Salmon Aquaculture-Derived Nutrients and Metals in Biota from Rocky Habitats in the Bay of Fundy

by

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Bachelors of Science in Biology, McGill University, 2016

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

Masters of Science in Biology

in the Graduate Academic Unit of Biology

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This thesis is accepted by the Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

September, 2018

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Abstract

Past studies have assessed the impact of metal and nutrient loading from aquaculture, but few have examined rocky bottom habitats or quantified effects at distances greater than 200 m from salmon pens. My goal was to assess metal contamination and feed reliance at two distances from salmon pens. I deployed 7 bio-collectors at 8 pairs of sites near (68-441 m) and away (260-2750 m) from salmon pen sites across three Bay Management Areas in the Bay of Fundy to assess exposure to copper, zinc and nutrients (using stable isotopes) in five benthic species: blue mussels (*Mytilus edulis*), vase tunicates (*Ciona intestinalis*), American lobster (*Homarus americanus*), shorthorn sculpin (*Myxocephalus scorpius*) and rock gunnel (*Pholis gunnellus*) in 2016 and 2017. Of the combined 41 species-site pair combinations across both years compared using t-tests, 8 and 6 showed significant differences in copper and zinc, respectively, between near and away sites, but the direction of difference was inconsistent. Some species-site pair combinations showed differences in isotope values, but only sulfur isotopes suggested a small shift towards reliance on aquaculture nutrients. Using a chi-square goodness of fit test, only sulfur in 2016 and zinc in 2017 showed significant directionality in the response to being near aquaculture. Overall, my results suggest limited impacts of aquaculture in terms of metal contamination and feed use in animals in rocky bottom habitats greater than 200 m from aquaculture pens.
Dedication

I would like to dedicate this to John Fischer, my grandfather, my mentor and my inspiration. We lost him while I was working on this thesis and I know that he would be so proud of me for sticking it out when times got rough and finishing this chapter of my journey. All he ever wanted was the best for us and I hope in some little way I can honor his legacy through my work. I would also like to dedicate this to my family and specifically my Grandmother, Catherine Fischer, for the continuous love, support, patience and mostly their drive as it is an inspiration to me to make the most of every day. If I have learned one thing it is sometimes we lose people who are major cornerstones in our lives, but it is in those moments that those who are still around become the support for the people who are still hurting until the pain goes away.
Acknowledgements

I would like to acknowledge all of the people who have made this research possible. I would like to first thank my supervisory committee, Dr. Karen Kidd, Dr. Heather Hunt, Dr. Rémy Rochette and Dr. Bruce MacDonald, for all of the amazing information, help and dedication over the last two years. It was not always easy, but they did their best to keep me on the right track. I would also like to thank our funding agencies and industrial partners who gave us the means to conduct this research and the information required to be as accurate as possible throughout the process; this includes Environment and Climate Change Canada (ECCC), Cooke Aquaculture Inc., Fundy North Fisherman’s Association and The New Brunswick Department of Agriculture, Aquaculture and Fisheries. I would like to specifically thank the lab technicians who permitted me to do the work required and who passed on their knowledge: Angella Mercer, Marie-Josée Maltais and Dr. Jennifer Thera. Lastly, I would like to thank my amazing lab mates who helped out with every step of the process, whether that was getting up at 5am for fieldwork or those who simply took the time to talk to me in stressful times. Some people who come to mind are Dr. Jennifer Loughery, Curtis Forbes, Tammy Bo, Dr. Bryan Morse and all of the undergraduate and MSc candidates who helped out with collectors.
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List of Symbols, Nomenclature or Abbreviations

$\Delta$: delta symbol used for calculating changes in stable isotope values between near and away sites
$\delta$: delta symbol used to express the relative difference of isotope ratios between samples and standards
‰: per mille -> parts per thousand
AIC: Akaike Information Criterion -> estimator of relative quality of statistical models
Bio-collector: Sampling device consisting of a lobster wire cage, lined with 1mm “pet screen” and filled with cobble creating artificial rocky habitat to encourage recruitment of benthic species
BMA: Bay Management Area
Cobble: rock particles between 64 and 256 mm
DFO: Department of Fisheries and Oceans Canada
Floc: an aggregation of fine suspended material
g: grams
GLM: generalized linear model
ICP OES: inductively coupled plasma- optical emission spectrometry
IMTA: Integrated Multi-trophic Aquaculture
m: metre
NIST: National Institute of Standards and Technology
QA/QC: Quality assurance and quality control
US EPA: United States Environmental Protection Agency
CCME: Canadian Council of Ministers of the Environment
Introduction

Background and importance

Salmon aquaculture has been present in the Bay of Fundy for over 40 years and is a cornerstone for the province of New Brunswick, creating employment opportunities and producing $270 million in annual revenue (Burridge et al., 2010). The industry has grown from a single salmon pen operation producing 1500 fish in its first year to roughly 92 marine finfish leases, 45 of which were active, and Department of Fisheries and Oceans Canada (DFO) reported that in 2017, salmon aquaculture generated $227 million (Chang et al., 2014). Growing salmon for market involves first rearing smolts in freshwater hatcheries to roughly 90 g and then moving them to floating marine pens. Over the next two years, the salmon are fed a specific engineered feed daily and will grow to roughly 5 kg before they are sent to market (Strain & Hargrave, 2005). At this point the pens are left empty – or fallow - for roughly a year to prevent disease transmission. However, growing large numbers of fish in a small area has had its share of opposition and controversy over the years.

Impacts of salmon aquaculture

Salmon aquaculture as an industry is only as healthy as the fish themselves and maintaining the health of livestock can be difficult and costly for owners. Any surface submerged in water is almost immediately encrusted by sessile organisms like mussels and barnacles (Ashraf & Edwin, 2016). This biofouling of the net pens can decrease the health of the fish. As the encrusting organisms grow and reproduce, they cause net
occlusion which impedes the flow of water and reduces oxygen availability, which suppresses immune function of the fish while creating a reservoir of pathogenic microorganisms causing increased risk of disease (Kalantzi et al., 2016). Biofouling also impedes proper waste dispersal further reducing water quality. Moreover, large numbers of these organisms can damage the cages, which in turn increases the likelihood of fish escape (Fitridge et al., 2012). Anti-fouling paints and regular cleaning are used to prevent species from establishing and ensure proper waste dispersal (Braithwaite et al., 2007). However, the former can contain contaminants such as metals that leach into the environment and may affect resident biota (see below for more details). For this reason, some aquaculture farms have switched to new forms of anti-fouling paints that do not use metals as their active ingredients or to frequent cleaning to scrape off fouling organisms with no use of paints.

Fish are fed a commercial feed that is made of a combination of fish meal (anchovy and herring), fish oil and plant material (Lander et al., 2013; Yokoyama et al., 2006). Up to 17% of feed is not consumed, and for each tonne of fish produced, there is an estimated 76 kg of feces released into the system (Strain & Hargrave, 2005). Dispersal rates of leftover food and feces are determined by the depth and current surrounding the farm, with excess organic material typically depositing in sediments within 50 m of the pen sites (Giles, 2008). Nutrient and organic loadings from the pens has been shown repeatedly to impact the benthic ecosystem through changes in sediment properties and reduced oxygen availability at a distance up to 70 m from aquaculture facilities (Strain & Hargrave, 2005; Giles, 2008). Lack of oxygen causes a loss of benthic macrofauna biodiversity as more hypoxic-resistant species become more abundant (Karakassis et al.,
The most common example is the domination of aquaculture-affected sediments by the polychaete worm *Capitella capitata*, which can exploit suboptimal environments rich in organic material (Grant et al., 1995). Newly-introduced, nutrient-rich organic material may act as an additional food source for benthic species and have positive effects on their growth (Olsen et al., 2012). For example, mussels grown on salmon farms grew significantly more than individuals grown 200 m away, with the largest differences occurring between groups in the fall and winter months (Lander et al., 2013). Therefore, there is the possibility that other species may begin to feed more on aquaculture waste. The addition of a new food source may also release some prey from predation, allowing those species to increase in abundance, potentially leading to competitive exclusion, trophic cascades and loss of diversity (Schmitz et al., 1997).

Most past studies of impacts of organic loading from aquaculture sites have focused on soft bottom habitats. Effects, such as deposits of organic material, changes in sediment condition and losses of diversity are known to decrease with distance, although some studies have shown effects 200 m away (Mazzola & Sara, 2001; Kalantzi & Karakassis, 2006). Little is known about whether species of nearby rocky bottom habitats and organisms living at greater distances (>200 m) from aquaculture pens are feeding on aquaculture wastes.

**Metals and their use in aquaculture**

Metals exist in both particulate and soluble forms in the marine environment and it is the soluble, ionized form that is of highest risk to marine species as it is readily incorporated into an organism’s tissues (Jezierska & Witeska, 2006; Costa et al., 2013).
Marine species accumulate metals through two main pathways: directly from the surrounding environment (water or sediments) or through their diet (Jezierska & Witeska, 2006). Normally, each species accumulates metals to different levels, even when exposed to the same conditions, because the importance of each route is dependent on the species and bioavailability of the metal in the water and diet of those individuals (Jezierska & Witeska, 2006). However, in general, higher environmental levels of metals lead to higher accumulation in species (Rainbow, 2002; Moiseenko et al., 2008). The fate of these metals - whether they are used in metabolic functions, excreted, stored or toxic - is dependent on the species’ physiology and whether the metals are essential or non-essential (Rainbow, 2002). Trace metals normally have a high affinity for sulfur and nitrogen, major components in amino acids, and can therefore alter the metabolic function of enzymes (Rainbow, 2002). For example, past studies have shown that increased levels of copper in rock crab (Cancer irroratus) can alter enzyme production, oxygen availability and primary metabolic pathways, leading to decreased growth and survival (Hansen et al., 1992). It is widely accepted that sediments surrounding aquaculture sites (up to 50 m away) can contain high levels of metals and there are studies that suggest that individuals living in highly contaminated sediments also have high levels of metals (Bryan & Hummerstone, 1971; Giles, 2008; Burridge et al, 2010). However, there is little information regarding metal levels in species living in rocky bottom habitats near cage sites or at distances greater than 200 m from pen sites.

Most contemporary antifouling paints use copper (Cu) as an active ingredient, along with organic or organometallic booster biocides. Copper-laden paints can flake off with age and during regular cleaning, but the element can also leach off of the pen over
time at rates specific to the surrounding temperature, salinity, pH and presence of biofilms (Singh & Turner, 2009). Ionic forms of metals will bind naturally to ligands in organic material and settle, along with particles from cage cleaning, to the bottom. This leads to increased levels of metals such as copper in nearby sediments (Nikolaou et al., 2014) and this can, in turn, have adverse effects on sediment-dwelling organisms (Chambers et al., 2006; Singh & Turner, 2009; Burridge et al., 2010; Salvo et al., 2014; Edge et al., 2015). Metal-bound sediments can also be resuspended, increasing the potential exposure time for suspension feeding organisms and transporting particles further from the cages (Edge et al., 2015). Metal deposition from aquaculture activities is normally constrained to a radius of 40 to 70 m from the pens (Giles, 2008). However, in some cases, high sediment metal levels have been recorded between 300 and 500 m away, likely due to resuspension (Jones & Iwama, 1991). Sediments from directly beneath the cage to about 100 m downstream can have up to 150 mg/kg dry weight of copper (Burridge et al., 1999), which is above what is considered “safe” (108 mg/kg) based on the threshold effects levels in the sediment quality guidelines by the Canadian Council of Ministers of the Environment (CCME). Moreover, Smith et al. (2005) have also shown that copper remains high in sediments for at least 5 years after a site stopped housing fish. Copper-laced organic material, or floc, present in sediments can be consumed by benthic organisms, leading to its bioaccumulation and toxicity in some marine species, most noticeably in mollusks, crustaceans and algae (Burridge et al., 2010). While there is limited information on copper levels in many benthic organisms surrounding aquaculture, one study in the Bay of Fundy noted that green sea urchin collected between 0 and 75 m away from aquaculture operations had significantly higher
levels of copper than reference individuals (5 μg/g dw versus 1 μg/g dw) (Chou et al., 2003). Unlike other metal contaminants, copper does not increase with increasing trophic level (Cardwell et al., 2013). Most effects of copper have been seen in laboratory studies and include reduced swimming speed in barnacle larvae (Lang et al., 1980), reductions - by at least 45% - in metabolic enzymes such as phosphofructokinase and pyruvate kinase in crabs (Hansen et al., 1992), and reduced survival in larval sea urchins (Burridge et al., 1999).

The second metal of concern is zinc, a required micronutrient present in salmon feed (Salvo et al., 2014). Zinc is thought to be less toxic than copper because its uptake is highly controlled in fish species and it has higher LC50 values, the minimum concentration required to cause 50% mortality, than copper (Clearwater et al., 2002; Newman & Unger, 2003; Burridge et al., 2010). However, zinc is often found in sediment under aquaculture pens at concentrations between 233 and 444 mg/kg dry weight, which is above the Canadian threshold effect level in the sediment quality guidelines (124 mg/kg ), and exceeds 260 mg/kg up to 75 m downstream of the farm (Brooks & Mahnken, 2003). Zinc levels decrease away from pen sites until they return to background levels (< 50 mg/kg) around 300 m away (Burridge et al., 2010). Elevated zinc in sediments may impair invertebrate recruitment (Watzin & Roscigno, 1997) and many scientists have reported its lethal and sublethal effects in laboratory experiments (e.g. Wisely & Blick, 1966; Avelelas et al., 2017). As an example, zinc affects the development of sea urchin embryos, with 50% not developing at concentrations of 386.8 mg/L (King & Riddle, 2001). Moreover, studies showed lobsters collected near
aquaculture sites have background levels of zinc in their tissues and, like copper, this metal does not biomagnify (Chou et al., 2002; Cardwell et al., 2013).

**Nutrient isotopes**

Stable isotopes of carbon (C; $^{13}$C, $^{12}$C), nitrogen (N; $^{15}$N, $^{14}$N) and sulfur (S; $^{34}$S, $^{32}$S) are alternate forms of the element with varying numbers of neutrons that affect their physical and chemical reactivity (Fry, 2008). Stable isotope ratios (expressed as $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S) are often used to assess the dietary habits of a species by comparing its value to those of potential prey items; in doing so, stable isotopes provide information on the primary sources of energy ($\delta^{13}$C and $\delta^{34}$S) supporting a species and its relative trophic level ($\delta^{15}$N) (Carlier et al., 2010). Together, these isotopes can be used to generate the food web structure of a community. The values of $\delta^{13}$C represents the ratio of the heavier isotope to the lighter one ($^{13}$C/$^{12}$C) and in primary producers it varies as a result of environmental conditions (boundary layers, CO$_2$ availability) and the photosynthetic pathways used by primary producers, with terrestrial plants normally having a $\delta^{13}$C around -28‰, while marine phytoplankton are about -21‰ (Schwinghammer et al., 2011). Moreover, dissolved marine organic matter is closer to -23‰ (Peterson & Fry, 1987; Fry & Chumchal, 2012). Carbon isotope ratios are used to assess reliance on benthic versus pelagic or marine versus terrestrial carbon (Peterson & Fry, 1987) because the ratio is conserved from prey to predator, with a shift of only about 1 per mil per trophic level (McCutchan et al., 2003). Sulfur isotopes can be used to infer the relative importance of marine, freshwater and terrestrial prey because marine sources tend to be higher in $\delta^{34}$S than the other two (Hoekstra et al., 2002) and, as for carbon, the ratio
changes little (<1.0‰) between trophic levels and can be used to identify food sources for predators (Moreno et al., 2010). Lastly, more 15N than 14N is retained during metabolism and tissue synthesis resulting in an enrichment of the predator when compared to its prey (Peterson & Fry, 1987; Michener & Kaufman, 2007). As a result, δ15N increases by ~ 3-4‰ with each successive trophic level and is used to identify the relative position of an organism within a food web (McCutchan et al., 2003).

The pellets fed to farmed salmon consist of marine based fishmeal (60%), fish oil (15%) and terrestrial plant materials (25%) (Carter & Hauler, 2000) and, therefore, they should have unique stable isotope values relative to that of marine prey. Feed samples have a δ13C ranging from -18‰ to -24‰, which is 13C-depleted compared to natural primary consumer prey items which average -15‰ (Redmond et al., 2010). Similarly, the δ34S of marine phytoplankton averages around 21‰, while terrestrial plant material has values between 2‰ and 8‰ (Peterson & Fry, 1987; Peterson & Howarth, 1987; Fry, 1988). Little information is available about the sulfur isotope ratio in salmon feed, but trout feed averages ~8‰ (Wellman et al., 2017). Comparing isotope values of organisms collected near and away from aquaculture sites should allow me to directly determine whether a species may be changing its diet to incorporate aquaculture-derived nutrients (Yokoyama et al., 2006).

Stable isotopes, specifically carbon and nitrogen, have been used to trace the effects of salmon feed use in a variety of systems. As examples, salmon fed solely commercial feed had significantly different isotope values than those of wild salmon (Dempson & Power, 2004) and in a laboratory experiment, blue mussels fed a diet subsidized with salmon feed shifted in δ13C by 1.5‰ and in δ15N by 2‰ towards the feed.
after only 28 days (Redmond et al., 2010). Also, at a site 200 m away stable isotopes have shown that benthic clams at a depth of 9 m predominantly fed on aquaculture-derived waste while mussels suspended at a depth of 3 m had isotope values reflecting greater phytoplankton consumption (Mazzola & Sara, 2001). Mussel isotope ratios did not suggest shifts in their diet towards fish feed when grown in an integrated multi-trophic aquaculture (IMTA) system compared to those collected 3 to 10 km away (Sanz-Lazaro & Sanchez-Jerez, 2017). Stable isotopes have not been used to assess whether salmon feed is a food source for communities in rocky-bottom habitats.

**Study species**

For this study I focused on species found in rocky habitats because, with the exception of a few studies on lobster, the impact of aquaculture on this benthic community is not known. I chose 3 invertebrate and 2 fish species; 4 of them colonized bio-collectors (artificial rocky substrates; see below) at most sites in this region in previous studies (Hunt et al., 2017). Blue mussels (*Mytilus edulis*), which were added to the bio-collectors due to their presence in the area but inconsistent colonization, and vase tunicates (*Ciona intestinalis*) were selected because they feed on organic particles in the water column and are thought to be effective at removing excess nutrients released from aquaculture activities (Sanz-Lazaro & Sanchez-Jerez, 2017). They are also commonly observed fouling salmon pens (Scheer, 1945). *Ciona intestinalis* is an invasive suspension feeder adapted to low oxygen environments (Sephton et al., 2014). The American lobster (*Homarus americanus*) was selected because it supports a highly valuable industry in eastern Canada and is believed to be an apex predator and scavenger
(Gendron et al., 2001; Cooper & Blanchard, 2016). Adults feed primarily on small rock crab, juvenile mollusks, polychaetes and fish remains, while juvenile lobster consume mostly sessile juvenile bivalves and meiobenthic crustaceans (Sainte-Marie & Chabot, 2002; Cardinale, 2008; Hanson, 2009). Juveniles shelter in cobble substrates where they are protected from predators (Palma et al., 1999). Another study species was the rock gunnel (*Pholis gunnellus*). It is a benthic fish primarily found in and around rocky substrates in the intertidal and subtidal zones and feeds on amphipods, isopods or other small crustaceans (Shorty & Ganon, 2013). The other benthic fish species, shorthorn sculpin (*Myoxocephalus scorpius*), regularly consumes amphipods, copepods, polychaetes and occasionally juvenile lobsters (Dick et al., 2009; Hanson, 2009).

**Objectives, Hypotheses and Predictions**

Aquaculture operations are a source of metals and organic matter to the benthos surrounding salmon pens, which include species in both muddy and rocky bottom habitats. However, little is known about how the inhabitants of rocky bottom habitats are accumulating aquaculture-derived metals or using aquaculture feed as a food source, as indicated by their shift in stable isotope values towards that of the feed. In addition, impacts of aquaculture facilities on biota have been seen up to 75 m away (Chou et al., 2003), but little is known about whether they persist at greater distances. Sediment samples have shown elevated levels of metals 200-500 m from salmon pens (Burridge et al., 2010). Therefore, I hypothesize that individuals at these distances may also be influenced by aquaculture wastes. To address these knowledge gaps, the main objective of this project was to assess the levels of metals and stable isotope values in rocky
bottom-dwelling invertebrates and fishes at sites near (68-441 m) and away (260-2750 m) from salmon lease sites in the Bay of Fundy. To accomplish this, I used cobble bio-collectors deployed near and away from the aquaculture sites to collect my target species and then analyzed them for metals (copper and zinc) and stable isotopes of C, N and S. I also hypothesize that feeding strategies will affect the uptake of metals and aquaculture nutrients. First, I predict that individuals collected near aquaculture sites show significantly more metals and shifts in isotope levels towards those of the feed when compared to individuals at the away sites. Second, I predict that these differences should be more pronounced in years in which sites are housing second year fish, because of the accumulation of waste from more than a year of operations. Third, I predict that suspension feeders (vase tunicates, blue mussels) will have the highest concentrations of metals and more similar isotope ratios to that of the feed due to their reliance on settling or resuspended particulates (Kach & Ward, 2008; Milligan & Law, 2013); this has been shown in a previous study on vase tunicates, C. intestinalis, from a contaminated harbour in Australia that accumulated elevated metals at highly contaminated sites compared to references (Arienzo et al., 2014). Moreover, in general, lower-trophic-level species have higher levels of metals than upper-trophic-level species because most metals do not biomagnify and are found at higher levels in organisms at the base of the food web (Cardwell et al., 2013). In contrast, I expect that the top predators lobster and sculpin will have the lowest metal levels and little similarity in isotopic values to the feed, given their reliance on larger-bodied prey (Rainbow & White, 1989; Laskowski & Hopkin, 1996). Lastly, scavenging rock gunnels feed on settled organic material (Shorty & Ganon, 2013), for example leftover salmon pellets, and therefore I predict that these organisms
will have isotope and metal levels that fall in between those of the predators and filter feeders, because of the diversity of their diets. Overall, this study will improve knowledge on the ecological characteristics that affect exposure of species in cobble bottom habitats to aquaculture wastes, and provide data to help inform management decisions to minimize impacts of this industry on the environment.

**Methods**

**Sampling sites**

Sampling took place in two consecutive years (2016 and 2017) and all sample sites were located in three Bay Management Areas (BMA) in the southwest Bay of Fundy, New Brunswick. Each BMA goes through the same three-year cycle (1\(^{st}\) year fish, 2\(^{nd}\) year fish, 3\(^{rd}\) year fallow), but are at different stages of this cycle in one particular year. This allows for rotations of active pen sites to reduce the potential for disease transmission and impacts on the environment. As described in more detail below, I used bio-collectors to sample 8 pairs of “near” (close to a salmon cage site) and “away” (far from a cage site) sites across the three BMAs (3 sites in each of BMA-1 and BMA-3 and 2 in BMA-2). Each one of the near sites was placed on average 240 m (SD ±112.82 m) from the edge of the rectangular portion of the leases belonging to Cooke Aquaculture; however, due to the shape and positioning of some lease sites, some near bio-collectors were placed within the lease area. Away sites were on average 1215 m (SD ±737.21 m) from the rectangular lease edge (Figure 1). The sites were selected in consultation with the DFO and lobster fisherman for consistency in depth and bottom composition, and near and away sites were paired to minimize environmental variation due to habitat and
maximize exposure at the near site based on an understanding of currents. In addition, bio-collectors were placed at 2 reference sites (~1000 m & 1700 m from nearest pen site) that are shown in Figure 1. Data for these reference site individuals are shown in the appendix (Figures A1 & A2) as they were not used in the statistical analyses presented herein because they could not directly be compared to any one site. Stocking plans within BMA 2A unexpectedly changed and this affected the proximity of two of my sites to active aquaculture leases. In 2016, unused lease sites close to Howard Island (originally an away site) were stocked in May of that year and those closer to Man-O-War and Limekiln (originally a near site as they were in use in 2015 and previously) were not restocked after harvest in 2015. In the results, I have labelled Howard Island as a “current near” and Man-O-War as a “historic near” site to highlight these issues as the results need to be interpreted separately from the normal “near” and “away” sites. Similarly, pens near Limekiln, also in BMA 2a, were not restocked as expected in 2016; however, in this case, Limekiln remained much closer to other active lease sites than the away site Deadman’s Harbour, which is quite removed from any aquaculture leases.
**Figure 1:** Map of the study sites located in 3 Bay Management Areas (BMAs) in the southwest Bay of Fundy in New Brunswick. Black circles represent the pen sites, Pink and blue symbols represent the near sites and away sites, respectively, and grey symbols represent my two reference sites in BMA 3a. Each site pair has a specific symbol (square, triangle, diamond) to make the associated sites easy to identify.

**Table 1:** Names and stage of production cycle for sites located "near" and "away" from aquaculture pens in three BMAs in 2016 and 2017. Site codes refer to the names of the two sites that belong to a pair. One near site (Man-o-war), whose pen sites had been stocked the years prior to this study, in BMA 2a, were not restocked as originally planned. Sites closer to the away site (Howard Island) were stocked instead during the study period. Therefore, site designations were slightly modified for this outlier pair, Man-o-war is called a “historic near” site and Howard Island is called a “current near” site. “*” indicate the aquaculture sites where stocking plans were changed for the duration of the study.

<table>
<thead>
<tr>
<th>BMA</th>
<th>Site</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Site code</th>
<th>Production cycle stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fairhaven</td>
<td>-67.015247</td>
<td>44.961622</td>
<td>FHRM</td>
<td>Near 2nd year fish</td>
</tr>
<tr>
<td>1</td>
<td>Round Marsh</td>
<td>-66.993127</td>
<td>44.938281</td>
<td>FHRM</td>
<td>Away 2nd year fish</td>
</tr>
<tr>
<td>1</td>
<td>Doctor's Cove</td>
<td>-66.979773</td>
<td>44.938192</td>
<td>DCII</td>
<td>Near 2nd year fish</td>
</tr>
<tr>
<td>1</td>
<td>Indian Island</td>
<td>-66.965861</td>
<td>44.937355</td>
<td>DCII</td>
<td>Away 2nd year fish</td>
</tr>
<tr>
<td>1</td>
<td>Boone Cove</td>
<td>-66.925872</td>
<td>45.002821</td>
<td>BCDI</td>
<td>Near 2nd year fish</td>
</tr>
<tr>
<td>1</td>
<td>Dinner Island</td>
<td>-66.947097</td>
<td>44.987383</td>
<td>BCDI</td>
<td>Away 2nd year fish</td>
</tr>
<tr>
<td>2a</td>
<td>Limekiln*</td>
<td>-66.823168</td>
<td>45.061183</td>
<td>LKDH</td>
<td>Near Fallow</td>
</tr>
<tr>
<td>2a</td>
<td>Deadman's Harbour*</td>
<td>-66.784366</td>
<td>45.047707</td>
<td>LKDH</td>
<td>Away Fallow</td>
</tr>
<tr>
<td>2a</td>
<td>Man-O-War*</td>
<td>-66.846524</td>
<td>45.031938</td>
<td>MOWHI</td>
<td>Historic Near Fallow</td>
</tr>
<tr>
<td>2a</td>
<td>Howard Island*</td>
<td>-66.831025</td>
<td>45.039152</td>
<td>MOWHI</td>
<td>Current Near Fallow</td>
</tr>
<tr>
<td>3a</td>
<td>Foley's Cove</td>
<td>-66.691266</td>
<td>45.066748</td>
<td>FCSC</td>
<td>Near 2nd/1st year fish</td>
</tr>
<tr>
<td>3a</td>
<td>Sand Cove</td>
<td>-66.668628</td>
<td>45.070001</td>
<td>FCSC</td>
<td>Away 2nd/1st year fish</td>
</tr>
<tr>
<td>3a</td>
<td>Seeley's Cove</td>
<td>-66.644671</td>
<td>45.087412</td>
<td>SECSEB</td>
<td>Near Fallow</td>
</tr>
<tr>
<td>3a</td>
<td>Seeley's Basin</td>
<td>-66.636784</td>
<td>45.088919</td>
<td>SECSEB</td>
<td>Away 1st year fish</td>
</tr>
<tr>
<td>3a</td>
<td>Welch's Cove</td>
<td>-66.487252</td>
<td>45.088175</td>
<td>WCNWC</td>
<td>Near 1st year fish</td>
</tr>
<tr>
<td>3a</td>
<td>North Welch's Cove</td>
<td>-66.496254</td>
<td>45.094553</td>
<td>WCNWC</td>
<td>Away 1st year fish</td>
</tr>
</tbody>
</table>
**Bio-collectors and deployment**

I used bio-collectors to collect species for elemental analyses. They were 91 cm by 61 cm by 15 cm rectangular cages made of plastic coated lobster trap wire with 37 mm mesh size, lined on the sides and bottom with 1 mm “pet screen” and each filled with roughly 100 kg of cobble stone (~10 to 20 cm in diameter). The top of the collectors did not have 1 mm mesh, to allow for the settlement of larval organisms and for access to the cage by mobile juvenile benthic species (Ellis et al., 2015). To meet this project’s objectives, I deployed these bio-collectors for 4 months during the summer (July-Oct) at the sites listed in Table 1 and collected 5 species that were present at most sites and that represent different phyla (rock gunnel, shorthorn sculpin, American lobster, vase tunicate and blue mussel) and feeding habits. This study was done as part of a larger project that assessed the effects of salmon aquaculture on biodiversity of cobble-dwelling species.

**Field sampling**

All bio-collectors were deployed in early July and retrieved between late October and early November in both 2016 and 2017. This time period was selected as it is outside of lobster fishing season, coincides with the larval settlement period of many species, and it allows ample time for species to colonize and grow. Seven bio-collectors were placed on cobble substrate at each near and away site each year. Geographic coordinates, water temperature and depth were collected at each site where bio-collectors were deployed. Upon retrieval, all collectors - unless they were lost or damaged - were processed and all target animals were removed and frozen for subsequent identification and counts to
assess biodiversity (results reported elsewhere). My target species were subsampled from the bio-collectors, placed in bags on ice, and then frozen the same day.

Because blue mussels are not normally abundant in the bio-collectors at all sites and there was interest in assessing impacts on another suspension feeder, I attached bags of mussels to the outside of the collectors in 2017. I could only place the mussels at 5 of the 8 paired sites as I was not allowed to put out mussels on collectors located within aquaculture lease sites. These blue mussels were collected from the pier in St. Andrews, NB, on the 29th of June 2017 and held in a flow-through sea water tank at the Huntsman Marine Science Centre for four days, during which time they were fed once with algal paste designed as feed for invertebrates kept in a laboratory setting. A sample of mussels was frozen immediately and another after the four days in the sea table to establish baselines for contamination. The morning of deployment, 40 to 50 mussels of similar sizes (15 to 30 mm in shell length) were placed in each bag (10 x 15cm) of 5 mm mesh size. Immediately before deployment of the bio-collectors at a site, one bag of mussels was placed on the top of each of the 7 collectors and secured on both ends with zip ties.

**Sample processing**

Sample processing began less than a week after collection. First, samples were thawed, tunicates were cleaned of external and internal sediments and weighed, while all fish and lobsters were cleaned, measured and weighed in the lab. I standardized individuals for my samples based on length and then only animals from sites with sufficient numbers of individuals (4 to 7) within a common size range were analyzed. Analyses for stable isotopes and metals were conducted on whole tunicates and mussels.
(shells removed), lobster tail muscle and fish (muscle and whole body, respectively).

Individual fish had their digestive tract removed, a muscle sample was taken for isotope analysis, and the rest of the body was used for metal analysis. Moreover, two different types of commonly-used feed from Cook Aquaculture were obtained in the summer of 2017 and analyzed for stable isotopes. Samples were freeze-dried to remove the moisture while avoiding contaminant loss and weighed before and after to calculate percent moisture, as this is often used to calculate wet weight contaminant levels and can be used to determine minimum wet weight for analysis in future studies. All samples were then homogenized either by grinding with a glass rod (tunicates, mussels, lobsters) or chopping using ceramic knives (fish). All tools were cleaned between each sample to avoid cross contamination. Depending on dry weight, individuals were either pooled to meet the mass requirements for metal analysis or analyzed individually; multiple tunicates and mussels were usually required to make up a sample, while fish and lobster were almost always analyzed individually. The same individuals or pooled samples were analyzed for both metals and isotopes.

Metal analyses

Four to seven samples were run per species per site for each year from across all collectors. Copper and zinc analyses were conducted using a standardized protocol in the Kidd lab at UNB Saint John. Subsamples (0.5 g) were digested using microwave digestion (CEM Mars 5) and 10 ml of metal grade nitric acid (Fisher Scientific, Canada) and then diluted with 40 ml of Milli-Q water to a standardized volume. A known quantity of lithium buffer and an internal standard (Yittrium (SCP Science, QC)) were
added for quality control. Samples were transferred to polypropylene or glass test tubes and then an aliquot was run on an inductively coupled plasma-optical emissions spectrophotometer (ICP OES, iCAP 6500 Duo, Thermo Fisher Scientific) using an internal standard calibration method to quantify the amount of Cu and Zn in the tissues. The detection limits were determined by running 20 repeats of a blank and adding 3 times the standard deviation to the average, based on the US EPA 200.7; for Cu and Zn detection limits were 0.19 µM and 0.08 µM, respectively (Van Geest et al., 2015). For quality assurance and control (QA/QC), each run included 11 samples, a method blank (MB), a mussel tissue sample as a certified reference material (SRM 2976) from the National Institute of Standards and Technology (NIST) and sample duplicates varied by an average of 9.68% for copper and 6.36% for zinc (n = 24). A set of blanks containing only 10 ml of nitric acid was run in the digester between each sample batch to eliminate any trace elements from the previous samples. At the beginning of each day, the ICP OES was calibrated, monitored for any changes throughout the analysis, and recalibrated when necessary.

**Stable isotope analyses**

A minimum of 4 to 7 samples were run per species per site in both years for stable isotopes. Carbon, nitrogen and sulfur isotopes were measured for each biotic sample as well as for the salmon feed. Although the feed was only collected in one year, I assumed the same isotopic values for salmon feed in both years. Dried fish and lobster tail muscle and whole tunicate and mussel tissues were powdered and weighed (1 mg) into tin capsules for carbon and nitrogen analysis. Samples were sent to the Stable Isotope in
Nature Laboratory at UNB Fredericton and run on a Costech 4010 elemental analyzer coupled with a Thermo-Finnigan DeltaPLus isotope ratio mass spectrometer. The SINLAB uses two laboratory standards certified by the International Atomic Energy Agency; the first is ammonium sulfate (20.45 ± 0.09‰) and the other is a polyethylene foil (-32.19 ± 0.05‰). The average deviation of duplicates was 0.75% for carbon and 1.25% for nitrogen (n = 26). For sulfur isotope analyses, enough mass to obtain 0.05 mg of sulfur, roughly 2 to 5 mg of tissue, was weighed into tin capsules and sent to the Hatch Lab at the University of Ottawa for analysis. In this lab they used an Elementar Micro Cube Elemental Analyzer coupled with DeltaPlus XP isotope ratio mass spectrometer and an in-house silver sulfide standard averaging between -0.42 ±0.31‰ and -0.78 ±0.18‰. The average deviation of duplicates for sulfur was 2.06% (n = 20). If elevated lipids are in a sample they may skew the isotope ratios because lipids often have highly negative δ¹³C values (Hoffman & Sutton, 2010). As a general rule, C:N ratios that are above 4 or show a negative linear relationship with δ¹³C require lipid correction (Hoffman & Sutton, 2010). The C:N ratios were below 4 (range in mean values of 3.16 to 3.58) for most species in both years (exceptions were tunicates with means of 4.88 and 4.99 in 2016 and 2017, respectively, and mussels with a mean of 4.31 in 2017); however, in 2016, but not 2017, there was a significant negative relationship between δ¹³C and C:N for lobster, gunnel and sculpin – but not tunicates or mussels - suggesting some influence of lipids on their δ¹³C values (see Table B1 for ratios and regressions; Figures B1 and B2). Moreover, feed samples had an average C:N ratio of 7.25 and a significant negative relationship with δ¹³C (p = 0.001 & R² = 0.76). Therefore, the 2016 δ¹³C data for those
three species and the feed were corrected (hereafter referred to $\delta^{13}C_{\text{adj}}$) for the effects of lipid using the equation for marine species from Post et al (2007). This correction removed the relationship between $\delta^{13}C_{\text{adj}}$ and C:N for both fish species and feed, but not for lobster (Table B1). Statistical analyses were done using $\delta^{13}C_{\text{adj}}$ data for lobster, gunnel and sculpin in 2016 and raw $\delta^{13}C$ data for tunicates in 2016 and for all species in 2017 (appendix B). $\delta^{13}C$ values are raw data unless otherwise indicated.

**Statistical analysis**

The means and standard deviations were calculated for all variables for each species at each site. I also calculated the difference between near and away sites by subtracting the away site average from the near site average; this left me with a positive delta ($\Delta$) value when the near site had more metal or heavier isotope values and a negative $\Delta$ value when it had less metal or lighter isotope values than the away site. With my isotopic data, I generated carbon-nitrogen, carbon-sulfur and sulfur-nitrogen bi-plots to visualize the trophic relationships between all species and the salmon feed.

For both metals and each of the isotopes in a given year, I ran generalized linear models (GLM) [family = Gamma (link=log)] for each species. For each species, I ran all possible models with combinations of the factors BMA (when data existed for multiple BMAs), site pairs (nested within BMA, when multiple BMAs were sampled), treatment (near and away), and interactions between the factors. GLMs could only be run for BMAs that included two or more paired sites for that species, which severely limited the number of possible species-metric combinations that could be tested. In most cases, GLMs were performed across two BMAs for a particular species; but in some cases,
GLMs were only run across all site pairs within 1 BMA. The best model for each species and year for a particular metric was selected using Akaike Information Criterion corrected for small sample size (AICc). The AICc ranks all possible models based on their probability of explaining the data, and the model with the lowest AICc value is considered to be the best fit. The best fit GLM models for my data often included statistically significant interaction terms (alpha value < 0.05). Due to the inconsistency in the best fit models among species and years, the high number of significant interactions between treatment and other factors, and the restricted number of BMA and site pairs that could be tested from my data set. I checked normality using a Shapiro-Wilks test and then compared near and away sites of each site pair for each metric and species using paired t-tests or Mann-Whitney/Wilcoxon 2 sample test depending on whether data was normal or not (p = 0.05). The best fit GLM models for each metric and species tested are presented in Appendix C, while t-tests are shown in the Results. Lastly, I conducted chi-square goodness of fit tests for each metric in each year across all species-site pair combinations to determine if the direction of differences was significantly different from what would be expected by chance (50:50 chance of near > away and away > near) as a way of identifying overall effects of aquaculture on each metric.

Results

General summary of species results

Percent moisture varied among species (Table 2). Whole tunicates had the highest moisture content, averaging 91%, while whole mussels were lower at 84%. Lobster
muscle, sculpin and gunnel whole body averaged 80%, 76% and 71% moisture respectively.

**Table 2:** Number of individuals (n), range of total lengths (mm), average wet (±SD) and dry weight (g ± SD) (after the gut was removed for fish and of strictly lobster tail muscle) and percent moisture (±SD) (%) for all five species collected in 2017 in cobble bio-collectors at all near and away sites combined in 3 Bay Management Areas in the Bay of Fundy.

<table>
<thead>
<tr>
<th>species</th>
<th>n</th>
<th>length range (mm)</th>
<th>Average wet weight (g)</th>
<th>Average dry weight (g)</th>
<th>% moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vase tunicate</td>
<td>62</td>
<td>NA</td>
<td>7.89 (±4.08)</td>
<td>0.61 (±0.15)</td>
<td>91% (±0.09)</td>
</tr>
<tr>
<td>Blue mussel</td>
<td>74</td>
<td>NA</td>
<td>3.95 (±0.85)</td>
<td>0.65 (±0.18)</td>
<td>84% (±0.02)</td>
</tr>
<tr>
<td>Rock gunnel</td>
<td>70</td>
<td>85-137</td>
<td>3.56 (±1.47)</td>
<td>1.01 (±0.40)</td>
<td>71% (±0.03)</td>
</tr>
<tr>
<td>Shorthorn sculpin</td>
<td>58</td>
<td>60-100</td>
<td>5.65 (±2.57)</td>
<td>1.45 (±1.25)</td>
<td>76% (±0.06)</td>
</tr>
<tr>
<td>American lobster</td>
<td>42</td>
<td>20-48</td>
<td>4.26 (±2.07)</td>
<td>0.86 (±0.47)</td>
<td>80% (±0.03)</td>
</tr>
</tbody>
</table>

There were large differences in copper among species, with the two fish species having the lowest levels of the five taxa analyzed (Table 3). Across both years, average copper levels in fish ranged from between 1.68 to 3.68 μg/g dw. Similarly, the two suspension feeders averaged 3 to 5 times higher Cu, with mussels in 2017 at 6.03 μg/g dw and tunicates ranging between 8.67 and 10.97 μg/g dw in both years. Lastly, American lobster had much greater levels of copper than the other species, ranging between 32.5 and 35.86 μg/g dw across both years. In contrast, zinc levels were less consistent between years, both within and across species. Shorthorn sculpin had the lowest levels of zinc, ranging between 32.91 and 42.52 μg/g dw. Gunnels had the smallest range of any species in zinc levels (56.95 to 58.77 μg/g dw). Mussels and
lobsters had similar zinc levels, while tunicates had the highest values and the largest range in zinc (64.89 to 91.17 μg/g dw).

**Table 3:** Mean Cu and Zn (μg/g dw) concentrations for each species collected near and away from aquaculture sites in bio-collectors deployed for 4 months in 2016 and 2017 in three Bay Management Areas in the Bay of Fundy.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cu 2016</th>
<th>Near</th>
<th>Away</th>
<th>Cu 2017</th>
<th>Near</th>
<th>Away</th>
<th>Zn 2016</th>
<th>Near</th>
<th>Away</th>
<th>Zn 2017</th>
<th>Near</th>
<th>Away</th>
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</thead>
<tbody>
<tr>
<td>Vase tunicates</td>
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<td>10.97</td>
<td>9.84</td>
<td>62</td>
<td>8.67</td>
<td>9.20</td>
<td>56</td>
<td>91.17</td>
<td>77.35</td>
<td>62</td>
<td>72.00</td>
<td>64.89</td>
</tr>
<tr>
<td>Blue mussel</td>
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<td>6.06</td>
<td>6.00</td>
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<td>82.09</td>
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<td>57.82</td>
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</tr>
</tbody>
</table>

**Copper**

In 2016, my sample collections yielded 15 species-site pair combinations, a comparison of individuals of a species collected at the near and away sites of a site pair, across the four species: rock gunnel (5), shorthorn sculpin (2), vase tunicates (5) and American lobster (3). Overall, 11 of the 15 species-site pairs indicated that animals collected near aquaculture sites had more copper and two of these was statistically significant in t-tests (see below for more details) (Table 4). In 2017, there was a total of 26 different species-site pair comparisons across the five species: rock gunnel (6), shorthorn sculpin (5), tunicates (6), lobsters (4) and mussels (5). Overall, 17 of the 26 combinations indicated that near individuals had higher metal levels, five of those t-tests were statistically significant, while 1 other was significant in the opposite direction (Table 4). The chi-square tests did not indicate that there were more near sites with
greater copper concentrations than away sites than the number expected by chance alone (Appendix D).

Table 4: Differences in mean copper (µg/g dw; n=4 to 7) concentrations for all species-site pair comparisons and results of t-tests for bio-collectors deployed for 4 months at sites near and away from salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy. Positive values represent site pairs where near/current near > away/historic near and negative values represent where away/historic near > near/current near. "*" represent site pairs where the difference between near and away sites were significantly different as identified in the paired t tests (p < 0.05). The stage of the production cycle is also included in the table.

![Table 4](https://example.com/table4.png)

Within species comparisons

There was little evidence that aquaculture increased copper exposure in the four or five species examined across years. In 2016, tunicates were the only species that
showed significant differences between near and away sites (Figure 2). In BMA 2a, tunicates from the “historic near” site (Man-O-War) had half (8.27 ± 1.07 μg/g dw) the copper levels of those at the “current near” site (Howard Island; 17.7 ± 5.78 μg/g dw) (p = 0.010). In BMA 1, tunicates from the near site (Doctor's Cove) had significantly higher copper than at the away site (Indian Island) (p = 0.046), with averages of 9.95 ± 2.62 μg/g dw and 6.78 ± 1.02 μg/g dw, respectively. Meanwhile, individuals collected in BMA 3 at the near (Foley's Cove, 16.17 ± 2.99 μg/g dw) and away (Sand Cove; 8.10 ± 2.97 μg/g dw) sites had a large difference in mean copper (8.07 μg/g dw) but I lacked sufficient sample sizes to find a statistical difference (p = 0.083). In 2017, all species had at least 1 site-pair showing significant differences between treatments (Figure 3 & Table 4).

Tunicates collected at the “current near” site in BMA 2a (Howard Island) (11.85 ± 2.13 μg/g dw) had significantly higher copper values (p = 0.003) than those collected at the “historic near” site of the pair (Man-O-War) (8.08 ± 1.24 μg/g dw). Mussels showed a significant difference (p = 0.011) at one site pair in BMA 1, where near individuals (Boone Cove) averaged 5.75 ± 1.38 μg/g dw and their away counterparts (Dinner Island) averaged 3.53 ± 1.00 μg/g dw. Lobster in BMA 3a from WCNWC showed significantly more copper in individuals collected near than away from aquaculture sites (p = 0.014); near collectors (Welch’s Cove) had lobsters with 41.74 ± 9.40 μg/g dw of copper, nearly double what was measured in away lobster (North Welch’s Cove) (24.60 ± 7.87 μg/g dw) in this site pair. Sculpin collected in BMA 3a at FCSC also showed a significant difference in their copper levels (p = 0.047); near individuals (Foley’s Cove) had higher copper (2.91 ± 1.37 μg/g dw) than those collected at the away site (Sand Cove; 1.63 ± 0.26 μg/g dw). In contrast, rock gunnels had significantly more copper in their tissues at
the away than at the near site at one site pair in BMAs 1 (Round Marsh) (1.89±0.53 μg/g dw) and at the near site at one site pair in BMA 2a Howard Island (current near) in BMA 2a (2.29 ± 0.80 μg/g dw) (p = 0.022 & 0.049, respectively).

**Figure 2:** Mean (± SD) copper (μg/g dw; n=4 to 7) concentrations of all species sampled in bio-collectors deployed for 4 months at near (orange), away (blue), historic near (yellow) and current near (red) sites around salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy in 2016. The stage of the salmon production cycle is shown above the bars. “**” represents a significant difference between the two
sites within a pair. Refer to methods for a description of the differences between current and historic near sites at MOW and HI.
Figure 3: Mean (±SD) copper (µg/g dw; n=4 to 7) concentrations of all species sampled in bio-collectors deployed for 4 months at near (orange), away (blue), historic near (yellow) and current near (red) sites around salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy in 2017. The stage of the salmon production
cycle is shown above the bars. “*” represents a significant difference between sites within a pair. Green bars in the figure for blue mussels represent the two controls, “pre” being immediately upon collection and “post” after 4 days being held in the sea table, both prior to deployment. Refer to methods for description of the difference between current and historic near sites at MOW and HI.

**Among species comparisons**

The direction and significance of t-tests of the species-site pair combinations is summarized in Table 4 to facilitate broader comparisons. In 2016 in BMA 1, the direction of differences was inconsistent across species, with the fish often showing opposite trends (away>near) to those of the tunicates (near>away). All 4 species collected at the MOWHI site pair had higher copper at Howard Island, which had nearby active pen sites, than at Man-O-War, which had nearby empty pen sites that had recently been active. Species-site pair combinations in BMA 3a also showed consistency in the direction of differences, with both tunicates and lobster from all near sites having more copper than away sites. Within BMA 1 in 2017, trends were similar to those found in 2016 as tunicates and blue mussels showed greater levels of copper at near sites while both fish species showed predominantly more copper at the away sites. Gunnel and mussels each had 1 site pair with a significant difference between near and away, but these differences were in opposite directions. In BMA 2a, LkDH once again showed higher levels at the away site for both lobster and tunicates, but MOWHI showed inconsistent directions of differences across species; all species except mussels showed higher levels near aquaculture. Finally, sites in BMA 3a had a mix of species with both directions of
differences. When considering all the BMAs together, there was no concurrence in how the species responded to aquaculture inputs. Vase tunicates were the species most likely to show higher Cu values at the near sites. In fact, all sites in BMA 1 and 3a showed higher levels in near site tunicates than their away counterparts in both 2016 and 2017. Similarly, those collected at MOWHI in BMA 2a showed the higher copper levels at the “current near” site opposed to the “historic near” site.

**Relationships to the stage of salmon production**

The stage of the production cycle nearest to each site-pair can be found in Figure E1 in the appendix. There were no clear patterns between copper levels in organisms and the stage in the salmon production cycle (Table 4). All sites in BMA 3a in 2016 and one site in BMA 1 (FhRM) in 2017 were fallow for the entire time collectors were deployed. Tunicates, mussels and lobsters exposed to fallow conditions in 2016 and 2017, respectively, showed higher copper at near than away sites, while fish showed higher levels at the away site in the fallow site pair in BMA 1 in 2017. The latter includes rock gunnels, where the difference was significant. Of the BMAs with first year fish (2 sites in BMA2a in 2016 and 3 sites in 3a in 2017), BMA 2a had 4 species-site pair combinations at MOWHI and 1 at LkDH; all those at MOWHI were had differences in the same direction (current near>historic near), with the difference in tunicates being significant. Both lobster and sculpin from BMA 3a each had one site pair with a significant difference (near>away), but overall no consistency in response across species in the presence of first year fish. Moreover, under the same conditions but in different years, tunicates showed significantly higher levels away at Howard Island in 2016, but higher
levels near at Foley’s Cove in 2017. Lastly, there were 2nd year fish in BMA 1 in 2016 and in BMA 2a and at 2 sites for part of the deployment period in BMA 1 (BCDI & DCII) in 2017. Tunicates at all three site pairs in BMA 1 in 2016 showed higher levels of copper near active pen sites than away and one was significant (DCII). For the two sites in BMA1 in 2017, the direction of difference remained the same for tunicates and was also consistent for mussels (near>away). Responses in BMA 2a were similar, with all species showing higher levels at the current near site than the historic near site (Howard Island versus Man-O-War). Stage of the production cycle showed little evidence of an effect with differences being seen during all stages.

Zinc

As for copper, the fish showed the smallest differences in absolute zinc values between near and away sites of a site pair while tunicates and lobsters showed the largest differences in this metal between near and away sites. Of the 15 species-site pair combinations in 2016, 10 had higher zinc near than away from aquaculture and, of those, 2 showed significant differences in t-tests (Table 5). In 2017, 20 of the 26 species-site pair combinations indicated that individuals near aquaculture had more zinc in their tissues than those at the away sites (Table 5). However, of these 26 pairs only four showed significant differences, three where near animals had higher metals and one with the opposite result. In 2016, the number of species-site comparisons for which near individuals had greater zinc concentrations than those at the paired away site did not differ significantly from the number of events expected by chance alone. However, in 2017 there were significantly more species-site pairs with greater zinc in near individuals
than would be expected by chance alone (significant and non significant results combined) (Appendix D).

**Table 5:** Difference in mean zinc (µg/g dw; n=4 to 7) concentrations for all species-site pair comparisons for bio-collectors deployed for 4 months at sites near and away from salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy. Positive values represent site pairs where near/current near > away/historic near and negative values represent where away/historic near > near/current near. “**” represent site pairs where the difference between near and away sites were significantly different as identified in the paired t tests (p < 0.05). The stage of the production cycle is also included in the table.

### Within species comparisons

A small proportion of samples collected in the two years had significant differences in zinc among site pairs in the t-tests. In 2016, tunicates were the only species to show any significant differences in zinc levels between near and away sites (Figure 4).
At one of the site pairs in BMA 1, zinc in tunicates was greater at the near (Fairhaven) than the away (Round Marsh) site, averaging 114.63±16.99 μg/g dw and 81.55±13.70 μg/g dw, respectively (p = 0.0043). This trend was also seen at one site pair in BMA 3a, with roughly 2-fold higher zinc levels in tunicates near (Foley’s Cove, 93.70±16.98 μg/g dw) than away (Sand Cove, 45.35±11.81 μg/g dw) (p = 0.035). The difference between mean zinc levels in individuals at the near and away sites was 33.08 μg/g dw and 48.35 μg/g dw for FhRM (BMA 1) and FCSC (BMA 3a), respectively. In 2017, each species except blue mussels had a significant difference in zinc levels at one site pair (Figure 5 & Table 5). Tunicates collected at the “current near” site in BMA 2a (Howard Island) had a mean zinc concentration of 71.13±9.71 μg/g dw, which was significantly higher (p = 0.017) than the mean at the “historic near” site (Man-O-War, 58.63±6.31 μg/g dw).

Lobster showed significantly more zinc (p = 0.0038) at the other away site in BMA 2a (Deadman’s Harbour, 66.63±1.71 μg/g dw) than its near site pair (Limekiln, 56.43±3.31 μg/g dw). The other two significant differences were found in BMA 1 at FhRM (p = 0.020) and DCII (p = 0.0025), where near individuals had more zinc in their tissues than those at the paired away site. Sculpin collected at the near site of one pair in BMA 1 (Fairhaven) had an average zinc level of 50.3±4.95 μg/g dw that was significantly greater (by 9.50 μg/g dw) than at the away site, Round Marsh (p = 0.020). Gunnels collected at another near site in BMA 1 (Doctor’s Cove) had a mean of 77.74±9.20 μg/g dw of zinc in their tissues, which was significantly higher (by 16.53 μg/g dw) than for gunnels collected at the away site, Indian Island (p = 0.0025). Mussels collected at one site pair in BMA 2a (Man-o-War and Howard Island) had the largest difference recorded - 30.44 μg/g dw; however, it was not statistically different due to the high variability (p = 0.13).
Figure 4: Mean (+/- SD) zinc (µg/g dw; n=4 to 7) concentrations of all species sampled in bio-collectors deployed for 4 months at near (orange), away (blue), historic near (yellow) and current near (red) sites around salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy in 2016. The stage of the salmon production cycle is shown above the bars. “*” represents a significant difference between sites within a pair. Refer to methods for description of the difference between current and historic near sites at MOW and HI.
Figure 5: Mean (+/- SD) zinc (µg/g dw; n=4 to 7) concentrations of all species sampled in bio-collectors deployed for 4 months at near (orange), away (blue), historic near
(yellow) and current near (red) sites around salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy in 2017. The stage of the salmon production cycle is shown above the bars. “*” represents a significant difference between sites within a pair. Green bars in the figure for blue mussels represent the two controls, “pre” being immediately upon collection and “post” after 4 days being held in the sea table, both prior to deployment. Refer to methods for description of the difference between current and historic near sites at MOW and HI.

**Among species comparisons**

In 2016, there were some inconsistent directional responses in zinc for taxa collected at the same site pairs (Table 5). In BMA 1, all species collected at two (FhRM & DCII) of the three site pairs had greater zinc levels near aquaculture leases, including a significant result for tunicates at FhRM, while at the third site pair (BCDI) all species except tunicates showed the same trend (near>away). In BMA 2a, all four species were sampled at only one of the site pairs (MOWHI) (the other had only one species sampled) and they were split in their responses to aquaculture. Lastly, in BMA 3a, there were only 3 species-site pair combinations across two site pairs (FCSC & SeCSeB) and two of the three showed more zinc at the near than away site. In 2017, BMA 1 had similar results to those of 2016, with the majority of species-site pair combinations having greater zinc levels at the near sites (2 combinations were significant; sculpin at Fairhaven and gunnels at Doctor’s Cove). More specifically, all species collected at one site pair in BMA 1 (FhRM) showed higher levels of zinc at the near site (Fairhaven). For BMA 2a, species collected at the MOWHI site pair, where stocking was changed, consistently showed
more zinc at the “current near” site (Howard Island). Lobsters collected at the away site of the other site pair in BMA2a (Deadman’s Harbour) was the only species-site pair combination to have significantly higher zinc at the away site than its near counterpart (Limekiln). Lastly, in BMA 3a, zinc in 7 of 10 species-site pair combinations showed the expected direction of response (near>away), yet none of them were significant.

**Relationships to the stage of salmon production**

BMA 3a and one site pair in BMA 1 (FhRM) were fallow in 2016 and 2017, respectively. Although BMA 3a was fallow in 2016, there was higher zinc at near than away sites for 2 of 3 species-site pair combinations, including a significant difference in tunicates from FCSC. In BMA 1, FhRM was fallow the entire time of deployment in 2017 yet all species collected there had higher levels of zinc near aquaculture. Moreover, this difference was statistically significant for sculpin at this site. First year fish were in BMA 2a in 2016 and BMA 3a in 2017. In both of these areas, I saw mixed results across species. Lastly, 2nd year fish were found for the full period of collector deployment at all sites in BMA 1 in 2016 and BMA 2a in 2017 and for part of the deployment period at two sites in BMA 1 in 2017. In BMA 1 in 2016, the fish showed higher zinc near aquaculture, while vase tunicates showed the same trend at two of the three site pairs, with one being significant (FhRM). In BMA 2a in 2017, all species collected at the MOWHI site pair showed higher levels of zinc at the “current near” site, including significant results for tunicates. The two sites in BMA 1 that had 2nd year fish for part of the deployment period in 2017 showed mixed responses, but gunnels had significantly more zinc near (Doctor’s Cove) than away (Indian Island) in one site pair. Focusing on
tunicates as they were common across most sites, no effect of production cycle was observed across years. Most tunicates collected near aquaculture had higher levels of zinc than those at the away site of a site pair regardless of whether they were exposed to fallow conditions or to 1st year or 2nd year fish.

Isotopes

The lipid adjustment shifted the \( \delta^{13}C \) values of feed, gunnel, sculpin and lobster by 3.85‰, 0.21‰, 0.02‰ and 0.07‰, respectively. Both adjusted and unadjusted \( \delta^{13}C \) feed values were included in the biplots but the former was used to assess potential use of feed by these species. In 2016, lobsters and the two fish species were grouped, but were distinct in their \( \delta^{13}C, \delta^{15}N \) and \( \delta^{34}S \) values from those of the tunicates. Carbon and nitrogen biplots indicated that in 2016, there was slight overlap in \( \delta^{13}C \) between feed (l lipid adjusted) and gunnels, and little to no overlap between feed (lipid adjusted), lobster and sculpin (Figures 6 & 7). Tunicate \( \delta^{13}C \) values were lighter than the adjusted feed values suggesting little reliance (Figure 6). In 2017, lobsters and the two fish species were distinct in their \( \delta^{13}C, \delta^{15}N \) and \( \delta^{34}S \) values from those of the tunicates and mussels. Tunicates and mussels had \( \delta^{13}C \) value that would indicate feeding on aquaculture feed \( \text{feed}_{\text{adj}} \) at certain sites based on fractionation, but there was little evidence of reliance when considering average values. Aquaculture feed has an average isotope values of -17.91‰ for \( \delta^{13}C_{\text{adj}} \), 3.47‰ for \( \delta^{15}N \) and 2.07‰ of \( \delta^{34}S \). If the species herein fed mainly upon aquaculture feed, their isotope values would be closer to \( \delta^{13}C = -16.91‰, \delta^{15}N = 6.47‰ \) and \( \delta^{34}S = 3.00‰ \), based on the current understanding of enrichment factors for these elements. However, their use of the feed was not similarly supported by their \( \delta^{34}S \) values.
There was also a slight overlap of gunnel $\delta^{13}$C values with the $\delta^{13}$C_{adj} of feed, but they had $\delta^{15}$N values that were ~ two trophic levels higher. Sculpin and lobster $\delta^{13}$C values remained distinct from values of the salmon feed (Figure 7). In both 2016 and 2017, vase tunicates had comparable $\delta^{13}$C values (-19.68‰ and -20.34‰) and $\delta^{34}$S values (19.71‰ and 20.38‰). These values were close to those of blue mussels from 2017 ($\delta^{13}$C - 19.42‰ and $\delta^{34}$S 20.97‰), suggesting that the two suspension feeders have similar energy sources. In both years the $\delta^{13}$C values for the fishes and lobster were higher (2016: $\delta^{13}$C_{adj} rock gunnels -16.56‰, shorthorn sculpin -15.53‰, lobster -15.84‰; raw $\delta^{13}$C 2017: rock gunnels -16.84‰, shorthorn sculpin -16.08‰, lobster -15.93‰) than for the suspension feeders. In addition, the $\delta^{34}$S values were lower for gunnel (14.74‰ and 18.00‰), sculpin (13.38‰ and 17.70‰), and lobster (16.43‰ and 17.47‰) over the two years than those for suspension feeders. Together the isotope ratios for these two elements suggest that energy sources supporting the fishes and lobster are different from those of the suspension feeders. Vase tunicates had higher $\delta^{15}$N (7.34‰) than the mussels (6.03‰) collected in the same year, suggesting that the former fed at a slightly higher trophic level. The species expected to be at a higher trophic level (fishes and lobster) had $\delta^{15}$N values roughly one trophic level (~3 to 4‰) above the suspension feeders, with overall mean values of 10.84 and 10.95‰, 9.70 and 9.87‰, and 10.78 and 11.04‰ in 2016 and 2017 for rock gunnel, American lobster and shorthorn sculpin, respectively.
**Figure 6:** Community biplots with mean values within sites for each of the three combinations of isotopes (‰): (A: δ^{15}N versus δ^{13}C (δ^{13}C_{adj} for sculpin, gunnel and lobster), B: δ^{34}S versus δ^{13}C (δ^{13}C_{adj} for sculpin, gunnel and lobster) and C: δ^{15}N versus δ^{34}S) for species collected in bio-collectors deployed for 4 months at sites near (closed symbols) and away (open symbols) from salmon aquaculture leases in three Bay Management Areas in the Bay of Fundy in 2016. Two feed types (CI & II) are represented by two different coloured circles. Both lipid adjusted and raw feed carbon values are included in each figure. Each point represents an average for a species collected at either near or away sites within one BMA.
**Figure 7:** Community biplots with mean values within sites for each of the three combinations of isotopes (‰) (A: $\delta^{15}$N versus $\delta^{13}$C, B: $\delta^{34}$S versus $\delta^{13}$C and C: $\delta^{15}$N versus $\delta^{34}$S) of species sampled in bio-collectors deployed for 4 months at sites near (closed symbols) and away (open symbols) from salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy in 2017. Two feed types (CI & II) are represented by two different coloured circles. Both lipid adjusted and raw feed carbon values are included in each figure. Each point represents an average for a species collected at either near or away sites within one BMA.

**Within species comparisons for carbon**

There were some differences in the $\delta^{13}$C values of species within the site pairs but the directions of the responses were inconsistent (Table 6). Differences were calculated by subtracting the average isotope value of the away site from that of the near site [positive $\Delta$ indicates near>away (away more similar to feed for fish/lobster & near more similar for filter feeders); negative $\Delta$ indicates away>near (near more similar to feed for fish/lobster & away more similar for filter feeders)]. In 2016, 6 of 19 species-site pair combinations were statistically different in $\delta^{13}$C values in t-tests but most differences were small in magnitude; these were for rock gunnel and shorthorn sculpin from 2 sites in BMA 1 [FhRM (gunnel$_{(adj)}$: $\Delta$ 1.05‰, $p = 0.00010$ & sculpin$_{(adj)}$: $\Delta$ 0.83‰, $p = 0.00010$) and BCDI (gunnel$_{(adj)}$: $\Delta$ -1.03‰, $p = 0.0017$ & sculpin$_{(adj)}$: $\Delta$ -1.58‰, $p = 0.0012$)] and for rock gunnel and American lobster from one site in BMA 3a [SeC-SeB (gunnel$_{(adj)}$: $\Delta$ 0.78‰, $p = 0.011$ & lobster$_{(adj)}$: $\Delta$ 0.74‰, $p = 0.031$)] (Figure 8 and Table 6). In 2017, there were 6 of 25 species-site pair combinations that showed significant differences in
\( \delta^{13}C \) (Table 6). Differences were still present in gunnel and sculpin in BMA 1 [FhRM (gunnel: \( \Delta 2.12\% \), \( p = 0.0001 \) & sculpin: \( \Delta 2.40\% \), \( p = 0.0001 \))]. Gunnels and lobsters in at another site in BMA 3a showed similar trends to the year before [FCSC (gunnel: \( \Delta 0.80\% \), \( p = 0.0093 \) & lobster: \( \Delta 0.65\% \), \( p = 0.0016 \)]) (Figures 9 & 10 and Table 6). BMA 3a had one site pair (WCNWC) where lobster had significantly lower \( \delta^{13}C \) at the near than the away site (\( \Delta -0.25\% \), \( p = 0.039 \)) and another (SeCSeB) where there was significantly lower \( \delta^{13}C \) in rock gunnel from away than near (\( \Delta 0.73\% \), \( p = 0.00024 \)) (Figures 9 & 10). Based on the chi-square analysis, neither near nor away species-site pair combinations showed depleted \( \delta^{13}C \) values more often than expected by chance alone (Appendix D).

**Table 6:** Difference in mean \( \delta^{13}C \) and \( \delta^{13}C_{adj} \) (\( \% \); \( n=4 \) to 7) for all species-site pair comparisons from bio-collectors deployed for 4 months at sites near and away from salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy. Positive values represent site pairs where near/current near > away/historic near and negative values represent where away/ historic near > near/current near. “*” represent site pairs where the difference between near and away sites were significantly different as identified in the paired t tests (\( p < 0.05 \)). The stage of the production cycle is also included in the table.
Within species comparisons for nitrogen

There were also significant differences in $\delta^{15}$N values within species, but they were small, ranging between 0.26‰ and 0.74‰ across site pairs, and were inconsistent in direction (Figures 8, 9 & 10). In 2016, gunnel were significantly different in $\delta^{15}$N between near and away sites at all three sites pairs in BMA 1 and at 1 site pair in each of BMA 2a and 3a (FhRM $\Delta -0.49\%$, $p = 0.0073$; BCDI $\Delta 0.50\%$, $p = 0.0034$; DCII $\Delta -0.55\%$, $p = 0.0064$; LkDH $\Delta 0.35\%$, $p = 0.037$; FCSC $\Delta 0.60\%$, $p = 0.045$) (Table 7). Moreover, tunicates at one site pair in BMA 1 (BCDI $\Delta 0.27\%$, $p = 0.011$) and sculpin at one site pair in BMA 2a (MOWHI $\Delta -0.42\%$, $p = 0.031$) also showed significant differences. In 2017, rock gunnels had significantly higher or lower $\delta^{15}$N values at the near sites in BMA 1 (FhRM $\Delta -0.51\%$, $p = 0.0014$; BCDI $\Delta 0.68\%$, $p = 0.0034$; DCII $\Delta -0.73\%$, $p < 0.0001$) (Table 7). The only other two species-site combinations in this year to show statistically significant differences were mussels at one site pair in BMA 2a
(MOWHI Δ -0.34‰, p =0.011) and sculpin at one site pair in BMA 3a (FCSC Δ -0.62‰, p = 0.044) (Table 7). Neither the near nor away species-site pair combinations showed higher $\delta^{15}$N values more often than expected by chance alone (Appendix D).

**Table 7:** Difference in mean $\delta^{15}$N (‰; n=4 to 7) values for all species-site pair comparisons from bio-collectors deployed for 4 months at sites near and away from salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy. Positive values represent site pairs where near/current near > away/historic near and negative values represent where away/ historic near > near/current near. “*” represent site pairs where the difference between near and away sites were significantly different as identified in the paired t tests (p < 0.05). The stage of the production cycle is also included in the table.

<table>
<thead>
<tr>
<th>2016</th>
<th>Vase Tunicate</th>
<th>American Lobster</th>
<th>Rock Gunnel</th>
<th>Shorthorn Sculpin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMA 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd year fish</td>
<td>Fairhaven &amp; Round Marsh</td>
<td>-0.34</td>
<td>0.27*</td>
<td>0.49*</td>
</tr>
<tr>
<td></td>
<td>Boone Cove &amp; Dinner Island</td>
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<td>0.50*</td>
<td>-0.47</td>
</tr>
<tr>
<td></td>
<td>Doctor’s Cove &amp; Indian Island</td>
<td>-0.27</td>
<td>0.55*</td>
<td></td>
</tr>
<tr>
<td>BMA 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st year fish</td>
<td>Howard Island &amp; Man-O-War</td>
<td>-0.02</td>
<td>0.09</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Linekin &amp; Deadman’s Harbour</td>
<td>0.02</td>
<td>0.35*</td>
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</tr>
<tr>
<td>BMA 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fallow</td>
<td>Foley’s Cove &amp; Sand Cove</td>
<td>0.03</td>
<td>-0.02</td>
<td>0.60*</td>
</tr>
<tr>
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<td>-0.02</td>
<td>0.46</td>
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</table>

<table>
<thead>
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<th>2017</th>
<th>Vase Tunicate</th>
<th>American Lobster</th>
<th>Blue Mussel</th>
<th>Rock Gunnel</th>
<th>Shorthorn Sculpin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMA 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fallow</td>
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<td>0.06</td>
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<td>2nd/fallow</td>
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<td>0.68*</td>
</tr>
<tr>
<td>2nd/fallow</td>
<td>Doctor’s Cove &amp; Indian Island</td>
<td>-0.02</td>
<td>-0.14</td>
<td>-0.34*</td>
<td>-0.73*</td>
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<tr>
<td>BMA 2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2nd year fish</td>
<td>Howard Island &amp; Man-O-War</td>
<td>-0.20</td>
<td>0.09</td>
<td>-0.14</td>
<td>-1.05</td>
</tr>
<tr>
<td></td>
<td>Linekin &amp; Deadman’s Harbour</td>
<td>0.20</td>
<td>0.14</td>
<td>-0.34*</td>
<td>-0.62*</td>
</tr>
<tr>
<td>BMA 3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st year fish</td>
<td>Foley’s Cove &amp; Sand Cove</td>
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<td>-0.15</td>
<td>-0.07</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Seeley’s Cove &amp; Seeley’s Basin</td>
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<td>-0.17</td>
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<td>-0.54</td>
</tr>
<tr>
<td></td>
<td>Welch’s Cove &amp; North Welch Cove</td>
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<td>0.30</td>
<td>-0.05</td>
<td>-0.21</td>
</tr>
</tbody>
</table>
Within species comparisons for sulfur

Sulfur isotopes showed the most consistent results within and among species across site pairs (Table 8). In 2016, there were 6 of 13 species/site pair combinations that showed significant differences between the near and away sites of a pair, all of which had the largest values at the away site, and the differences ranged from $\Delta-0.83\%$ to $\Delta-2.78\%$ (Figures 8, Table 8). In 2016, gunnels ($p = 0.023$), sculpin ($p = 0.0028$) and tunicates ($p = 0.012$) from one site pair in BMA1 (FhRM) had consistently higher values at the away than near site: $\Delta-2.16\%$, $\Delta-2.78\%$ and $\Delta-2.04\%$, respectively. In addition, gunnels at one site pair in BMA 2a (LkDH $\Delta -1.18\%$, $p = 0.011$), lobster at the other (HIMOW $\Delta -1.14\%$, $p = 0.029$) and tunicates at one site pair in BMA 1 (DCII $\Delta-0.83\%$, $p= 0.001$) showed lower values at near sites. In 2017, 9 of 25 species/site pair combinations had significant differences and all the away sites, except for mussels at Seeley’s basin, had higher $\delta^{34}$S than their paired near sites (Figures 9 & 10, Table 8). In BMA 1, rock gunnel collected at 2 site pairs (DCII $\Delta-1.84\%$, $p = 0.001$; FhRM $\Delta-2.09\%$, $p = 0.001$) and shorthorn sculpin at one site pair (FhRM $\Delta-3.58\%$, $p = 0.0001$) showed significantly different $\delta^{34}$S values. In BMA 2a, only mussels at 1 site pair (MOWHI $\Delta-1.25\%$, $p = 0.010$) had significant results with the “current near” site individuals being more similar to feed than the “historic near” site. Lastly, there was only one site pair in BMA 3a (FCSC) which showed significant differences; however, the results were consistent across all 4 species (away > near) (tunicates $\Delta-2.68\%$, $p = 0.0001$; lobsters $\Delta-1.45\%$, $p = 0.0039$; sculpins $\Delta-1.30\%$, $p = 0.048$; gunnels $\Delta-0.92\%$, $p = 0.013$). Mussels at Seeley’s Cove was the only statistically significant species-site pair with higher sulfur at the near site rather than the away site (SeCSeB $\Delta 0.42\%$, $p = 0.028$). In 2016, 11 of the 13
species-site pair combinations had near individuals that had lower $\delta^{34}$S, which was significantly different than the 50% expected by chance alone ($p = 0.013$); however, 2017 did not show a significant difference ($p = 0.16$) (Appendix D).

**Table 8:** Difference in mean $\delta^{34}$S (‰; $n=4$ to 7) values for all species-site pair comparisons from bio-collectors deployed for 4 months at sites near and away from salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy. Positive values represent site pairs where near/current near > away/historic near and negative values represent where away/historic near > near/current near. “*” represent site pairs where the difference between near and away sites were significantly different as identified in the paired t tests ($p < 0.05$). The stage of the production cycle is also included in the table.
Figure 8: Mean (±SD) values for all three isotopes ratios ($\delta^{13}$C (adj), $\delta^{15}$N, $\delta^{34}$S) (%; n=4 to 7) of fish species sampled in bio-collectors deployed for 4 months at near (orange), away (blue), historic near (yellow) and current near (red) sites around salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy in 2016. The stage of the
salmon production cycle is shown above the bars. “*” represents a significant difference between sites within a pair. Refer to methods for description of the difference between current and historic near sites at MOW and HI.
Figure 9: Mean (±SD) values for all three isotopes ratios ($\delta^{13}$C (adj), $\delta^{15}$N, $\delta^{34}$S) (‰; n=4 to 7) of invertebrate species sampled in bio-collectors deployed for 4 months at near (orange), away (blue), historic near (yellow) and current near (red) sites around salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy in 2016. The
stage of the salmon production cycle is shown above the bars. “*” represents a significant
difference between sites within a pair. Refer to methods for description of the difference
between current and historic near sites at MOW and HI.

Figure 10: Mean (± SD) values for all three isotopes ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) (‰; n=4 to
7) of both fish species sampled in bio-collectors deployed for 4 months at near (orange),
away (blue), historic near (yellow) and current near (red) sites around salmon aquaculture
sites in three Bay Management Areas in the Bay of Fundy in 2017. The stage of the
salmon production cycle is shown above the bars. “*” represents a significant difference
between sites within a pair. Refer to methods for description of the difference between
current and historic near sites at MOW and HI.
**Figure 11:** Mean (± SD) values for all three isotopes ratios ($\delta^{13}$C, $\delta^{15}$N, $\delta^{34}$S) (‰; n=4 to 7) of American lobster, blue mussel and vase tunicates of all species sampled in bio-collectors deployed for 4 months at near (orange), away (blue), historic near (yellow) and current near (red) sites around salmon aquaculture sites in three Bay Management Areas in the Bay of Fundy in 2017. The stage of the salmon production cycle is shown above the bars. "*" represents a significant difference between sites within a pair. Green bars in the figure for blue mussels represent the two controls, “pre” being immediately upon collection and “post” is after 4 days being held in the sea table, both prior to deployment.
Refer to methods for description of the difference between current and historic near sites at MOWHI.

**Relationships to the stage of salmon production**

Because tunicates were found across most sites they were used to examine whether production cycle affected the isotopic ratios; results suggested that isotope values in tunicates were unrelated to stage of the production cycle in either year. In both years there were no significant differences in tunicate $\delta^{13}C$ between near and away sites in any site pairs (Table 6), suggesting no production cycle effects for this isotope. Moreover, for $\delta^{15}N$ only 2016 tunicates from Boone Cove (BMA 1), where they were exposed to second year fish, had higher but small shifts in $\delta^{15}N$ than its paired away site ($\Delta0.27\%o$, $p = 0.011$) and no differences were observed in the 2017 data. Similarly, for $\delta^{34}S$ there was little evidence of production cycle effects for tunicates; of the 30 site pair/year combinations for tunicates, significant differences were only found in two site pairs in BMA 1 in 2016 (2nd year fish: FhRM $\Delta-2.04\%o$, $p = 0.012$; DCII $\Delta-0.83\%o$, $p = 0.001$) and in one site pair in BMA 3a in 2017 (FCSC; 1st year fish; $\Delta-2.68\%o$, $p = 0.00036$).
Table 9: Percentage of t-tests that were statistically significant for all species in both years for all 5 metrics measured.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cu Near</th>
<th>Cu Away</th>
<th>Zn Near</th>
<th>Zn Away</th>
<th>C Near</th>
<th>C Away</th>
<th>N Near</th>
<th>N Away</th>
<th>S Near</th>
<th>S Away</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vase tunicate</td>
<td>2016 40%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 50%</td>
<td>0%</td>
</tr>
<tr>
<td>Blue mussel</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
</tr>
<tr>
<td>American lobster</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 50%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
</tr>
<tr>
<td>Shorthorn sculpin</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 40%</td>
<td>0%</td>
<td>2016 20%</td>
<td>0%</td>
</tr>
<tr>
<td>Rock gunnel</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 0%</td>
<td>0%</td>
<td>2016 14%</td>
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<td>9%</td>
<td>3%</td>
<td>19%</td>
<td>8%</td>
<td>9%</td>
<td>10%</td>
<td>2%</td>
<td>35%</td>
</tr>
</tbody>
</table>

**Discussion**

Overall, my study did not detect a consistent and significant influence of aquaculture on metals or stable isotopes of five benthic species inhabiting rocky bottom habitats located more than 200 m away from pen sites. While there were some significant results indicating near sites to be more heavily influenced by copper, zinc concentrations or stable isotopes values of carbon, nitrogen and sulfur, the majority of t-tests of species-site pair combinations yielded no significant differences. In addition, there was little consistency in the direction of differences, with some pairs showing greater levels of metals and isotopes near and others away from aquaculture. Lastly, in all cases except for sulfur in 2016 and zinc in 2017, the number of species-site pair combinations for which values at near compared to away sites were in the direction predicted due to aquaculture.
exposure did not exceed the number expected by chance alone. Both metals had more significant differences indicating more metals at near sites, while carbon and nitrogen isotopes showed few significant differences which were inconsistent in direction, and sulfur provided the most significant differences between treatments (near and away individuals) and indicated that near individuals had slightly more similar isotope values compared to salmon feed than those at away sites. Overall, while there were large differences in concentrations of metals between species there were no consistent increased levels of metals at near sites or during specific phases of the production cycle, and my isotope data did not indicate that any of the species I sampled fed predominantly on aquaculture feed.

In this study, copper was not consistently higher at the near than away sites across the taxa I examined. This could be because the bio-collectors were outside of the area that is impacted by aquaculture, copper-containing paints are being phased out, or other local sources of this metal were affecting levels in organisms at the away sites. Other studies have found that sediments are typically elevated in copper within 40 to 70 m of pens, but occasionally up to 300 m, and that sediment-dwelling invertebrates are elevated in copper up to 75 m from the pens (Jones & Iwama, 1991; Chou et al., 2003; Giles, 2008). However, at the distances I used in this study (> 200 m), no information existed about whether this metal is elevated in benthic organisms near aquaculture sites. My results suggest that waste accumulation is dependent on the production and dispersal rates (Cromey et al., 2002), which in turn is determined by distance from pen sites and site depth (Kalantzi & Karakassis, 2006). The increased distances between my sites and active pen sites may be why there was not consistent elevated exposure of these taxa.
(within or among species) to copper. Copper-containing paints are also being phased out of the industry and were last use at these sites in 2013 (Michael Szemerda, Cooke Aquaculture, Pers. Comm.) and this too may explain the lack of impacts at the near sites. However, elevated metals can persist in sediments for at least 5 years after salmon production is terminated, so biotic exposures can continue past active aquaculture periods due to the sediment reservoir that exists and that can be resuspended by waves and currents (Smith et al., 2005). Indeed, in the current study elevated Cu was found in rock gunnel at an away site (Round Marsh) in 2017, an area that had a long history of producing salmon up until 2013, and this may be due to legacy contamination as was seen in Smith et al. (2005). Lastly, the lack of near site effects on copper found herein may be due to other copper sources affecting the animals at away sites. More specifically, copper is in anti-fouling paints used on boat hulls and therefore areas with high boat activity such as harbours may lead to increased metals in biota collected near these sites (Warken et al., 2004). To determine if our bio-collectors were too far away from the salmon cages to be influenced by them or closer to other sources of metals, analysis of surface sediment or core samples would be useful as this would identify areas with higher copper inputs. Finally, there may have been elevated copper at the near sites but it could have been unavailable for uptake because environmental conditions, hydrodynamics and biogeochemical processes may have reduced its bioavailability (Eggleton & Thomas, 2003).

Zinc also showed little or no evidence of impacts at the near sites in the current study; this may be due to the organisms being outside of the area impacted by salmon cage culture (as discussed above), other sources of Zn in these BMAs that are masking
the effects of aquaculture (as discussed above), the more efficient use of Zn by salmon because of changes in diet formulations and subsequent reduced inputs to the area surrounding the cage, or the ability of animals to regulate Zn levels in contaminated environments. Few studies have assessed Zn in sediments and sediment-dwelling organisms near salmon aquaculture. Of those that did, elevated levels were found in sediments up to 300 m away and in lobster as far as 50 m from pen sites (Chou et al., 2002; Burridge et al., 2010). As for copper, the larger distances between my sites and active pen sites means they may have been outside the area of influence of salmon production. Sediment samples at near and away sites, but ideally at a series of distances from pens, would have helped assess spatial trends in zinc levels and their potential for influence on the benthic community. This metal may be elevated in biota at some away sites because of additional local sources of zinc; more specifically, ships and other watercraft can be a source of metals like zinc which are present in hull paints (Chen et al., 2007; Singh & Turner, 2009). This could explain why animals from Deadman’s Harbour, a site that is the most removed from aquaculture influence but that does have a fair bit of boat activity, had higher zinc levels than those from its near counterpart. Zinc is an essential nutrient in fish feed and formulations have been changed in recent years to make it more available for uptake by salmon, increasing growth efficiency and leading to the production of the same amount of fish with a third less zinc (Watanabe et al., 1997; Maage et al., 2001; Taylor et al., 2019). The subsequent reductions in waste zinc around cages may also explain the lack of impacts observed in my study. Finally, because Zn is an essential nutrient, the animals I analyzed may be able to regulate its levels to maintain correct zinc levels for proper metabolic functions regardless of elevated exposures. It is
not known if the species examined herein have this ability, but other lobster, fish and to a lesser extent tunicates have been reported to maintain constant internal zinc levels even when exposed to a range of water concentrations (Bryan, 1964; Papadopoulou & Kania, 1977; Canli & Furness, 1993; Andres et al, 2000).

The carbon isotope data, but not nitrogen, suggested some use of aquaculture feed by the rocky-habitat species examined in this study; however, this overlap in values was seen in both near and away individuals. This indicates either widespread contamination of the area by the feed, or more likely a lack of distinction between the carbon isotopic composition of the feed and other potential prey for these species. Otherwise, the lack of similarity in isotope values between individuals and feed may be explained by a greater diversity of food sources in rocky habitats that reduces the importance of any one prey item, or by the fact that the bio-collectors were located too far away to show heavy reliance on aquaculture waste. The C and N isotope values of these species were different from what would be expected if they fed predominantly on feed, based on known fractionation. Even when there were statistically significant differences between near and away individuals in either carbon or nitrogen isotope ratios, the shifts were normally less than 1.00‰ which is typically within analytical error (O’Leary, 1988), meaning there is little or no evidence of reliance of salmon feed. The isotopic values of the species more closely resembled other natural prey that are found in the area, such snails and polychaetes (δ\(^{13}\)C range between -12‰ and -16.5‰) (Chaston-Vickers, 2015). Moreover, rocky bottom habitats are diverse ecosystems that contain a large number of substrate-specific species (Danovaro & Fraschetti, 2002; Stål et al., 2007). Given that the breath of a species’ diet is directly related to prey diversity, with high biodiversity resulting in a
more diverse diet (Stål et al., 2007), this may explain the lack of strong reliance of rocky-habitat organisms on aquaculture feed. Of the three elements examined, sulfur isotope values had the most consistent and largest shifts in near individuals towards that of the feed. Marine phytoplankton obtain sulfate from sea salt and have a sulfur value around 21‰ (Peterson & Howarth, 1987; Fry, 1988). The feed used at my sites had a sulfur isotope ratio close to 2.00‰, which is more similar to that of wheat (4.00‰), plant material often used in salmon feed (Monaghan et al., 2009). Each species had individuals that showed shifts in sulfur isotope values towards the feed at some sites and all species collected at one site pair (Foley’s Cove/Sand Cove) showed consistent shifts. The difference between species values and feed values were still too large (between 15.46‰ and 19.00‰, greater than 1.00‰) to conclude that the feed plays an important role in the diet of these species. Of the three elements, stable isotopes of sulfur appear to be the most useful for assessing reliance of organisms on aquaculture feed due to the consistency of results, the very distinct sulfur values of the feed and the larger differences detected between near and away individuals (Table 9). More recently, researchers have begun to use a combination of carbon stable isotopes and fatty acid markers to trace the assimilation of salmon feed into organisms and it has yielded promising results (Redmond et al., 2010). For my study it would have been useful to collect and analyze other potential prey for isotopes to be able to run mixing models to assess the relative importance of aquaculture feed to these organisms. However, this was not done because it would have required extensive sampling over time to adequately characterize the composition of potential prey items across the sites I examined.
Of the 5 taxa examined, it is my opinion that tunicates are the best choice to monitor if organisms are taking up metals and using nutrients released from aquaculture sites because they are resident (non-mobile), short-lived, invasive and able to colonize diverse habitats, known to accumulate metals, and occupy low positions in the food web. Tunicates settle and grow on introduced hard surfaces and being sessile, fast-growing and short-lived allows for better defined exposure periods and spatially accurate sampling. Using tunicates, we can collect data that represent exposures over a specific period of time in a precise location and we do not need to worry about carry over (legacy) effects, unlike with other species. Both juvenile fish and lobster are central place foragers and remain in small areas for the first few years of life, making mobility a non-factor in this study; however, their true exposure time to aquaculture wastes is unknown (Ojeda & Dearborn, 1991; Moring, 1993; Morse & Rochette, 2016). Vase tunicates are invasive and have the ability to inhabit hypoxic and anoxic environments allow us to collect and analyze animals from habitats unlikely to contain other species. Suspension-feeders are often susceptible to metal contamination because they filter contaminated waters and suspended sediments and take up dissolved and particulate metals into their tissues (Rainbow, 1995; Hill et al., 2009); as a result, they are known to accumulate copper and to a lesser degree zinc (Papadopoulou & Kanias, 1977; Roberts et al., 2008). In contrast, copper and zinc do not biomagnify and higher-trophic-level predators often have lower levels of these metals (Zhang & Wang, 2012), a result supported by my findings. The exception in my study was lobster, which had the highest levels of Cu despite their position in the food web; this is likely due to their use of an enzyme known as hemocyanin, a Cu-containing molecule, as their primary oxygen transport molecule in
their cells (Tarrant et al., 2012). This concept is also supported by the percentage of paired-sites where tunicates showed significant differences (table 9). Specifically, for zinc and sulfur, tunicates showed a high percentage of sites with a significant difference in both years compared to other species.

Although I examined whether there were greater effects in BMAs with active aquaculture when compared to those that are fallow, no general trends were observed across production cycles, which may be due to legacy effects. I found the largest number of significant differences at sites exposed to second year fish, however, elevated metals were found in organisms near pens in fallow years as well. While it is current practice to reduce the number of feeding events as fish grow larger, faeces and food pellets will have accumulated in the sediment over the production cycle, and metals do not degrade over time (Morris et al., 2003; Roussiez et al., 2011). Overall my results suggest that legacy effects mask any production-related differences in impacts of aquaculture.

Most of the previous studies assessing the impacts of aquaculture have been conducted in muddy or sandy habitats, with little known about effects on organisms living on hard bottom substrates. While the primary reason for not finding a significant effect on metals and nutrients in organisms in my study is likely the spatial scale at which I sampled being greater than 200 m, while most other studies focus on the area within 100 m, it is my opinion that substrate also influences the exposure of organisms to contaminants. Rocky habitats may be less susceptible to the effects of aquaculture because of their increased biodiversity and fewer resuspension events. First, rocky bottoms have much greater habitat diversity than soft bottoms, leading to greater diversity of meiofauna (Danovaro & Fraschetti, 2002; Stål et al., 2007) and a larger
selection of potential food sources that reduces the reliance of a species on one food source (Stål et al., 2007). Second, resuspension of contaminated sediments is much more common in soft sediment than rocky habitats, increasing the bioavailability of contaminants to organisms in the former sites (Hill et al., 2009). A suite of environmental factors can alter the frequency of resuspension events including current speeds, site depth, bottom topography and how sheltered it is. Resuspension is a result of water moving over the bottom, lifting particles back into the water column, with sheltered and deep water sites having far less resuspension events due to protection from wave action (Kalantzi & Karakassis, 2006; Erm et al., 2011). Substrate type and grain size greatly alter the force of moving water over the bottom with larger grain sizes, such as cobble, generating a lot of turbulence between rocks, while sand and mud bottom are smooth allowing for more laminar flow (Jönsson et al., 2005). This consistent flow parallel to the substrate allows for greater shear stress in the water-sediment boundary layer, whereas the turbulence generated by large obstructions greatly reduces the amount of force directed on sediments (Jönsson et al., 2005). When I consider all of these factors, the sites that would be the least vulnerable to aquaculture influence are deep water, rocky bottom sites with strong currents that allow for maximum dispersal of wastes (Cromey et al., 2002; Jönsson et al., 2005; Kalantzi & Karakassis, 2006).

While this project allowed me to assess several poorly understood areas of aquaculture’s impact on benthic communities, there were several factors that limited the explanatory power of my results. First, the paired design required similar substrates at the near and away sites and there were a limited number of sites with appropriate substrate available near aquaculture operations. This directly impacted the power of my analysis as
I only had 8 site pairs. Moreover, because I needed rocky habitats for the collectors and there was high heterogeneity of the substrates in this area, I could not use consistent distances from pens for collector deployment across all sites and this lead to inconsistencies in the distances (and therefore exposures) of near and away sites across locations. Second, due to natural variability in species distributions, not all species colonized the bio-collectors at all sites, leading to an incomplete dataset and an inability to conduct GLM analyses across species and BMAs. It was for this reason that I used individual t-tests to compare paired sites; however, the probability of identifying a difference incorrectly (type 1 error) increases with the increasing number of tests. In my analysis, I conducted 199 separate t tests, meaning that with an alpha value of 0.05, I would expect ~10 of the 52 significant differences to have occurred by chance alone. In addition, I used chi-square goodness of fit tests to assess whether values were greater at the near than the away site of a pair more often than expected by chance. Because the data used in the chi-square test included multiple data points per species, these data are not fully independent, and should be interpreted with caution. However, both the chi-square goodness of fit tests and the t-tests showed similar results, with some evidence of differences due to aquaculture, particularly for sulfur and zinc, but not a great deal of consistency in differences between near and away sites across site pairs and BMAs.

I believe there are a few major questions that still remain to be answered regarding aquaculture effects on the benthic community in rocky habitats in the Bay of Fundy, but more specifically in other regions not as exposed to strong currents. First, we need to determine how large a footprint is generated by each farm. This information will allow us to optimize placement of future farms to areas of low diversity to further reduce
its impacts. As mentioned earlier, research has mainly been conducted within 100 m of pen sites and this was the first study to my knowledge to assess contaminant and feed influence at distances greater than 200 m. I suggest a systematic approach to assess how metal accumulation and feed reliance change with increasing distance from active pen sites. I would put ceramic plates and sediment traps on the bottom every 25 m downstream of pen sites for 300 m. This would allow tunicates to colonize and grow under treatment conditions as well as provide information on sediment condition, deposition rate and metal levels. Second, we should continue to examine how the production cycle effects environmental conditions as well as the longevity of impacts after sites have been decommissioned. By sampling all three stages of the production cycle (three consecutive years) at a site and obtaining information on stocking density, we could more precisely examine how the different times in the production cycle affect metal and nutrient exposures. Moreover, we should continue to collect data from sites after they have been decommissioned to better understand the risks of legacy effects. Third, we should continue to investigate the contribution of aquaculture to the diets of commercially valuable species. Once we have identified the most heavily impacted sites, we could use bio-collectors, as in this project, to subsample the community and collect as many prey species as possible to assess the relative importance of multiple prey species through sulfur isotopes and a combination of carbon isotopes and fatty acids analysis (Redmond et al., 2010). With most if not all potential food sources, we could do a more complex examination of the relative importance of salmon feed in the diets of higher trophic level predators.
Conclusion

In conclusion, aquaculture seems to have little effect on metals and stable isotopes in benthic organisms in rocky bottom habitats greater than 200 m away from active lease sites in these BMAs of the Bay of Fundy. There were a small percentage of differences in metals between near and away sites which were significant. Moreover, these cases were sporadic across species, sites and years. The vast majority of my species-site pair comparisons yielded non-significant results and had inconsistent direction of increases. The scarcity of significant results and their inconsistency could indicate that those sites were influenced by other anthropogenic factors and not solely aquaculture. Moreover, other factors such as currents and bio-collector positions relative to the cages may influence the exposure and lead to differences between sites. The industry as a whole is improving their practices and technologies to reduce their influence on the environment. The distance from pen sites, decommissioning of copper based anti-fouling paints and improvements in feed formulation are also likely involved in the reduced influences seen in this study relative to what was expected in the past. Also, the isotope data indicated that species do not have high reliance on feed as a food source. While sulfur isotopes did indicate a shift towards the value of feed in individuals from near sites, the difference between their values and those of the feed were much larger than would be expected if feed made up a major portion of their diet. This study also supported previous work regarding the susceptibility of some species to contamination and trophic positions of marine benthic species from this region. It gave indirect information regarding the biomagnification of metals in marine benthic food webs. It provides some of the first information regarding contamination and feed dependence in a habitat type (rocky-
bottom), as well as at a larger spatial scale (>200 m), and also examines how the influence of aquaculture may change throughout the production cycle. Lastly, this study showed indications that aquaculture waste is being consumed, even if in small amounts, by a variety of benthic organisms on rocky bottom habitats. While, there was no obvious increase in metal contamination, there are other contaminants such as pesticides that may still be affecting organisms. Therefore, this study suggests that we need to look at other contaminants from aquaculture operations and the scale at which they may be affecting organisms.

**Bibliography**


Chang, B. D., K. A. Coombs, and F. H. Page. 2014. The development of the salmon aquaculture industry in southwestern New Brunswick, Bay of Fundy, including steps


Figure 12: Mean (+/- SD) copper (µg/g dw; n= 3 to 4) concentrations of American lobster and vase tunicates sampled in bio-collectors deployed for 4 months at reference sites in Bay Management Area 3a in the Bay of Fundy in 2016. Lobsters collected at reference sites averaged 18.4 (± 4.75) µg/g dw, while tunicates averaged 10.43 (± 2.48) µg/g dw copper levels. Therefore, tunicates at a reference site had comparable average levels of copper to away individuals, but lobster only had on average 57% of the copper found in away individuals in BMA 3a.
Figure 13: Mean (+/- SD) zinc (µg/g dw; n= 3 to 4) concentrations of American lobster and vase tunicates sampled in bio-collectors deployed for 4 months at reference sites in Bay Management Area 3a in the Bay of Fundy in 2016. Lobster and tunicates averaged 79.70 (± 21.92) µg/g dw and 66.00 (± 11.82) µg/g dw, respectively. Therefore, lobsters zinc levels were comparable to those of BMA 3a away individuals, while tunicates had lower levels compared to the average from away individuals across all BMAs.
Appendix B

Figure 14: $\delta^{13}C_{\text{adj}}$ (%) versus C:N ratios for each of the 4 target species collected in the Bay of Fundy during the summer of 2016 (see Table B1 for regression analyses).
Figure 15: $\delta^{13}C$ (‰) versus C:N ratios for each of the 5 target species collected in the Bay of Fundy during the summer of 2017 (see Table B1 for regression results).
Table 10: Mean C:N ratios (±SD) and δ^{13}C (‰, ±SD) and linear regression equations for raw δ^{13}C (A) or δ^{13}C_{adj} (B) versus C:N and associated R^2 and p values for each species in each year.

### A

<table>
<thead>
<tr>
<th>Species</th>
<th>Average C:N (±SD)</th>
<th>Average δ^{13}C (±SD) (unadjusted)</th>
<th>Regression equation</th>
<th>R^2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016 Rock gunnel</td>
<td>3.58 ±0.17</td>
<td>-16.69±0.74</td>
<td>y = -1.79x-10.29</td>
<td>0.16</td>
<td>3.19*10^{-5}</td>
</tr>
<tr>
<td>Shorthorn sculpin</td>
<td>3.33 ±0.08</td>
<td>-15.56±0.84</td>
<td>y = -2.87x-5.99</td>
<td>0.079</td>
<td>0.029</td>
</tr>
<tr>
<td>American lobster</td>
<td>3.28 ±0.13</td>
<td>-15.98±0.65</td>
<td>y = -3.23x-5.37</td>
<td>0.42</td>
<td>1.23*10^{-5}</td>
</tr>
<tr>
<td>Vase tunicate</td>
<td>4.88 ±0.54</td>
<td>-19.52±0.78</td>
<td>y = 0.40x-21.48</td>
<td>0.078</td>
<td>0.042</td>
</tr>
<tr>
<td>2017 Rock gunnel</td>
<td>3.41±0.11</td>
<td>-16.81±0.83</td>
<td>y = -0.33x-15.69</td>
<td>0.0019</td>
<td>0.67</td>
</tr>
<tr>
<td>Shorthorn sculpin</td>
<td>3.26 ±0.054</td>
<td>-16.08±0.82</td>
<td>y = -2.92x-6.56</td>
<td>0.038</td>
<td>0.081</td>
</tr>
<tr>
<td>American lobster</td>
<td>3.16 ±0.063</td>
<td>-16.69±0.80</td>
<td>y = 0.24x-16.69</td>
<td>0.0012</td>
<td>0.83</td>
</tr>
<tr>
<td>Vase tunicate</td>
<td>4.98 ±0.61</td>
<td>-20.38±1.12</td>
<td>y = 0.12x-20.98</td>
<td>0.0043</td>
<td>0.65</td>
</tr>
<tr>
<td>Blue mussel</td>
<td>4.31 ±0.32</td>
<td>-19.75±0.61</td>
<td>y = 0.036x-19.91</td>
<td>0.0004</td>
<td>0.95</td>
</tr>
</tbody>
</table>

### B

<table>
<thead>
<tr>
<th>Species</th>
<th>Average C:N (±SD)</th>
<th>Average δ^{13}C (±SD) (adjusted)</th>
<th>Regression equation</th>
<th>R^2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016 Rock gunnel</td>
<td>3.58 ±0.17</td>
<td>-16.48±0.69</td>
<td>y = -0.789x-13.618</td>
<td>0.036</td>
<td>0.054</td>
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<tr>
<td>Shorthorn sculpin</td>
<td>3.33 ±0.08</td>
<td>-15.58±0.83</td>
<td>y = -1.879x-9.3125</td>
<td>0.036</td>
<td>0.15</td>
</tr>
<tr>
<td>American lobster</td>
<td>3.28 ±0.13</td>
<td>-16.05±0.57</td>
<td>y = -2.24x-8.69</td>
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<td>0.0012</td>
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<tr>
<td>Vase tunicate</td>
<td>4.88 ±0.54</td>
<td>-18.01±1.07</td>
<td>y = 1.39x-24.80</td>
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<td>-16.75±0.83</td>
<td>y = 0.66x-19.01</td>
<td>0.0076</td>
<td>0.48</td>
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<td>y = -1.93x-9.88</td>
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<td>0.38</td>
</tr>
<tr>
<td>American lobster</td>
<td>3.16 ±0.063</td>
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<td>y = 1.23x-20.01</td>
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<td>0.28</td>
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<td>4.98 ±0.61</td>
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<td>y = 1.11x-24.30</td>
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<td>6.53*10^{-5}</td>
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<td>Blue mussel</td>
<td>4.31 ±0.32</td>
<td>-18.80±0.69</td>
<td>y = 1.03x-23.23</td>
<td>0.23</td>
<td>6.22*10^{-5}</td>
</tr>
</tbody>
</table>
Appendix C

Most best fit GLMs of the metals and stable isotope data had significant interactions between BMA and treatment or between site pairs and treatment (Tables C1, C2). There were a few species for each metal where BMA and treatment explained the most variation, although BMA was usually the significant factor (Table C1). Similarly, a model including site pair and treatment was the best model in some cases when only one BMA was analyzed (Table C1). Similar to the metals data, the GLMs that best fit the isotope data often included an interaction between Site Pair and Treatment or BMA and Treatment (Table C2).
**Table 11:** Best fit GLM model for each metal for each species in both 2016 and 2017 as determined using AIC. P-values are indicated for each factor. Significant factors are indicated by colour with interaction terms being yellow, BMA being blue, Site Pair being orange and Treatment being green.

<table>
<thead>
<tr>
<th>2016</th>
<th>2017</th>
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</thead>
<tbody>
<tr>
<td><strong>Vase tunicate</strong></td>
<td><strong>BMA 1</strong></td>
</tr>
<tr>
<td><strong>Cu</strong></td>
<td><strong>Zn</strong></td>
</tr>
<tr>
<td>Pair</td>
<td>0.21</td>
</tr>
<tr>
<td>Treatment</td>
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<tr>
<td><strong>Rock gunnel</strong></td>
<td><strong>BMAs 1&amp;2a</strong></td>
</tr>
<tr>
<td><strong>Cu</strong></td>
<td><strong>Zn</strong></td>
</tr>
<tr>
<td>BMA</td>
<td>0.24</td>
</tr>
<tr>
<td>Treatment</td>
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<tr>
<td><strong>Cu</strong></td>
<td><strong>Zn</strong></td>
</tr>
<tr>
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<td>BMA</td>
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<tr>
<td><strong>BMA 1&amp;3a</strong></td>
<td><strong>BMA 1&amp;3a</strong></td>
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<td><strong>Cu</strong></td>
<td><strong>Zn</strong></td>
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<td>Pair</td>
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<td>Treatment</td>
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<tr>
<td><strong>Shorthorn sculpin</strong></td>
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<td>Pair</td>
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Table 12: Best fit GLM model for each isotope for all possible species-year combinations determined using AIC. P-values are indicated for each factor and interactions. Significant factors are colour coded with significant interaction terms being yellow. BMA is blue, Pair is orange and Treatment is green.
<table>
<thead>
<tr>
<th>Year</th>
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<th>BMAs</th>
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<tr>
<td>2015</td>
<td>Vase tunicate</td>
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<td></td>
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<tr>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Rock gunnel</td>
<td>BMAs 1&amp;2a</td>
</tr>
<tr>
<td></td>
<td>$\delta^{13}C$</td>
<td>$\delta^{15}N$</td>
</tr>
<tr>
<td></td>
<td>Pair Treatment</td>
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</tr>
<tr>
<td></td>
<td>Pair</td>
<td>4.39*10^-11</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>4.72*10^-5</td>
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<tr>
<td>2017</td>
<td>Vase tunicate</td>
<td>BMAs 1&amp;2a</td>
</tr>
<tr>
<td></td>
<td>$\delta^{13}C$</td>
<td>$\delta^{15}N$</td>
</tr>
<tr>
<td></td>
<td>Null</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>American lobster</td>
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</tr>
<tr>
<td></td>
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<td>$\delta^{15}N$</td>
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<td></td>
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<td></td>
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<td>BMAs 1&amp;3a</td>
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<tr>
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<td></td>
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<td>Treatment</td>
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<tr>
<td></td>
<td></td>
<td>0</td>
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<td></td>
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<tr>
<td></td>
<td>$\delta^{13}C$</td>
<td>$\delta^{15}N$</td>
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<td>Pair Treatment</td>
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<td>Treatment</td>
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<td>BMAs 3a</td>
</tr>
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<td>$\delta^{15}N$</td>
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<td></td>
<td>Null</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>
Appendix D

Table 13: The number of species-site pair combinations where either near or far individuals were more heavily influenced, either through elevated metal levels or more similar isotope levels to feed. P-values were generated by a chi-square Goodness of Fit test comparing the number of near and away sites with higher metals or more similar isotope levels to a 1:1 split that would be expected by chance. Two of the 5 metrics tested in both years—sulfur in 2016 and zinc in 2017—showed significantly more in one category than would be expected by chance alone.

<table>
<thead>
<tr>
<th></th>
<th>2016 near influenced</th>
<th>away influenced</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>11</td>
<td>4</td>
<td>0.07</td>
</tr>
<tr>
<td>Zinc</td>
<td>10</td>
<td>5</td>
<td>0.2</td>
</tr>
<tr>
<td>Carbon</td>
<td>8</td>
<td>11</td>
<td>0.49</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>10</td>
<td>9</td>
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</tr>
<tr>
<td>Sulfur</td>
<td>11</td>
<td>2</td>
<td>0.013*</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>2017 near influenced</th>
<th>away influenced</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>17</td>
<td>9</td>
<td>0.12</td>
</tr>
<tr>
<td>Zinc</td>
<td>20</td>
<td>6</td>
<td>0.006*</td>
</tr>
<tr>
<td>Carbon</td>
<td>10</td>
<td>15</td>
<td>0.32</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>16</td>
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<tr>
<td>Sulfur</td>
<td>16</td>
<td>9</td>
<td>0.16</td>
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</table>
Figure 16: Stocking cycle for the nearest aquaculture site to each of our sample sites between 2015 and 2017. Each BMA moved fish to its marine pens at different times and in some cases each site pair was stocked in a different month. Green and blue squares represent months were first year and second year fish were present in the nearest aquaculture site respectively. Black boxes surrounding July to October in each year represent the time in which bio-collectors were present at these sites. White boxes are months where pen sites were fallow.
Curriculum Vitae

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University attended: McGill University, 2013-2016, Bachelors of Science in Biology

Publications:


Conference Presentations: