

Master's thesis



# The effects of Atlantic salmon aquaculture on decapod crustaceans and fishes in the Quoddy region of the Bay of Fundy

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*The effects of Atlantic salmon aquaculture on decapod crustaceans and fishes in the Quoddy region of the Bay of Fundy*

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## **Declaration**

I hereby confirm that I am the sole author of this thesis and it is a product of my own academic research.

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# Abstract

The Food and Agriculture Organization (FAO) reported in 2016 that aquaculture production had doubled since 2001, indicating exponential growth in the industry that has a global value of more than \$200 billion CAD. In New Brunswick, Canada, there is concern that the activities of the aquaculture industry are having a detrimental effect on benthic communities, with an emphasis on the commercially-important American lobster. The aim of this thesis was to monitor the impacts of the aquaculture industry on decapod crustaceans and fishes in the Quoddy Region, New Brunswick. Cobble-filled collectors were deployed at paired sites near and away from aquaculture cages at shallow subtidal locations in three Bay Management Areas (BMAs) in the Quoddy region in 2015 and 2016, where near sites were approximately 200 m in distance from aquaculture operations, compared to 1200 m for the away sites. No significant effect of proximity to aquaculture on fish and decapod communities in terms of individual abundance, species richness and species evenness was found; while a significant interaction between year and BMA was observed in a PERMANOVA of decapod and fish data. Abundance of lobster was not significantly different between treatments, apart for one site pair. At present, there is no evidence to suggest that there is an effect of aquaculture on fish and decapod crustaceans on cobble habitat ~200 m away from aquaculture pens. Due to the three year production cycle of Atlantic salmon in the Quoddy region, another year of data is required to confirm this finding.

Keywords: Decapods, fish, Quoddy region, aquaculture, individual abundance, species richness, New Brunswick.



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## **Acronyms and factors**

ANOVA	Analysis of variance
AQ (BM)	Aquasite (BMA)
CAD	Canadian dollars
BMA	Bay Management Area
DFO	Department of Fisheries and Oceans
MDS	Multidimensional scaling
PERMANOVA	Permutational analysis of variance
SIMPER	Similarity percentages
swBoF	South-west Bay of Fundy
TR	Treatment
YE	Year

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# **1 Introduction**

## **1.1. Growth of the aquaculture industry on the world stage**

Since its inception as a commercial industry in Asia during the 1960s (White et al., 2004), aquaculture (the breeding, rearing and harvesting of seafood species) has quickly proliferated and taken hold as an important means of food production around the globe. In 2014, 73.8 million tonnes of fish were produced by aquaculture activities worldwide, for a value of more than \$200 billion CAD. This production in 2014 constituted 40% of the global seafood supply (including aquaculture and wild harvest) for that year, up from 7% in the 1980s (World Bank, 2013). Even more recently, the Food and Agriculture Organization reported in 2016 that aquaculture production had doubled the levels of 2001 (FAO, 2016), indicating exponential growth in the industry. This is in contrast to the traditional wild-catch fishing industry, which has remained relatively stable in production since the early 1990s (FAO, 2016) (Fig. 1.1).

The stagnation of the traditional fishing industry combined with a similar trend in agriculture has led to an ever-increasing demand for food in the face of the soaring global population, and changes in income and diet requirements – especially in Asian countries (Ausubel et al., 2013; The World Bank, 2013). This demand for protein is expected to continue to significantly increase in coming years (Bolland et al., 2013). Aquaculture is well poised to meet this increasing demand due to its high efficiency when compared to other protein sources such as agriculture. The feed conversion ratio in aquaculture (the amount of feed required to gain a kg of body weight) is more profitable than in other protein production systems. Certain fish species can gain up to 1 kg in weight from the consumption of just 1.15 kg of feed for a feed conversion ratio of 1:1.15, which is considerably more efficient than that of pork production, which boasts a feed conversion ratio of 1:2.63 (Bjorkli, 2002). This translates to a lowered input cost for the production of the same output (1 kg of protein), leading to higher profits for business owners promoting business growth.

## Aquaculture Is Expanding to Meet World Fish Demand

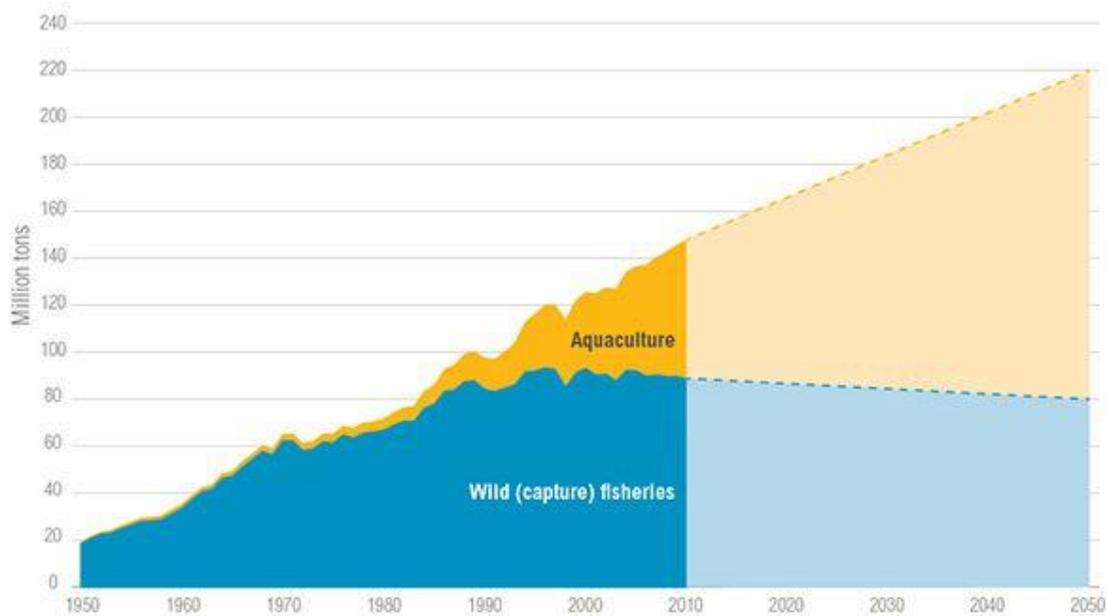


Figure 1.1: The aquaculture industry has grown significantly since the early 1990s and is expected to grow to a point where it produces more fish than capture fisheries that are expected to decline at a gradual pace throughout the next 30 years. Source: FAO, 2016.

## 1.2. Seafood industries of Atlantic Canada

Aquaculture has played an important role throughout the coastal environments of Atlantic Canada – an area inclusive of the provinces of Newfoundland and Labrador, Prince Edward Island, Nova Scotia and New Brunswick – since the 1970s. The industry in this region saw its inception with the introduction of Atlantic salmon farming in the Bay of Fundy, New Brunswick (DFO, 2015a). Throughout this time, the southwest Bay of Fundy (swBoF) in New Brunswick - the location of this study - was the most popular area for the industry (Chang et al, 2014). In 1986, the industry had pockets of pen farms (composed of floating enclosures that allow for water exchange through the use of mesh netting (Beveridge, 1984) throughout New Brunswick, Nova Scotia, Prince Edward Island (PEI) and Newfoundland and Labrador for the production of both finfish and shellfish. In the same year, these farms produced 5, 093 metric tonnes of seafood, bringing in \$15.7 million CAD to the region (DFO, n.d). Growth in production since 1986 has been considerable: the most recent statistics yielding an Atlantic Canadian production of 59,650 metric tonnes of finfish and shellfish in 2014, valued at \$279.9 million CAD (DFO, n.d). This period of growth in the aquaculture industry has resulted

in the creation of over 7500 jobs in Atlantic Canada (Aquaculture Industry Alliance, 2015).

Given its success within farmed conditions, Atlantic salmon (*Salmo salar*) is the primary finfish species farmed throughout Canada. Production of the species accounts for 84.3% of all finfish produced (in tonnes) in the country (DFO, 2014a). The production process for this species begins with freshwater hatcheries, followed by the transfer of smolts to cages (pen farms) that are typically located in the marine environment. From here, the salmon smolts are raised until they gain a sufficient body size that is profitable for market (Mikkelsen, 2006). The production of Atlantic salmon has increased in recent decades as a result of advances in technology, including changes to the composition of fish feed and disinfectants, decreasing production costs while increasing production, further encouraging the expansion of the industry in the area (Asche et al., 2009).

For centuries throughout Atlantic Canada, fisheries have arguably been at the heart of coastal communities where they have provided high economic profits and regular employment that ensures stability for families in difficult times. Atlantic Canada harvests a variety of groundfish (e.g. Atlantic cod, haddock and Atlantic halibut), pelagic fish (e.g. Atlantic herring and Atlantic mackerel) and shellfish (American lobster, snow crab and northern shrimp), that all contribute to the overall value of the fishery in the region (Charles, 1997). During the early 1990s, groundfish species contributed almost 50% of the total landed seafood, yet by 2008 this had fallen drastically to less than 15%, partly as a result of the decline of Atlantic cod stocks throughout this period (DFO, 2014b). Consequently, the fishery turned to shellfish as the main target species, which contributed over 85% of the total value of commercial landings for 2013 (DFO, 2014b). Landings of American lobster (*Homarus americanus*) have grown from approximately 15,000 metric tonnes in 1975 to 74,581 metric tonnes in 2014 in Atlantic Canada. This provides roughly \$1 billion CAD to the economy of the Canadian east coast as a valuable export (DFO, 2015b) and justifies the status of American lobster as a critically important commercial species for the region. Between 1990 and 2008, New Brunswick consistently experienced approximately 7,500 tonnes of lobster landings per year, yet these landings have recently begun to increase exponentially: as of 2011, landings began to be reported at double this level by the

Department of Fisheries and Oceans (DFO), peaking at over 20,000 tonnes in 2014 (DFO, 2017). The explanation for this increase is twofold. First, the shift from a groundfish to a shellfish-dominant fishery has increased the intensity of lobster fishing effort, increasing catches. Second, groundfish species serve as an important predator for juvenile lobsters and the observed reduction in their population since the 1990s (particularly in the case of Atlantic cod) could result in increased lobster survival rates, giving rise to larger lobster population sizes. The growth of the wild capture shellfish fishery (especially *Homarus americanus*), combined with the aquaculture industry has provided economic and social stability for small rural communities that were once at risk of emigration following the lack of employment from the collapse of the Atlantic cod fishery (Mason, 2002).

### **1.3. Environmental impacts of aquaculture**

Aquaculture offers an efficient means of helping to meet the growing protein demand as global populations rise to approximately 9 billion by 2050 (UN, 2004). Aquaculture has the potential to provide resources for the development of sustainable food production and enhance food security and alleviate poverty (Torrissen et al., 2011). Further, the aquaculture industry is able to support industrial growth in coastal communities that are facing the loss of traditional wild-capture fisheries as a source of income. Additionally, farming commercially important species is often seen as a method of reducing the pressure on overexploited wild fish stocks (Naylor et al, 2000).

Despite these benefits, the environmental impacts associated with aquaculture have raised concerns. Aquaculture can contribute to the decline of certain wild fish stocks when large volumes of wild pelagic fish (including sardines and herring) are harvested (and in some cases overexploited) in order to create fishmeal (NOAA, n. d). Furthermore, the expansion of the industry in coastal environments throughout Atlantic Canada and around the world has resulted in the loss of important nursery grounds, important for the recovery of wild fish stocks, as well as the degradation of these coastal environments caused by nutrient and chemical discharges from pens (Naylor & Drew, 1998). As a result, the aquaculture and fishery industries have often clashed over numerous issues, ranging from conflicts over marine space, to a fear of job losses, to

environmental effects and damage to wild populations (Abreu et al., 2011; Wiber et al., 2012).

One of the main discussions surrounding the industry concerns the impacts on the biodiversity of ecosystems within close proximity of farm sites (Burrige et al., 2010; Kullman et al., 2006). The nature of finfish aquaculture requires the organisms to remain tightly packed in a confined space for the entirety of their lives. These conditions can be ideal for the spread of disease and parasites, having a profound impact upon the health of the species and, consequently, the quality of the product. The most common parasitic organism in aquaculture is sea lice, which attach themselves to the skin of the fish causing lesions, increased stress and further vulnerability to infection (Langford et al., 2014). To counter this, farmers use a range of pesticides, antifoulants, anaesthetics and disinfectants to treat infected fish (Bravo et al., 2008). Chemicals used in aquaculture activities enter the natural environment as a result of the open-containment nature of pen farms which allows for the free exchange of water within the marine environment and have been known to impact the environment and the natural biota (Diana, 2009; Martinez-Porchas & Martinez-Cordova, 2012). These factors, among others, have created a situation whereby the management of the aquaculture industry is under intense pressure to make the industry sustainable in an effort to limit the negative effects on the environment.

The negative environmental impacts of the chemicals used in aquaculture were seen in 2013 in the Quoddy Region of New Brunswick when illegal pesticides introduced to the marine environment resulted in the mortality of hundreds of lobster (Riley, 2013). As previously mentioned, lobster are an important source of income to coastal communities in the region and it is therefore important that damage to the benthic environment caused by pen farms in the region is better understood. Degradation of the benthic habitat caused by the presence of aquaculture activities can be expected to be evidenced by a change in benthic community assemblages within close proximity of pen farms. The aim of this thesis is therefore to use cobble-filled collectors as a means of monitoring the environmental impacts of the aquaculture industry in the Quoddy Region of New Brunswick.



## 2 Literature review

This section will review the literature detailing the development of the aquaculture industry and the effects that marine farming has on the environment, biodiversity and society. Section 2.1 will discuss the feed and waste generated from salmon farming and how a change from traditional fish feed to a plant-based diet can impact on the environment and biodiversity. Section 2.2 will focus on the issues surrounding aquaculture in terms of sea lice and the use of pesticides in the industry. Section 2.3 discusses the use of heavy metals in aquaculture and the effects on the biodiversity throughout the water column, whilst considering the benthic community at the forefront of discussions. More specifically, section 2.4 will discuss the changes in benthic community structures in terms of abundance of individuals, species richness and species evenness as a result of aquaculture operations. Finally, section 2.5 will give a detailed description into the current state of aquaculture in the southwest Bay of Fundy, where the issues concerning the industry in the region will be examined.

### 2.1. Fish feed and waste

Producing high quality fish is essential to a successful aquaculture business, something that is facilitated by ensuring each individual fish receives enough protein, energy and nutrients to grow to a marketable and profitable size. Herbivorous fish, such as tilapia (*Oreochromis spp.*) and grass carp (*Ctenopharyngodon idella*), consume solely plant-based feed that originates from agriculture by-products, while Atlantic salmon and other carnivorous fish rely upon feed that is a mixture of fish meal and fish oil, ensuring that fish retain high muscle growth and remain in good health (Fry et al., 2016). However, as a result of a fluctuation in the supply of fish meal, fish meal in the feed has predominantly - but not completely - been replaced by agricultural products including wheat, corn, beans, soy, sunflower and rapeseed oil (Tacon & Metian, 2008; Marine Harvest, 2016). The process to create the feed begins by first grinding plant and animal-based contents together which can then be broken down into small-sized pellets for consumption (Fry et al., 2016). This substitute to a more plant-based feed for farmed

carnivorous fish has relieved the pressure on global pelagic fish stocks traditionally used for fish meal and has reduced the reliance on a finite resource that would have otherwise led to overfishing and the eventual decline in fish feed to sustain the aquaculture industry (Turchini et al., 2009). Transferring to more plant-based sources of feed produces higher fish stocks as the feed conversion ratio becomes more efficient and is therefore an attractive option for farm owners (Crampton et al., 2010). As a result, the aquaculture industry has made significant efforts to increase the amount of agricultural-based feed in the diets of farmed fish (Naylor et al., 2009). Even so, the increase to a more plant-based diet in farmed salmon has reduced muscle and omega-3 fatty acids that are a characteristic of seafood and important for human consumption (Torpy, 2006). Further, the changes from fish oil to other types of oil has reduced the value and nutritional benefits of fish consumption in humans and can also affect aspects of the environment (Brandsen et al., 2003; Bell et al., 2003; Menoyo et al., 2005).

Much of the ingredients used for a plant-based diet contain mycotoxins, a toxic compound produced by fungi that are present in animal feed (Jakic-Dimic et al., 2005). Cereals are the main ingredient in salmon fish feed (up to 90%) and can become contaminated with fungi anytime throughout the supply chain (Rajic, 1993). This has been known to prompt disorders in individual fish such as cancerogenic, mutagenic and teratogenic effects (Jakic-Dimic et al., 2005). Further, there have been notable incidences whereby changes have been evident in nutrient resorption, cell and organ mutations and, in severe cases, mortality of farmed fish (Tolosa et al., 2014). As the feed is processed with the incorporation of a range of different ingredients, a new profile is produced that increases the disease risk and susceptibility to fish (Binder et al., 2007), whereby mycotoxins within fish feed can result in indirect losses such as a decrease in productivity and an increase in secondary diseases (Jakic-Dimic et al., 2005).

One of the most researched effects of aquaculture activities is the enrichment of organic sediments near the pens themselves (Hall et al., 1990; Holby and Hall, 1991; Hargrave et al., 1993; Karakassis et al., 1998 and Karakassis et al., 2002). The zone of detectable effects of fish farming on benthic communities and other macrofauna throughout the water column varies in range up to several hundred metres, yet the impacts caused by carbon and nitrogen from fish feed and waste can be detected up to

1000 m from the pens (Sara et al., 2004). The effects that aquaculture has on benthic communities can depend upon a range of factors such as water currents and the number of pens. The spatial range of localised impacts vary considerably among studies, with Pohle et al. (2001) identifying a range of more than 200 m in comparison to Karakassis et al. (2000) who found that effects did not exceed more than 25-30 m. The detection range seems to differ due to variation in the natural environment, especially site location and currents, which can alter the direction flow and distribution of organic material. Even so, differing reports justify the need for further research into the distance over which aquaculture operations impact on benthic communities.

It is stated that fish feed is the primary source of waste and is responsible for the majority of environmental impacts associated with aquaculture (Roque d'Orbcastel et al., 2009). It is estimated that 162 g of faeces is produced for every kg of Atlantic salmon that is produced (Bergheim & Asgard, 1996; DFO, 2017), yet this depends upon a range of factors including feed composition, fish species and temperature (Amirkolaie, 2011). Extrapolating this relationship to the local context and considering that 23,391 tonnes of Atlantic salmon were produced in New Brunswick throughout 2015, it can be estimated that approximately 3-4 million kg of faecal waste directly entered the marine environment as a result of Atlantic salmon aquaculture activities. Most of this waste accumulates just below the sea cage, but can be dispersed up to 1.2 km away from a farm site depending on currents (Holmer, 1991). With this introduction of organic input, high levels of nitrogen are released into the environment through several pathways including fish feed, faecal particles and faeces itself (Lee et al., 2015). A study into sea bream aquaculture in the Mediterranean found that the organic matter that is derived from excess food and fish waste caused an immediate decline in seagrass (Delgado et al., 1998). The increased primary production results in changes to plant composition that creates very dense algal blooms, reducing the sunlight necessary for seagrass to grow (McGlathery, 1995), which has a profound effect on the biodiversity and the marine environment as a whole. Further, algal blooms can create hypoxic and anoxic conditions whereby biodiversity is unable to thrive (GESAMP, 1990).

By growing marine life in a controlled environment, the aquaculture industry is arguably relieving the pressure on wild fish stocks. Yet it can be said that switching the feed from wild-caught fish to a plant-based diet does not eliminate environmental

pressure but directly transfers it from one ecosystem to another. The literature suggests that aquaculture activities impact upon biodiversity in a range of ways, including interactions between farmed and wild fish (Diana, 2009; Verspoor et al., 2015; DFO, 2016a), disease transfer (Christiansen et al., 2014) and pollution (Sliskovic et al., 2011). However, this section will solely focus upon the impacts that aquaculture has on the benthic habitat upon which considerable research has been focused (Gowen et al., 1988; Yokoyama et al., 2006; Kalantzi and Karakassis, 2006; Mente et al., 2010). If a fish farm is poorly sited or managed, then there is significant potential for impact on local marine environments by the use of fish feed and pesticides which, along with fish faeces, drop down through the water column and gather on the seafloor, where wild species feed, altering communities through the addition of nutrients from aquaculture operations (Fortt et al., 2007; Sorum, 2006).

As discussed, extensive input of organic material can create anoxic conditions that can have detrimental effects for the surrounding environment. The increase of organic matter from aquaculture results in a highly eutrophic environment whereby aerobic bacteria and microorganisms consume the nutrients, hence reducing the oxygen levels below the sediment (Costa-Pierce, 1996; Burd, 1997). Further, with an increase in nutrient loads, anaerobic bacteria become more prominent, resulting in anoxic sediments in which most infauna are unable to survive, creating an environment that lacks species richness. Many studies are in agreement that communities directly below pen sites are generally reduced to only two taxa (*Capitella capitata* and certain nematodes) (Pearson and Rosenberg, 1978; Levings, 1994; Findlay et al, 1995; Pohle and Frost, 1997; Mazzola et al., 2000; Yucel-Gier et al., 2007; Bascinar et al., 2014). Even so, the level of enrichment and hence the effects on benthic communities can differ depending upon the farm size, hydrographic conditions and husbandry methods (Mente et al., 2006). Long term studies into the effects of fish farming on benthic communities have been few and far between. One such study by Pohle et al. (2001) that spanned 6 years found that loss of species diversity was significant along the benthic floor where high levels of nutrient pollution were evident in Limekiln Bay, swBoF.

## 2.2. Pesticides

Like most livestock animals, fish in farmed conditions can be susceptible to disease and parasites that have the potential to impact upon entire fish assemblages in the pens and can cause significant damage to the business of a farm (BurrIDGE et al., 2010). Conditions inside the pens can be subpar - due to poor water quality, low space, crowding and high stress levels – giving parasites and bacteria the opportunity to thrive which greatly increases the risk of disease transmission (Langford et al., 2014). With a large amount of farmed fish being contained in close proximity resulting in raised stress levels, the effectiveness of the immune system of the fish decreases, leaving them susceptible to bacterial and parasitic infections (Barton & Iwama, 1991; Naylor & Burke, 2005). One of the main parasites that exploit these conditions is the planktonic crustacean, *Lepeophtheirus salmonis* (salmon lice) (BurrIDGE et al., 1999a). Sea lice attach themselves to the skin of the host to feed on mucous and blood which in turn causes wounds, resulting in stress and making fish susceptible to infection (Langford et al., 2014). Global indirect and direct losses in salmonid farming as a result of sea lice infection is estimated to be more than \$100 million US annually (Johnson et al., 2004). To tackle this, farmers use a range of treatments to reduce and prevent the spread of sea lice within and between pens. The treatments are classed as insecticides that are used for the control of parasites in a range of industries such as agriculture and medicine, and are also available for use in salmonid aquaculture to treat sea lice infestations (Bravo, 2013). The industry uses benzoylphenylurea insecticides, azamethiphos and pyrethroids which are administered via fish feed or injection. These pesticides interfere with sodium and potassium channels of the parasite and eventually result in the paralysis and death of sea lice (Langford et al., 2014). Although as resistance to the pesticide grows, usage is becoming ever-more prominent in the industry, as seen in Scotland where a 110% increase in treatment occurred in 2012 (Carrell, 2012). With the spread of disease, pesticide usage and plant-based fish feed occurring in farming environments, there is growing alarm about the known and unknown effects that these practices could have.

Like fish feed, extensive use of pesticides in farmed conditions can result in a range of direct and indirect impacts that can have detrimental effects on human nutrition. The pesticides that enter the marine environment biodegrade in particles that pass into the flesh of farmed fish (Bjorlund et al., 1990), which raises fear regarding the

effects on human health, given that fish are bred for human consumption (Cabello, 2006). Further, the compounds that remain in targeted fish have the potential to make the fish a vector for antibiotic resistance in human consumers (Kruse and Sorum, 1994; Sørnum, 2006). The Food and Drug Administration of the USA temporarily banned the sales of five Chinese aquaculture products in 2007 as a result of salmonella contamination that developed from the use of antibiotics during production (Martin, 2007). The consumption of contaminated fish poses the risk of altering human digestive organs and allowing for toxic ingestion to take place, influencing human immune systems (Cabello, 2003; Cabello, 2006). The use of pesticides in the marine environment have the potential to disrupt ecosystems with the release of bacterial pathogens through an open-water farming system, whereby the contamination poses a great risk to the natural environment (Sørnum, 2006).

In relation to the natural environment, there are several ways in which they enter the important ecosystems. Firstly, between 60-85% of the drug can be excreted via fish faeces which drop down the water column and are consumed by scavenger species such as lobsters (Alderman et al., 1994; Samuelsen, 1994; Weston, 1996) Secondly, as pesticides are given through fish feed, infected fish with reduced appetites are less inclined to consume feed, meaning that a significant proportion of pesticides accumulate at the benthic floor (Lotze & Milewski, 2004). Even though many chemical pesticides have low toxicity, the fact that they do not break down but remain active long after release can result in widespread biological concerns (Lotze & Milewski, 2004; Capone et al., 1996; Aarestrup, 2006).

Carvalho and Hance (1993) explain how pesticides interact with the marine environment and state that each pesticide behaves differently when coming into contact with water and other ambient conditions such as light, pH and humidity, making it difficult to generalise. Research is lacking into the effects pesticides have on the abiotic marine environment, yet the effects on the terrestrial environment can be used to make assumptions. Treating soil with pesticides can result in a considerable decline in soil microorganisms (Aktar et al., 2009). This loss of microorganisms translates to a loss of vital nutrients present in the soil, therefore degrading the soil quality (Aktar et al., 2009). With this, it can be assumed pesticides that interact with the marine environment would have a similar effect, whereby the degradation of sediment would result in the

loss of important algal species throughout the marine environment and hence impact upon biodiversity in the area. Pesticides used to eradicate sea lice are relatively non-toxic to fish and algae, yet can have detrimental effects on many non-target organisms, especially those whose growth is particularly dependent on the successful synthesis of chitin, such as decapod crustaceans, which include lobsters, shrimp and crabs (Macken et al., 2015). The compounds found in sea lice pesticides constrain the enzyme chitin synthase during the molting stages, which can be detrimental to the health of crustaceans and if exposed for long periods of time can result in mortality (Langford et al., 2011).

In southwest New Brunswick, an infestation in 1994 brought the introduction of three pesticides, Excis®, Salmosan® and Salartect®, yet sea lice resistance to the pesticides grew and by 2000 the products were unable to impact the sea lice. This brought an emergency registration of a new product, SLICE® (Burrige & Van Geest, 2014). SLICE® was used globally as it was capable of working against all stages of the lice, and was the only product available to tackle parasites in Canada until resistance to the product grew in 2009 and the product could no longer be applied. (Burrige & Van Geest, 2014). Immediately following, the Pest Management Regulatory Agency (PMRA) then registered Salmosan®, Paramove®50 and AlphaMax® to tackle the pest. The new pesticides were capable of reducing male and pre adult sea lice by 88-98% throughout the southwest Bay of Fundy (Whyte et al., 2014), however a directive was issued one year later that prevented the usage of AlphaMax® for fish treatment due to its effect on the environment (Burrige & Van Geest, 2014). Today, only Salmosan® and Paramove®50 are used throughout New Brunswick. Salmosan®, the most commonly used pesticide for the farming of Atlantic salmon, uses azamethiphos, an organophosphate insecticide, to eradicate sea lice through bath treatments on infected fish (Haya et al., 2001).

Commercially important to the region of the Bay of Fundy, *Homarus americanus* are a decapod species that are vulnerable to the effects of chemical pollutants that enter the marine environment through the use of Salmosan® and Alphamax® (Burrige et al., 1999b; Burrige et al., 2008; Fairchild et al., 2010; Burrige et al., 2014). A study by Burrige et al (1999b) found that when exposed to high levels of azamethiphos, an active ingredient used in Salmosan®, for less than one

hour, lobster mortality was 50%. In reality however, the amount of pesticides and rate of dispersion is unknown, meaning that the risk to lobsters and other decapod crustaceans is difficult to estimate (Haya et al., 2001). Even so, a rare example of mortality as a result of pesticide-use occurred in 2013 whereby Cooke Aquaculture Inc. was fined \$500,000 CAD after causing the death of hundreds of lobsters throughout the swBoF with the illegal use of cypermethrin, a pesticide that is toxic to lobster (Riley, 2013). Even though this is an example of illegal pesticide use, the extensive practice of legal pesticides are also a cause for concern given the known effects they have on important commercial species, the general biodiversity and the ecosystem as a whole. In addition to BurrIDGE et al (1999), there have been several other studies that have identified the detrimental effects that pesticides have on stage I through to adult *Homarus americanus* (Abgrall et al., 2000; BurrIDGE et al., 2014a).

The effects of pesticides on the environment has resulted in the development of non-pharmaceutical, bio-control approaches in an effort to reduce the number of sea lice infecting salmon in the pens and reduce the use of pesticides in the industry. One strategy introduces a co-culture of cleaner fish within the pens, which remove sea lice from the salmon (Treasurer, 2002). The use of several species of wrasse have proved successful in delousing salmon in fish farms in Europe and this strategy has grown in recent years (Treasurer, 2005; Skiftesvik et al., 2013). However, wrasse experience winter dormancy, limiting their use as a cleaner fish (Kelly et al., 2014), and hence prompting interest in other cleaner fish such as lumpfish that is now the most commonly used (Powell et al, 2017; Norwegian Directorate of Fisheries, 2015). Cleaner fish have proved to be a positive addition for farmers as they are potentially more cost-effective than the use of pesticides (Liu & Vanhauwaert Bjelland, 2014). These new bio-control methods are an example of the continuous development of new methods in aquaculture, and the efforts which are being made to reduce environmental impacts.

## **2.3. Metals**

The complexities of aquaculture operations result in a range of inputs into the marine environment whereby heavy metals can arguably be the most harmful to natural ecosystems. Metals, including copper, zinc, iron and manganese, along with others, are introduced to aquaculture sites from antifoulant paints or as an element of fish feed for

nutritional purposes (Burrige et al., 2010). Heavy metals are an ever-present and perpetual contaminant that have the potential to cause widespread damage to biological systems as a result of their toxicity and accumulation. Metals are non-biodegradable in water environments and would either accumulate on sediment particles or be absorbed by marine animals that come into contact with the substances (Kaoud, 2015). The high quantities of metals in the marine environment and their effects on biodiversity have been widely studied (Debourg et al., 1993; Burrige et al., 1999a; Brooks and Mahnken, 2003; Dean et al., 2007; Cheng et al., 2013; Jiang et al., 2014; Squadrone et al., 2016). Given the release of heavy metals into the aquatic environment, there is great potential to cause widespread damage.

Copper antifoulant paints have been employed on cages in an effort to reduce the buildup of epibiota organisms that would otherwise restrict the open flow of water through the cages (Braithwaite et al., 2007). Copper is a metal that has a low solubility in water where it will likely accumulate in sediments on the seafloor (Burrige et al., 2010). Studies in the 1990s found that levels of copper found near salmon farms in Canada were around 100-150 mg kg<sup>-1</sup> dry weight, figures that exceeded the levels deemed 'safe' by authorities (Debourg et al., 1993). A study in Norway show similar results with levels around 165 mg kg<sup>-1</sup> (Solberg et al., 2001), although a recent study in Greece showed concentration levels at 98.73 mg kg<sup>-1</sup> (Nikolaou et al., 2014). The rate at which the metals are released into the environment depends upon the nature of the toxic compounds, water temperature, the speed of water currents and the specified location of the pen farms (Singh and Turner, 2009). Research regarding metals predominantly focuses on the known effects on biodiversity. Yet, it is known that metallic compounds also decrease water and sediment quality, hence resulting in degradation to the surrounding ecosystem (Giddings et al., 2001; Fremion et al., 2016). High levels of copper have been present under active fish farms in the past (Chou et al., 2002; Brooks and Mahnken, 2003), raising the issue as to what effects this could have on the environment and biodiversity. Regardless of the unknown effects to the environment, a group of NGO's have expressed their desire to see copper based antifoulants reduced to protect ecosystems from aquaculture operations (MCS, 2013). It is important to state that aquaculture operations in some areas, including New Brunswick, are transitioning away from the use of copper as an antifoulant (Hoyle, 2017)

Copper, one of the most used metals in the industry, has a significant effect on phytoplankton whereby toxic effects can decrease the diversity of this important primary producer (Le Jeune et al., 2006), creating widespread implications for the rest of the food web. Additionally, the use of copper has been shown to delay molt stages of shrimp larvae (Young et al., 1979), change enzymatic behaviour in crabs (Hansen et al., 1992), remain in digestive glands of lobster (Chou et al., 2002) and impact upon a range of other crustaceans (Lang et al., 1980) and fish (Anderson et al., 1980; Bellas et al., 2001; Burridge and Zitko, 2002). Further, it is evident that *Homarus americanus* are a highly-rated bioindicator in the monitoring of metals in the aquatic environment as they have a greater capacity and accumulation of metals than other species along the benthic floor (Chou et al., 2002; Chou et al., 2003).

Another heavy metal in use throughout the aquaculture industry is zinc, which is present as an additive in salmon feed (Burridge et al., 2010). Developments in fish farming revealed high zinc concentrations in sediments near salmon pens (Long et al., 1995; Brook, 2000). As a result of this, fish farms reduced the amount of zinc used in fish feed to significantly decrease the levels of zinc that enter the marine environment (Nash, 2001). Zinc has also been introduced as an alternative antifoulant application to replace tributyltin (TBT), a product once used as a biocide as paint on fish cages (Turley et al., 2000). TBT is known to be highly toxic to marine organisms (Marshall et al., 2002) and has been banned by the International Maritime Organisation (IMO Resolutions A. 895 21, 25/11/1999). As a result, zinc pyrithione has been introduced as a substitute to the coating and is often combined with copper to reduce the growth of marine organisms that get attached to the enclosures (Townsin, 2003). Like copper, zinc can be toxic to the aquatic environment yet requires higher concentrations to reach similar lethality. Even so, zinc has shown to cause serious harm to biodiversity throughout the water column (Arnott and Ahsanullah, 1979; Sunda et al., 1987; Stauber and Florence, 1990; Bellas, 2005).

## **2.4. Abundance, richness and evenness**

This thesis examines the impacts that aquaculture operations have on benthic communities in terms of abundance of individuals, species richness and species evenness by comparing sites near and away from fish farming activities. Studies have

shown that the accumulation of organic nutrients can change the physical and chemical structure of the sediments and biodiversity of benthic communities (Karakassis et al., 2000; Mazzola et al., 2000; Neofitou et al., 2010). Changes to the benthic floor, whereby a pattern gradient of organic enrichment is evident, mirrors the model described by Pearson and Rosenberg (1978). The model proposes that with a slight increase in nutrients, abundance gradually rises and even more so with an even further increase in organic matter, until it reaches the ‘peak of opportunists’ which is followed by a sudden decrease in abundance as a result of a decline in oxygen concentration. Further, the model argues that the closer to the area of organic input, the lower amount of species are present. Several studies have found that negative impacts decrease with distance from aquaculture. Neofitou et al. (2010) found that species abundance, richness and biomass increased away from farm sites, and that there were temporal changes in abundance that indicated a range of possibilities such as a decrease in oxygen intake or restocking of fish farms elsewhere. Bascinar et al. (2014) also found that species diversity increased away from aquaculture operations. A recent study on lobster suggested that egg-bearing lobsters migrate away from an area following the introduction of a finfish farm, where a high rate of lobster mortalities was also reported as a result of the use of pesticides in the area (Wiber et al, 2012). In contrast, a study by D’Amours et al (2008) shows the unintended consequences that aquaculture can have for the non-target organisms along the benthic floor. Species were observed in higher numbers close to cage sites than further away, hence outputs from aquaculture were indirectly supporting an abundance of life, which supports the model of Pearson and Rosenberg (1978). This indicates that the industry could impact upon biodiversity in a positive way whereby organic matter drops to the bottom of the seafloor, providing food to benthic communities. However, as evidence has shown, high levels of organic matter can produce harmful effects on benthic communities and could alter their diet and therefore the food web (Pohle et al., 2001; Macken et al., 2015).

Research into how the abundance of individuals, species richness and species evenness of certain decapod crustaceans and fishes differ throughout the enrichment gradient from aquaculture sites is lacking. It is essential to understand how fish farming changes the composition of benthic communities in terms of abundance, richness and evenness (Yucel-Gier et al., 2007), which are critical indicators in defining the health of

an ecosystem (Noss, 1990; Henderson & Ross, 1995; Rice, 2003). Further, in aquatic ecosystems it is important to understand the effects that anthropogenic activities are having on the surrounding biodiversity as this could have profound unknown effects on the critically important seafloor, which is vital to protecting economic and ecological functions. With this, many studies focus on the effects that aquaculture has on the infauna in sediments (Levings, 1994; Findlay et al., 1995; Pohle and Frost, 1997; Mazzola et al., 2000; Yucel-Gier et al., 2007; Bascinar et al., 2014), yet knowledge is lacking into aquaculture effects on rocky habitats and the species in these areas. Hence, the purpose for this thesis is to identify the effects that aquaculture has on the biodiversity of non-target species, with a focus on fish and decapod crustaceans colonizing cobble sites near and away from aquaculture pens.

## **2.5. The Quoddy region of the Bay of Fundy as a study site**

The Quoddy region is located in the mouth of the Bay of Fundy, on the southwest coast of the Atlantic Canadian province of New Brunswick along the border with Maine, USA (Fig. 2.1). The Quoddy region is located in the waters of the Bay of Fundy which is known for having some of the highest tidal range in the world that, along with its currents and back eddies, makes it a suitable area to farm salmon (Chang, 1998). Furthermore, given that approximately 60% of all salmon produced in New Brunswick is exported to the United States, the region is notably close to the Eastern Seaboard of the United States, giving New Brunswick an advantage in providing seafood to this area in quick time (Government of New Brunswick, 2012). Salmon farming in this region began in 1978 with one commercial site, but has grown substantially since then with over 90 aquaculture sites in 2012 and is now the largest single food commodity in New Brunswick with a valuation of \$117.3 million CAD in 2013 (Government of New Brunswick, 2012). Further, the industry employ an estimated 1,500 people - accounting for one in every five jobs in Charlotte County, New Brunswick (Government of New Brunswick, 2012). The constant growth of the aquaculture industry in the southwest Bay of Fundy has brought a range of economic and societal benefits to the province. Also, there are pilot projects ongoing that oversee Atlantic sturgeon as a possible commercial export from the aquaculture industry (DFO,

2014c). Yet as the industry expanded, ecological impacts have been shown to have an adverse effect on the marine environment (BurrIDGE et al., 1999; Chou et al., 2002, 2003; Government of New Brunswick, 2012).

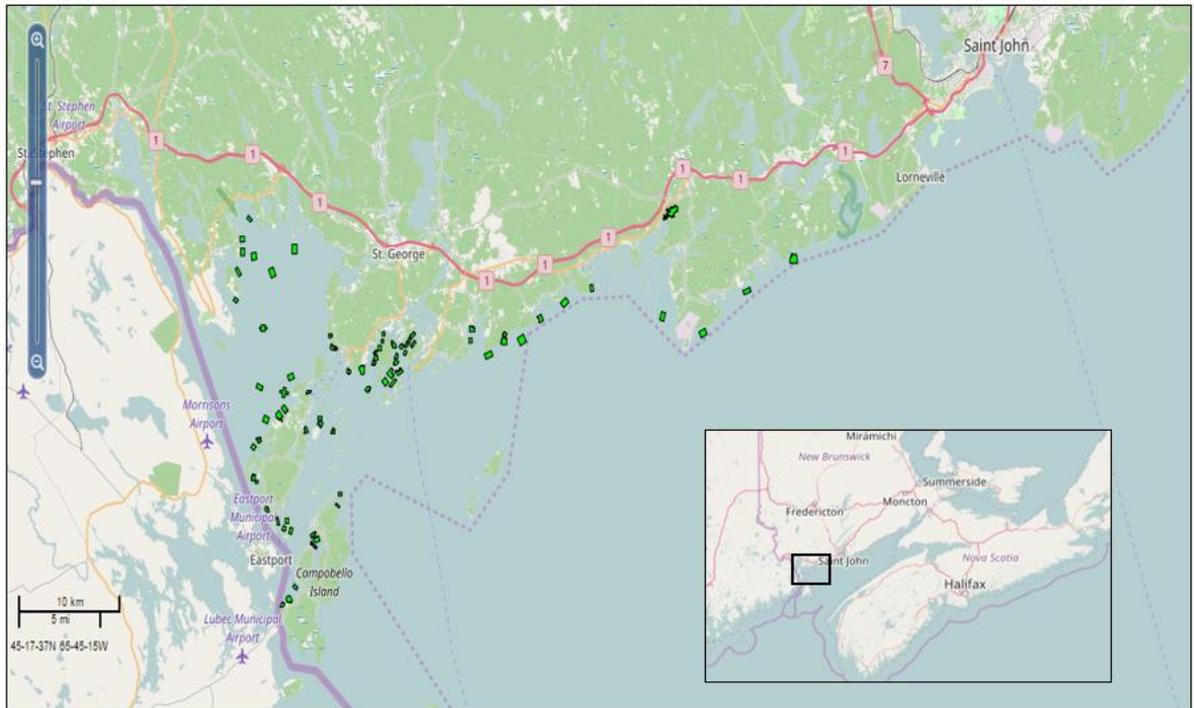


Figure 2.1: Map of the Quoddy region in the southwest Bay of Fundy with the location of leased aquaculture sites in the area highlighted in green. Source: Government of New Brunswick, 2017.

As a result of an outbreak of salmon anaemia in 1996, a management strategy in New Brunswick introduced Bay Management Areas (BMAs) in an effort to prevent further economic loss from infected stocks (McGeachy and Moore, 2003; NBDFA, 2000). The aim of this was to improve cooperation and connections between fish farms to protect fish health and the management of aquaculture practices (Chang et al., 2006). The area was divided into separate BMAs, where 22 were designated throughout the region to account for all licensed finfish farms. The thought process concerning the BMA boundaries took fish health, economic and oceanographic conditions into account (Halse, 2002). Salmon aquaculture operations are obliged to function as single year class operations, on a 3-yr rotation cycle (Chang et al., 2007). The initial outbreaks of disease resulted in the northern part of the Quoddy region being allocated as odd-year farms, and those in the southern part allocated even-year operations (Chang et al., 2006).

The southwest Bay of Fundy area has been the focus of much research given that it is home to 30% of the entire Canadian salmon aquaculture production, making it a suitable case study when identifying impacts on benthic communities (Hellou et al., 2005). Older reports focusing upon the state of aquaculture in this region identified high levels of heavy metals and persistent organic pollutants (POPs) in sediments (Burridge et al., 1999). In addition, metal concentrations were measured near Atlantic salmon pens, with high levels of copper and zinc concentrations present in *Homarus americanus* (Chou et al., 2002, 2003).

However, it is important to state that aquaculture companies have been making changes to the way that they operate, with an emphasis on reducing the impacts on the environment. As discussed, new methods are being developed to treat sea lice, such as the introduction of cleaner fish inside the pens (although cleaner fish are not yet used in pens New Brunswick). There have also been efforts to reduce to use of antifoulant paints that aim to minimise the amount of biofouling on the pens. There has been a large effort to find a substitute to replace antifoulant paints, with some success being reported with biological control in the form of herbivorous fish and invertebrates (Fitridge et al., 2012). In New Brunswick, Cooke Aquaculture has switched to using remotely-operated net cleaners to scrape off pens (M. Szemerda, pers. com.).

Despite the improvements to its environmental footprint over time, the aquaculture industry has come under scrutiny from a range of groups. In the Bay of Fundy, tensions have arisen between the aquaculture industry and capture fisheries (predominately lobster, *Homarus americanus*). Studies of commercial fishers show the divide between the two industries is growing and trust is fragile (Walters, 2007). Wiber et al (2012) used the knowledge of fishers to conclude that aquaculture inputs result in crab, shrimp and lobster mortalities, with the latter being a commercially important species in the region. Given the changes in aquaculture industry practices and the tensions between coastal industries, there is a need for new research to understand the effects of salmon aquaculture on the marine environment, and crucially decapod crustaceans.

## 2.6. Research Questions

This thesis examines the impacts that aquaculture operations have on decapod crustaceans and fishes by comparing assemblages colonizing cobble-filled bio-collectors (see Methodology) that are in close proximity (~200 m) to aquaculture sites, to those at least 1 km from aquaculture operations.. In order to quantify the impacts of aquaculture on the benthic communities of cobble habitat in the Quoddy region, New Brunswick, the following research questions were developed:

1. How do decapod crustacean and fish assemblages colonizing cobble-filled collectors compare between sites located near (~200 m) aquaculture sites to those collectors deployed away (~1200 m) from aquaculture sites?
2. As temporal variability is common in marine communities and salmon aquaculture is done on a 3-year production cycle, does any effect of nearness to aquaculture pens on decapod crustacean and fish assemblages differ between years (2015 samples and 2016 samples)?
3. Does the abundance of juvenile American lobsters differ between cobble-filled collectors located near aquaculture sites and collectors at sites away from aquaculture pens?



## **3 Methodology**

### **3.1. Sampling tool: bio-collectors**

To investigate the impacts that aquaculture activities have on decapod crustacean and fish assemblages, settlement collectors were deployed during the summers of 2015 and 2016 throughout the Quoddy region of the Bay of Fundy, New Brunswick. Settlement collectors are a sampling tool developed by Wahle et al. (2009). Wahle et al. (2009, 2013) developed the collectors to measure American lobster recruitment but researchers have found that they also provide useful information on other species of invertebrates and fishes (e.g. Ellis et al., 2015, Hunt et al., 2017). The collectors measure 61 cm x 91 cm x 15 cm and are made of 37 mm wire mesh lined on the sides and bottom with a 1 mm screen. The settlement collectors are filled with river cobble that normally range between 15-25 cm in diameter.

### **3.2. Study area and sampling strategy**

As discussed previously, the Bay Management Areas (BMAs) were introduced for salmon aquaculture management in southwestern New Brunswick in 1996. This was as a result of a disease outbreak and to reduce future economic losses for the area through a 3-year rotation of fish (year 1 fish, year 2 fish, fallow period). My thesis research was carried out within 3 of these BMAs: BMAs 1, 2a, and 3a (Fig. 3.1). Study locations were chosen as a result of recent studies that assessed the impact of aquaculture activities on egg-bearing *Homarus americanus*, as well as tidal excursion particle tracking models. The collectors were deployed at paired sites to reduce the effects of spatial variability. Each site pair had an area exposed to aquaculture-related activities (Near) and the other away from known aquaculture-related activities for 3+ years (Away) (Fig. 3.1). 8 pairs of sites were chosen, 3 each in Bay Management Areas (BMA) 1 and 3a, and 2 pairs in BMA2a (Table 1). The difference in pair numbers between BMAs was due to difficulties in locating suitable sites away from aquaculture sites in BMA2a. On average, the near sites were 200 m from the nearest aquaculture site while the away sites were an average 1200 m away (see Appendix A and B).

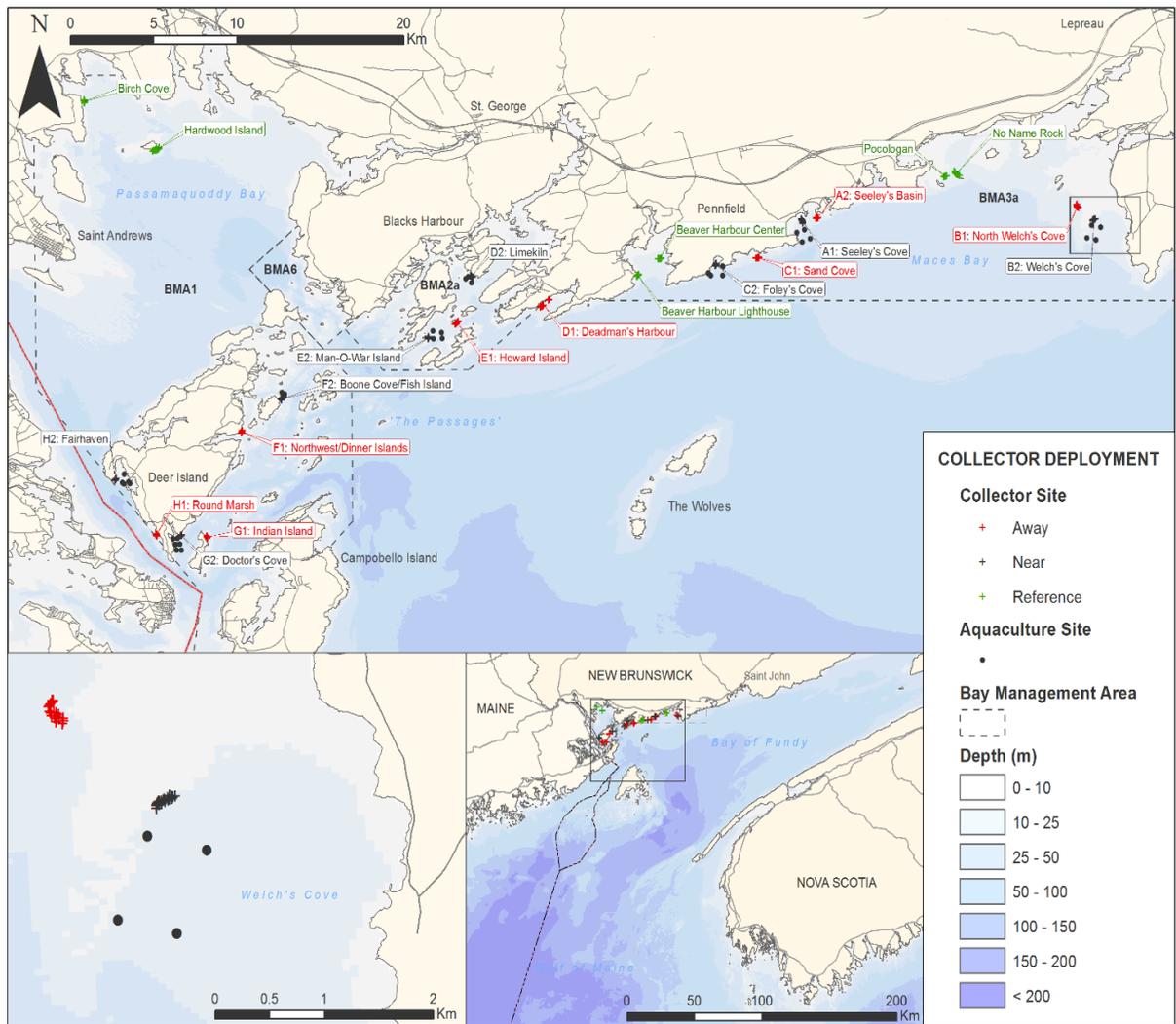


Figure 3.1: Map of the study site Quoddy region in the Bay of Fundy. Near sites are noticeable in black and away sites in red.

Table 1: A table showing the paired sites in their designated BMA. The near sites are highlighted in red and the away sites in blue.

BMA	FAIRHAVEN (NEAR)	DOCTOR'S COVE (NEAR)	BOONE COVE/FISH ISLAND (NEAR)
1	ROUND MARSH (AWAY)	INDIAN ISLAND (AWAY)	NORTHWEST/DINNER ISLANDS (AWAY)
BMA	MAN-O-WAR (NEAR)	LIMEKILN (NEAR)	
2	HOWARD ISLAND (AWAY)	DEADMAN'S HARBOUR (AWAY)	
BMA	FOLEY'S COVE (NEAR)	SEELEY'S COVE (NEAR)	WELCH'S COVE (NEAR)
3	SAND COVE (AWAY)	SEELEY'S BASIN (AWAY)	NORTH WELCH'S COVE (AWAY)

A total of 160 collectors (10 collectors per site) were deployed at the paired sites during July 2015 to overlap with the lobster settlement period in the region which takes place over the summer. Each collector was labelled for identification purposes and linked to a line with an attached buoy during deployment that assisted during the retrieval process. The collectors were then left for 4-5 months to be colonized by benthic biodiversity. Retrieval took place in November, 2015, whereby collectors were stacked onto the boat, returned to shore and then brought to the Huntsman Marine Science Centre in Saint Andrews, New Brunswick, for processing.

For the 2016 season, the same procedure was repeated for deployment and retrieval of collectors. Due to time limitations, only 7 collectors per site were deployed. This meant that 14 collectors per pair were deployed in 2016 compared to 20 during 2015.

Once on shore, the collectors were rinsed down with sea water in an effort to keep marine organisms alive and to rinse them to the bottom of the collector, making it easier to process. A team of up to 20 individuals assisted in processing the collectors. Upon opening a collector, each rock was carefully examined and any organism (not including encrusting organisms) located on the rock was removed and placed in a Ziplock® bag with the nametag of the collector placed inside. This process was repeated until all rocks were removed. Any larger organisms on the bottom of the collector identifiable with the naked eye were also placed in the bag. The collector was then rinsed and all remaining organisms and sediment were collected and gathered into the bag. The bags were placed in an ice cooler and transferred to the UNB Saint John campus and stored frozen. Following this, the bags were thawed and processed in the laboratory. The larger organisms which could be identified with the naked eye (fishes, crabs, lobster) in each bag were removed, identified, measured and placed back into the bag. The smaller organisms that could not be identified with the naked eye were placed into a sample jar with ethanol for preservation. For this thesis, smaller decapod crustaceans were later identified and measured under a microscope and kept preserved in ethanol. As a result of time limitations, only 5/7 collectors from 12/16 sites (4 from each BMA) in 2016 could be fully processed during the course of this thesis. In total, 60 collectors were fully processed for decapod crustaceans and fishes in 2016.

### 3.3. Statistical analysis

Univariate analyses was performed using PRIMER version 6 and focused on the following metrics for decapod crustaceans and fishes: total abundance, species richness and Pielou's evenness (Pielou, 1966), as they have shown to be critical indicators in defining the health of an ecosystem (Noss, 1990; Henderson & Ross, 1995; Rice, 2003). Three-way factorial ANOVAs were conducted using the dependent variables: total abundance, species richness and species evenness. Independent variables were treatment (near vs away) and Bay Management Area (BMA), which were both fixed factors, and aquasite (site pair), a random spatial factor that was nested in 'BMA'. The univariate analyses were done using an ANOVA which produces the same F-statistic as the traditional parametric univariate F-statistic for univariate data (Anderson, 2001). Euclidean resemblance matrices were used. Individual univariate analyses were done separately for total abundance, richness and evenness of decapods and fish. ANOVAs were conducted separately for 2015 and 2016. In order to determine whether effects were consistent between years, a four-way ANOVA was used year as an additional fixed factor. Two site pairs from the 2015 samples (BMA1 - Doctor's Cove/Indian Island; BMA3a - Seeley's Cove/Seeley's Basin) were excluded from the combined dataset as time limitations restricted the processing of those pairs in 2016. Consequently, analyses of the 2016 data and of the combined dataset included two pairs from each BMA.

A univariate analysis of abundance of juveniles for the commercially important lobster (*Homarus americanus*) was carried out by running an ANOVA using the PERMANOVA software. The factors were treatment (near vs away) and BMA, both fixed factors, and aquasite, a random spatial factor nested in BMA.

Multivariate analyses of the assemblage of decapod crustaceans and fishes were also performed using PRIMER v6. Analyses were done separately for untransformed as well as square-root transformed, logarithm transformed and presence/absence transformed data. The purpose of transformation for multivariate analyses is to explore how much abundant species affect the results. For each dataset, a resemblance matrix was then created based on Bray-Curtis similarities, which are most appropriate for species data (Clarke & Gorley, 2006). Non-metric Multidimensional Scaling (nMDS) ordination was performed to create a 2D plot that visually show the similarities between

collectors grouped by the chosen factors. Stress is a measure of how well the ordination represents the similarities between the samples and values below 0.2 represent a reasonable fit. The MDS ordination in PRIMER uses the Kruskal stress formula 1 to standardise stress to make similarities a better fit. For each transformation, similarities in the assemblage of decapod crustaceans and fishes were compared between fixed (BMA and treatment) and random (aquasite) factors using a three-way permutational analysis of variance (PERMANOVA). This procedure was run for the 2015 and 2016 samples separately. Factors that proved to be statistically significant ( $p < 0.05$ ) were analysed further using pair-wise tests that determine which levels differed. Similarly to the univariate analyses, a combined multivariate analysis of 2015 and 2016 data was then produced to compare years. The taxa that had the highest contribution to similarities between treatments and BMAs were identified using a similarity percentage (SIMPER) test.



## 4 Results

### 4.1. 2015 samples

A total of 3,610 individual decapod crustaceans and fishes were identified from the 160 samples. The species that contributed the most to the total abundance of the decapod and fish assemblage were the fish species, *Pholis gunnellus* (18.7%) and the decapod species *Cancer irroratus* (crab) (11.0%), *Homarus americanus* (lobster) (10.7%) and *Eualus pusiolus* (shrimp) (9.8%). The maximum number of individuals found at one site ranged from 415 (pooled across 10 bio-collectors) at Fairhaven in Bay Management Area 1 (near) to 120 at Doctor's Cove, also a near site in BMA1. (Fig. 4.1). Sites near the aquaculture operation had a mean abundance of 23 whilst the collectors located away from aquaculture pens had a mean abundance of 21 individuals per collector. PERMANOVA revealed a significant interaction between aquasite (nested in BMA) and treatment for total abundance of individuals of decapod crustaceans and fishes (Table 2). No significant effect of BMA or other significant interactions were observed (Table 2).

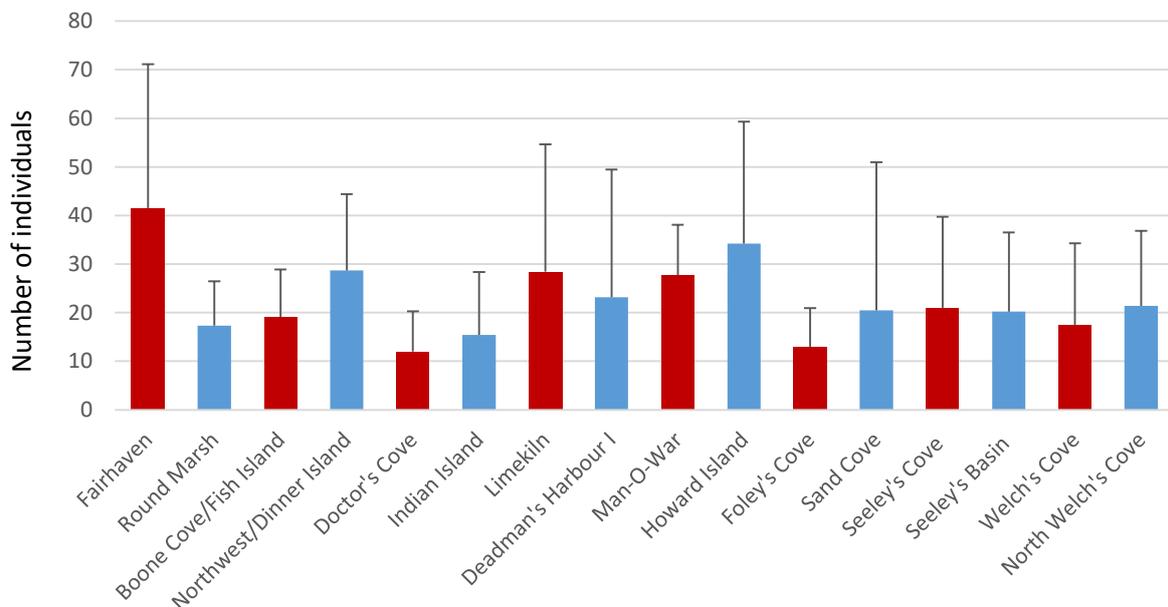


Figure 4.1: Average (+SE) abundance of decapod crustaceans and fish at each site in 2016. The near sites are highlighted in red and the away sites are highlighted in blue.

Table 2: Univariate results for total abundance of individuals, species richness and species evenness for decapods crustaceans and fishes for 2015. The factors were BMA (BM), treatment (TR) and aquasite nested in BMA (AQ (BM)).

Source of variation	df	IND. of ABUNDANCE			SPECIES RICHNESS			PIELOU'S EVENNESS		
		MS	F	P	M	F	p	M	F	p
BM	2	698.81	0.93303	0.444	44.452	5.1732	0.009	1.1312E-2	0.62502	0.574
TR	1	64.241	7.8442E-2	0.816	5.1126E-2	1.0573E-2	0.909	8.2738E-3	0.23231	0.67
AQ (BM)	5	740.72	2.1206	0.071	8.4277	2.0063	0.074	1.7977E-2	2.3219	0.041
BM x TR	2	251.01	0.27656	0.788	19.888	1.3122	0.364	1.8389E-2	0.51081	0.646
AQ(BM) x TR	5	915.44	2.6208	0.024	14.903	3.5477	0.005	3.5557E-2	4.5925	0.001
RESIDUAL	154	349.29			4.2007			7.7424E-3		

The mean abundance of fish pooled across collectors at a site ranged from 16 at Howard Island in Bay Management Area 2a (away) to 3 at Sand Cove, an away site in BMA3a. PERMANOVA revealed a significant effect of aquasite (nested in BMA) for total abundance of fishes (Table 3). There was no significant effect of BMA, treatment or other significant interactions for total fish abundance (Table 3).

Table 3: Univariate results for total abundance of individuals for decapods, fish and lobster for 2015. The factors were BMA (BM), treatment (TR) and aquasite nested in BMA (AQ (BM)).

Source of variation	df	IND. of ABUNDANCE (DECAPODS)			IND. of ABUNDANCE (FISH)			IND. of ABUNDANCE (LOBSTER)		
		MS	F	p	MS	F	p	MS	F	p
BM	2	64.875	0.17815	0.732	609.21	3.8385	0.071	271.54	17.983	0.026
TR	1	0.62976	1.0001E-3	0.973	1.0714E-2	2.5093E-4	0.986	22.671	1.2233	0.316
AQ (BM)	5	364.17	1.1923	0.309	158.71	6.8611	0.001	15.1	3.3943	0.007
BM x TR	2	301.21	0.47836	0.653	17.204	0.40292	0.701	29.154	1.5731	0.278
AQ(BM) x TR	5	629.67	2.0616	0.06	42.698	1.8459	0.107	18.533	4.1661	0.002
RESIDUAL	154	305.42			5591.8			4.4486		

386 juvenile American lobster were present in the samples in 2015. The maximum number of individuals found at one site (pooled across 10 collectors) ranged from 83 at Welch's Cove (near) in Bay Management Area 3a to 0 at Fairhaven (near), Round Marsh (away), Doctor's Cove (near) and Indian Island (away) in BMA1. Sites near an aquaculture operation had a mean abundance of 31 per site whilst the collectors located further away had a mean abundance of 27 (Fig. 4.2). The mean abundance was higher in BMA3a (33) and BMA2a (31) than BMA1 (3) (Fig. 4.3). PERMANOVA revealed a significant difference among BMAs and a significant interaction between

aquasite (nested in BMA) and treatment for total abundance of juvenile American lobster (Table 3). There was no significant effect of treatment (near vs away) or between the interaction of Bay Management Area and treatment.

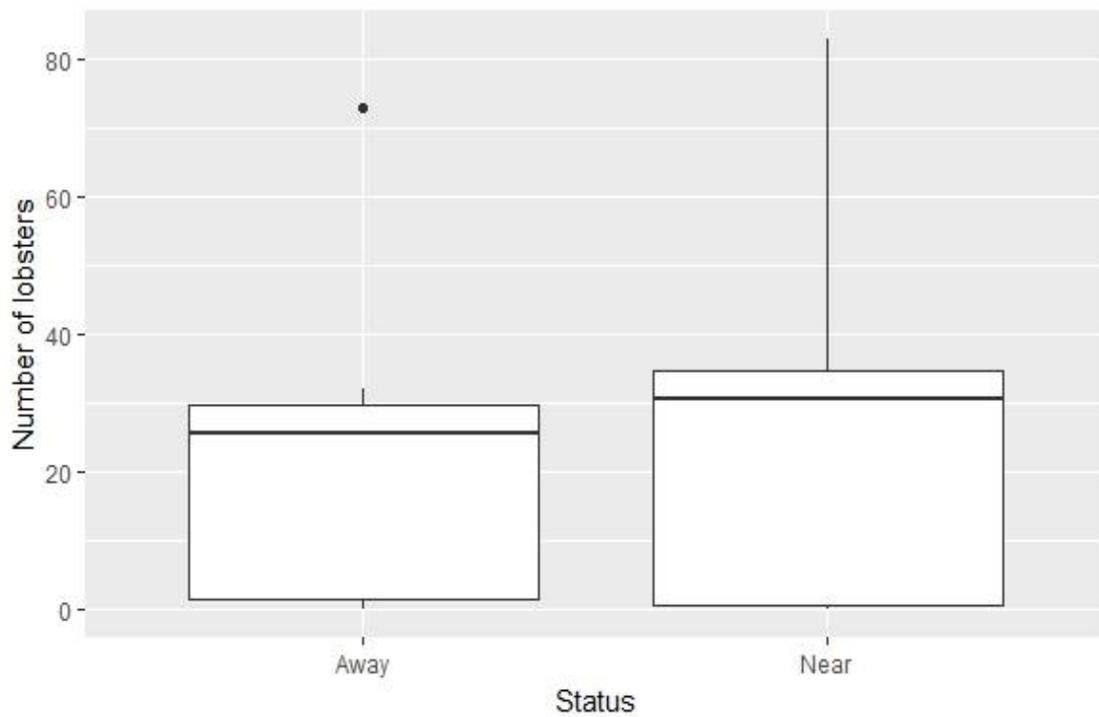


Figure 4.3: Boxplot of total abundance of juvenile American lobsters per site at near and away sites in 2015. Lobsters were pooled across 10 collectors for each site.

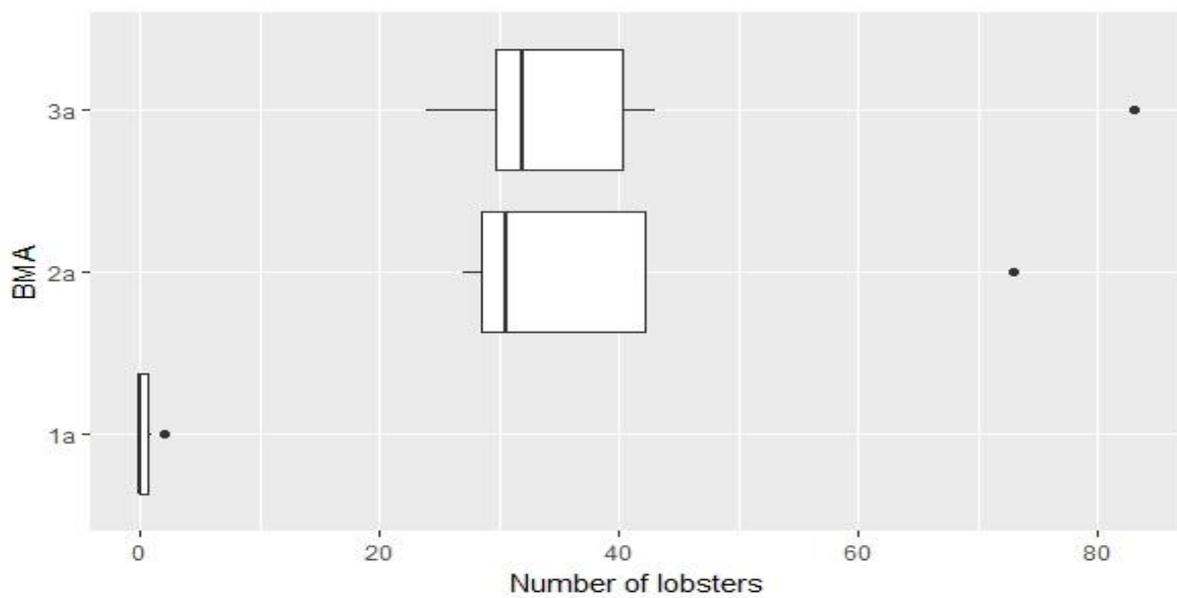


Figure 4.2: Boxplot of total abundance of juvenile American lobsters per site between BMAs in 2015. Lobsters pooled across collectors for each site, where each site has 10 collectors and the average per site is given per BMA.

26 species of decapod crustaceans and fishes were present at the sites sampled. The maximum number of species found at one site ranged from 17 at Howard Island (away site) in Bay Management Area 2a, to 10 at Fairhaven, a near site in BMA1. The mean species richness was greatest at Man-O-War (near) in BMA2a and least at Welch's Cove (near) in BMA3a (Fig. 4.4). Collectors in the three BMAs had similar mean species richness per site (BMA2a: 7; BMA3a: 5; BMA1:5). PERMANOVA revealed a significant interaction between aquasite (nested in BMA) and treatment for species richness of decapod crustaceans and fishes (Table 2). There was no significant effect of treatment, aquasite or BMA nor any significant interactions (Table 2).

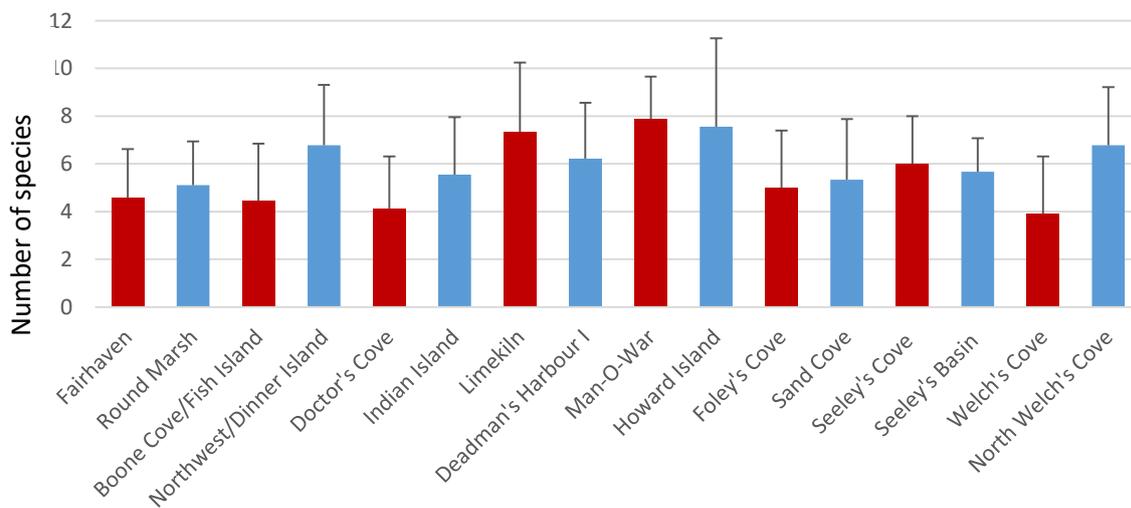


Figure 4.4: Average (+SE) species richness for decapod crustaceans and fish at each site in 2015. The near sites are highlighted in red and the away sites are highlighted in blue.

Similarly, when decapod crustaceans and fishes were considered separately, no significant differences occurred in species richness between treatments (Table 4). The mean number of decapod crustacean species was significantly higher in Bay Management Area 2a (4 per site) than BMA3a (3) and BMA1 (3) but there was no significant effect of treatment or any significant interactions (Table 4). The mean number of fish species per site ranged from 4 at Man-O-War (near) in BMA2a to 1 at Doctor's Cove (near) in BMA1. For fishes, there was a significant difference in richness between pairs of sites but no significant effect of treatment, BMA, and no significant interactions between the factors.

Table 4: Univariate results for separate analyses of richness of decapod and fish species for 2015 data. The factors were BMA (BM), treatment (TR) and aquasite nested in BMA (AQ (BM)).

Source of variation	df	SPECIES RICHNESS (DECAPODS)			SPECIES RICHNESS (FISH)		
		MS	F	p	MS	F	p
BM	2	6.6802	25.051	0.009	22.614	5.6067	0.081
TR	1	5.5048	1.4067	0.278	1.219	1.1015	0.331
AQ (BM)	5	0.26667	8.7016E-2	0.993	4.0333	3.2213	0.008
BM x TR	2	6.8385	1.7475	0.273	2.4302	2.196	0.208
AQ(BM) x TR	5	3.9133	1.277	0.268	1.1067	0.88386	0.489
RESIDUAL	154	3.0646			1.2521		

For Pielou's evenness index, Round Marsh (0.92) in BMA1 was the site with the greatest species evenness of decapod crustaceans and fishes, with Welch's Cove (0.73) in BMA3a as the least even in terms of abundance of species (see Appendix C, Table 2). PERMANOVA revealed a significant interaction between aquasite (nested in BMA) and treatment for Pielou's evenness index (Table 2). There was no significant effect of BMA, treatment or interaction between these two factors (Table 2).

Multivariate MDS plots generally showed that the decapod crustacean and fish assemblages in collectors in BMA2a and BMA3a were similar to each other and differed from those from BMA1. This pattern emerges for both untransformed (not shown), square-root transformed (not shown), log-transformed (Fig. 4.5) and presence/absence data (Fig. 4.6), showing that this trend is driven more by species identity than by species abundance. The stress values for 2D MDS plots were somewhat high (0.2), whereas the 3D MDS plots showed lower stress values (0.15), with similar patterns (not shown). Untransformed, square-root, log-transformed and presence/absence transformed data had similar PERMANOVA results (Table 5). There was a significant effect of BMA and a significant interaction between treatment and aquasite (nested in BMA) (Table 5), indicating that there are differences between treatments at some of the paired sites but not others. The interaction between BMA and treatment was not significant (Table 5). Pair-wise tests indicate that BMA2a is significantly different from BMA1 (log-transformed data:  $t = 2.25$ ,  $p < 0.001$ ) and BMA3a (log-transformed data:  $t = 1.45$ ,  $p < 0.001$ ), yet BMA1 and BMA3a did not differ significantly (log-transformed data:  $t = 3.20$ ,  $p > 0.093$ ).

Table 5: PERMANOVA results for untransformed, square-root transformed, logarithmic transformed and presence/absence transformed data for 2015. The factors were BMA (BM), treatment (TR), aquasite nested in BMA (AQ (BM)).

Source of variation	Df	Untransformed			Square-root transformation			Log (X + 1) transformation			Presence/absence transformation		
		MS	F	p	MS	F	p	MS	F	p	MS	F	p
<i>BM</i>	2	34050	4.7179	0.002	30596	6.60609	0.001	29391	6.1758	0.007	24242	7.4183	0.001
<i>TR</i>	1	2909.6	0.6071	0.673	2045.2	0.65085	0.656	1694.6	0.5839	0.669	1980.2	1.1613	0.356
<i>AQ (BM)</i>	5	7066.3	4.1127	0.001	4941.3	4.1063	0.001	4658.3	4.1799	0.001	3198.4	3.8371	0.001
<i>BM x TR</i>	2	7737	1.6043	0.163	5251.8	1.661	0.158	5078.5	2.5803	0.131	3454.5	2.0231	0.094
<i>AQ(BM) x TR</i>	5	4739.9	2.7587	0.001	3108.1	2.5829	0.001	2875.6	2.5803	0.001	1679	2.0142	0.008
<i>RESIDUAL</i>	154	1718.2			1203.3			1114.4			833.56		

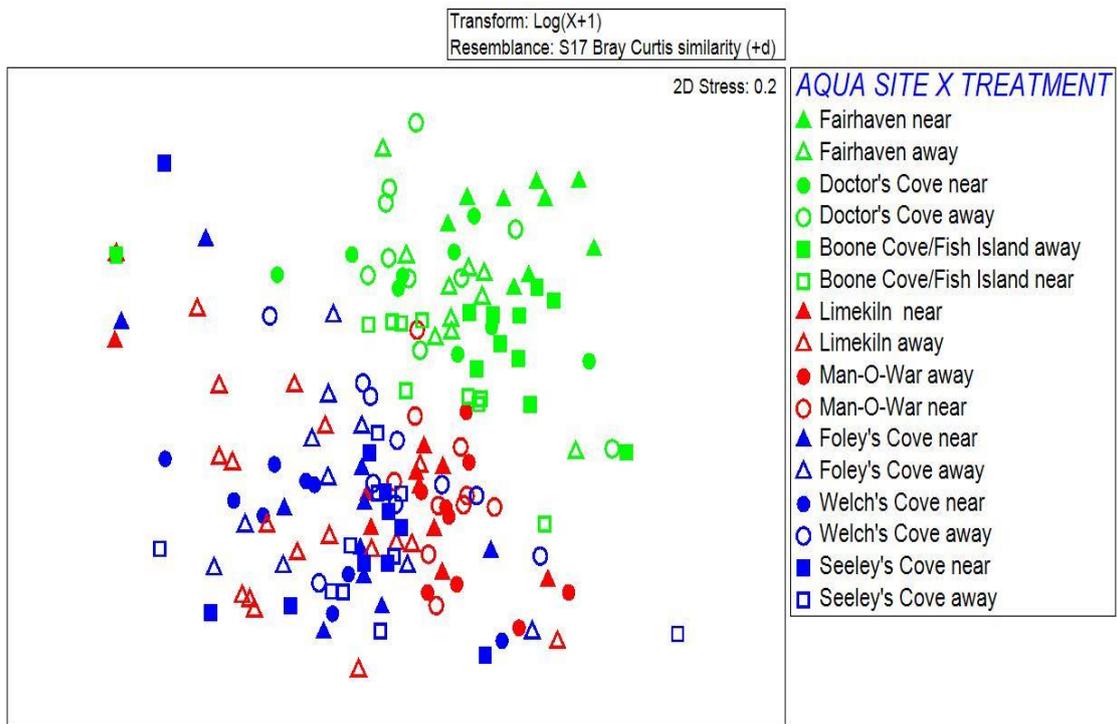


Figure 4.5: Two-dimensional MDS plot of logarithmic ( $\log(X + 1)$ ) transformed data for 2015 based on Bray-Curtis similarities. Each symbol represents one collector, with the shaded symbols as a near collector and the non-shaded symbols represent the away collectors. The green symbols represent those collectors from BMA1, red symbols represent BMA2a and blue represent BMA3a. The stress value is given in the upper right corner of the MDS.

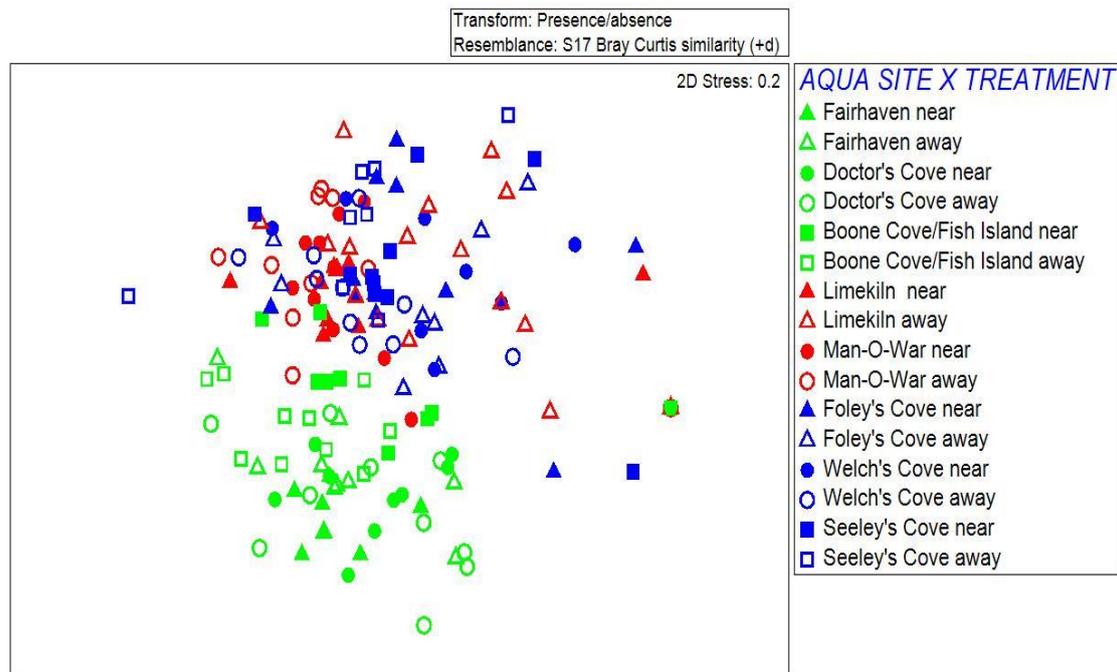


Figure 4.6: Two-dimensional MDS plot of presence/absence transformed abundance data for 2015 based on Bray-Curtis similarities. Each symbol represents one collector, with the shaded symbols as a near collector and the non-shaded symbols represent the away collectors. The green symbols represent those collectors from BMA1, red symbols represent BMA2a and blue represent BMA3a. The stress value is given in the upper right corner of the MDS.

## 4.2. 2016 samples

A total of 7,762 individuals of decapod crustaceans and fishes were identified from the 60 collectors. The species that contributed the most to the total abundance of decapods and fishes were the shrimp species *Eualus spp.* (86.35%), *Pandalus montagui* (2.28%) and *Coridian gordini* (2.09%), and the fish species, *Pholis gunnellus* (2.34%). The maximum number of individuals found at one site ranged from 1,749 at Deadman's Harbour in Bay Management Area 2a (away site) to 80 at Boone Cove/Fish Island, a near site in BMA1 (Fig. 4.7). Sites near the aquaculture pens had a mean abundance of 108 whilst the collectors located further away had a mean abundance of 151 individuals per collector. PERMANOVA revealed a significant interaction between aquasite (nested in BMA) and treatment for total abundance of individuals of decapod crustaceans and fishes (Table 6). There was no significant effect of BMA, treatment or interaction between these two factors (Table 6).

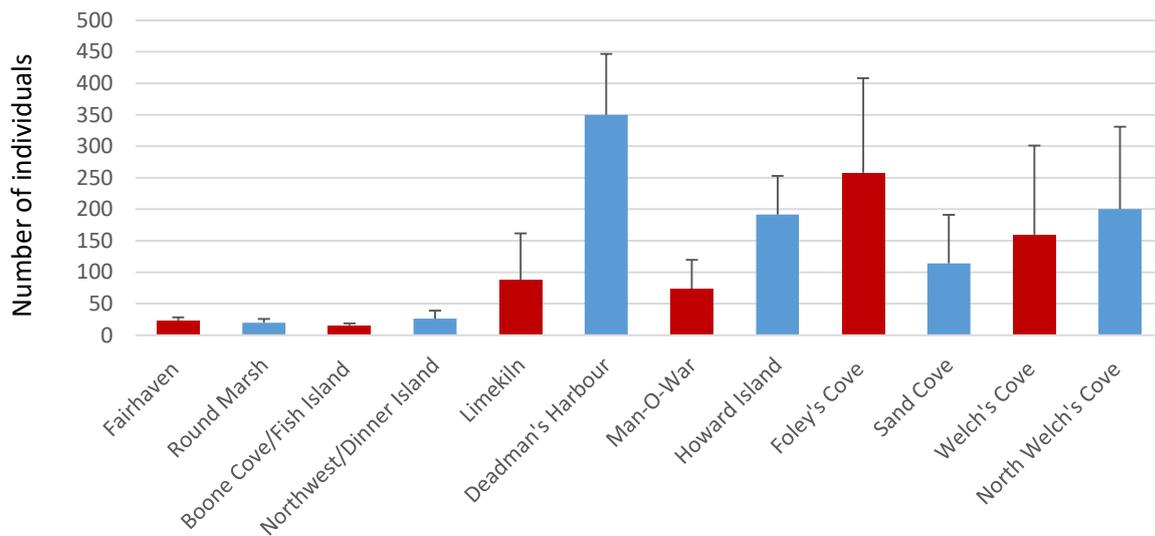


Figure 4.7: Average (+SE) abundance of decapod crustaceans and fish at each site in 2016. The near sites are highlighted in red and the away sites are highlighted in blue.

Table 6: Univariate results for individual abundance, species richness and Pielou's evenness for decapod crustaceans and fishes in collectors in 2016. The factors were BMA (BM), treatment (TR; near vs away) and aquasite nested in BMA (AQ (BM); pairs of sites).

Source of variation	df	IND. of ABUNDANCE			SPECIES RICHNESS			PIELOU'S EVENNESS		
		MS	F	p	MS	F	p	MS	F	p
BM	2	1.6656E5	13.445	0.215	14.067	2.1313	0.318	0.85484	8.3786	0.08
TR	1	27136	1.3428	0.326	13.067	2.0206	0.2573	4.311E-4	2.3554E-2	0.817
AQ (BM)	3	12388	1.8446	0.131	6.6	2.9663	0.054	0.10203	15.448	0.001
BM x TR	2	86709	4.2907	0.142	4.8667	0.75258	0.5566	0.15953	8.7162	0.059
AQ(BM) x TR	3	20208	3.0091	0.038	6.4667	2.9064	0.036	01.8303E-2	2.7713	0.045
RESIDUAL	48	6715.9			2.225			6.6044E-3		

The mean abundance of fish at a site ranged from 10 at Fairhaven in Bay Management Area 1 (near) to 0 at Sand Cove, an away site in BMA3a. PERMANOVA revealed a significant effect of aquasite for individual abundance of fishes (Table 7). There was no significant effect of BMA, treatment or any significant interactions (Table 7). The abundance of decapod crustaceans per collector ranged from 338 at Deadman's Harbour in Bay Management 2a (away) to 6 at Boone Cove/Fish Island, a near site in BMA1. PERMANOVA revealed a significant interaction between aquasite (nested in BMA) and treatment for total abundance of decapod crustaceans (Table 7). There was no significant effect of BMA, treatments or other significant interactions (Table 7).

Table 7: Univariate results for the abundance of decapods, fish and lobster for 2016. The factors were BMA (BM), treatment (TR) and aquasite nested in BMA (AQ (BM)).

Source of variation	df	ABUNDANCE of INDIVIDUALS (DECAPODS)			ABUNDANCE of INDIVIDUALS (FISH)			ABUNDANCE of INDIVIDUALS (LOBSTER)		
		MS	F	p	MS	F	p	MS	F	p
BM	2	1.851E5	14.171	0.209	267.52	6.7187	0.191	13.117	23.848	0.134
TR	1	28297	1.5211	0.296	0.41667	6.1125E-2	0.822	0.81667	0.92453	0.4344
AQ (BM)	5	13062	1.9473	0.133	39.817	5.938	0.002	0.55	0.55932	0.621
BM x TR	2	81580	4.3854	0.134	28.817	4.2274	0.1451	2.2167	2.5094	0.231
AQ(BM) x TR	5	18602	2.7734	0.039	6.8167	0.87207	0.467	0.88333	0.89831	0.445
RESIDUAL	154	6707.5			7.8167			0.98333		

59 juvenile American lobster were present in the samples in 2016. The maximum number of individuals found at one site (pooled across 7 collectors per site) ranged from 12 at Welch's Cove (near site) in BMA 3a to 0 at Fairhaven (near), Round Marsh (away) and Boone Cove Fish Island (near) in BMA1. Sites near the aquaculture operation had a mean abundance of 6 juvenile lobster whilst the collectors located further away had a mean abundance of 5 (Fig. 4.8); and no significant effect for treatment was observed (Table 7). The mean abundance of juvenile lobster per site ranged from 6 in BMA2a and BMA3a to 0 in BMA1 (Fig. 4.9). However, PERMANOVA revealed there was no significant effect of BMA or aquasite or significant interactions (Table 7).

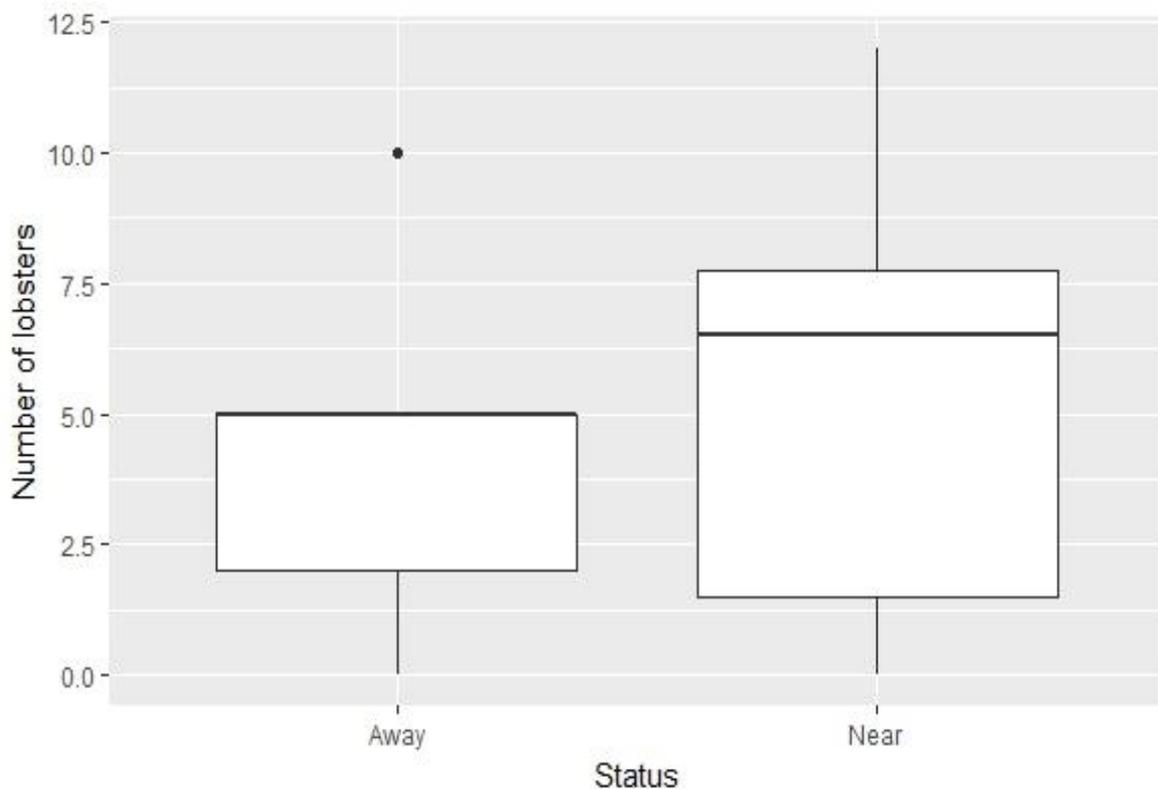


Figure 4.8: Boxplot of total abundance of juvenile American lobsters per site at near and away sites in 2016. Lobsters pooled across collectors for each site, where each site has 7 collectors and the average per site is given.

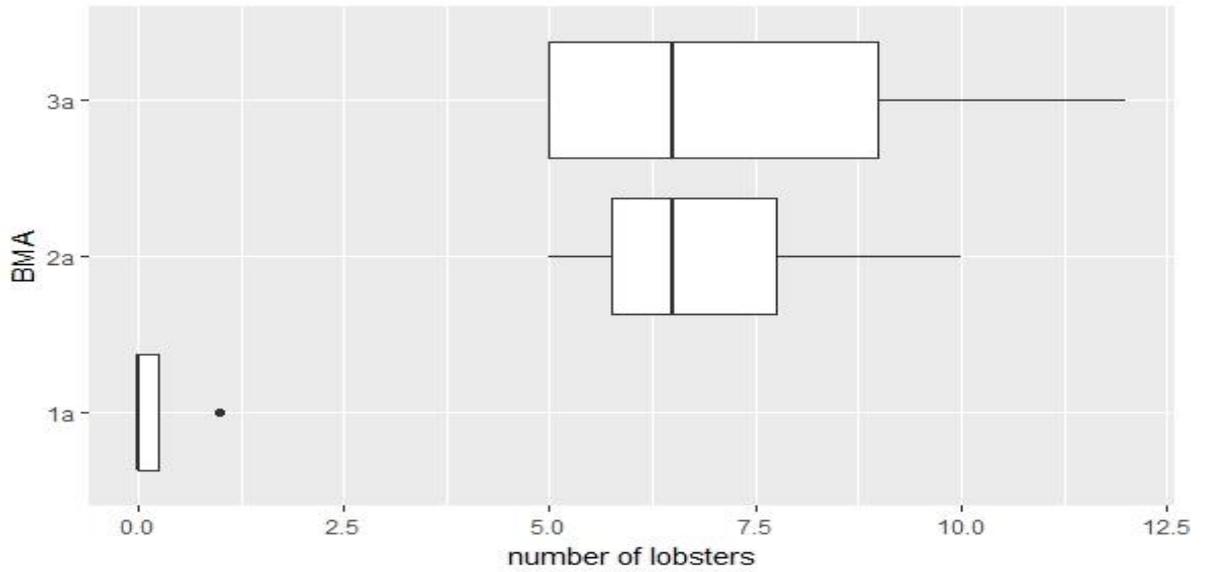


Figure 4.9: Boxplot of total abundance of juvenile American lobsters per site between BMAs in 2016. Lobsters pooled across collectors for each site, where each site has 7 collectors and the average per site is given per BMA.

34 species of decapod crustaceans and fishes were present in collectors sampled in 2016. The maximum number of species found at one site ranged from 16 at Howard Island (away site) in Bay Management Area 2a and Foley’s Cove (near) in BMA3a, to 10 at Welch’s Cove, a near site in BMA3a. The species richness per site (pooled across 7 collectors per site) ranged from 10 at Howard Island (away) in BMA2a to 5 at Welch’s Cove (near) in BMA3a (Fig. 4.10). PERMANOVA revealed a significant interaction between aquasite (nested in BMA) and treatment for mean species richness of decapod crustaceans and fishes (Table 6). There was no significant effect of treatment or BMA and no significant interaction between these two factors (Table 6).

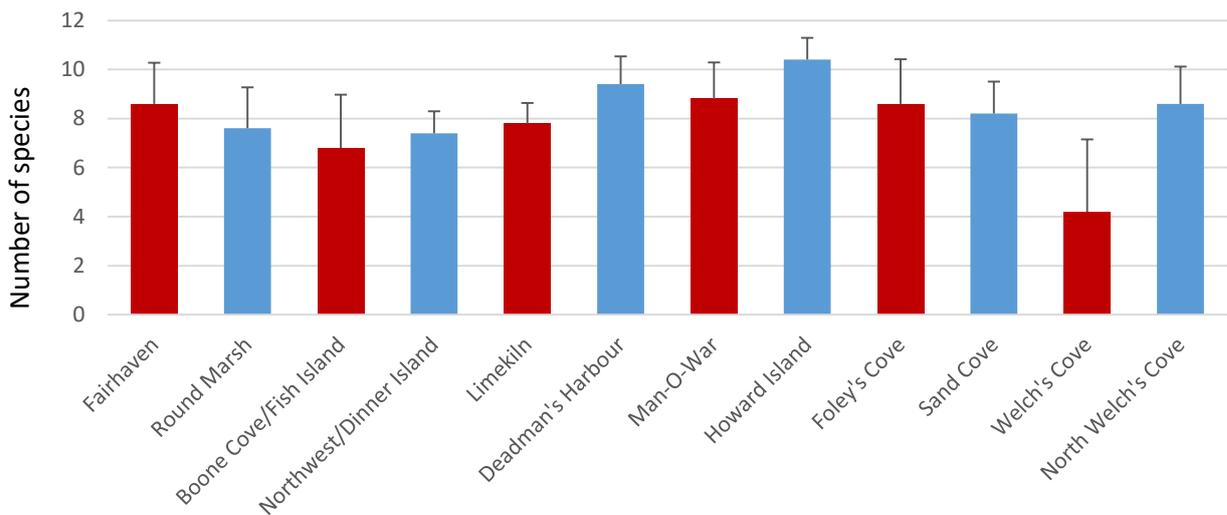


Figure 4.10: Average (+SE) species richness for each collector in 2016. The near sites are highlighted in red and the away sites are highlighted in blue.

The mean number of fish species per site ranged from 3 at Howard Island in Bay Management Area 2a (away) to 0 at Sand Cove in BMA3a (away). PERMANOVA revealed a significant effect of aquasite (nested in BMA) for richness of fishes (Table 8). There was no significant effect of BMA, treatment or other significant interactions for fish richness. The mean number of decapod crustacean species per site ranged from 7 at Foley’s Cove (near site), Sand Cove (away) in BMA3a, and Deadman’s Harbour (away) in BMA2a, to 3 at Boone Cove/Fish Island (near) in BMA1. PERMANOVA revealed a significant effect of aquasite (nested in BMA) for richness of decapod crustaceans (Table 8). There were no significant effect of BMA or treatment or significant interactions for the richness of decapod species (Table 8).

*Table 8: Univariate results for richness of decapod and fish species for 2016. The factors were BMA (BM), treatment (TR) and aquasite nested in BMA (AQ (BM)).*

		SPECIES RICHNESS (DECAPODS)			SPECIES RICHNESS (FISH)		
Source of variation	df	M	F	p	M	F	p
BM	2	39.517	5.5268	0.22	12.717	2.18	0.331
TR	1	12.15	9.9863	0.056	0.26667	0.2963	0.632
AQ (BM)	5	7.15	5.3292	0.007	5.8333	8.75	0.001
BM x TR	2	0.15	0.12329	0.8739	1.0167	1.1296	0.438
AQ(BM) x TR	5	1.2167	0.90683	0.434	0.9	1.35	0.264
RESIDUAL	154	1.3417			0.66667		

For Pielou’s evenness index, Round Marsh (0.90) in BMA1 had the greatest species evenness, while Welch’s Cove (0.22) in BMA3a had the least species evenness (see Appendix D, Table 6). PERMANOVA revealed a significant interaction between aquasite (within BMA) and treatment (Table 6). There was no significant effect of BMA, treatment or the interaction between these two factors (Table 6).

In multivariate MDS plots, decapod and fish assemblages in collectors in BMA2a and BMA3a were similar to one another and separated from the assemblages in collectors from BMA1. This pattern occurred for untransformed and square-root transformed data (not shown), as well as for log-transformed (Fig. 4.11) and presence/absence transformed data (Fig. 4.12), showing that this pattern is driven more by species identity than abundance of individuals. The MDS plots suggest no obvious differences between near and away sites across BMA2a and BMA3a. The stress values

for 2D MDS showed that both the MDS plots for log transformed data (0.14) and presence/absence transformed data (0.18) were reasonable representations of the similarities between collectors; the 3D MDS plots showed similar patterns with lower stress values. For both transformed and untransformed data, PERMANOVAs indicate a significant interaction occurred between treatment and aquasite, with aquasite nested in BMA (Table 9). This indicates that there are significant differences between some of the paired sites, but not for other site pairs. There was no significant effect of BMA (although this difference was only marginally non-significant for untransformed and presence-absence data) or interaction between treatment and BMA (Table 9).

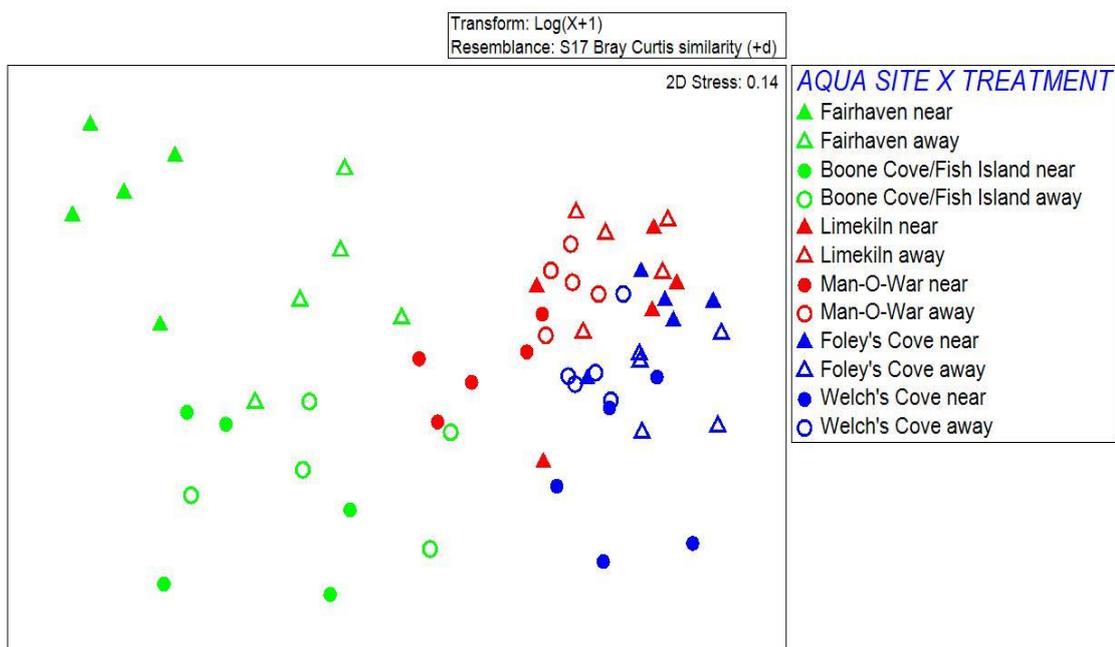


Figure 4.11: Two-dimensional MDS plot of a  $(\text{Log}(X + 1))$  transformed abundance data for 2016. Each symbol represents one collector, with the shaded symbols as a near collector and the non-shaded symbols represent the away collectors. The green symbols represent those collectors from BMA1, red symbols represent BMA2a and blue represent BMA3a. The stress value is given in the upper right corner of the MDS.

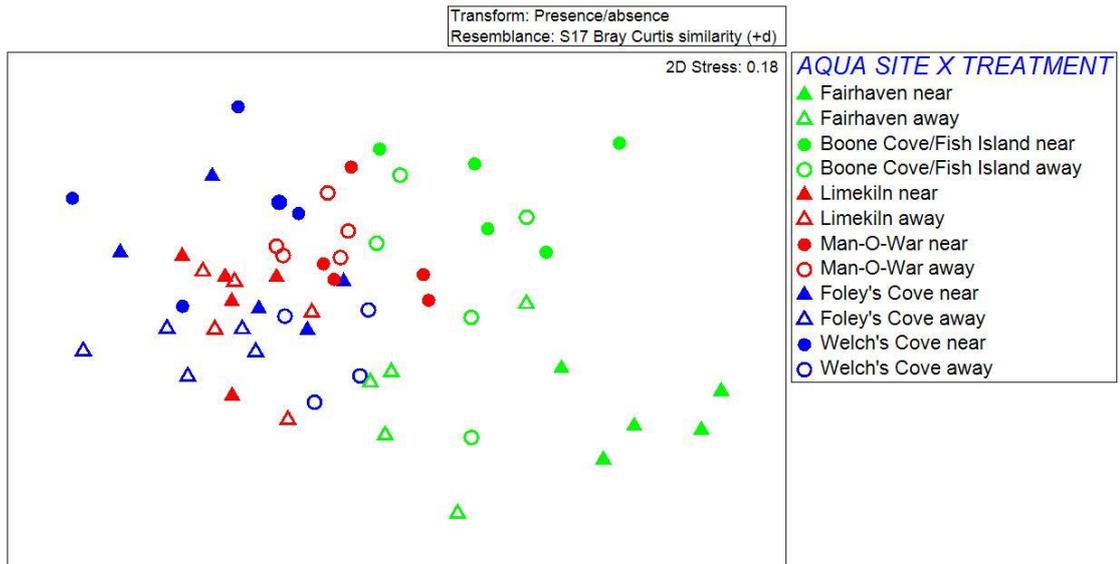


Figure 4.12: Two-dimensional MDS plot of a presence/absence transformed abundance data for 2016. Each symbol represents one collector, with the shaded symbols as a near collector and the non-shaded symbols represent the away collectors. The green symbols represent those collectors from BMA1, red symbols represent BMA2a and blue represent BMA3a. The stress value is given in the upper right corner of the MDS.

Table 9: PERMANOVA results for untransformed, square-root transformed, logarithmic transformed and presence/absence transformed decapod and fish data for the 2016 samples. The factors were BMA (BM), treatment (TR) and aquasite nested in BMA (AQ (BM)).

Source of variation	df	Untransformed			Square-root transformation			Logarithm transformation			Presence/absence transformation		
		MS	F	p	MS	F	P	MS	F	p	MS	F	p
<b>BM</b>	2	4947.2	3.8047	0.065	21170	6.1886	0.144	18374	5.7117	0.68	10228	3.5887	0.069
<b>TR</b>	1	356.34	0.78271	0.4715	2273.1	0.92176	0.47	2015.6	0.88557	0.471	1779.1	0.99402	0.414
<b>AQ (BM)</b>	3	1300.3	13.18	0.001	3420.9	5.7752	0.001	3216.9	6.243	0.001	2850.1	5.7989	0.001
<b>BM x TR</b>	2	1424.1	3.1282	0.118	3459.9	1.403	0.271	2392.3	1.0511	0.47	1559.7	0.87142	0.598
<b>AQ(BM) x TR</b>	3	455.26	4.6147	0.001	2466	4.1633	0.001	2276	4.4169	0.001	1789.8	3.6417	0.001
<b>RESIDUAL</b>	48	98.655			592.33			515.29			491.48		

### 4.3. Comparison of years

The maximum number of individuals of decapod crustaceans and fishes for the combined years found at one site ranged from 1749 at Deadman’s Harbour in Bay Management Area 2a to 271 in Boone Cove/Fish Island in BMA1. The mean abundance per site observed in the samples was 24 in 2015, and 128 in 2016. PERMANOVA revealed a significant interaction between BMA and year, and between aquasite (nested in BMA), treatment, and year for total individual abundance of decapod crustaceans and fishes (Table 11).

The total abundance of juvenile *Homarus americanus* was greater in the collectors in 2015 (325) than 2016 (58) (Table 10). However, these totals do not take into account the differences in collector numbers between years (Table 10). Mean number of juvenile lobsters per collector did not show any significant effect of BMA treatment or aquasite as well no significant interactions (Table 10).

Table 10: PERMANOVA univariate results for individual abundance for American lobster in collectors in both 2015 and 2016. The factors are BMA (BM), treatment (TR), aquasite nested in BMA (AQ (BM)) and year (YE).

Source of variation	df	ABUNDANCE OF HOMARUS AMERICANUS		
		MS	F	p
BM	2	130.06	16.933	0.121
TR	1	17.336	1.9209	0.256
YE	1	121.34	40.558	0.026
AQ (BM)	3	7.6806	1.8637	0.156
BM x TR	2	23.786	2.6356	0.2312
BM x YE	2	32.803	10.965	0.052
TR x YE	1	8.4028	1.6796	0.285
AQ (BM) x TR	3	9.025	2.1899	0.098
AQ (BM) x YE	3	2.9917	0.72593	0.548
BM x TR x YE	2	7.0861	1.4164	0.333
AQ (BM) x TR x YE	3	5.0028	1.2139	0.312
RESIDUAL	156	4.1212		

The mean number of species of decapods and fish per site was greater in 2016 (8.0) (Fig. 4.10) than in 2015 (5.6) (Fig 4.4, Table 11). Species richness ranged from 9 at Deadman’s Harbour (away) in Bay Management Area 2a to 4 in Welch’s Cove. PERMANOVA revealed a significant effect of year and a significant interaction between aquasite (within BMA) and treatment for species richness of decapod

crustacean and fishes (Table 11). There was no significant effect of BMA or significant interaction between BMA and treatment (Table 11).

For Pielou's evenness index, Round Marsh (0.92) in BMA1 was had the greatest species evenness with Deadman's Harbour (0.47) with the least evenness. The mean species evenness was greater in 2015 (0.957) than 2016 (0.877). PERMANOVA revealed significant interactions occurred between aquasite (within BMA) and year, aquasite (within BMA) and treatment, and BMA and year (Table 11). No other interactions were significant (Table 11).

Table 11: PERMANOVA univariate results for individual abundance, species richness and Pielou's evenness for decapod crustaceans and fishes in collectors in both 2015 and 2016. The factors are BMA (BM), treatment (TR), aquasite nested in BMA (AQ (BM)) and year (YE).

Source of variation	df	ABUNDANCE OF INDIVIDUALS			SPECIES RICHNESS			PIELOU'S EVENNESS		
		MS	F	p	MS	F	p	MS	F	p
BM	2	1.0227E5	13.818	0.195	68.047	8.4157	0.201	0.5183	6.207	0.125
TR	1	20854	1.3925	0.333	39.229	2.3464	0.226	3.0993E-3	0.13801	0.664
YE	1	4.267E5	45.918	0.018	129.6	19.481	0.024	1.4775	67.793	0.013
AQ (BM)	3	7401.2	2.9514	0.037	8.0859	1.6619	0.175	8.2875E-2	12.259	0.001
BM x TR	2	54076	3.6107	0.165	5.5291	0.33069	0.754	0.13301	5.9179	0.098
BM x YE	2	1.1276E5	12.134	0.039	1.7582	0.26428	0.795	0.53452	24.505	0.021
TR x YE	1	21406	1.2823	0.338	0.44845	0.33695	0.602	3.0028E-2	2.4268	0.21
AQ (BM) x TR	3	14977	5.9723	0.001	16.72	3.4365	0.02	2.2495E-2	3.3275	0.022
AQ (BM) x YE	3	9292.6	3.7057	0.012	6.6528	1.3674	0.262	2.1831E-2	3.2292	0.03
BM x TR x YE	2	54895	3.2884	0.179	11.012	8.2753	0.063	4.4704E-2	3.6109	0.17
AQ (BM) x TR x YE	3	16693	6.6569	0.002	1.3306	0.27347	0.853	1.2387E-2	1.8323	0.146
RESIDUAL	156	2507.7			4.8654			6.7604E-3		

For multivariate data for decapods and fishes in 2015 and 2016, MDS plots show differences in the assemblage of decapods and fish colonising collectors between the years. This is more evident for log-transformed (Fig. 4.13) than untransformed and square-root transformed data (plots not shown for the latter two), yet differences are still present between the years for all data. For presence/absence transformed data (Fig. 4.14), the points are closer together for the different years than for the other transformations, indicating that species composition is more similar between years than abundance patterns. Differences between BMAs are also apparent in the MDS plots, where samples from BMA1 are separated from those in the other two BMAs and

differences between years are evident for BMA 2a and 3a but not BMA1 (Fig. 4.13). This is more evident for the presence/absence transformed data (Fig. 4.14). Overlap of symbols representing collectors at near and away sites suggest no differences between assemblages of decapods and fishes near aquaculture sites from those away (Fig. 4.13 & Fig. 4.14). The stress values for the 2D MDS plots for all transformations were moderate (0.15-0.21). Using the untransformed, square-root, or log-transformed data, PERMANOVA results showed a significant 3-way interaction between aquasite, treatment, and year (Table 10). Untransformed and square-root transformed data also had a significant interaction between BMA and year. For presence/absence transformed data, significant effects were observed for BMA and year, and a significant interaction between aquasite and year but no other significant interactions. For all transformations, pairwise tests indicate that the similarity of the assemblage in the collectors in each BMA differs between years ( $t = 2.21 - 3.87$ ,  $p < 0.001$  for all comparisons).

SIMPER analysis revealed 75.92% dissimilarity in the assemblage of decapods and fishes in the collectors between years for square-root transformed abundance data. Results show that *Eualus pusiolus* had the largest contribution to the dissimilarity (diss/SD = 1.59, contribution = 26.22%). *Eualus fabricii* (diss/SD = 1.29, contribution = 12.83%) and *Pholis gunnellus* (diss/SD = 0.98, contribution = 5.64%) also made large contributions to the dissimilarity between years. The dissimilarity between BMAs was 72.05% for square-root transformed data, with *Eualus pusiolus* again having the largest contribution to this figure (diss/SD = 1.56, contribution = 27.94%). For log transformed data, the dissimilarity between BMAs was 70.99%, with *Eualus pusiolus* having the largest contribution (diss/SD = 0.95, contribution = 12.27%). For presence/absence transformed data, a dissimilarity of 63.47% was observed between the years. Once again, *Eualus pusiolus* contributed highly to the variability in dissimilarity (diss/SD = 1.75, contribution = 10.25%), followed by *Eualus fabricii* (diss/SD = 0.99, contribution = 8.30%) and *Homarus americanus* (diss/SD = 0.87, contribution = 6.25%).

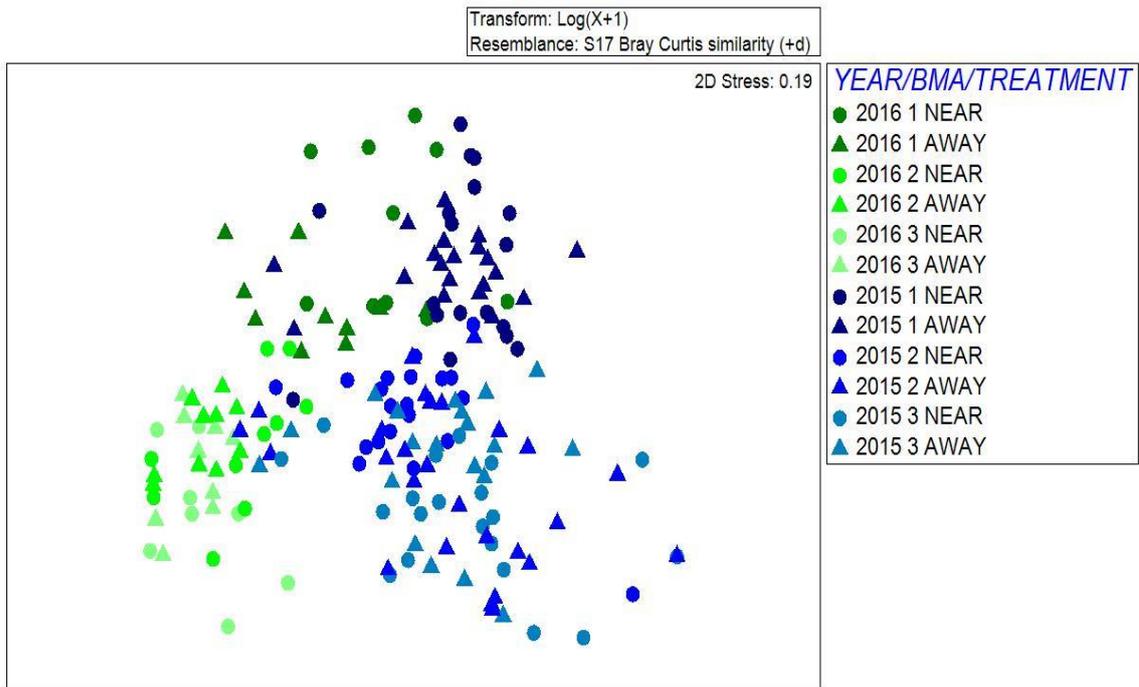


Figure 4.13: Two-dimensional MDS plot of logarithmic ( $\text{Log}(X + 1)$ ) transformed abundance decapod and fish data for 2015 and 2016. Each symbol represents one collector, with the circled symbols as a near collector and the triangle symbols representing the away collectors. The blue symbols represent those collectors from 2015, with green symbols representing 2016 collectors. The colour gradient represents the BMAs, with the darker shade as BMA1, the lighter shade as BMA3a and the middle shade as BMA2a. The stress value is given in the upper right corner of the MDS.

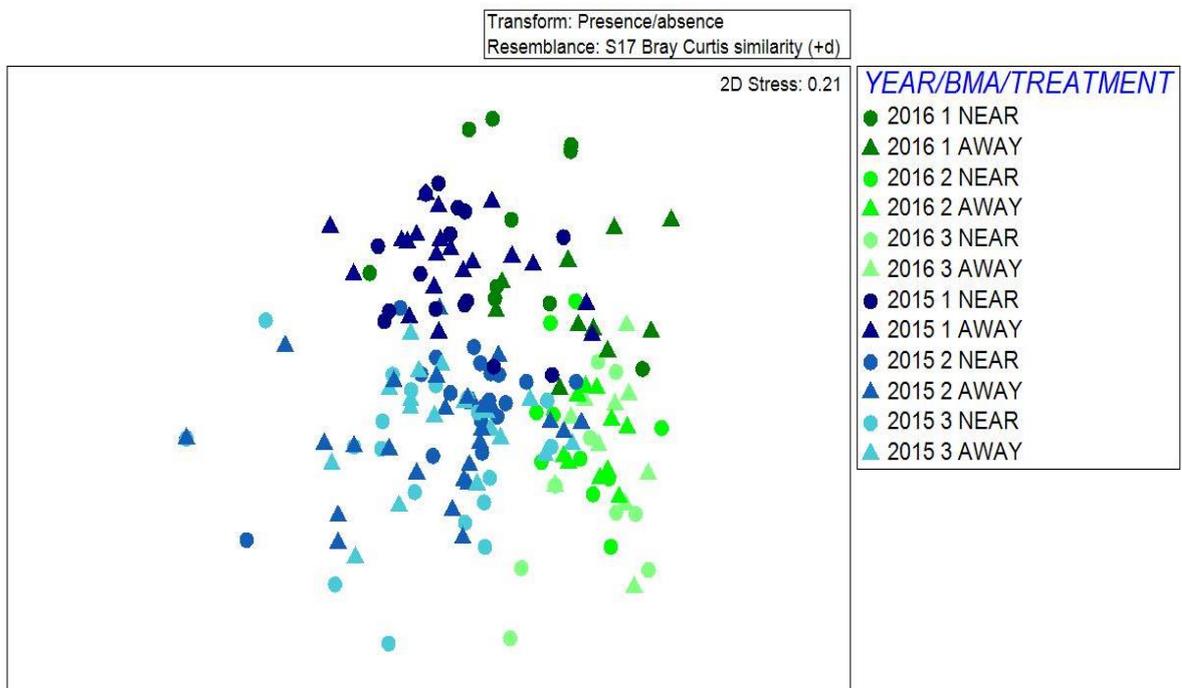


Figure 4.14: Two-dimensional MDS plot of presence/absence transformed abundance decapod and fish data for 2015 and 2016. Each symbol represents one collector, with the circled symbols as a near collector and the triangle symbols representing the away collectors. The blue symbols represent those collectors from 2015, with green symbols representing 2016 collectors. The colour gradient represents the BMAs, with the darker shade as BMA1, the lighter shade as BMA3a and the middle shade as BMA2a. The stress value is given in the upper right corner of the MDS.

Table 12: PERMANOVA results for untransformed, square-root transformed, logarithm transformed and presence/absence transformed data for decapod crustaceans and fishes in collectors in both 2015 and 2016. The factors are BMA (BM), treatment (TR), aquasite nested in BMA (AQ (BM) and year (YE).

Source of variation	df	Untransformed			Square-root transformation			Logarithm transformation			Presence/absence transformation		
		MS	F	p	MS	F	p	MS	F	p	MS	F	p
BM	2	36478	5.0238	0.056	31582	4.9104	0.064	30073	4.9183	0.073	22212	3.9847	0.047
TR	1	4065	0.6967 1	0.583	3535	0.7525	0.556	3217	0.7112	0.562	3423.3	0.95666	0.46
YE	1	88265	31.975	0.011	70925	41.269	0.019	64039	40.101	0.013	37261	41.098	0.017
AQ(BM)	3	7268.1	4.7373	0.001	6432.8	6.1231	0.001	6115.6	6.3682	0.001	5575.4	7.5237	0.001
BM x TR	2	9095.5	1.5586	0.233	4816.8	1.0253	0.483	3902.7	0.8627	0.603	2543.4	0.71066	0.67
BM x YE	2	20048	7.262	0.019	9947.6	5.7877	0.047	7539.2	4.7206	0.074	1576.8	1.7391	0.319
TR x YE	1	3156.1	1.0181	0.438	1707.5	1.0309	0.425	1381.1	0.9836	0.463	797.79	1.3934	0.306
AQ(BM) x TR	3	5836.4	3.8073	0.001	4698.7	4.4725	0.001	4524.3	4.7111	0.001	3579.6	4.8304	0.001
AQ(BM) x YE	3	2761	1.8011	0.013	1718.9	1.6361	0.037	1597.2	1.6632	0.033	906.72	1.2236	0.29
BM x TR x YE	2	6658.9	2.1478	0.127	3056.4	1.8452	0.197	2374.2	1.6909	0.207	1648.1	2.8788	0.081
AQ(BM) x TR x YE	3	3100.7	2.0227	0.003	1656.5	1.5767	0.069	1404.2	1.4622	0.09	572.46	0.77251	0.694
RESIDUAL	166	1533			1050.6			960.34			741.04		



# 5 Discussion

## 5.1. Implications of findings

In general, the results from this thesis show that there were no significant differences between sites near (200 m) and away (1200 m) from salmon aquaculture cages on fish and decapod communities in the bio-collectors in terms of individual abundance, species richness and species evenness as well as for multivariate abundance data. For the multivariate analysis, the results show that significant differences occurred between some of the pairs of sites. Significant univariate and multivariate differences in the fish and decapod assemblages were observed between years. SIMPER analysis identified that multivariate differences were largely due to the shrimp species, *Eualus pusiolus*.

The lack of differences between near and away sites suggests that salmon aquaculture pens do not have an effect on decapod crustacean and fish assemblages at a distance of 200 m. However, PERMANOVA did detect a significant interaction between treatment and aquasite, with pair-wise tests identifying that Welch's Cove site pair did not differ significantly for the square-root transformed data, whereas the opposite is true for presence/absence data. This means that there are differences in individual abundance patterns for all paired sites except Welch's Cove, but only the Welch's Cove pair exhibited differences in species identity. The higher number of juvenile American lobster in the Welch's Cove paired site compared to others could be a factor that influences the assemblages in the collectors. The lack of differences between treatments occurred for both 2015 and 2016. However, a year of further monitoring is essential for confirmation of this as salmon aquaculture in the Quoddy region runs on a three-year rotation cycle.

BMAs were shown to differ significantly for all data transformations for 2015 and for presence/absence data only for the multivariate comparison of 2015 and 2016. Differences between BMAs are likely the result of larger-scale spatial differences related to habitat preferences for differing species and are, in all likelihood, not related to aquaculture operations. Differences were also evident between the two years

analysed in terms of individual abundance and species evenness, but not in species richness. In all likelihood, the differences in years and the interactions that involve years are as a result of normal temporal variability in species abundance in the Quoddy region due to abiotic factors (e.g. temperature). Because differences between treatments or interactions between treatment and year or BMA were not detected, the Quoddy region's 3-year production cycle is not likely to have caused differences in the assemblage in the bio-collectors between years. Differences in the number of collectors deployed and processed during this thesis may have contributed to differences between years in the detection of the effects of some factors, such as BMA. 160 collectors were processed for the 2015 samples, yet only 60 were fully processed in 2016, raising the possibility that differences between years in terms of which factors were significant (e.g. BMA) could have been due to differences in sample size.

Most previous studies have examined impacts of aquaculture on infaunal organisms at sites under or close to aquaculture cages. There have been a few previous studies examining fish and decapod communities. The results from this thesis are consistent with those of Tanner and Williams (2015). Their study used underwater video to compare rocky substrate sites located 5 m and over 200 m from finfish aquaculture operations and found that aquaculture operations did not appear to have any local or regional impact on benthic fish and crustacean assemblages. In contrast to the results from this thesis and the findings of Tanner and Williams (2015), Bacher et al (2012) found that numbers of individuals of fish in dive surveys over rocky substrate did differ in proximity (30-200 m) to aquaculture operations. However, the sites surveyed in both these previous studies were much closer to aquaculture cages than those used in my thesis (near sites ~200 m from cages).

My thesis focused on near sites that were, on average, 237 m (near) and 1215 m (away) from areas of aquaculture activities. Many studies argue that major effects of aquaculture are localised and normally occur up to 80 m away (Heinig, 2001; Costa-Pierce 2002; Mente et al., 2006; Neofitou et al., 2010). This localized impact could be a reason why no significant difference was observed between near and away sites in my thesis. However, the benefit of my study is that it can detect changes in community structure outside of aquaculture lease sites, if they occurred. Because of this focus on far-field effects, it is difficult to compare with other studies as most are done 0 – 1000

m from aquaculture cages and are able to trace bio indicators from either directly below fish farms or in close proximity. One study which worked at similar or greater distances from salmon aquaculture pens as my thesis was Mente et al. (2010). This study found no difference in infaunal and epifaunal communities in soft sediment habitat in grab samples and beam trawls along transects <2000 m and >2000 m from aquaculture operations. There have been limited studies that focus on ranges up to and over 200 m from the area of operations, with many studies ranging in closer proximity to aquaculture pens.

Given that Atlantic lobster are a valuable fishery in this region, it is of particular interest to determine whether salmon aquaculture has an impact on the recruitment of this species in the Quoddy region. Differences in the abundance of juvenile *Homarus americanus* were evident between years, but no other factors or interactions were significant. Juvenile American lobster were only significantly different in abundance between the near and away site Welch's Cove. Paired sites in BMA 1 (Man-O-War, Boon Cove/Fish Island, and Fairhaven) yielded total juvenile lobster abundances under ten individuals indicating that they are not likely to be important lobster settlement areas. My results do not suggest any impact of salmon aquaculture on juvenile lobsters outside lease sites (200 m away from pens). Considering that lobsters are benthic detritivores it is likely that nutrients in the form of lost feed and faecal matter can provide an important source of nutrients, supporting a higher number of juvenile lobsters (Wang & McGaw, 2016). Previous literature (Chou et al., 2002; DFO, 2016b) suggests impacts occur on American lobster closer to the salmon cages up to a range of 50 m, but other previous studies have not examined whether impacts on lobster occur at a distance of 200 m or greater from cages (outside of lease sites). In the present study, I found differences in juvenile lobster abundance between years. The differences between years could be due to seasonal variations of a range of factors, such as temperature.

## **5.2. Limitations**

Salmon aquaculture operations in the Quoddy region are obliged to function as single year class operations, on a 3-year rotation cycle (Chang et al., 2007), and hence, it will take 3 years to fully assess whether there are any impacts of proximity to aquaculture in this study (this thesis presents the first 2 years of this study). Sampling in

two separate years does provide results that identify temporal variation in the assemblage colonizing bio-collectors. Inconsistencies between years in terms of differing sample sizes limited my thesis to some extent, as differing number of sites and bio-collectors per site would have the potential to skew results and alters the statistical power available to detect differences between treatments in the two years. This point is important as the study was intended to be consistent between years in order to identify if patterns change between years. This restriction is as a result, in part, of time and collector limitations when the collectors were being deployed in the summer of 2016. Further, time limitations when I was processing the bio-collectors in fall 2016 restricted the number of processed 2016 collectors to 60, in comparison to 160 in 2015.

The sampling method used in this thesis was cobble-filled collectors deployed in June/July of each year, with retrieval taking place in October/November of the same years – a range of 4-5 months whilst the collectors are present on the seafloor. This may introduce a seasonal bias in terms of which species are sampled as natural variations in the marine environment caused by seasonal fluctuations in temperature, nutrients and other abiotic factors can affect species composition and the recruitment of different species. However, a recent thesis compared the efficiency of cobble-filled collectors that were deployed in May to those deployed in July (Wittig, pers. com.). It was found that deploying earlier in the season did not make much difference to the number of species of settlers compared to those deployed in July, which indicates that most species settle between July and October, the time period that collectors were deployed in my study.

Cobble-filled collectors are a useful tool that provides a snapshot into the status of the organisms living in cobble habitat. This thesis is focused, however, on decapod crustaceans and fishes and does not provide a full assessment into the entire suite of species that colonize cobble-filled collectors. Further assessments of other cobble-dwelling species that were found in the collectors would provide a greater understanding into the effects of aquaculture on the wider ecological system. Previous research using the bio-collectors has identified >500 species from 14 phyla in the Quoddy region (Hunt and Rochette, unpubl. data). The susceptibility of invertebrate species to impacts of aquaculture is likely to be species specific. For example, decapod

crustaceans, such as lobsters, that rely on the synthesis of chitin are susceptible to pesticides that could impact on their growth (Macken et al., 2015).

### **5.3. Management recommendations**

Monitoring benthic macroinvertebrates is crucial to understanding changes in physical, chemical and ecological characteristics of the marine environment (Milbrink, 1983). Given the growth in the farming of Atlantic salmon and the activities involved with their cultivation, monitoring benthic communities would provide further evidence into the effects that aquaculture has on the wider environment as the effects of aquaculture operations may be detected on smaller species than those focused upon in this thesis. Given that no significant differences occurred in community structure between treatments yet some paired sites do differ, it would be important to monitor aquaculture impacts by taking spatial variation into account, such as was done in this project by using sites matched as much as possible in characteristics other than proximity to aquaculture.

Cobble-filled collectors are a valuable sampling method for monitoring cobble habitat in the Quoddy region and would be useful in other regions given the right environmental settings. The sampling method improves our knowledge into critically-important ecosystems and the use of collectors should continue in this region to develop our understanding into how anthropogenic activities impact on the environment.

When focusing on the aquaculture industry on a regional scale, the management strategy should focus on a clear goal where sustainability is at the forefront of decision-making with a range of management strategies. The main incentive would be economic security and social wellbeing, where a healthy environment benefits all species. In New Brunswick, one strategy being implemented is an Integrated Multi-Trophic Aquaculture (IMTA) practice that aims to use the by-products from one species to become an input for another species (Barrington et al., 2009). This strategy combines fish aquaculture with organic and inorganic extractive aquaculture to achieve environmental sustainability through biomitigation, economic stability and better social management practices, which aims to increase productivity and decrease pollution (Cranford et al., 2013). In the Bay of Fundy, there have been successful trials of using IMTA

(Barrington et al., 2009; Troell et al., 2009), however there have been concerns that the production of species in IMTA is lower than monoculture, resulting in less economic gains and a less attractive option for farmers (Thomas, 2010).

A final management recommendation would be increasing research into the development of farming techniques that reduces the environmental impact of salmon farming and specifically the impact of sea lice on the stocks. One study found that a more dynamic approach to management thresholds should be implemented that take more consideration into biological and farm management factors (DFO, 2014d). Along with this, using an integrated sea lice management strategy that incorporates non-chemical approaches, such as cleaner fish, IMTA and immunostimulatory fish feed, could combine to reduce the numbers of sea lice inside fish pens. However, more research is needed to improve the value of these combined pest control measures.

## **5.4. Future research**

To further our understanding into the effects that aquaculture has on benthic communities, research is required to identify the positive and/or negative influences on benthic organisms. In this study, decapod crustacean and fish assemblages differed between some pairs of sites, as well as differing between the two years, but I found no overall difference between sites near and away from aquaculture. To confirm these results, further research would be needed that identifies whether similar patterns occur in the third year of the production cycle. In addition to decapod crustaceans and fishes, future research should expand to include other types of organisms found in the collectors as effects of aquaculture on species other than decapod crustacean and fishes could exist. In the literature, there has been a wide range of spatial scales that have been used for studies of impacts of aquaculture. In order to fully understand the local effect of aquaculture, it would be interesting to compare the effects on the benthic community directly below aquaculture operations, to those at distances up to several kilometres. Such research could be conducted by using collectors deployed in a trajectory line away from aquaculture pens at multiple sites to identify how community structures change with distance from an aquaculture site. Such a study should be conducted over several

years to take into account any effects of the 3-year production cycle that occurs in the Quoddy region.

Growth of the aquaculture industry is widespread throughout the world and is making a large contribution to meeting humanity's demand for seafood. However, ecosystems are sensitive to changes in their environment and substantial growth in global aquaculture, without understanding the true effects of the industry, puts important communities at risk. Like any industry, striving toward sustainability is essential to maximise economic profits and social wellbeing, whilst preventing ecological depletion. With continued research and development from academics and private aquaculture companies, achieving sustainability is a possibility for the industry.



## 6 Conclusion

This thesis has aimed to identify how decapod crustacean and fish communities in cobble-filled collectors compare between sites near (~200 m) aquaculture pens to those ~1200 m away from aquaculture pens. It was found that there was no significant effect of proximity to aquaculture (near vs away) on fish and decapod communities in terms of individual abundance, species richness and species evenness or multivariate assemblage structure. The abundance of juvenile American lobster also did not differ significantly between treatments. However, there were significant differences between sites within some site pairs and further investigation is required to understand the differences that were observed. Significant differences were observed between 2015 and 2016 samples, largely due to the shrimp species, *Eualus pusiolus*, which was in high abundance in the 2016 collectors, and, in some cases, between BMAs.

Aquaculture is a growing industry that has the potential to provide protein to much of world and become an economically sustainable industry. There are many benefits associated with aquaculture. However, past research has shown that in some cases the industry can cause severe indirect effects for commercially and ecologically-important species ranging up to 2000 m in proximity. The management of fish farms is critically important, and the use of continual research and monitoring will assist farmers in creating a sustainable industry that protects their interests, as well as the protection of marine environments. Therefore, this thesis recommends continued research in the region that focuses on the benthic communities. To further our understanding into whether aquaculture effluents have impacts on cobble habitat, further monitoring with cobble-filled collectors should be conducted for the final year of the 3-year rotation cycle that occurs in the Quoddy region. Continued monitoring would improve our knowledge of the degree of impact of aquaculture on the environment and capture fisheries, a subject that has caused conflict between different social groups, and support sustainable use of the environment.



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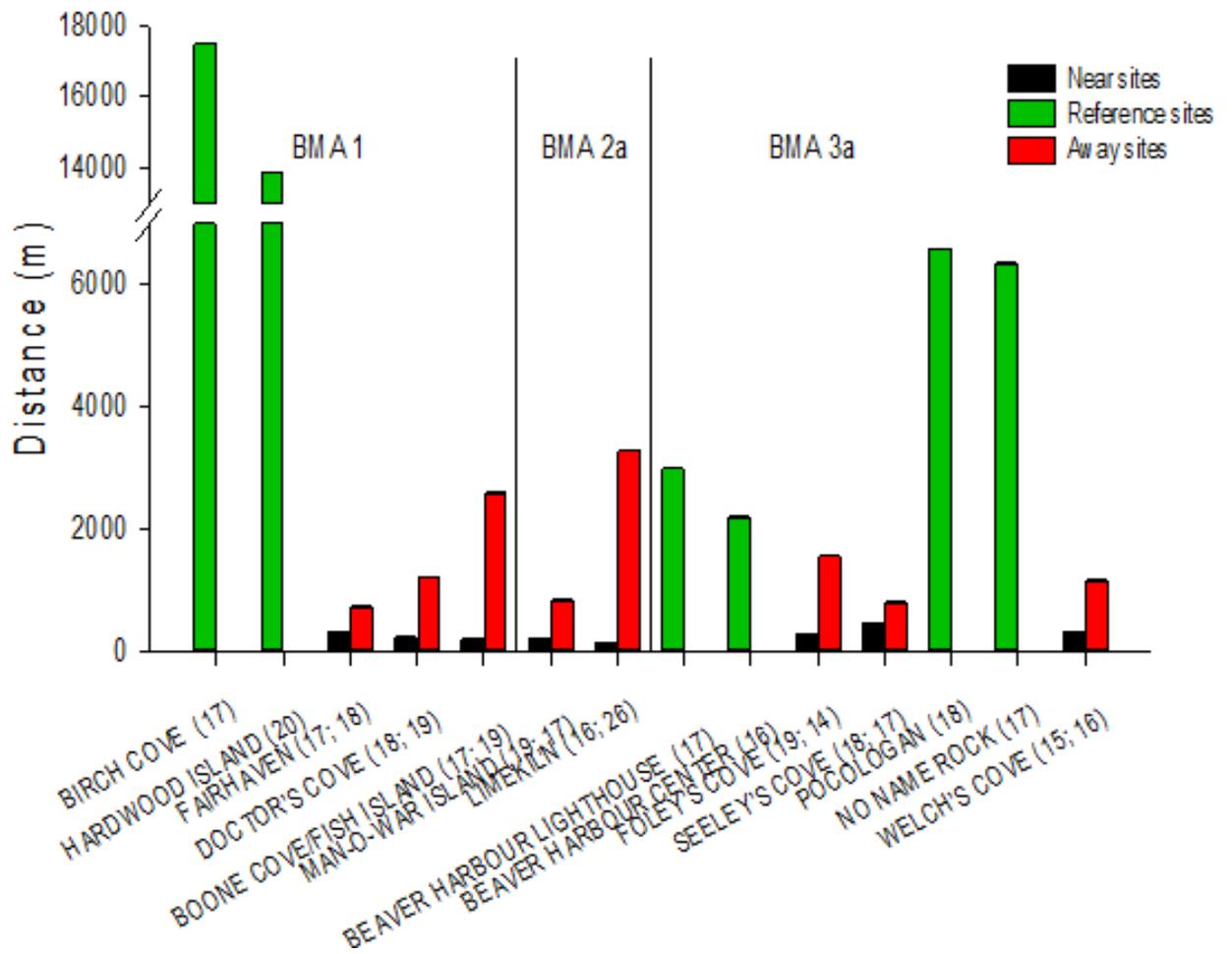
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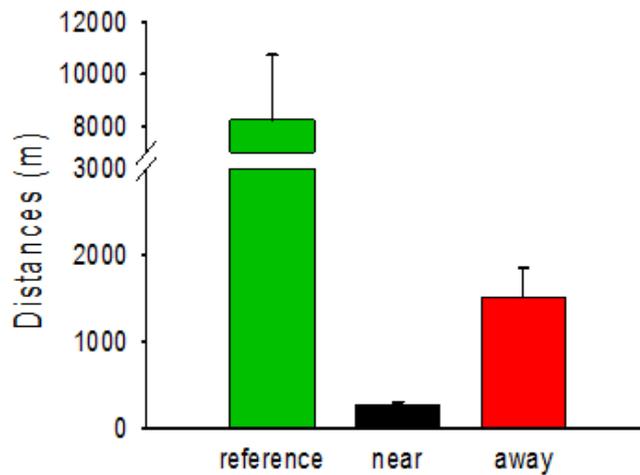
# Appendix A: Distance between paired sites

Mean (+SE) distance between collectors and aquaculture pens in 2015  
 Number of collectors retrieved are in brackets (near; away)

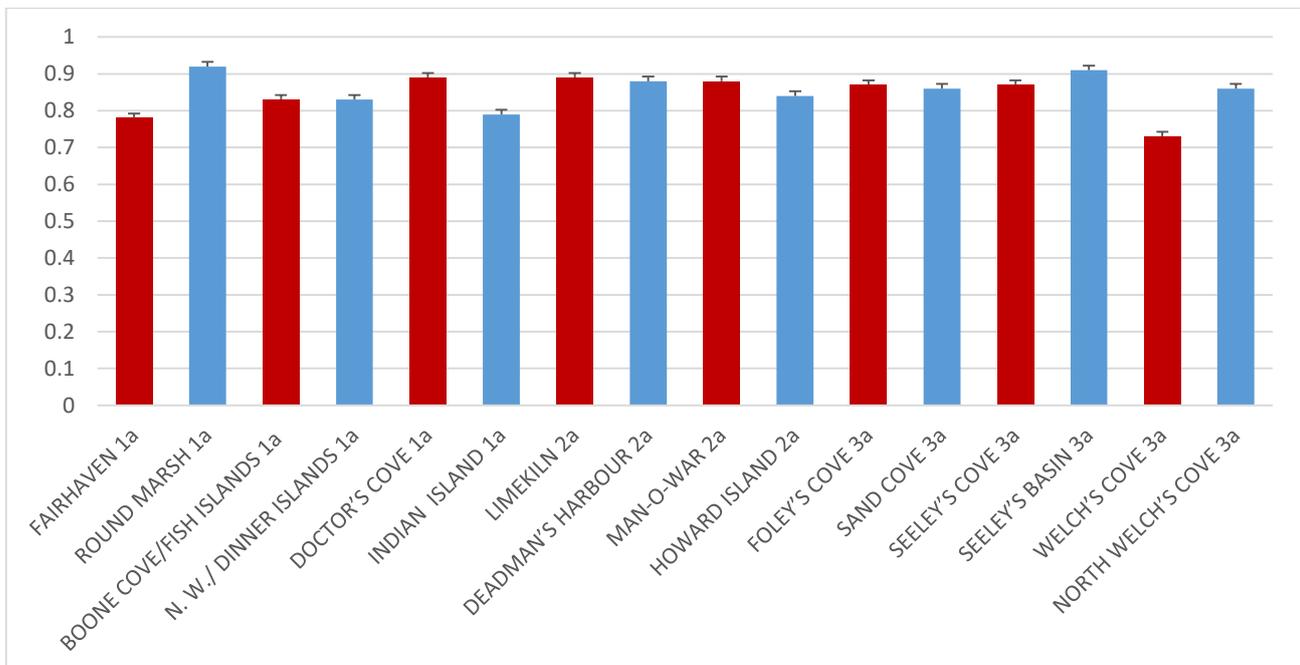




## Appendix B: Average distance between near and away sites



## Appendix C: Average species evenness for 2015 sites





## Appendix D: Average species evenness for 2016 sites

