biofouling organisms at each site. A: Arnold's Cove, B: Royal Newfoundland Yacht Club, C: Little Bay, D: Burin.

There was a significant difference in the accumulation of biofouling among the sites (ANOVA, $p<0.05$). The difference of the coating types, as well as the relationship of growth over time were both significant (binomial, $p< 0.05$) for both). As shown in Figures 2.3.2 - 2.3.5, over time, there was an increase of biofouling growth on the different panels. Due to the differences in the chemical and physical properties of the coatings, some coatings started accumulating biofouling two to three months post-deployment while other coatings were deployed for over a year and over-wintered before biofouling began to accumulate. For statistical purposes, all coatings and all data were used, but to represent the data visually, one of each type of antifouling property/ component was chosen (e.g., copper biocide, zinc biocide, FRC, etc.).

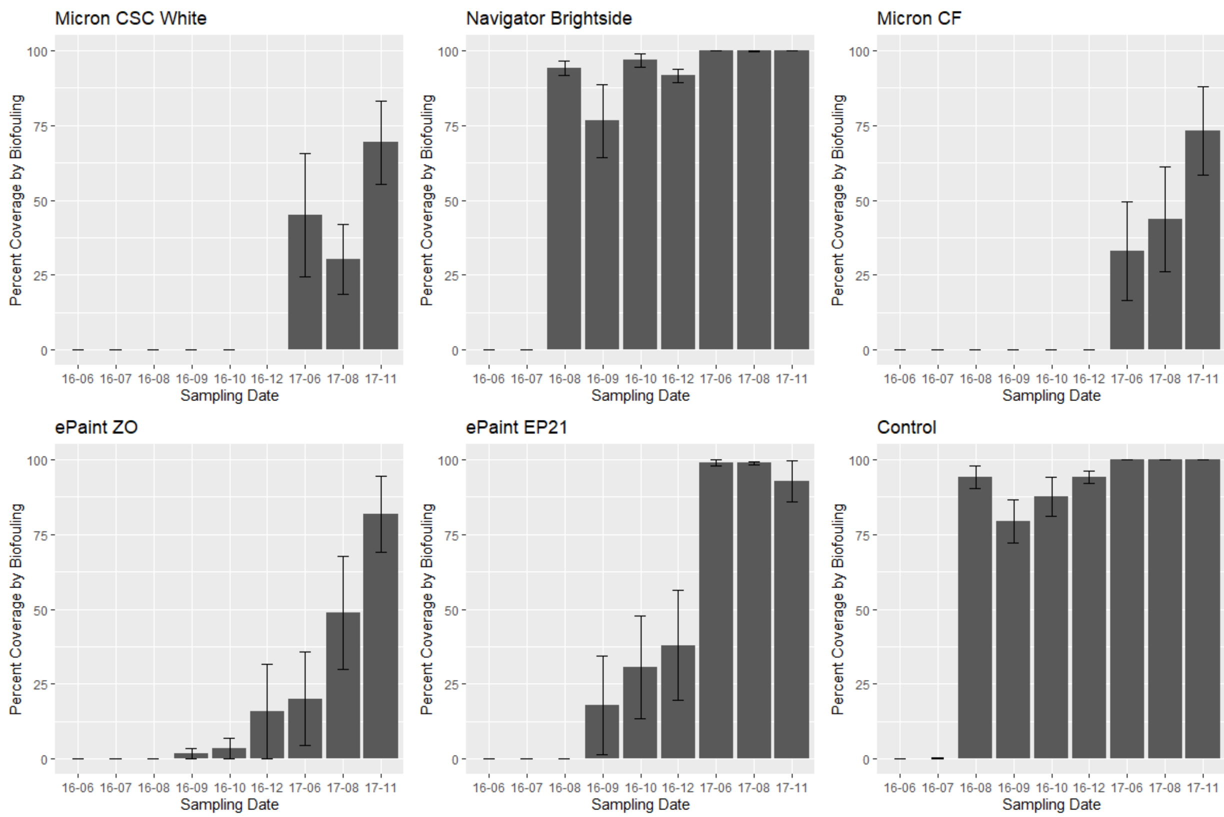


Figure 2.3.2: Total biofouling coverage of different types of coatings at the Royal Newfoundland Yacht Club, Conception Bay. The type of biofoulant coating is displayed in the top left-hand corner of each panel. The X-axis is represented as Year-Month (Y-M). Bars represent mean +/- S.E.M. of n = 5, n= 10 (control only).

Some non-antifouling coatings such as Navigator Brightside were fouled within the third month post-deployment with over 90% biofouling coverage by that time and 100% coverage by the end of the 17-month trial, at the RNYC. Coatings like Micron CSC, which contained copper biocide, did not accumulate fouling until after the over winter period, and by the end of the study had less than 70%

biofouling coverage. Micron CF was the only coating which contained the biocide Econea®, this coating also started to accumulate fouling in the second year of the field trial and was less than 75% covered with biofouling by the end of the field trial. FRCs such as EP21 showed accumulated biofouling in the first year of the study, with less than 40% biofouling coverage in the first year, but before the end of the trial the surface had been 100% covered with biofouling. Zinc pyrithione was another biocide tested in this study; Micron ZO accumulated a small amount of biofouling organisms late in the first field season (less than 15% coverage). In the second field season, it accumulated more fouling at each sampling period but only had approximately 80% coverage at the end of the 17 months. As for control panels (untreated wood), organisms fouled this substrate as early as the third month of the study, and fouling growth and coverage increased until 100% of the surface was covered, one-year post-deployment.

Information about the amount of biocide present in each coating was obtained from the safety data sheets. The coating BottomKote, contained 25-50% cuprous oxide by weight, which is more copper biocide than Tri-Lux II which only contained 10-25% cuprous thiocyanate by weight. Also, coatings that contained zinc biocides contained approximately 1-5% zinc pyrithione by weight. The biocide coatings, by recommendation from the manufacturer labels should be cleaned and/or repainted after one year in salt water. None of the coatings in RNYC or any site were cleaned or repainted; testing the limits of the coatings, though there were some coatings such as Micron CF that did not foul until a year had passed, suggesting that reapplying this coating yearly could potentially prevent biofouling growth.

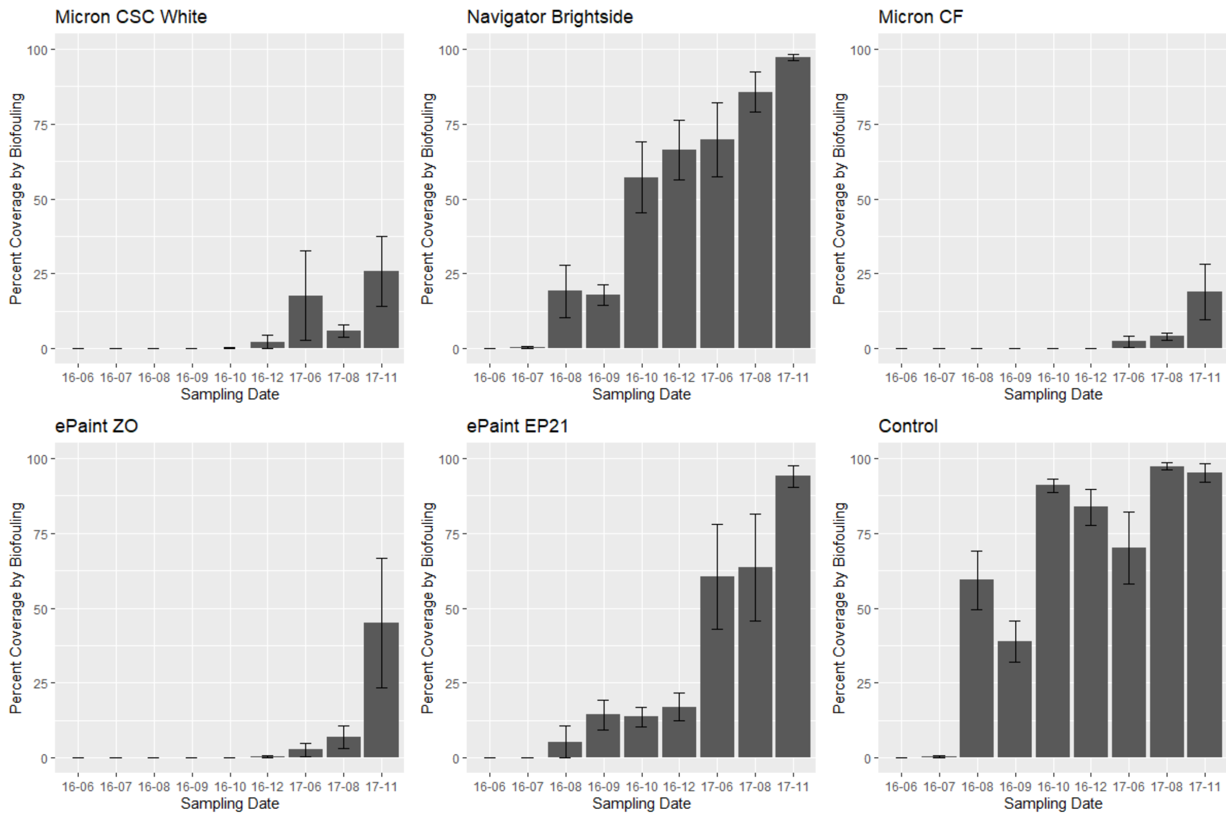


Figure 2.3.3: Total biofouling coverage of different types of coatings in Arnold's Cove, Placentia Bay. The type of biofoulant coating is displayed in the top left-hand corner. The X-axis is represented as Year-Month (Y-M). Bars represent mean +/- S.E.M. of n = 5, n=10 (control only).

Biofouling in Arnold’s Cove, Placentia Bay (Fig. 2.3.2), was very similar to biofouling at the RNYC (Fig. 2.3.3), Conception Bay, where the non-antifouling coatings, such as the control and Navigator Brightside, were the first coatings to foul since they had no chemical or physical properties to defend against biofouling organisms.  Micron CSC did deter fouling in the first year of deployment, and even in the second year the percent biofouling coverage was less than 30%.  Non-antifouling coatings, such a Navigator Brightside, had approximately 60-65% coverage by the end of the first deployment season and was 100% covered with biofouling by the end of the field study. Micron CF did not foul until the second year, with less than 25% coverage by November 2017. Zinc coatings like ePaint ZO also did not have fouling growth in the first year, and by the end of the trial it was less than 50% covered. FRCs fouled in both seasons of the field study but did accumulate approximately 90% coverage by the end of the second. But there were also coatings such as Micron CSC White that continued to have a lower amount of biofouling growth, less than 30%, during the duration of the deployment.

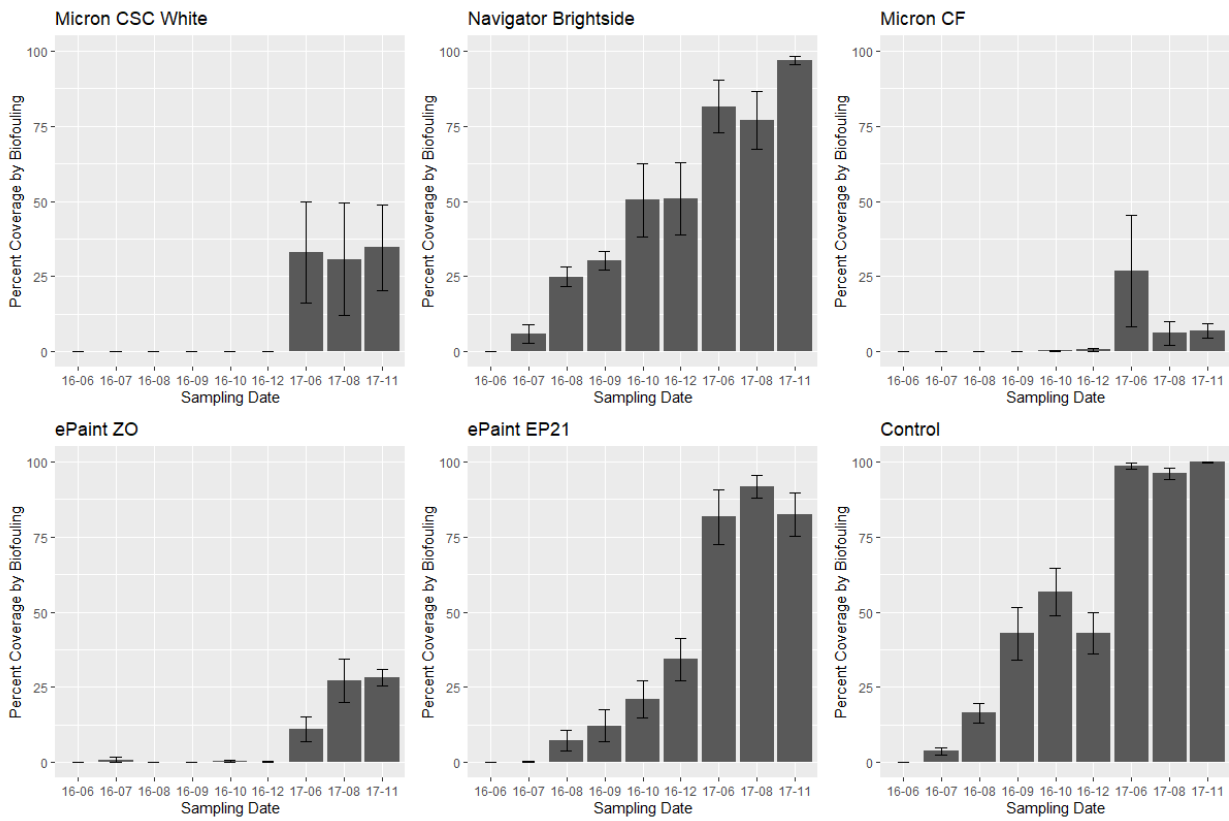


Figure 2.3.4: Total biofouling coverage of different types of coatings in Little Bay, Placentia Bay. The type of biofoulant coating is displayed in the top left-hand corner. The X-axis is represented as Year-Month (Y-M). Bars represent mean +/- S.E.M. of n = 5, n=4 (after June 2017), n=10 & 8 for control (before and after June 2017 respectively).

Growth in Little Bay, Placentia Bay (Figure 2.3.4) was more gradual, with higher amounts of growth occurring after a year of deployment. Non-antifouling coatings showed higher amounts of fouling than coatings that contained biocides such as copper but were very similar to foul release coatings by the end of the 17 months. Coatings such as Micron CSC, a copper-based biocide, had minimal growth in the first year of the field trials. Although they fouled early in the second growing season and stayed consistence during the sampling period, with approximately 35% coverage of the coated surface. Brightside, and similarly other non-antifouling marine coatings fouled within the first month of deployment and consistently accumulated biofouling, by the end of the study there was 96% growth on the panel. Micron CF, with the Econea® biocide, did not foul until after overwintering and biofouling did not exceed 26% of the surface. The zinc pyrithione coating, ePaint ZO, also did not foul until the second-year post-deployment and had less than 30% of its surface covered with biofouling by the end of the field trial. Unfortunately, the FRCs did develop biofouling in the first growing season but accumulated less than 35% coverage in the first year. In the second year the fouling quickly attached to these coatings with more than 90% coverage during that season. Little Bay was unique as, Fisheries and Oceans Canada had previously completed a mitigation study at that site (McKenzie et al., 2016b). The floating docks where the panels were deployed were treated with antifouling coatings, two of the same coatings that were used in the current study, Epoxycop and Micron CSC Shark White. Even though the mitigation was for Vase tunicate, measures taken to prevent growth of Vase tunicate would also prevent fouling by other organisms as well.

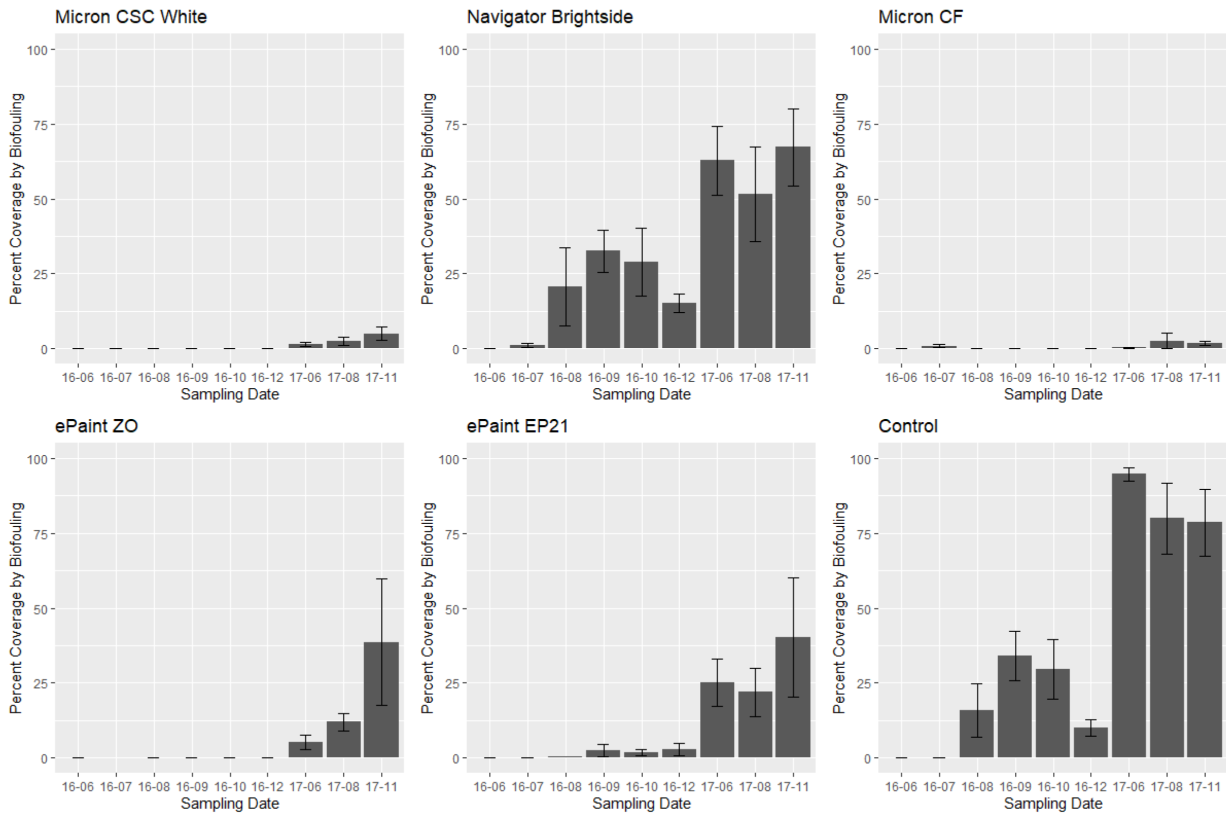


Figure 2.3.5: Total biofouling coverage of different types of coatings in Burin, Placentia Bay. The type of biofoulant coating is displayed in the top left-hand corner. The X-axis is represented as Year-Month (Y-M) Bars represent mean +/- S.E.M. of n = 5, n= 4 after June 2017, n= 10 and 8 for control (before and after June 2017 respectively).

Burin, Placentia Bay (Figure 2.3.5) was the only site where some experimental panels were attached to a large wharf as well as a floating dock. This was done based on personal communication with Fisheries and Oceans AIS team in St. John's, who noticed on their PVC settlement plates for AIS detection, there was a part of the main wharf where there was vast settlement by Vase tunicates. Therefore, it was suggested that this area should be covered as well, instead of just one small floating wharf that was very sheltered. The growth of fouling in Burin was similar to Little Bay, Placentia Bay, (where Vase tunicate was the dominant invasive tunicate species) in that most of the biofouling growth occurred after one year of deployment. Copper biocide-based coatings such as Micron CSC, had minimal percent coverage, even in the second growth season. Even at the end of the field experiment it had less than 5% of its surfaced fouled with organisms. Alternative biocide coatings, like Micron CF, also showed minimal fouling throughout the field study, with no more than 1.8% of the surface covered with biofouling. Non-antifouling marine coatings, such as Navigator Brightside, had growth occur within the first two months of deployment. By the end of the trial over 65% of the surface of the coating had accumulated fouling growth. The FRCs, such as ePaint EP21, also had growth in the first year, but within that time the growth was less than 1% of the surface area of the panel. By the last month of the trial, it had accumulated biofouling, but the surface was still less than 40% covered with biofouling organisms.

Overall, at all sites there was an increase in the amount of biofouling over time. There was also a trend among all sites, even though the growth was different at each site, such that coatings without antifouling properties had higher levels of percent biofouling coverage than coatings that contained a biocide. Coatings that had physical properties (e.g., FRC) to prevent fouling performed similarly to the wooden control. At most sites, Micron CSC (copper biocide) performed better than ePaint ZO (zinc biocide). Also, a trend among the sites was that the Micron CF, Econea® biocide, performed better than all other biocides. Therefore, the ranking of the biocides, in effectiveness to prevent biofouling, would be Econea®, Copper, and Zinc. The non-antifouling marine coatings did not prevent fouling, much the same as the wooden control panels. The FRCs, did not prevent the accumulation of biofouling over time on stationary structures. Therefore, on structures such as docks and very slow-moving vessels, biocide coatings would likely prevent biofouling growth more effectively.

Fouling at the RNYC site was the fastest growing, growing earlier than the other sites. Temperature data were recorded at each site using VEMCO data loggers, and RNYC appeared to have higher temperatures than the other sites (Figure 2.3.6), though there was not a statistically significant difference (ANOVA, p=0.98) in the temperature among the sites. RNYC was the most protected study site and the only site in a Conception Bay, which may explain the more rapid growth of fouling at this site. RNYC peak temperature in the 2016 field season was 17.9°C, only slightly higher than Arnold's Cove which was 17.8°C.

Temperatures at Little Bay and Burin for the same study season were 14.7°C and 13.9°C, respectively. In 2016 the peak temperatures all occurred at about the third month of deployment, about the same time a lot of the non-biocide coatings started to experience biofouling growth. In 2017, Little Bay had a spike in temperature earlier than the other sites (20.2°C) later in the season RNYC, Arnold's Cove and Burin had peak temperatures of 17.3°C, 16.8°C and 14.0°C respectively.

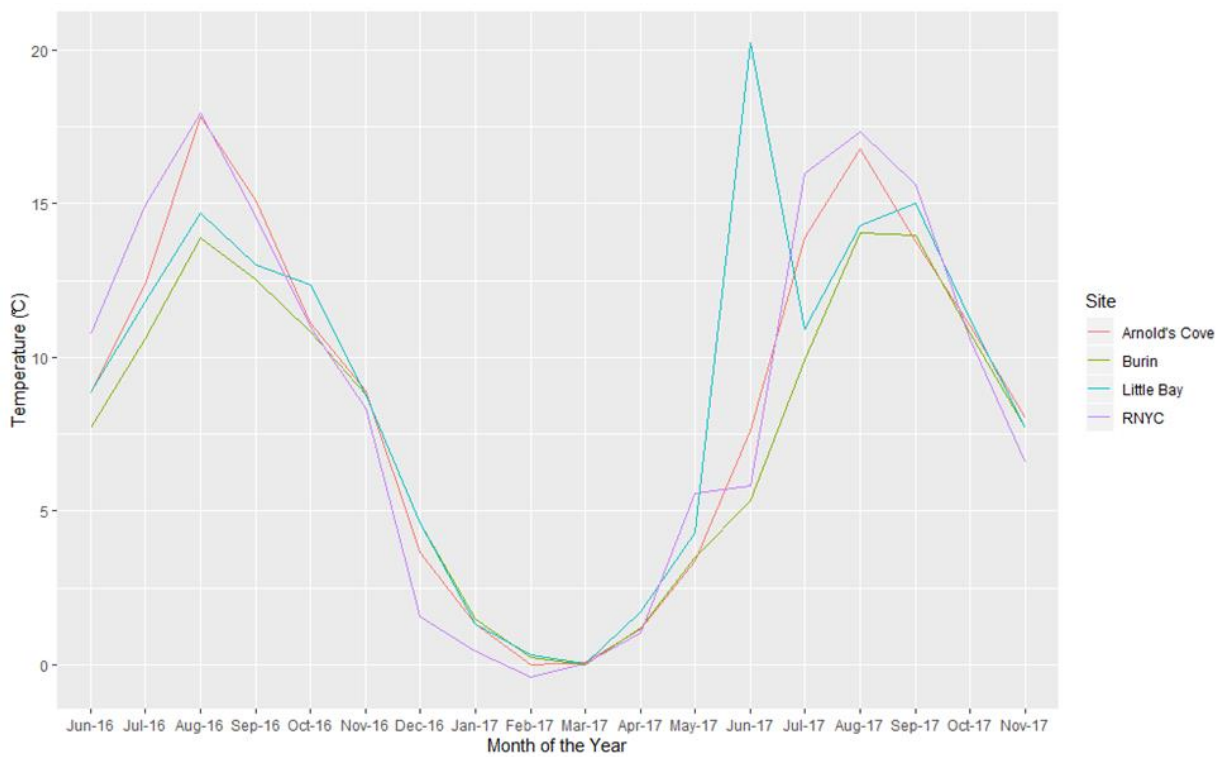


Figure2.3.6: Average monthly seawater temperatures for the deployment period of June 2016 to November 2017 for sites in Arnold's Cove, Burin, Little Bay, and the Royal Newfoundland Yacht club (RNYC). Data collected by Vemco immersion thermographs.

Most of the sites were similar in the progression of fouling settlement over the duration of the trial (see Appendix I for raw data). At RNYC, Conception Bay, Golden star tunicate was the first to settle on the panels, while at the other sites in Placentia Bay invasive species were amongst the last organisms to settle on the panels.

During the field trials, Golden star tunicate and Vase tunicate were recorded settling on the experimental panels. The same model was used on AIS data as it was on total biofouling, where the binomial component of the model which looks for differences in accumulation between locations, showed that there was a significant difference in the site with respect to coverage by AIS ($p<0.05$), this was expected due to the nature of the AIS at the different sites and how they attach. The colonial tunicate will attach itself and spread over the surface whereas Vase tunicates being solitary will attach at its base and grow away from the surface, therefore when measuring Vase tunicate, it is only the base that was measured and not the whole organism. Unlike total biofouling, none of the coatings were more effective at preventing AIS growth ($p> 0.05$) when compared to one another. There was a significant relationship with coverage with respect to time, as time increased there was increased growth by AIS on the panels ($p<0.05$). Because there was other biofouling growth at the same time as AIS growth, it was difficult to calculate a percent coverage for the coatings. The statistical model was based on presence/ absence data.

The RNYC had two types of tunicates present, Golden Star, and Violet tunicate, but only Golden Star settled on the panels. For coatings that did not have antifouling properties, Golden Star attachment occurred within one month of deployment, at an average water temperature of 10.7°C (Table 2.3.2). Coatings that did contain biocides were deployed for 5 to 6 months before AIS began to grow. After one year of deployment there were too many native fouling species accumulated on the surface to calculate growth by AIS alone, though AIS did continue to grow after this time.

Arnold's Cove only had one invasive tunicate species, Golden star tunicate. There were some coatings at this site that did not have any AIS settle (Table 2.3.2, BottomKote, Expoxycop) during the whole field trial. While other coatings such a Navigator Brightside had invasive species growth in August 2016, during the first field season.

Table 2.3.2: First record of aquatic invasive tunicate settlement on each of the different coatings at each of the sites.

| Coating | RNYC | Arnold's Cove | Little Bay | Burin |
|---|---|---|---|---|
| BottomKote | Sept 2016 | None | Nov 2017 | Nov 2017 |
| Epoxycop | Aug 2017 | None | Nov 2017 | Aug 2017 |
| Micron CSC (White) | Aug 2017 | None | Nov 2017 | Nov 2017 |
| Tri-Lux II | June 2017 | Nov 2017 | Nov 2017 | Nov 2017 |
| Micron CSC (Black) | Dec 2016 | None | Nov 2017 | June 2017 |
| Navigator Brightside | July 2016 | Aug 2016 | Sept 2016 | Sept 2016 |
| SuperMarine | Aug 2016 | Sept 2016 | Dec 2016 | Sept 2016 |
| Micron CF | June 2017 | None | Aug 2017 | Nov 2017 |
| ePaint ZO | Dec 2016 | None | Nov 2017 | Nov 2017 |
| ePaint Ecominder | June 2017 | Aug 2017 | June 2017 | Aug 2017 |
| Hullspeed 3000 | July 2016 | Aug 2016 | Sept 2016 | Sept 2016 |
| ePaint EP 21 | December 2016 | None | Aug 2017 | Nov 2017 |
| Control (wood) | July 2016 | Aug 2016 | Sept 2016 | Sept 2016 |

In Little Bay, Placentia Bay, NL there were two species of invasive tunicates, Golden Star, and Vase tunicate. Of these two species, Vase tunicate was the dominant fouler, being recognized as attached to the substrate a month earlier, as well as being present on more coatings than the Golden

Star tunicate. In December 2016, the average Golden Star accumulation on all panels was 1.2%, for Vase tunicate it was 1.7%. Since the solitary tunicate was the dominant and only the bases of these were measured, the percent coverage is similar than that of Golden Star where the entire organism was measured, even though Vase tunicate was observed on more coating panels than Golden star. As seen in Table 2.3.2 most of the growth by AIS in Little Bay occurred after the over-winter period, in 2017. Some panels such as the control did have AIS growth in 2016.

Vase tunicate was the only invasive tunicate reported in Burin, Placentia Bay, Newfoundland. At this site divers have observed large amounts of this tunicate growing on wharf pilings and other structures in the water (pers. comm., C. McKenzie 2018). Some of the areas where the panels were placed were heavy with tunicate settlement, more than 90% of the fouling observed was by Vase tunicates. While other areas, such as the floating docks, had less settlement, less than 85% was Vase tunicate settlement by the end of the field trial. Similarly, in Little Bay Vase tunicate mostly did not settle until the panels were deployed for a year, coatings which did accumulate fouling by Vase tunicate in 2016, saw less than 2% of the total fouling being Vase tunicate. In 2017 by the end of the season, panels which had Vase tunicate fouling had more than 20% percent coverage, with some panels such as the control being over 95% covered with Vase tunicates. (Table 2.3.2).

**2.4 Discussion**

Biofouling species, mussels, algae, and AIS can all cause issues within the aquaculture industry, either from competition for food, or space, or smothering organisms. They can create problems with the aquaculture species and infrastructure as explained in the introduction. They have cost aquaculture industries in other provinces millions of dollars to control the growth of these organisms (Cordell et al., 2013). Prevention and early detection provide an important advantage to prevent the introduction and spread of biofouling organisms, including AIS, to aquaculture sites in this province, as opposed to the cost associated with the control and mitigation of these species once, they are established.

Adams et al. (2011) conducted a survey in various parts of the United States for shellfish growers, to determine the type of biofouling control the growers utilized. There were many different controls used, which included mechanical and hand cleaning, including scraping/scrubbing/brushing, power washing equipment, gear cycling and fresh water washing (Adams et al., 2011). They also reported shellfish growers using brine and lime dips as a control method (Adams et al., 2011). For finfish culture in NL, nets are coated with an antifouling coating containing copper biocide or cleaned regularly using in-situ cleaners (personal communication NL Aqua Services, personal observations). The use of antifouling net coatings is common practice in other areas where finfish are cultured as well (Braithwaite et al., 2007). Fish farms often include regular net cleaning and changing routines to help combat biofouling on the cages (Braithwaite et al., 2007). If AIS have not been reported to settle on an aquaculture site there is an advantage of being able to prevent the transfer of these species to sites, rather than mitigating these species once they are on site. Therefore, a part of this study showed the effectiveness of the antifouling coating to prevent the settlement and growth of AIS.

Coatings which contain biocides, such as Micron CSC and Micron CF, work better to prevent growth of biofouling organisms than coating that do not contain chemical properties or components for biofouling prevention. Though Micron CF had the lowest percentage of biocide by weight, it still had the greatest effectiveness, less than 60% biofouling coverage at all sites.

The FRC used in this study were not used for their intended purpose. These coatings need a force, strong current and/or vessel movement, to remove the fouling organisms from the surface of the coating, which, due to the smooth and hydrophobic surface of the coatings, should remove the fouling with ease. On vessels that are moving frequently, foul release coatings may be effective at preventing fouling from being transferred from one location to another. That could not be demonstrated in this study as the panels that used FRCs were kept stationary, attached to a dock, and were not cleaned for the duration of the deployment, therefore based on these findings it would not be useful to apply an FRC to a stationary structure such as a dock.

Other coatings which can be purchased at marine stores are marine paints intended to be used on the deck of a vessel, and not on the hull (non-antifouling coatings). The above results show that these types of coating, do not prevent any form of fouling and perform similarly to untreated wood. The effectiveness of a particular coating is related to its chemical properties. Coatings which contain biocides may work better on stationary structures, as demonstrated in this study, compared to coatings which rely on a smooth surface and drag forces. But also, the type of biocide is important, as not all biocides are equally effective, and they may have different efficacy depending on the fouling species. Also, each coating has a different amount of biocide, so a coating with less biocide may not last as long as another containing higher levels.

Violet tunicate was located at only one site that was used for the field study. It was on the other side of the RNYC, Conception Bay, that due to the low infestation and short larval life stage, they did not migrate or attach to any of the experimental panels. Golden Star and Vase tunicates were the only invasive tunicate species which settled and therefore could be studied in the field trial.

There were also differences in the growth between the deployment sites themselves. Three sites were in Placentia Bay, Newfoundland and separated from each other, by as much as 128 km along the coastline. Little Bay and Burin are approximately 125 km and 128 km along the coast from Arnold's Cove, respectively. Burin and Little Bay are approximately 25 km apart along the coastline. The RNYC was in a different bay, Conception Bay. The difference in the growth pattern and rate between the two bays could be due to slight differences in temperature, depth of the bay, food availability and turbidity of the site, however other parameters other than temperature were not measured during the field experiment. It is also likely that there was a difference in the amount of protection, or how sheltered the panels were for settlement recruitment.

In Little Bay, the first signs of AIS occurred in September 2016 on wood, non-antifouling marine coating, and a foul-release coating. For zinc containing antifouling coatings, invasive species were noted in June and August of 2017. The copper biocides did not accumulate invasive species until the end, November 2017. In a previous study by Reid et al. (2016) it was demonstrated that *C. intestinalis* larvae may undergo pre-attachment metamorphosis. Therefore, if a substrate is not suitable to support growth and development of this organism, it is possible that it would choose not to settle on a toxic substrate such as copper antifouling paint. These organisms may choose to settle on wood, or another substrate while antifouling coatings still contain biocides. It is possible that after a biocide is completely leached out of a coating, after being in the water for a year, new larvae will settle on the then more suitable substrate.

Golden Star tunicate was the only tunicate present in Arnold's Cove, Placentia Bay. Throughout the field trial it was only reported on one copper biocide coating, Tri-Lux II, in November 2017 before the panels were removed. ePaint Ecominder was the only zinc-based coating that also had settlement by Golden star in August 2017. No other biocide-based coating reported AIS settlement. In August and September 2016 AIS was recorded on the non-antifouling coatings Navigator Brightside and Super Marine. For the foul release coatings, Hullspeed 3000 had Golden Star reported in August 2016, whereas ePaint EP21 did not have any reports of Golden Star. The control panel, which was just untreated wood, also had Golden Star settlement in August 2016 shortly after the panels were deployed. The settlement pattern of most Golden Star settling in August, was expected. In a study by Ma et al. (2017), it was reported that in Arnold's Cove, the recruitment window was from August to October. Also, from this previous research it was determined that settlement occurred mostly at 1 m depth, therefore all panels in this study were deployed at 1 m (Ma et al., 2017). Ma et al. (2017) deployed multiple substrates, PVC, aluminum, and wood, all of which are used for various aquaculture infrastructure. It was observed that recruitment was greater on PVC than on aluminium or wood. Wood was chosen for this research to represent the highest risk of introducing AIS to aquaculture sites in Newfoundland, wharves, and docks. Aquaculture sites, both finfish and shellfish, contain a lot of PVC material, therefore, if harmful invasive species can be prevented from being transported to these locations, then the higher recruitment on this type of substrate can be avoided.

In Burin, Placentia Bay, and the RNYC in Conception Bay, Vase tunicate and Golden Star tunicate were the only non-indigenous species reported, respectively. In both sampling sites, all panels had recruitment by the end of the field trials. RNYC had very early settlement by Golden star, with first settlement occurring in July 2016 on Navigator Brightside, Hullspeed 3000 and wood control. The last coating to be settled by golden star was Epoxycop and Micron CSC (white). In Burin Vase tunicate was first reported in September 2016 on Navigator Brightside, Hullspeed 3000 and wooden control. The last coatings to have invasive recruitment were BottomKote, Micron CSC (white), Tri-Lux II, ePaint ZO and ePaint EP21 in November 2017. At both sites, Micron CSC had a different settlement pattern based on colour. The black coloured coating had settlement by invasive species before the white colour coating, though overall there was no difference in settlement occurrence between the white and black colours of Micron CSC ($p>0.05$). These results support previous findings that suggest fouling species may be attracted to different colours, or brightness, darker colours being more attractive than lighter colours (Dobretsov et al., 2009; Ells et al., 2016; Satheesh and Wesley., 2010). Ells et al. (2016) noted that Vase, Golden Star and Violet tunicates showed no significant difference in settlement for colours blue, red and green. They did report that these tunicates did respond significantly to difference in substrate brightness, with lower settlement on lighter plates (Ells et al., 2016).

Even though there were significant differences in biofouling between sites, coatings that were not meant to be used as hull coatings did not prevent fouling effectively and had fouling covering over 80% of their surface by the end of the trial, very similar to the control wooden panels. Of all the copper biocide coatings, cuprous oxide-based paints performed better than cuprous thiocyanate (Tri-Lux II). The cuprous thiocyanate appeared to have a greater coverage than the cuprous oxide (Epoxycop, Micron CSC white) at the majority of the field sites. Coatings which contain toxic biocides worked to prevent biofouling for multiple months, but none worked more than a year, suggesting that vessels or other structures such as dock would have to be cleaned and recoated yearly.

There were variations in the performance amongst deployment of coatings containing cuprous oxide as the biocides. One cuprous oxide coating, Micron CSC white, had some of the lowest coverage by fouling at all four sites, with coverage below 75% at all sites. Econea®, the eco-friendly biocide in Micron CF, performed well in marine water, having biofoulant coverage below 60% at all sites. Coatings containing zinc pyrithione had varying performance between sites, with coverage below 80% at all sites. At all but one site, Burin, zinc pyrithione coatings accumulated more fouling coverage than coatings which contained cuprous oxide. This may have been the result of the nature of the fouling community, with Burin having more solitary fouling organisms present than colonial tunicate species.

The foul release coatings, ePaint EP 21 and Hullspeed 3000, did not prevent biofouling well in the trial. But these coatings were kept on stationary panels and were not cleaned during the duration of the study. These coatings are meant to be used on vessels that are moving frequently, such that when the vessel is underway the force of the water against the fouling would remove the organism from the hull. The use of these coatings on a stationary structure, such as a floating dock or wharf, would not be appropriate to prevent the settlement of biofouling.

## 2.5 Conclusion

Coatings which did contain a biocidal chemical, appeared to prevent biofouling more effectively and for a longer time than coatings that did not contain biocides. Among all sites, Micron CF had very little biofouling accumulation the first year (less than 5%) and by the end of the trial had less than 60% of the area settled by fouling organisms. Micron CF is a coating which contained the organic biocide Econea®. Copper biocides coatings had less than 75% of the surface covered by the end of the field trial, while zinc pyrithione coatings had higher accumulation than the copper biocides at most sites, but less than 80% coverage at the end. There was variation in the effectiveness of the different copper-based coatings as well as the zinc pyrithione based coatings, which could be explain with the variation of biocide (by weight) present in the different coatings.

There were also coatings which did not contain a chemical repellent or biocide. Fouling release coatings were a type of non-biocide coating that was used in this study. They did not prevent biofouling from attaching to the stationary substrate for more than one month post deployment, and by the end of the trial had completely failed to prevent biofouling over any of the surface area of the panel. This is important, as it demonstrates the lack of effectiveness these types of coatings would have on a stationary structure such as a dock or floating fixed platforms, such as feed barges used in the finfish industry. Coatings which use chemical deterrents would be better at preventing organism settlement and growth on these structures.

Another type of non-biocide coating was the non-antifouling coatings. Two coatings, polyurethane, and enamel were used to demonstrate that some marine coatings are not effective or recommended on the hulls of vessels, or on other submerged surfaces such as docks. These coatings did not prevent biofouling for even a full month of being submerged and showed no difference than the untreated wooden panel used as a control in the field experiments.

Recommendations from the results of this study would be to use a coating which contains Econea® biocide, such as Micron CF, which was the most effective at preventing biofouling in this study. If Micron CF or Econea® coatings are not available, copper coatings, such as Micron CSC are more effective than zinc pyrithione coatings like ePaint ZO. Polyurethane and enamel coatings are not effective at preventing the attachment of biofouling organisms and are not recommended to be used as a hull coating. Foul release coatings are not effective on stationary structures, a coating with a chemical biocide is more effective for this type of structure.

# Chapter 3: The importance of surface structure topography in antifouling coating applications

## 3.1 Introduction

Biofouling, the growth of unwanted aquatic organisms on submerged substrates, can create issues for different industries. For example, biofouling on vessels used in the shipping industry, causes more friction, thus increased fuel consumption for speed maintenance, and increased time dry docking to remove fouling (Chen et al., 2015; Perez et al., 2015). In the aquaculture industry, biofouling can reduce flow through netting used for fin fish pens, resulting in lower nutrient and waste exchange (Bazes et al., 2006; Braithwaite et al., 2007; Fitridge et al., 2012). Fouling organisms can also compete with shellfish for food, oxygen, and space, causing problems for shellfish aquaculture (Fitridge et al., 2012; Paetzold et al., 2012).

Antifouling technologies, in the form of antifouling coatings, work to prevent the settlement of biofouling species on submerged surfaces (IMO. 2020). Of the different types of antifouling coatings available, biocide-based coatings may be the most commonly used (Early et al., 2014; Wallström et al., 2011). These coatings rely on the use of chemical biocides to deter fouling organisms from settling on a surface. But there are other types of coating that rely on other properties, such as smoothness, to reduce attachment of organisms on a surface (Buskens et al., 2013).

Figure 3.1.1, a diagram modified from Rosenhahna. (2008) and Hellio and Yebra. (2009), explains how biofouling attaches to a surface. There are four main categories which are used; structure, mechanical, chemistry and polarity; chemistry and mechanical properties were explored in the previous chapter. In this chapter, structure of the surface will be studied in more detail. For structure there were three parameters, which included, the topology of the surface, porosity of the surface, and the pattern or periodicity of the surface. These surface parameters may help explain why some coatings may appear to prevent more biofouling than others.

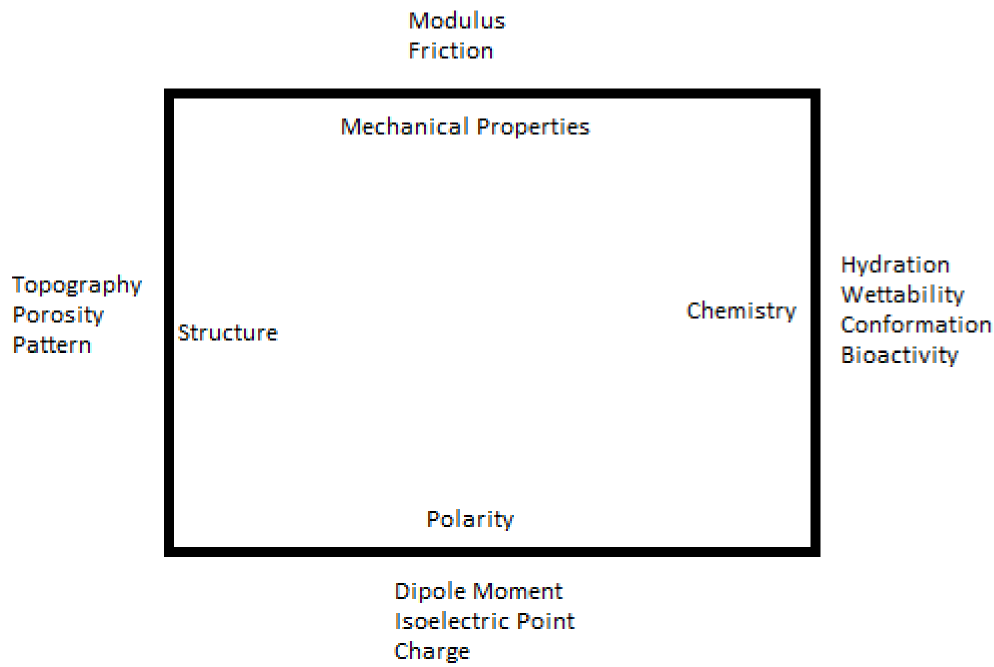


Figure 3.1.1: Diagram representing properties of antifouling coatings (modified from Hellio and Yebra., 2009; and Rosenhahna., 2008).

Settlement of fouling organisms is influenced by many different factors including, chemical cues, surface texture or type, surface energy/wettability and roughness (Brown et al., 2005; Callow et al., 1994; Dalhström et al., 2003; Holm et al., 1997; James et al., 1994; Rittschof et al., 1984). Surface properties are of interest, as they are based on the settlement mechanisms of the different fouling species. Organisms foul surfaces through the secretion of adhesive polymers, these interactions are determined within a few nanometres of the surface, therefore making the micro and nanostructure of a coating important (Smith., 2006). Kerr and Cowling. (2003) noted the influence of topography on attachment of bacteria; the almost instantaneous adhesion is influenced by imperfections on the surface at the nanometre scale, such that the size and shape of the imperfections is equal to the size and shape of the adhesion molecule.

Settlement of fouling species can be influenced by the complexity of the microstructure, micro-topographies of the surfaces. Textures that are similar to the size of the larva, promote settlement. Surface topographies smaller than the larva minimize the amount of attachment points available to the organisms, reducing the chance of settlement (Berntsson et al., 2000; Chen et al., 2015; Gribben et al., 2011; Whalan et al., 2015). Therefore, the topography of an antifouling surface is an important property, when determining the effectiveness of an antifouling technology.

Surface structure parameters include topography, porosity, and pattern. These pores and patterns act as the crevices, which, depending on shape and size can promote or deter settlement of antifouling coatings (Whalan et al., 2015). Using scanning electron microscopy (SEM), the surface topography, particularly patterns, or textures of the coatings, and the porosity of the coatings can be viewed and measured. SEM uses an electron microscope which produces images of a surface with the use of a beam of electrons. It is able to produce high resolution and high detail images of surfaces for further analysis. With the use of SEM images surfaces of materials can be studied at the micrometre scale. Working on the micrometre scale surfaces can be studied for parameters such as porosity and periodicity, as well as affording the ability to examine aspect ratio of any particles on the surface of the coating. Patterns on the surface and pores in the surface can be explained further with the use of aspect ratios of these particles or pores, which could help explain the shape and size of these surface structures (Rosenhahna., 2008). These structures can be important in determining the effectiveness of different antifouling coatings.

### 3.2 Methodology

To study the microscopic surface structure topography, SEM was performed, under laboratory conditions, on samples of the antifouling coatings. Paint was applied to small wooden crafting circles for SEM analysis. Two samples of each coating were imaged using a FEI MLA 650 FEG scanning electron microscope. Sample type A was a painted sample, where two coats of paint were applied via brush to the circles 24hrs apart. Sample type B entailed dipping the circles into the paint twice, 24hrs apart to allow for the first coat to dry. This method was used as a proxy to mimic spray applications used on some vessels or structures. Once the samples were completely dry, a month after painting, SEM was performed. There were 13 coating surfaces, 12 of which were coatings and one wooden blank control. The type of coating and the physical and chemical properties of each are shown in Table 3.2.1.

Table 3.2.1: Antifouling coating used for imaging and the antifouling property/ compound they possess.

| Antifouling Coating | Biocide or Non-Biocide | Antifouling Property/ Compound |
|---|---|---|
| Interlux BottomKote<br>Interlux Epoxycop<br>Interlux Micrcon CSC (two colours) | Biocide | Cuprous oxide |
| Interlux Tri-lux II | Biocide | Cuprous thiocyanate |
| Interlux Brightside<br>Matchless Super Marine | Non-biocide | No antifouling properties |
| Interlux Micron CF | Biocide | Econea®/ Zinc Pyrithione |
| ePaint ZO<br>ePaint Ecominder | Biocide | Zinc Pyrithione |
| Hullspeed 3000<br>ePaint EP21 | Non-Biocide | Foul release coating |
| Control | Non-biocide | Untreated wooden control |

One of each coating from sample A and B were mounted on the base using a double-sided carbon adhesive (Figure 3.2.1). The SEM was run at high vacuum 10,000 and spot 3.5 and the images collected were secondary electron images. For each sample an image was taken at 120X, 5,000X, 10,000X and 20,000X magnification. Images taken at 5,000X magnification were the most useful for determining the pattern and porosity of the surfaces. Along with the qualitative information collected, the aspect ratio for each particle or artefact on the surface was also calculated. A general linear model was used to investigate significance differences in the aspect ratio between the different coatings (Equation 3.2.1 below). Using Image J (ver. 1.5i) imaging software, the length and the width of each particle was calculated and then the ratio between them calculated (Rosenhahna et al. 2008).

Figure 3.2.1: Coating samples mounted on base ready for SEM imaging.

Equation 3.2.1: Linear model used to determine relationship between aspect ratio and coating type:

$$Aspect=\beta_0+ \beta_{coating} X\ Coating$$

Chapter 2 discussed the details of a field trial, where the twelve antifouling coatings were deployed at different deployment sites for seventeen months. The growth that accumulated on the panels during that time, was collected by placing the antifouling panels into individual bag with sea water to be transported that the laboratory. The biofouling organisms were removed from the panels and each organism removed was identified to the lowest taxonomic level possible.

### 3.3 Results

The aspect ratio was used to describe the shape of cavities on or in the surface of the paints. Figures 3.3.1 and 3.3.2 shows the aspect ratio for each sample type, A and B respectively. For each coating the aspect ratio was calculated for each feature, meaning there may be more than one aspect ratio for the coating depending on how textured the surface was. Some coatings had particles or patterns on the surface, others had pores in the surface and some coatings had both.

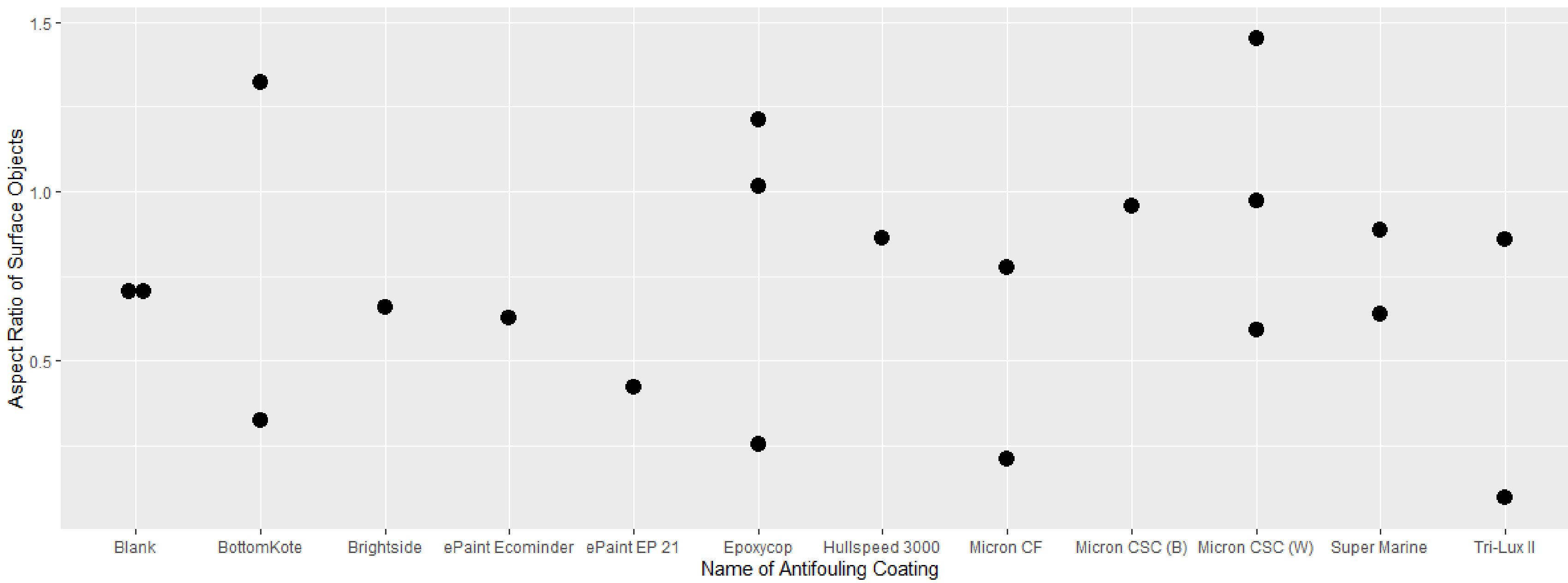


Figure 3.3.1: Plot of the aspect ratio of objects on the coating surface for each coating (sample type A brushed application) Micron CSC (B) is Micron CSC black and Micron CSC (W) is Micron CSC white.

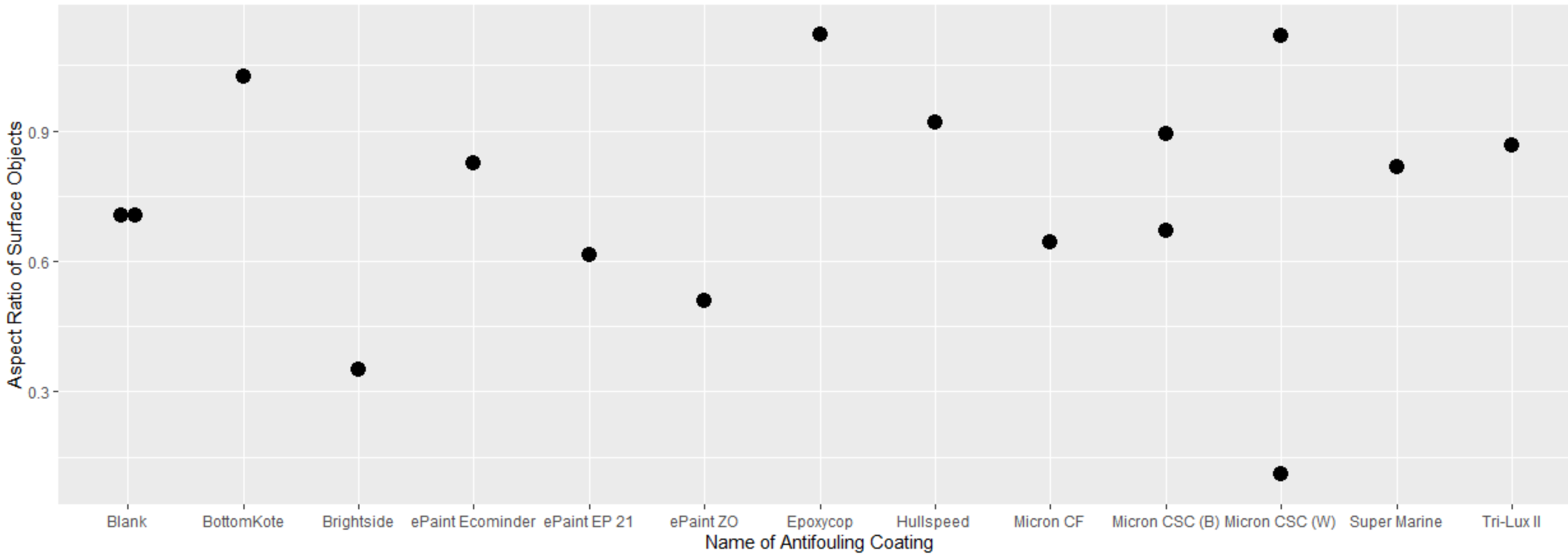


Figure 3.3.2: Plot of the aspect ratio of objects on the coating surface for each coating. (Sample type B dipped application). Micron CSC (B) is Micron CSC black, and Micron CSC (W) is Micron CSC white.

Aspect ratios were calculated at 5000X magnification, only ePaint ZO did not have an aspect ratio when the coating was applied with a paint brush. This was because the objects or texture were too small to calculate an aspect ratio. Some coatings had more than one aspect ratio because there was more than one type of texture or object on the surface of the coating. There was no significant difference in the aspect ratios between the coatings (ANOVA, p>0.0.05) when applied with a brush.  For dipped coatings, aspect ratios were also calculated at 5000X magnification. Some coatings had more than one aspect ratio because there was more than one type of texture or object on the surface of the coating. There were no significant differences in aspect ratios between the coatings (p>0.0.05), for this type of application.

Another view of the surface structure of the coatings came from looking at the SEM images. From these images the porosity and the pattern of the surface could be qualitatively described. The images at 5,000X magnification were the images that gave the most detail to make inferences about the structure of the coatings.

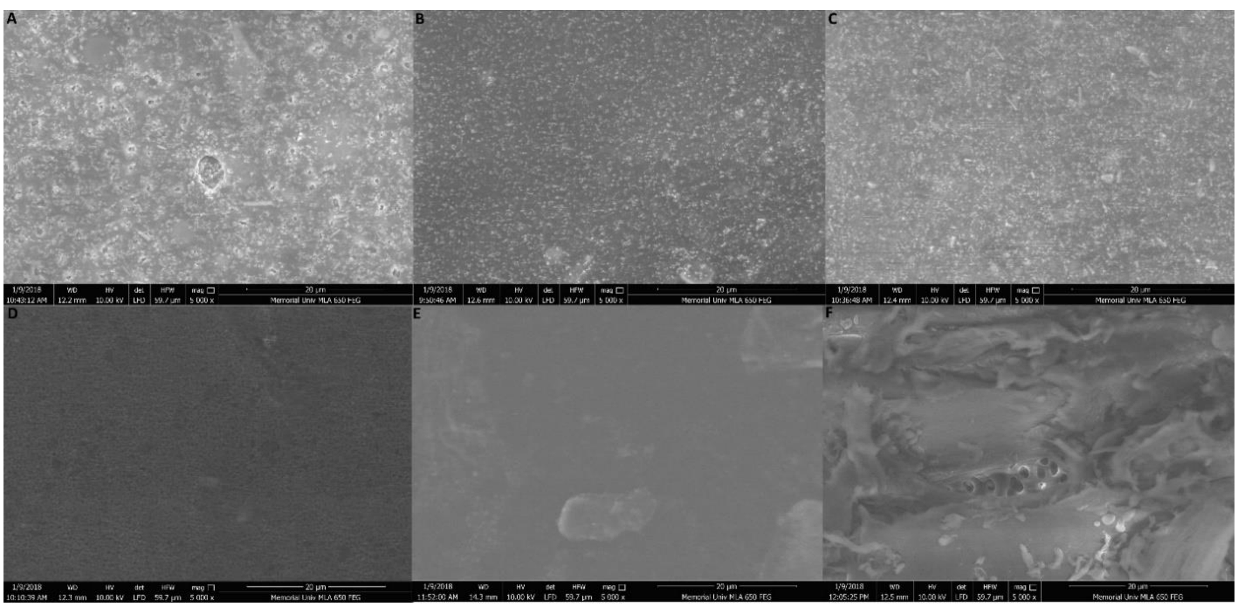


Figure 3.3.3: SEM images of coatings applied via brush. A-Micron CSC (white), B- Navigator Brightside, C-Micron CF, D-ePaint ZO, E-ePaint EP21, F- Wood.

MicronCSC Shark white (Figure 3.3.3A) showed that there were bubbles (AR=0.59) or pores appearing through the surface of the coating. There were also small particles (AR= 0.97) shining on the surface, that were non-uniform in shape and size, but average aspect ratio shows them to almost circular in shape. There was also a large bubble in the middle of this image. Small pores were not uniform in size or shape, some were spherical, and others were elongated. Figure 3.3.3B showed many tiny specks over the surface of the coating, creating a textured pattern. Some of these specks were too small to measure, therefore they were not uniform in size, and from the measurements they were not uniform in shape either (AR=0.65). Figure 3.3.3C (Micron CF) showed rods throughout the coating, that were long and thin (AR=0.21). There were small particles all throughout the coating that were uniform in size and shape (AR=0.78). Both these particles create a pattern on the surface of the coating.

When ePaint ZO (Figure 3.3.3D) is applied with a brush, the coating was covered in many tiny particles on the surface. These particles were too small to measure at this magnification. For ePaint EP21, (Figure 3.3.3E) applied with a brush, there was a large irregular shaped particle on the coating (AR=0.42). There was only one full particle shown in the image, and there appeared to have been a couple more at the edge of the image. Other than these sparsely occurring particles, the coating appeared to be smooth, which was expected for a foul release coating. Figure 3.3.3F shows the wood control. The surface of wood was very textured, there was a wave like pattern in the wood as well as pores (AR=0.72) of various shape and size in addition to some particles on the smoother part of the wood which were irregular in shape and size (AR=0.69). Pores were non-uniform in shape and size. Particles were irregular in shape and size.

Most of the other copper-based coatings had similar surface topography to Micron CSC (white), containing both pores and objects on the surface, though the size of the pores varied between the different coatings. Epoxycop was an exception, it had a very smooth surface when it was brushed onto the substrate. SuperMarine was also very similar visually to Brightside, as they are both non-biocide marine coatings. The other zinc pyrithione coating also had a surface topography that was comparable to ePaint ZO, above. When EP21 was brushed, it had a smooth surface, but Hullspeed 3000, was very textured, with objects on the surface, when applied with a paint brush.

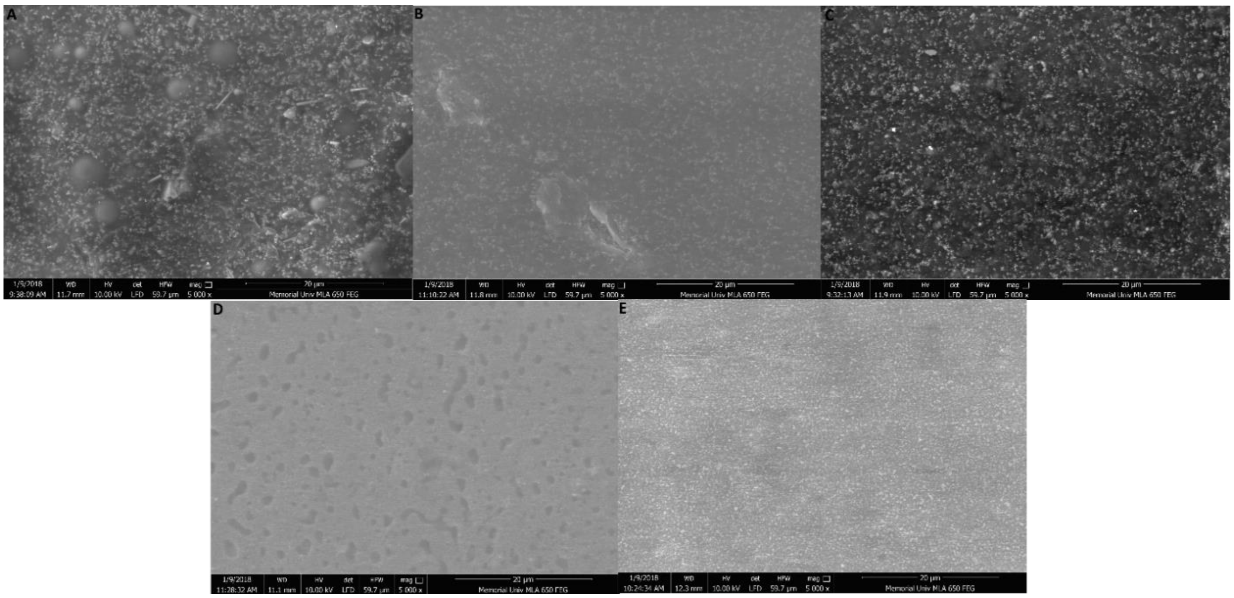

Figure 3.3.4: SEM images of coatings applied via dipping. A-Micron CSC (white), B- Navigator Brightside, C-Micron CF, D-ePaint ZO, E-ePaint EP21.

The dipped application of Micron CSC (white) had a lot of surface texture at 5000X magnification (Figure 3.3.4A). There were a lot of circular particles (AR=1.12) on the surface as well as some rods (AR=0.11). There appeared to be some pores in the coating, but they were not in full focus in the image. Navigator Brightside coating showed blemishes on the surface (AR=0.13), these were irregular in shape but were similar in size (Figure 3.3.4B). There were also tiny specks in the image that were too small to measure at this magnification, creating a texture pattern on the surface, after being dipped into the coating. Micron CF (Figure 3.3.4C) showed many small particles over the entire surface of the coating. These particles are very small and are uniform in size, though they had a slight variation in shape (AR=0.64). These small particles continued over the full surface of the coating.

ePaint ZO (Figure 3.3.4D) when it was dipped it had a lot of pores over the surface (AR=0.51). These pores were irregular in shape and non-uniform in size. There were many tiny particles over the surface of ePaint EP21 (Figure 3.3.4E), these particles were similar in shape and size (AR=0.61). These particles were very small and did not appear to create a large textured pattern.

Most of the copper-based coatings were very similar visually, having texture on the surface containing circular and rod-shaped particles. Epoxycop also had texture on the surface when the coating is applied by dipping the substrate into the paint. Other non-antifouling coatings, like Super Marine, had very similar texture as that of Brightside, with very small objects over the surface. ePaint Ecominder, zinc pyrithione, was more textured with more pores, than ePaint ZO. The fouling release coating, Hullspeed 3000, was very smooth when it was dipped, which differed visually from the coating ePaint EP21, which had very small, tight texture on the surface.

For the purpose of this thesis, porosity and pattern along with chemical makeup of the coating was used to attempt to explain the difference in effectiveness of the coatings at preventing biofouling growth. Figure 3.3.5 showed the growth of fouling on each coating over the duration of the field experiment. In the field experiment, discussed in the previous chapter of this thesis, all coatings were applied to wooden panels and deployed at four different sites. Three of these sites were in Placentia Bay NL (Arnold's Cove, Little Bay, and Burin) and one was in Conception Bay NL (Royal Newfoundland Yacht Club). There was a difference in the biofouling growth between the sites. There was also a difference in growth over time and between the different coatings. In this section, only the difference between the coatings will be discussed with respect to the surface structure of the coatings analyzed from SEM images above, as the difference in the effectiveness to prevent biofouling settlement on the coatings was discussed in Chapter 2 of this thesis.

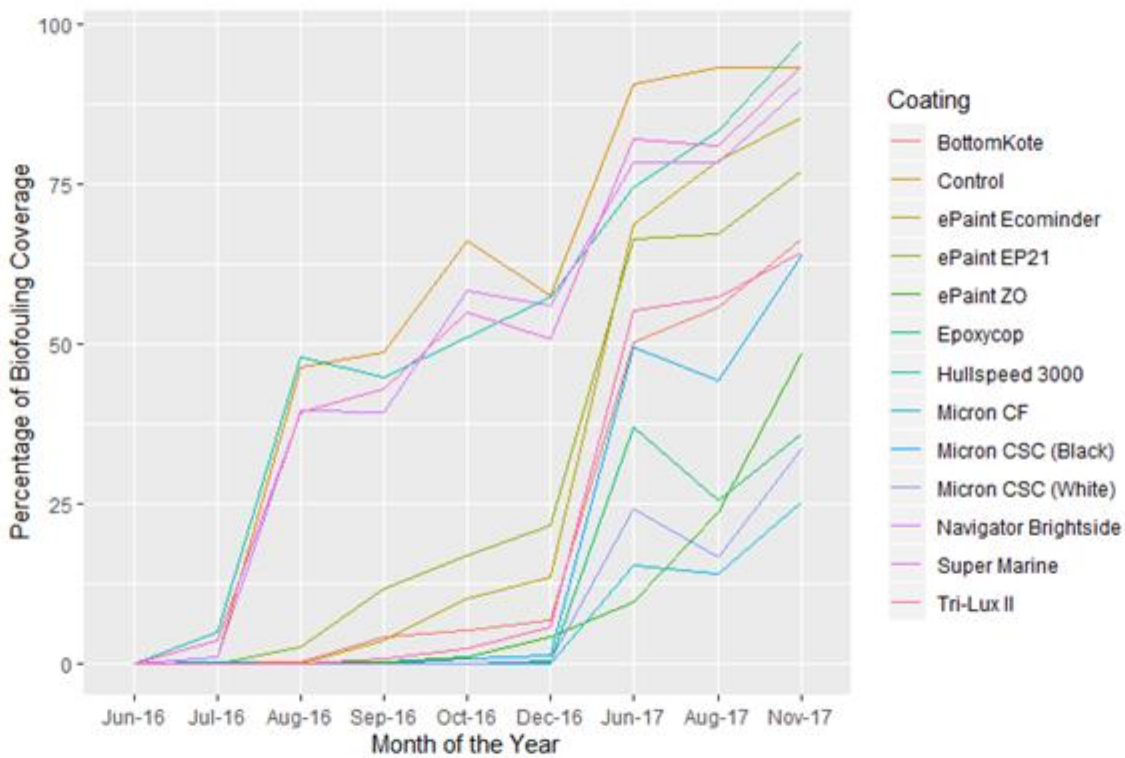


Figure 3.3.5: Percent biofouling coverage of each antifouling coating over a 17-month field trial.

Most of the coatings, whether they contained a biocide or not, had a pattern or texture on the surface whether they were applied with a brush or were dipped into the coating. Coatings such as Micron CF and ePaint Ecominder showed the same pattern when they were applied by both methods. When the coatings were applied via brush Tri-LuxII, Micron CSC (Black) and ePaint ZO had pores on the surface without a textured pattern. ePaint EP21 was mostly smooth when applied with a brush, with not many particles on the surface to create a pattern. The rest of the coatings did have a textured pattern from this application method. Brush application is the most applicable to the field experiment discussed in the previous chapter.

Surface texture is not the only property which attributes to an antifouling coating's effectiveness, chemical, mechanical, and other properties contribute to the make up of a coating. The relative biofouling accumulation with respect to surface texture warrants further discussion. For instance, the results of the previous chapter demonstrated that Hullspeed l had the highest amount of biofouling growth, on average, at the end of the field trial (97%). Hullspeed had large globular particles creating a pattern on the surface of the coating. SuperMarine, Wood (control panel) and Navigator Brightside also had large amounts of biofouling growth, on average 94%, 93% and 91%, respectively. Both Navigator Brightside and SuperMarine had very tiny particles on surface creating a pattern, while the controls (wood) had a wave-like pattern and pores in the surface structure. Ecominder had small particles over the surface, creating a textured pattern; it was the same for both application methods. ePaint EP 21 was a foul release coating; when it was applied by brush it did apply relatively smooth, but it still had accumulated biofouling during the field trial (79% biofouling coverage). Micron CF had small particles over the surface similar to that of Ecominder, but on average it accumulated the least amount of biofouling during the field trial. Micron CF had an average percent coverage of 33% at the end of the field trial whereas Ecominder had 85%. Because some of these coatings contained biocides and some did not, and there was a difference in the type and amount of biocide in the different coatings, it was impossible to rely on one property of antifouling coating, or surface structure, to explain the difference in their effectiveness to prevent biofouling.

When coatings were applied via dipping the substrate into the paint there were some differences in how the coating structure looked compared to brush application. Micron CSC (White) had some pores in the structure when brushed, but when dipped it did not have any pores. With Micron CSC (Black) the pores in the coating appeared and measured smaller when dipped compared to brush applications. ePaint EP21 was mostly smooth when applied with a paint brush but had a textured pattern when applied by dipping the substrate into the paint. The opposite was seen for Epoxycop, it was smoother when dipped into the coating material and more textured when it was applied using a paint brush, which was also true for Hullspeed 3000.

Colour or brightness of a substrate can act as a cue for settling organisms. Ells et al. (2016) noted that tunicates such as *Ciona intestinalis* (Vase tunicate) and *Botryllus schlossseri* (Golden Star tunicate), showed significantly less settlement on bright substrates than darker substrates. Other fouling organisms follow the same pattern, settling on black substrate over white in trials (Dobretsov et al., 2016). In this experiment, there were two shades of the same coating which were used to investigate any differences in settlement due to color or brightness. Micron CSC, in both white and black were used at each site. Over the duration of the trial, it was determined that there was no significant difference in the settlement of Micron CSC white over that of Micron CSC black (GLMM, binomial, $p>0.05$). The lack of significance may be attributed to the differences in fouling organisms, especially tunicate species, present at different sites.

The SEM analysis above shows the microstructure of the coating and not the nanostructure. The nanostructure, which is on a molecular level, would be useful for determining which type of biofouling organisms settled on which type of coating, though important to note would also be the antifouling property of the coatings to strengthen this argument. Nevertheless, the organisms that settled onto the coating were noted at the end of the field trial, for each of the different sites: Royal Newfoundland Yacht Club (Conception Bay), Arnold’s Cove, Little Bay, and Burin (Placentia Bay). The organisms identified to have settled to the panels are in Tables 3.3.1-3.3.4 (obtained from Results of Chapter 2). The taxonomic identification of the organism was completed to the lowest taxonomic level possible. Algae was identified into types (red, green, brown), from photographs taken during the field trial, when possible. For all other organisms some level of identification was provided.

Table 3.3.1: Identification of biofouling organisms at the Royal Newfoundland Yacht Club (RNYC), X indicates the presence of the species.

| | | Fouling Species | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mussels *Mytilus sp.* | Tube worm Terebellidae/ Trichobranchidae | Scale worm *Lepidonotus sp.* | Golden Star Tunicate *Botryllus schlosseri* | Clam *Macreidae sp.* | Bryozoan *Bugula sp.* | Flat worm *Nereis sp.* | Red algae | Algae (general) |
| Coating | BottomKote | X | X | X | X | | | | | X |
| | Epoxycop | X | | | X | | | | | |
| | Micron CSC (white) | X | | | | X | | | X | |
| | Tri-Lux II | X | X | X | X | | X | | | X |
| | Micron CSC (black) | X | | X | X | | | X | | |
| | Navigator Brightside | X | X | | X | | | | | X |
| | SuperMarine | X | X | X | X | X | | X | X | |
| | Micron CF | X | | X | X | | | X | X | |
| | ePaint ZO | X | X | | X | X | X | | | |
| | ePaint Ecominder | X | X | X | X | | | | X | |
| | Hullspeed 3000 | X | X | | X | | | | X | |
| | ePaint EP 21 | X | X | | X | | | X | | X |
| | Control | X | X | X | X | X | | | | |

Table 3.3.2: Identification of biofouling organisms in Arnold's Cove, X indicates the presence of the species.

| | | **Fouling Species** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mussels *Mytilus sp.* | Scale worm *Lepidonotus sp.* | Golden Star Tunicate *Botryllus schlosseri* | Branched Bryozoan *Bugula sp.* | Sea Grape *Mogula sp.* | Colonial Bryozoan *Electra sp .* | Jingle Shell *Anomiidae* | Sea Star *Asterias sp.* | Red algae | Algae (general) |
| **Coating** | BottomKote | | | | | | | | | | X |
| | Epoxycop | | | | | | | | | | X |
| | Micron CSC (white) | | | | | | | | | | X |
| | Tri-Lux II | | | X | X | | | | X | | X |
| | Micron CSC (black) | | | | X | | | | X | | X |
| | Navigator Brightside | | X | X | X | X | X | X | | | X |
| | SuperMarine | X | X | X | | | X | X | | | X |
| | Micron CF | | | | | | | | | | X |
| | ePaint ZO | | | | | | | | | | |
| | ePaint Ecominder | | | X | X | | X | | X | | X |
| | Hullspeed 3000 | | | | X | X | X | X | X | | X |
| | ePaint EP 21 | X | | | X | | | | X | | X |
| | Control | X | | | X | | X | | X | X | X |

Table 3.3.3: Identification of biofouling organisms in Little Bay, Placentia Bay, X indicated the presence of the species.

| | | **Fouling Species** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mussels *Mytilus sp.* | *Flat worm Nereis sp* | Scale worm *Lepidonotus sp.* | Vase Tunicate *Cinoa intestinalis* | Coffin Box Byrozoan *Membranipora membranacea* | *Clam Macreidae sp.* | Sea Grape *Mogula sp.* | Jingle Shell *Anomiidae* | Sea Star *Asterias sp.* | Green algae | Red algae | Algae (general) |
| **Coating** | BottomKote | X | | | | | | | | | | | X |
| | Epoxycop | | | | | | | | | X | | | X |
| | Micron CSC (white) | | | | | | | | | | | | |
| | Tri-Lux II | X | | | X | | | | | X | | | X |
| | Micron CSC (black) | X | | | X | | | | | | | | X |
| | Navigator Brightside | X | X | X | X | | X | | X | X | | | X |
| | SuperMarine | | X | X | X | X | X | | X | | | | X |
| | Micron CF | X | | | | | | | | | | | |
| | ePaint ZO | X | | | X | | | | | | | X | X |
| | ePaint Ecominder | X | X | X | X | | X | X | | X | | | X |
| | Hullspeed 3000 | X | | X | X | | | | X | X | | | X |
| | ePaint EP 21 | X | X | X | | | | | | X | | | X |
| | Control | X | X | X | X | | | | | X | x | | X |

Table 3.3.4: Identification of biofouling organisms in Burin, Placentia Bay, X indicates the presence of the species.

| | | Vase Tunicate *Cinoa intestinalis* | *Clam Macreidae sp.* | Jingle Shell *Anomiidae sp.* | Green algae | Algae (general) |
|---|---|---|---|---|---|---|
| Coating | BottomKote | | | | X | |
| | Epoxycop | | | | | X |
| | Micron CSC (white) | | | | | |
| | Tri-Lux II | X | | | | X |
| | Micron CSC (black) | X | | | | |
| | Navigator Brightside | X | | | | X |
| | SuperMarine | X | | | X | X |
| | Micron CF | | | | | |
| | ePaint ZO | X | | | | |
| | ePaint Ecominder | X | | | | |
| | Hullspeed 3000 | X | | X | X | X |
| | ePaint EP 21 | X | | | | X |
| | Control | X | X | | | X |

Each site had a unique fouling community. Mussels (*Mytilus sp.*) were present on panels at three of the sites, but it was confirmed by the dive team that mussels were growing in areas at all the sites. At the RNYC, in Conception Bay, Golden Star tunicate grew on most of the panels, as well as different types of worms, which may have been a result of the sediment build up on the panels. In Arnold's Cove Jingle shells (*Anomiidae* sp.) were among the fouling species as well as some colonial and branched bryozoans *(Electra sp.,* and *Bugula sp.* respectively). Both Little Bay and Burin, Placentia Bay, had vase tunicate as a major fouling species. Another invasive species was attached to Super Marine in Little Bay, Coffin Box Byrozoan (*Membranipora membranacea*). This bryozoan was not found growing on any other panels at this site or any other site, though it has been reported growing at all the sites and observed by divers. Burin had extensive growth by vase tunicate species which fouled almost the full panels, not leaving room for other fouling organisms, this is possibly the reason this site does not have a large biodiverse fouling community on the panels. Each of these species would use mechanism of adhesion to attach to a surface (Chen et al., 2015; Kerr and Cowling. 2003; Smith. 2006; Whalen et al., 2015). For the coatings, which were painted using a brush, to have accumulated biofouling these organisms must have had a positive interaction with the surface, meaning that their adhesive mechanism must have fit the shape and the size of the pores and other textures which made up microtopography of each coating.

**3.4 Discussion**

Biofouling is an important consideration for industry, particularly in the aquaculture industry fouling organisms can cause problems, with the farmed animals and the farm infrastructure (Adams et al., 2011; Fitridge et al., 2012; Floerl et al., 2016; Guenther et al., 2011). Antifouling coatings are important to prevent the spread of biofouling species to aquaculture sites in Newfoundland and Labrador. To understand how antifouling coatings work, it is important to understand how biofouling occurs, when a surface is submerged in marine environments. Chemical, biological, and physical events occur, on the surface of the substrate, where a complex community of micro and macro-organisms attach to the surface (Kerr and Cowling. 2003; Martín-Rodrígues et al., 2015). Therefore, an important aspect for antifouling coating performance is surface topography.

Smith. (2006) noted that fouling organisms, foul surfaces through a secretion of adhesive polymers, which occurs on a nanoscale. The attachment of marine bacteria to a surface is almost instantaneous; the adhesion to these bacteria is strengthened by imperfections on a nanometre scale, meaning that the size and shape of an imperfection can be similar to the size and shape of the molecules that an organism uses to attach to a submerged substrate (Chen et al., 2015; Kerr and Cowling. 2003; Whalen et al., 2015). If settlement of organisms occurs on a nano or microscale, then it is important to study the micro and nanostructures of antifouling coating systems.

The surface structure of a coating would include any roughness, patterns, pores or any changes in size and shape of the surface. Rougher surfaces tend to foul more quickly than smooth surfaces (Kerr and Cowling. 2003). Rosenhahna. (2008) included topography, porosity, and periodicity as examples of physical parameters which could change the effectiveness of an antifouling system. These parameters are under surface characteristics. There are other properties such as chemical, mechanical and polarity which also need to be accounted for inclusively to determine the effectiveness of an antifouling coating. In this chapter, the micro-structure of the coatings used in this experiment were discussed based on their texture or periodicity and porosity, though the use of SEM imaging.

Without knowing the nanoscale structure of the antifouling coatings and the molecular makeup of the adhesive mechanisms of the above organisms, the interaction of these organisms and the surface cannot be discussed in detail. From the SEM images, the surface of coating applied with a brush, which was the application used for the field study, appeared to have resulted in more pores and patterns present than when the substrate was dipped into the paint and left to dry. These patterns, grooves, and pores could have provided more ideal location for larva to settle. At the end of the trial, if any antifouling chemical properties were still active, they would have been weak. Most coating manufactures suggest recoating vessels every year (in November 2017, the panels deployed in the study from Chapter 2, would have been deployed for seventeen months). Therefore, any imperfections in the surface structure such as texture patterns or pores would be utilized by the organism for settlement.

There are some physical structures which prevent fouling settlement in nature. Laser abrasion and photolithography are used to produce microtextured surface that mimic the surface topography of mollusc shells and shark skin, since these materials naturally inhibit fouling growth naturally (Lejars et al., 2012; Maréchal et al., 2009). These materials and properties, once further developed, may become the future for antifouling technologies. New micro-structure manipulation could prevent the settlement of organisms on vessels and other structures which may be submerged for various industries.

### 3.5 Conclusion

The application method of a fouling coating is important. There are different ways to apply a coating to a surface, they may be applied with a paint brush, sprayed onto the surface or if only small substrates are used, they may be poured on the surface, or the substrate dipped into the coating. From the results of the current study, it can be concluded that for some coatings the method of application can influence the surface structure of the coating, which in turn can negatively impact the effectiveness of the coating to prevent the settlement of biofouling organisms.

A coating applied with a paint brush may create grooves or brush marks on the surface of the coating. It is possible that these grooves could be of the right shape and size to promote the settlement of fouling species. With the use of SEM imaging, it was confirmed that paint brushes do create more texture on the surface of a coating, but also when compared to coatings that were applied by a dipping method, the coatings were more porous.

A single parameter cannot be used to explain the effectiveness of these coatings to prevent fouling settlement. Most of the coatings used in the field trials contained some form of biocide, copper, zinc or organic. Other coatings were selected based on their physical parameters to reduce the attachment strength of fouling organisms to their surface. The chemical, physical and microtopography of a surface, as well other parameters such as wettability, roughness, and charge of the surface should also be considered.

Aside from the coatings themselves, the fouling community is also important to understand. At each of the four sites discussed in this chapter, there were many overlapping species present at each, but these sites also had their own unique fouling communities. Such as Burin, which had extensive growth by Vase tunicate species which fouled almost the full panels, not leaving room for other fouling organisms, this is possibly the reason this site does not have a large biodiverse fouling community on the panels.  These species all have different mechanisms for settlement and understanding how they settle could be important in choosing the most effective coating or developing a new coating. Further investigation into the adhesive chemicals of fouling species is needed, then the microtopography of the coatings can be discussed in relation to these species. Learning more about this mechanism can give insight into how to manipulate a surface to deter the settlement of these species.

Some of the decisions regarding choice or development of new effective antifouling technologies relies on the surface micro-structure. The use of these technologies, such as a new antifouling coating, would benefit from a smooth surface, regardless of the presence of biocide, with a preferred application method. This would decrease the interaction between the antifouling surface and the attachment mechanisms of fouling organisms.

# Chapter 4: Discussion

## 4.1 Discussion

For the aquaculture industry, biofouling can be detrimental due to increase weight causing damage to infrastructure, competition for resources and reduction in water flow (Braithwaite et al., 2007; Fitridge et al., 2012; Fleorl at al., 2016; Paetzold et al., 2012). This can cost the industry millions of dollars annually to control (Cordell et al., 2013). In other Atlantic provinces, controlling the growth of these species is the best defence against them. In NL where transport costs, for exporting products, are so important and the additional cost of cleaning would be unlikely to be cost effective, preventing tunicates from being transferred to the sites is critical.

There are controls already established to mitigate biofouling communities in the aquaculture industry. Mechanical and hand cleaning, power washing, gear cycling, and water and chemical baths are some of these control methods used (Adams et al., 2011; Hodson et al; 1997). The use of antifouling coating on finfish cage netting is also used in the aquaculture industry, but increasingly to a lesser extent with the advent of mechanized net washing robots.

Antifouling coatings are one way to prevent biofouling of invasive species and the excess spread of native biofouling species from spreading to these sites. On vessels that are moving frequently, foul release coatings may be effective at preventing fouling from being transferred from one location to another. In this study, both foul release coatings (FRC) and self-polishing co-polymer coatings (SPC) were deployed in different Bays in Newfoundland to determine their effectiveness in coastal waters. The FRC did not show results that were equivalent to a real-life situation since the panels were maintained in a stationary position on the dock and not cleaned during the field trial. Coatings which contain toxic biocides worked to prevent biofouling for multiple months, but none worked more than a year, suggesting that vessels or other structures such as docks would have to be cleaned and repainted yearly to ensure longer-term protection.

To understand how antifouling coatings work, it is important to try and understand how biofouling occurs. When a surface is submerged in sea water, chemical, biological, and physical events occur where a complex community of micro and macro-organisms attach to the surface, a bacteria population adheres to the surface first creating a biofilm which creates an environment for macro-organisms to settle (Kerr and Cowling., 2003; Martín-Rodrígues et al., 2015). Micro-topographies of substrate surfaces with textures that are similar to size of the larva, promote settlement, surfaces smaller minimize the amount of attachment points available to the organisms, reducing the chance of settlement (Berntsson et al., 2000; Chen et al., 2015; Gribben et al., 2011; Whalan et al., 2015). Therefore, an important aspect for antifouling coating performance is surface topography along with the chemical makeup of the coating, as mentioned above.

Physical or structural properties of a coating would include the roughness, periodicity, porosity and topology of the surface of the coating (Hellio and Yebra. 2009; Rosenhahna. 2008). It is known that rougher surfaces tend to foul more quickly than smooth surfaces, though there have been exceptions (Crisp. 1960; Kerr and Cowling. 2003). In the microstructure study of this thesis, the porosity and pattern, referred to above as texture, were the only parameters that could be analysed from the SEM images collected.

A single parameter should not be used to explain the effectiveness of these coatings to prevent fouling settlement. Most of the coatings used in the field trials contained some form of biocide, copper, zinc or organic. Other coatings were selected based on their physical parameters to reduce the attachment strength of fouling organisms to their surface. The chemical, physical and microtopography of a surface, as well other parameters such as wettability, roughness, and charge of the surface should also be considered.

There are also new technologies being investigated using different properties such as, the use of chemical extracts from organisms, such a sponges, which naturally do not become fouled, though there is work needed to be completed to make these types of technologies work in real life situations (Ribeiro et al. 2013, Yebra et al. 2004). Creating oxidizing environments, using vibration, bubble streams and suspended particles to deter settlement, or creating new or mimicking surface textures are also being explored as new upcoming antifouling technologies (Cao et al., 2011; Lejars et al., 2012; Lowen et al., 2016; Maréchal et al; 2009).

It is important that research continues into the development of these technologies, as more information is being collected on the effective of metal biocides on the environment. The most effective antifouling coating was developed in the 1960s containing the biocide tributyltin (TBT), which was proven to have negative effects on non-target species such as oysters and dog whelks (IMO. 2020). In the late 1980s the harmful environmental effects of TBT were recognized by the International Maritime Organization (IMO). The Marine Environment Protection Committee (MEPC) of IMO recommended that Governments adopt measured to eliminate the use of antifouling coatings containing TBT, and in 2008 a complete prohibition of TBT containing paints was put into effect (IMO. 2020). If it has happened with one type of biocide it can happen with other types as well, including copper-based biocides.

# Chapter 5: Conclusion

## 5.1 Conclusions

When choosing an antifouling technology, it is important to look at many aspects, including situation for use and the properties of the technologies available. Results of the field trial concluded that coatings which contained biocides did perform better at preventing biofouling than coatings that did not contain a biocide when applied in the marine environment on stationary wooden blocks. Of the coatings that did contain biocide, Micron CF did have the least fouling accumulation at each site at the end of the 17-month field trial, even though it contained the least amount of biocide by weight. On a dock, a more chemical biocide approach may be useful to prevent organisms from attaching to the surface. The field trial was completed on stationary structures, therefore the effectiveness of the FRC used in the trial may not be accurate since they are meant to be used on vessels underway. Of the coatings that did not contain biocides, the ones that did not have any antifouling properties (Navigator Brightside, Super Marine and Control) did foul earlier than the FRC coatings (Hullspeed 3000 and ePaint EP21).

All properties of antifouling technologies are important. For instance, a biocide coating will only be effective until the biocide is depleted; once there is no biocide left in the coating the pores or pattern in the surface may then create ideal areas for adhesive compounds from fouling organisms to adhere.

The application method of a fouling coating is also important, as there are different ways to apply a coating to a substrate. The results of the current study suggest that for some coatings the method of application can influence the surface structure of the coating, which in turn can negatively impact upon the effectiveness of the coating to prevent the settlement of biofouling organisms.

Choosing the correct antifouling coating to prevent the settlement, growth and spread of biofouling and aquatic invasive species is important. Choosing coatings with biocides for vessels and infrastructure which stays mostly stationary or is slow moving would be more appropriate than using a foul release coating. Vessels underway, frequently experiencing higher speeds, will benefit from the use of FRCs. Using a painting method which reduces the amount of surface topography would aid in preventing the settlement of species to the surface.

In the aquaculture industry, where vessels are not moving great distances between the docks and the farms and where operations have the vessels moving slowing to complete various work on the farm sites, the use of ecofriendly biocide antifouling coatings would be the best defense against the movement and spread of fouling organisms and aquatic invasive species. Docking areas would also benefit from the use of biocide coating treatments, to prevent the establishment and growth of these potentially harmful species where aquaculture farm vessels and barges are tied between visits to farms.

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Appendix I: Major fouling species settled on the antifouling coatings at each site, at different sampling periods.

| **Royal Newfoundland Yacht Club** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Coating | July 2016 | Aug 2016 | Sept 2016 | Oct 2016 | Dec 2016 | June 2017 | Aug 2017 | Nov 2017 |
| BottomKote | No fouling | No fouling | -Algae[1]<br>-Golden Star (*Botryllus schlosseri*) | -Algae<br>-Golden Star | -Algae<br>-Golden Star | -Algae | -Algae<br>-Mussels (*Mytilus spp.)* | -Algae<br>-Mussels |
| Epoxycop | No fouling | No fouling | -Algae | No fouling | -Mussels | -Algae | -Algae<br>-Mussels | -Algae<br>-Mussels |
| Micron CSC (White) | No fouling | No fouling | No fouling | No fouling | -Mussels | -Algae | -Algae<br>-Mussels | -Algae<br>-Mussels |
| Tri-Lux II | No fouling | No fouling | -Algae | -Algae | -Algae | -Algae<br>-Mussels | -Algae<br>-Mussels<br>-Golden Star | -Algae<br>-Mussels<br>-Golden Star<br>-Branched Bryozoa (*Bugula spp.*) |
| Micron CSC (Black) | No fouling | No fouling | No fouling | -Algae | -Algae<br>-Golden Star | -Algae<br>-Golden Star<br>-Mussels | -Algae<br>-Mussels | -Mussels |
| Navigator Brightside | -Branched Bryozoa<br>-Golden Star | -Branched Bryozoa<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Mussels | -Mussels | -Mussels |
| Super Marine | -Branched Bryozoa | -Branched Bryozoa<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Branched Bryozoa<br>-Golden Star | -Mussels<br>-Algae | -Mussels | -Mussels |
| Micron CF | No fouling | No fouling | No fouling | -Algae | No fouling | -Algae | -Mussels<br>-Algae<br>-Branched Bryozoa | -Mussels |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ePaint ZO | No fouling | No fouling | -Algae | -Algae-<br>Mussels | -Algae<br>-Mussels | -Algae -<br>Mussels | -Algae<br>-Mussels<br>-Branched Bryozoa | -Algae<br>-Mussels |
| ePaint Ecominder | No fouling | No fouling | -Algae | -Algae | -Algae | -Algae | -Algae<br>-Mussels<br>-Golden Star | -Mussels |
| Hullspeed 3000 | -Branched Bryozoa<br>-Golden Star | -Golden Star | -Algae<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Golden -Star | -Algae<br>-Mussels | -Mussels | -Mussels |
| ePaint EP21 | No fouling | -Golden Star | -Algae<br>-Golden Star | -Algae | -Algae | -Algae | -Algae<br>-Mussels | -Algae<br>-Mussels |
| Control | -Branched Bryozoa<br>-Golden Star | -Branched Bryozoa<br>-Golden Star | -Algae<br>-Branched Bryozoa<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Branched -<br>Bryozoa<br>-Golden Star | -Algae<br>-Mussels | -Algae<br>-Mussels | -Algae<br>-Mussels |
| **Arnold's Cove** | | | | | | | | |
| BottomKote | -Algae | -Algae | No fouling | -Algae | -Algae | -Algae | -Algae | -Algae |
| Epoxycop | No fouling | No fouling | No fouling | No fouling | -Algae | -Algae | -Algae | -Algae |
| Micron CSC (White) | -Algae | No fouling | No fouling | -Algae | -Algae | -Algae | -Algae | -Algae<br>-Branched Bryozoa |
| Tri-Lux II | No fouling | No fouling | No fouling | -Algae | -Algae | -Algae | -Algae | -Algae |
| Micron CSC (Black) | No fouling | No fouling | No fouling | -Algae | -Algae | -Algae | -Algae<br>-Branched Bryozoa | -Algae |
| Navigator Brightside | -Algae | -Branched Bryozoa<br>-Algae | -Algae<br>-Branched Bryozoa<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Branched Bryozoa | -Algae |
| SuperMarine | -Algae<br>-Branched Bryozoa | -Algae<br>-Branched Bryozoa | -Algae<br>-Branched Bryozoa<br>-Golden Star | -Algae<br>-Branched Bryozoa<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Branched Bryozoa | -Algae |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Micron CF | No fouling | No fouling | No fouling | No fouling | No fouling | -Algae | -Algae<br>-Branched Bryozoa | -Algae |
| ePaint ZO | No fouling | No fouling | No fouling | No fouling | -Algae | -Algae | -Algae | -Algae |
| ePaint Ecominder | -Algae | No fouling | No fouling | -Algae | -Algae | -Algae | -Algae<br>-Branched Bryozoa | -Algae |
| Hullspeed 3000 | -Algae | -Algae<br>-Branched Bryozoa | -Algae<br>-Branched Bryozoa<br>-Golden Star | -Algae<br>-Branched Bryozoa<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Golden Star | -Algae<br>-Branched Bryozoa | -Algae |
| ePaint EP21 | No fouling | No fouling | -Algae<br>-Branched Bryozoa | -Algae | -Algae | -Algae | -Algae | -Algae |
| Control | -Algae | -Algae<br>-Branched Bryozoa | -Algae<br>-Branched Bryozoa<br>-Golden Star | -Algae<br>-Branched Bryozoa | -Algae<br>-Branched Bryozoa - Golden Star | -Algae<br>-Mussels | -Algae<br>-Branched Bryozoa | -Algae |
| **Burin** | | | | | | | | |
| BottomKote | No fouling | -Algae | -Algae | -Algae | -Algae | -Algae | -Algae | -Algae<br>-Vase (*Ciona intestinalis*) |
| Epoxycop | No fouling | No fouling | No fouling | No fouling | -Algae | -Algae | -Algae<br>-Vase | -Algae |
| Micron CSC (White) | -Algae | No fouling | No fouling | No fouling | No fouling | -Algae | -Algae | -Algae<br>-Vase |
| Tri-Lux II | No fouling | No fouling | -Algae | No fouling | -Algae | -Algae | -Algae | -Algae<br>-Vase |
| Micron CSC (Black) | No fouling | No fouling | -Algae | -Algae | -Algae | -Algae | -Algae | -Algae<br>-Vase |
| Navigator Brightside | -Algae | -Algae | -Algae<br>-Branched Bryozoa<br>-Vase | -Algae<br>-Branched Bryozoa<br>-Vase | -Algae<br>-Branched Bryozoa<br>-Vase | -Algae<br>-Vase | -Algae<br>-Vase | -Algae<br>-Vase |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| SuperMarine | -Algae | -Algae | -Algae<br>-Branched Bryozoa<br>-Vase | -Algae<br>-Branched Bryozoa<br>-Vase | -Algae -Vase | -Algae<br>-Vase | -Algae<br>-Vase | -Algae<br>-Vase |
| Micron CF | -Algae | -Algae | No fouling | No fouling | No fouling | -Algae | -Algae | -Algae<br>-Vase |
| ePaint ZO | -Algae | No fouling | No fouling | No fouling | No fouling | -Algae | -Algae | -Algae<br>-Branched Bryozoa<br>-Vase |
| ePaint Ecominder | No fouling | No fouling | No fouling | No fouling | -Algae | -Algae | -Algae<br>-Vase | -Algae -Vase |
| Hullspeed 3000 | -Algae | -Algae | -Algae<br>-Branched Bryozoa<br>-Vase | -Algae<br>-Branched - Bryozoa<br>-Vase | -Algae<br>-Vase | -Algae -Vase | -Algae<br>-Vase | -Algae<br>-Vase |
| ePaint EP21 | No fouling | -Algae | -Algae | -Algae | -Algae | -Algae | -Algae | -Algae<br>-Vase |
| Control | -Algae | -Algae | -Algae<br>-Branched Bryozoa<br>-Vase | -Algae<br>-Branched Bryozoa<br>-Vase | -Algae<br>-Branched Bryozoa<br>-Vase | -Algae<br>-Vase | -Algae<br>-Vase | -Algae<br>-Vase |
| **Little Bay** | | | | | | | | |
| BottomKote | -Algae | -Algae | -Algae | -Algae<br>-Mussels | -Algae - Mussels | -Algae | -Algae | -Algae<br>-Mussels<br>-Vase |
| Epoxycop | No fouling | No fouling | -Algae - Mussels | -Algae - Mussels | -Algae - Mussels | -Algae | -Algae | -Algae<br>-Vase |
| Micron CSC (White) | No fouling | No fouling | No fouling | -Algae<br>-Mussels | No fouling | -Algae | -Algae | -Algae<br>-Mussels<br>-Vase |
| Tri-Lux II | -Algae | No fouling | -Algae | -Mussels | -Algae<br>-Mussels | -Algae | -Algae | -Algae<br>-Vase |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Micron CSC (Black) | -Algae | No fouling | No fouling | -Mussels | -Mussels | -Algae | -Algae<br>-Mussels | -Algae<br>-Mussels<br>-Vase |
| Navigator Brightside | -Algae<br>-Branched Bryozoa | -Algae | -Branched Bryozoa<br>-Mussels<br>-Vase | -Mussels<br>-Vase | -Algae<br>-Mussels<br>-Golden Star<br>-Vase | -Algae | -Algae<br>-Mussels | -Algae<br>-Mussels<br>-Vase |
| SuperMarine | -Algae | -Algae | -Algae<br>-Branched Bryozoa<br>-Mussels | -Algae<br>-Mussels | -Algae<br>-Mussels<br>-Golden Star<br>-Vase | -Algae | -Algae | -Algae - Mussels<br>-Vase |
| Micron CF | -Algae | No fouling | -Mussels | -Mussels | -Mussels | -Algae<br>-Mussels | -Algae<br>-Vase | -Algae |
| ePaint ZO | -Algae | No fouling | -Mussels | -Mussels | -Algae<br>-Mussels | -Algae<br>-Mussels | -Algae | -Algae<br>-Vase |
| ePaint Ecominder | No fouling | No fouling | -Mussels | -Algae<br>-Mussels | -Algae<br>-Mussels | -Algae | -Algae | -Algae<br>-Mussels<br>-Vase |
| Hullspeed 3000 | -Algae | -Algae | -Branched Bryozoa<br>-Mussels<br>-Vase | -Algae<br>-Mussels<br>-Vase | -Algae<br>-Mussels<br>-Vase | -Algae - Mussels | -Algae<br>-Mussels | -Algae<br>-Vase |
| ePaint EP21 | -Algae | -Algae | -Algae | -Algae<br>-Mussels | -Algae | -Algae | -Algae | -Algae<br>-Mussels |
| Control | -Algae Branched Bryozoa | -Algae<br>-Branched Bryozoa | -Algae<br>-Branched - Bryozoa<br>-Mussels<br>-Vase | -Branched Bryozoa<br>-Mussels<br>-Golden Star<br>-Vase | -Algae<br>-Branched - Bryozoa<br>-Mussels<br>-Golden Star<br>-Vase | -Algae | -Algae - Mussels | -Algae<br>-Mussels<br>-Vase |

[1] Algae was not speciated