ASSESSING IMPACTS OF HISTORICAL PULP MILL EFFLUENT ON COASTAL FOOD WEB STRUCTURE AND IDENTIFYING SUITABLE BIOINDICATORS FOR WASTEWATER: A STABLE ISOTOPE ($\delta^{13}C$ AND $\delta^{15}N$) ANALYSIS

by

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Abstract

Boat Harbour, Nova Scotia was a tidal estuary that was converted into a wastewater treatment facility for pulp mill effluent in 1967. Treated effluent from Boat Harbour was discharged through a dam into the coastal Northumberland Strait. Effluent release ceased in January 2020 and remediation of Boat Harbour will soon commence with a goal to restore Boat Harbour to its pre-industrial state tidal estuary. To ensure successful completion of this remediation goal, effective characterization of the ecosystem in Boat Harbour and Northumberland Strait is required before and after remediation. Discharge of effluent contributed significant nutrient and freshwater inputs into the coastal environment, potentially impacting local biogeochemistry and ecosystem structure. This study used stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of Northumberland Strait taxa to assess spatial variability in nutrient sources and trophic dynamics. Results identified stable isotope variation along a gradient of historical effluent exposure, with depleted $\delta^{13}C$ and $\delta^{15}N$ values in taxa near Boat Harbour. Additionally, differences in trophic dynamics were observed with stable isotope values suggesting many organisms occupy lower overall trophic positions near the former effluent outflow. Findings of this study may be governed by residual pulp mill effluent-derived nutrients or differences in marine versus terrestrial nutrient sources, with a pronounced coastal salinity gradient. Certain taxa exhibited pronounced spatial variation in $\delta^{13}C$ and $\delta^{15}N$. Blue mussel (*Mytilus edulis*) and mummichog (*Fundulus heteroclitus*) were the most suitable bioindicators within the coastal environment. This finding contributed to a review of bioindicators for wastewater in stable isotope studies and an assessment of their perceived suitability. Suitable bioindicators exhibited low mobility, a well-defined life history, and limited diet complexity. Stable isotope analysis was effective at delineating nutrient sources and elucidating trophic dynamics and is recommended as a monitoring technique following remediation of Boat Harbour.

List of Abbreviations Used

ANOVA……...Analysis of Variance
BGI……Big Island
BHETF……Boat Harbour Effluent Treatment Facility
BHO...……Boat Harbour Outflow
BLP…….Black Point
BOD..……Biological Oxygen Demand
CFIA...……Canadian Food Inspection Agency
CNH...…….Chance Harbour
CRI.……Caribou Island
DFO.……Fisheries and Oceans Canada
DIC……Dissolved Inorganic Carbon
DIN…..……Dissolved Inorganic Nitrogen
EEM……Environmental Effects Monitoring
GDP……Gross Domestic Product
HRL…..……..Harbour Lights
HWP……...Halfway Point
LHB…….....Lighthouse Beach
LSI…….Liver Somatic Index
MKH……...Mackenzie Head
MLB……...Melmerby Beach
NB……New Brunswick
NRCAN……...……Natural Resources Canada
NS……...Nova Scotia
PAHs…….Polycyclic Aromatic Hydrocarbons
PCDD/Fs……..…….....Dioxins and Furans
PDB……..PeeDee Belemnite
PEI……...Prince Edward Island
PLFN……..…...Pictou Landing First Nation
POM……....Particulate Organic Matter
PPER……Pulp and Paper Effluent Regulations
SINLAB…….…….Stable Isotopes in Nature Laboratory
SVC……..…….…...Seaview Cemetery
TSS……..…….....Total Suspended Solids
$\delta^{13}C$…….Carbon Stable Isotope
$\delta^{15}N$……..…Nitrogen Stable Isotope

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Chapter 1: Introduction

1.1 Environmental Impacts of Pulp and Paper Mills

Coastal marine ecosystems face myriad anthropogenic pressures. Wastewater inputs from municipal and industrial operations release nutrients and toxins into coastal environments, frequently impacting ecosystem structure and function (Costanzo et al., 2001; Courtenay et al., 2002). Specifically, Canadian pulp and paper mills discharge effluent into aquatic environments, deleteriously impacting aquatic biota (Munkittrick et al., 1991; Parrot et al., 2006). Historically, pulp and paper mill effluent contained high levels of contaminants including metals, metalloids, and dioxins and furans (PCDD/Fs) (Chaudhary et al., 2020; Hoffman et al., 2017). Despite legislated parameters for pulp and paper mill effluent established under the *Pulp and Paper Effluent Regulations* (PPPER, 1992) pursuant to the *Fisheries Act* (1985), effluent contamination and its persistent impacts continue to affect aquatic ecosystems (Hewitt et al., 2006). In addition to metal, metalloid, and PCDD/F contamination, pulp and paper mill effluent is rich in both nutrients and organic matter; therefore, its discharge into waterbodies is often associated with numerous adverse ecological changes (Culp et al., 2003). A variety of physiological and population-level responses have been observed in aquatic biota exposed to pulp and paper mill effluent (McMahon et al., 2020; McMaster et al., 2006). Nutrient subsidies from effluent discharged into aquatic ecosystems are assimilated by primary producers and conserved by higher trophic level species, which alters the ecosystem's food web dynamics (McClelland et al., 1997). Specifically, nutrients from effluent can enhance algal productivity and alter consumer species composition (Culp et al., 2000). In cases of severe organic enrichment from pulp and paper mill effluent, a decline in species diversity has been observed, whereas increased productivity and diversity is typically observed under moderate enrichment (Culp et al., 2000;

Swanson et al., 1996). Understanding the extent and spatial variability of effluent impacts within a receiving aquatic ecosystem is therefore fundamental in assessing effluent's potential legacy effects on aquatic ecosystem biodiversity (Aguilar et al., 2008). Furthermore, due to the interconnectivity of biotic and abiotic ecosystem factors, effluent release into aquatic environments likely alters critical ecosystem functions, such as the exchange of energy and nutrients between organisms (Mor et al., 2022). Therefore, understanding the impacts of wastewater on biogeochemical conditions that support the foundation of coastal ecosystem processes is essential in gaining a comprehensive understanding of potential legacy impacts of pulp and paper mill effluent.

The discharge of pulp and paper mill effluent into aquatic receiving environments has been associated with a variety of adverse ecological effects on native biota such as impacts on fish reproductive development and nutrient enrichment in fish and invertebrates (Munkittrick et al., 1992; Parrot et al., 2006). Additionally, shifts in macroinvertebrate community structure have been observed in response to pulp and paper mill effluent exposure, resulting in the dominance of pollution-tolerant organisms in eutrophic conditions (Sibley et al., 1997). While adverse impacts of pulp and paper mill effluent exposure on aquatic biota have been observed, knowledge gaps remain in our understanding of effluent impacts on entire food web structure. The current research has predominantly focused on the effects of pulp and paper mill effluent enrichment on individual aquatic biota (Arciszewski et al., 2014; Dubé et al., 2005; Skinner et al., 2012). Additionally, most studies that evaluate pulp and paper mill effluent impacts on individual biota have been conducted in freshwater environments (McMahon et al., 2020; Wayland & Hobson, 2001). However, it is important to evaluate conditions in coastal marine contexts where factors such as mixing, tidal effects, salinity, organic matter inputs, and dilution

in varying receiving environment volumes can create conditions that deviate greatly from freshwater environments. Consequently, effects of pulp and paper mill effluent on biota across both freshwater and marine environments may not be analogous. As such, research assessing the impacts of marine biota exposed to pulp and paper mill effluent is required. Previous ecosystem-wide studies of effluent enrichment have focused on the impacts of sewage wastewater on food web dynamics, with considerably less research on pulp and paper mill effluent impacts on ecosystem structure and function. Anthropogenic disturbances such as sewage wastewater discharge into aquatic waterbodies can increase baseline productivity and facilitate an increase in abundance and diversity of primary consumers (de Carvalho et al., 2021; Freedman et al., 2012). Municipal sewage wastewater release has also been associated with a decoupling of benthic and pelagic food webs, with an increased reliance on pelagic nutrients in impacted ecosystems since benthic productivity becomes diminished (Freedman et al., 2012). Therefore, effects of wastewater enrichment on aquatic food webs have been observed; however, further research is required to assess how the dynamic interactions among organisms in food webs respond to pulp and paper mill effluent exposure.

1.2 Project Background

1.2.1 Boat Harbour

Beginning in 1967, a bleached kraft pulp mill in Nova Scotia, Canada discharged industrial effluent into a former tidal estuary, Boat Harbour (Figure 1.1) (Hoffman et al., 2017). Effluent went through a series of treatment stages in the Boat Harbour Effluent Treatment Facility (BHETF), and the final destination for wastewater discharge was the Northumberland Strait (Figure 1.1) (GHD Limited, 2018). Upon effluent release into the BHETF, a dam was

constructed at the mouth of the Boat Harbour estuary, preventing tidal flow into the Northumberland Strait and converting the estuary to a freshwater lake (Province of Nova Scotia, 2022). Mandated under the provincial *Boat Harbour Act* (2015), effluent discharge into Boat Harbour ceased in January 2020 and subsequent remediation will return Boat Harbour to pre-industrial conditions (Eichinger & Walker, 2020). Remediation of Boat Harbour will restore connection with the Northumberland Strait and return the waterbody back to tidal influence (GHD Limited, 2018). Prior to remediation, baseline characterizations of Boat Harbour and the adjacent marine environment have included comprehensive studies on surrounding wetlands and sediment (Hoffman et al., 2019; Quanz et al., 2021). However, previous studies on coastal marine organisms provide only partial baseline characterizations, as the specific contaminants and species studied do not provide a holistic representation of ecosystem health (Chaudhary et al., 2020; Fraser et al., 2021; Maltby, 2021). Subsequently, knowledge gaps exist in our comprehensive understanding of the link between historical pulp mill effluent impacts and the biological interactions between organisms within the coastal marine environment.

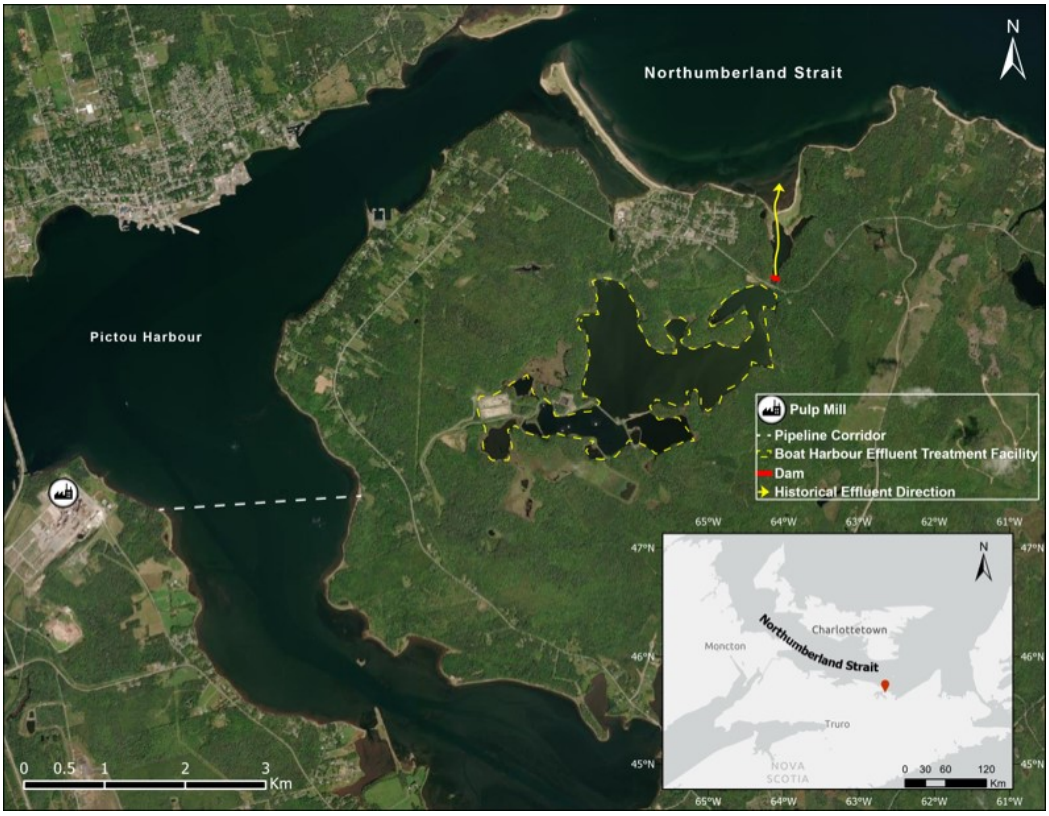

Figure 1.1 Location of the Boat Harbour Effluent Treatment Facility, illustrating the former direction of historical pulp mill effluent release into the Northumberland Strait.

Previous studies have shown that wastewater inputs affect an ecosystem's species richness and food web dynamics by altering energy availability (Freedman et al., 2012; Vander Zanden & Rasmussen, 1996). Over fifty years of pulp mill effluent release from Boat Harbour into the Northumberland Strait may have impacted the nutrient regimes within this receiving environment, ultimately influencing the trophic structure of native marine biota. While it has been historically documented that pulp mill effluent can severely impair aquatic organism health (Culp et al., 2000; Parrot et al., 2006), less research exists on how pulp mill effluent's impacts permeate throughout an entire ecosystem. Evaluating pulp mill effluent's impacts on food web structure in the Northumberland Strait, and the spatial extent of the impacts, prior to remediation will be essential to determine current baseline conditions. Understanding the current ecosystem

health of the Northumberland Strait adjacent Boat Harbour will be crucial in assessing future remediation's effectiveness in restoring natural ecosystem processes.

1.2.2 Stable Isotope Analysis in Ecology

Stable isotope analysis can be utilized for identifying and assessing wastewater impacts in aquatic environments. Ecological research is increasingly employing stable isotope analysis as a method of tracing the source, pathways, and fate of organic matter within ecosystems (Fry, 2006; Peterson & Fry, 1987). Stable isotope analysis compares the amount of a heavy isotope (an element with one additional neutron in the nucleus, *e.g.* C^{13}) to a light isotope (an element with one less neutron in the nucleus, *e.g.* C^{12}) in a sample relative to international standards. Stable isotope values are represented using delta notation (δ) and are expressed in parts per thousand (‰) (Fry, 2006). Stable isotope values are calculated according to the following equation (Eq. 1):

$$\delta X‰ = [(R_{sample} - R_{standard}) - 1] \times 1000 \qquad \text{[Eq. 1]}$$

Where X is the heavier isotope, R_{sample} is the ratio of the heavy isotope to the light isotope within the sample being analyzed, and $R_{standard}$ is the ratio of the heavy isotope to the light isotope within the standard. An increase in the δ value indicates the presence of an increased amount of the heavier isotope relative to the lighter isotope; this sample is considered *enriched* (Risk et al., 2009). Alternatively, values with lower δ values are termed *depleted*.

Two commonly applied stable isotopes in ecological research are nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) (Fry, 2006). These two isotopes are valuable tools for studying ecological communities as they can be used to evaluate nutrient dynamics and delineate trophic interactions

between organisms (Arciszewski et al., 2014). $\delta^{13}C$ is a useful measure of the origin and pathway of organic matter in food webs, and it permits the determination of the ecosystem's energy source (DeNiro & Epstein, 1981). For example, the $\delta^{13}C$ value of marine algae averages around -22‰ (Duarte et al., 2018), whereas terrestrial organic matter is typically isotopically lighter, approximately <-25‰ (Koziorowska et al., 2016). $\delta^{15}N$ values may be used to assess two separate but fundamentally linked aspects of food webs. First, the $\delta^{15}N$ of primary producer organisms assist in the delineation of specific sources of nitrogen supporting the base of the food web (Vander Zanden & Rasmussen, 2001). This is based on the premise that different nitrogen sources have distinct ranges of $\delta^{15}N$ values (McClelland et al., 1997). Second, offsets in $\delta^{15}N$ values between a consumer and its prey may be used to quantify the trophic position of an organism within a food web (DeNiro & Epstein, 1981). Therefore, a dual stable isotope analysis of both $\delta^{13}C$ and $\delta^{15}N$ can provide valuable ecosystem information regarding both an organism's energy source and food web interactions.

Stable isotope analysis can be used to trace wastewater effluent in aquatic receiving environments (Gearing et al., 1991; Tucker et al., 1999). Several studies have successfully used stable isotope analysis to trace exposure to effluent from municipal and industrial operations such as sewage wastewater (Loomer et al., 2015; Savage, 2005), wastewater from aquaculture facilities (Vizzini & Mazzola, 2006), oil sand operations (Farwell et al., 2009), and pulp and paper mills (Arciszewski et al., 2014; Dubé et al., 2005; McMahon et al., 2020; Wayland & Hobson, 2001). This method relies on the premise that wastewater inputs have distinct stable isotope signatures from the stable isotope signatures naturally occurring in receiving waterbodies (Wayland & Hobson, 2001). Therefore, the relative abundances of stable isotopes of carbon and nitrogen can be used as tracers of terrestrial carbon and nutrients that are released in wastewater

and incorporated into the tissues of aquatic biota that inhabit receiving waterbodies (Loomer et al., 2015). For example, previous studies have identified the $\delta^{13}C$ of pulp mill effluent as isotopically depleted, reflecting the stable isotope signature of terrestrial C_3 plants (-28‰) used in the pulp manufacturing process (Arciszewski et al., 2014; Mendoza, 2016). The $\delta^{15}N$ of pulp mill effluent is also typically depleted relative to aquatic receiving environments, often a result of fertilizer addition to optimize effluent treatment and chloroliginin, a by-product of wood pulp bleaching (Freedman et al., 2012). Therefore, measuring both $\delta^{13}C$ and $\delta^{15}N$ values of aquatic biota inhabiting pulp mill effluent receiving environments can provide a quantitative measure of the dietary reliance on effluent-derived nutrients (Wayland & Hobson, 2001). Additionally, since $\delta^{15}N$ values can be used to quantify an organism's trophic position within a food web, stable isotope analysis can identify changes in food chain length in ecosystems exposed to wastewater (Cabana & Rasmussen, 1994; Post, 2002a). As a fundamental ecosystem property, food chain length can be used to evaluate anthropogenic disturbance effects on ecosystem structure, providing a quantitative measure of ecosystem degradation (Hussey et al., 2014). Prior to remediation, applying stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$ to biota inhabiting this area that had previously received pulp mill effluent will provide a baseline of pre-remediation ecosystem structure by identifying the impacts of historical effluent exposure from Boat Harbour on the trophic dynamics of coastal biota.

1.3 Thesis Objectives

This thesis aims to evaluate the spatial variability of nutrient sources and assess trophic structure along a coastal marine environment, adjacent to a former wastewater treatment facility

for pulp mill effluent prior to ecosystem remediation. Specifically, the objectives of this study are to:

1. Assess variability in sources of carbon and nitrogen along a spatial gradient historically exposed to pulp mill effluent along the coastal Northumberland Strait prior to ecosystem remediation,
2. Evaluate potential impacts of pulp mill effluent exposure on trophic dynamics along the coastal Northumberland Strait prior to ecosystem remediation and
3. Evaluate the suitability of bioindicator taxa for assessing wastewater inputs in aquatic receiving environments using stable isotope analysis.

1.4 Methodology Overview

To complete objectives 1 and 2, bulk stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were conducted on a suite of coastal marine biota along a spatial gradient with historical pulp mill effluent exposure from Boat Harbour, Nova Scotia. Sampling locations were selected along the former receiving environment for effluent from Boat Harbour, the coastal Northumberland Strait. To represent varying degrees of effluent exposure, organisms were collected along a 15 km transect to the northeast and a 15 km transect to the northwest of the former effluent outflow. With representation of organisms from multiple trophic levels and feeding guilds, a holistic representation of the coastal ecosystem within the region was sampled. Organisms analyzed in the study include particulate organic matter (POM), macroalgae (*Fucus vesiculosus*), periwinkles (*Littorina littorea*), blue mussels (*Mytilus edulis*), mummichog (*Fundulus heteroclitus*), rock crab (*Cancer irroratus*), and American lobster (*Homarus americanus*). Organisms were collected in the field and processed in the laboratory for stable

isotope analysis. POM was filtered from seawater samples onto glass fibre filters and freeze-dried. All plant and animal tissues were freeze dried, ground into fine, homogenous powders, and weighed into small aliquots in tin capsules. Isotope ratio mass spectrometry was completed by the Stable Isotopes in Nature Laboratory (SINLAB), University of New Brunswick, Fredericton. Stable isotope values of $\delta^{13}C$ and $\delta^{15}N$ were used to assess variations in nutrient sources and potential reliance on effluent-derived nutrients. Additionally, to identify potential variation in trophic dynamics along a spatial gradient of historical effluent exposure, $\delta^{15}N$ values were used to estimate individual organisms' trophic position at each sampling location. A further, detailed description of the methodology applied in this study is described in Chapter III. To complete objective 3, a systematic literature review was conducted to determine the frequency of various taxa in stable isotope studies assessing wastewater (pulp mill effluent and/or sewage wastewater) inputs in aquatic receiving environments. Further, their perceived suitability as bioindicators was evaluated and a set of criteria was developed for the selection of suitable bioindicators for assessing wastewater inputs in stable isotope studies. Further details on this methodology are described in Chapter IV.

1.5 Thesis Structure

Four chapters follow the introduction of this thesis. Chapter II provides a literature review relating to the main topics of this thesis. Chapter III presents the background, methodology, results, and discussion for the study of spatial variability in $\delta^{15}N$, $\delta^{13}C$, and trophic dynamics along the coastal Northumberland Strait. With recommendations for future studies tracing wastewater inputs in aquatic ecosystems, Chapter IV contextualizes the findings from Chapter III and evaluates the suitability of taxa employed in previous stable isotope studies

tracing wastewater inputs as bioindicators. The final chapter, Chapter V, provides thesis conclusions and recommendations for future environmental monitoring.

Chapter 2: Literature Review

2.1 Environmental Impacts of Pulp and Paper Mill Effluent

Wastewater inputs to aquatic environments have deleterious impacts on aquatic biota and alter the structure and function of ecological systems (Courtenay et al., 2002). In particular, the manufacturing processes employed by the global pulp and paper industry utilize large volumes of water and discharge considerable pollutant loadings to aquatic ecosystems (Cabrera, 2017). Effluents from pulp and paper mills pose a risk to aquatic environments due to their associated organic and inorganic contaminants, high biological oxygen demand (BOD) and total suspended solids (TSS) loadings (Colodey & Wells, 1992). Pulp and paper mill effluent contamination frequently impairs water quality conditions and can have detrimental impacts on the health of aquatic organisms (Culp et al., 2003; Hewitt et al., 2006). For example, nutrients found in pulp and paper mill effluent such as ammonia and nitrate can cause eutrophication of waterbodies, subsequently impacting community composition within the aquatic environment (Pearson, 1980). In addition to nutrient enrichment from pulp mill effluent, metal, metalloid, and dioxin and furan (PCDD/F) contamination of aquatic systems has also been historically linked to pulp and paper mill effluent (Chaudhary et al., 2020; Fraser et al., 2021; Hoffman et al., 2017). Industrial wastewater discharge has been shown to negatively impact aquatic environments by introducing excess nutrients and depleting oxygen levels, effectively dominating the hydrological characteristics and ecosystem composition within the receiving environment (Cabana & Rasmussen, 1994). Prior to mitigating adverse ecological effects caused by pulp and paper mill effluent, proper evaluation of receiving environment conditions is essential to further our understanding of wastewater impacts on aquatic ecosystems.

Aquatic ecosystems exposed to pulp and paper mill effluent have exhibited myriad responses ranging from physiological effects in biota to population and community-level alterations (Sibley et al., 1997). The negative environmental impacts of pulp mill effluent were first recognized in the mid-1980s when Swedish researchers determined that pulp mill effluent posed a significant risk to aquatic ecosystems (Södergren, 1989). Researchers observed negative impacts to aquatic organisms even at low concentrations of effluent within the receiving environment (Munkittrick, 1992; Södergren, 1989). Furthermore, Canadian studies confirmed that fish similarly exposed to pulp mill effluent were exhibiting negative biological responses (McMaster et al., 1991; Munkittrick et al., 1991). The results of these studies indicated that wild fish populations exposed to pulp mill effluent experienced alterations in reproductive endpoints, including delayed sexual maturity, reductions in gonad size, changes in fecundity, and reduction in the development of secondary sexual characteristics (McMaster et al., 1991; Munkittrick et al., 1991). Numerous studies have since been conducted that confirm reproductive effects of wild fish populations in pulp mill effluent receiving environments (McMaster, 1995; Munkittrick et al., 1994). Furthermore, indicative biological responses, such as the altered composition of macroinvertebrate community structure, have been observed in response to effluent exposure. Such responses shift community composition towards more pollution-tolerant species (Sibley et al., 1997). Since these foundational studies assessing the impacts of pulp mill effluent on aquatic organisms, federal regulations have been established to provide more stringent measures for pulp and paper mills in Canada (Courtenay et al., 2002). Despite this progress in characterizing impacts of pulp mill effluent on aquatic biota and subsequent improved regulations for effluent discharge, pulp mill effluents continue to affect aquatic environments (van den Heuvel et al., 2010). To further understand how effluent impacts overall food web structure, continued

research is required to assess how effluent impacts a diversified set of aquatic habitats. This improved understanding of the impacts of pulp mill effluent on aquatic ecosystems will guide the evidence-based measures required to mitigate adverse ecological changes resulting from the Canadian pulp and paper industry.

2.2 Boat Harbour

2.2.1 History

Boat Harbour (Figure 2.1), Nova Scotia (NS), a former tidal estuary located on the Northumberland Strait, was the receiving location for bleached kraft pulp mill effluent beginning in 1967 (Hoffman et al., 2017). Also known as A'se'K ("the other room") in Mi'kmaq, Boat Harbour is located in the community of Pictou Landing First Nation (PLFN) (Figure 2.1). Prior to 1967, members of PLFN used Boat Harbour as a sacred land for refuge, recreation, fishing, hunting, and food gathering (Castleden et al., 2016). A'se'K was a sacred, culturally significant place where exchange of food, knowledge, and skills occurred between generations and among family members (Bennett, 2013; Hoffman et al., 2017). Following an agreement reached between the Government of Nova Scotia and a US pulp company to operate a pulp mill in northern Nova Scotia, the cultural significance of A'se'K was compromised when the provincial government decided that it was the ideal location for the effluent treatment facility.

More commonly referred to today as Boat Harbour, A'se'K was subsequently converted into a wastewater treatment facility for effluent from the pulp mill, located 4 km southwest in Abercrombie Point (Figure 2.1). Additionally, a nearby chlor-alkali plant also discharged wastewater into Boat Harbour from 1971-1992 (Hoffman et al., 2017; Quanz et al., 2021). The bleached kraft pulp mill in Abercrombie Point operated for over fifty years, producing ~280,000

tons of pulp annually (Ogden, 1972). Over its history, ownership of the pulp mill changed several times, with the provincial government maintaining ownership of the effluent treatment facility (Hoffman et al., 2017). The province operated the Boat Harbour Effluent Treatment Facility (BHETF; Figure 2.1) from 1967 until 1995, when the mill owners assumed operational duties (Hoffman et al., 2017). Additionally, treatment processes at the BHETF evolved over time from raw effluent discharge to secondary effluent treatment (Quanz et al., 2021). However, from the inception of effluent release into the BHETF, effluent had high BOD levels and near-complete mortality of native fish and invertebrates was observed in Boat Harbour (Castleden et al., 2016). Once a location used for traditional and recreational purposes, Boat Harbour was altered by pulp mill effluent into a waterbody with significant negative environmental impacts.

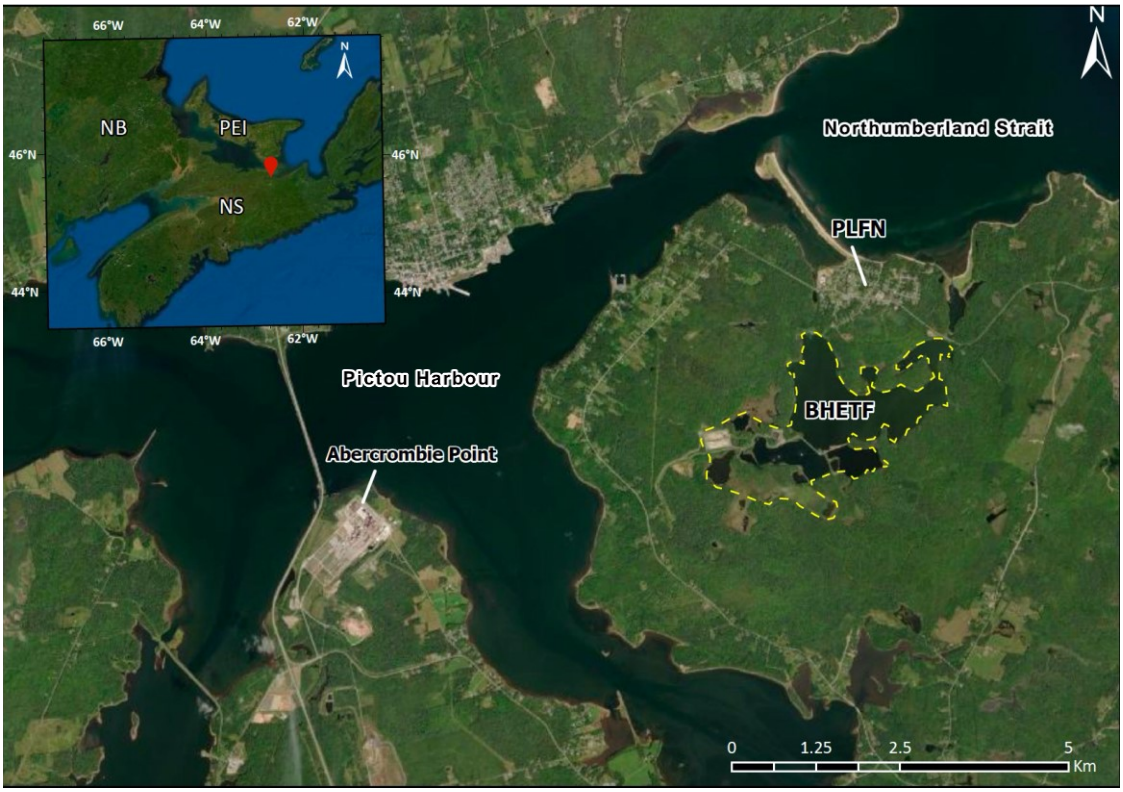

Figure 2.1 Map of Pictou, Nova Scotia, indicating the location of the pulp mill at Abercrombie Point, the BHETF, PLFN community, and the location for treated pulp mill effluent from the BHETF: Northumberland Strait (Adapted from Eichinger & Walker, 2020).

2.2.2 Pulp Mill Effluent Treatment

Throughout the history of pulp mill effluent release into Boat Harbour, periodic upgrades were made to the wastewater treatment procedures (Appendix A). Early effluent treatment consisted of raw effluent releasing from the mill (>70,000 m^3/day), travelling under the East River via pipeline (~3 km), with release into ditches and surrounding wetlands at Boat Harbour (Quanz et al., 2021). Effluent was then collected in settling ponds and discharged into the Boat Harbour Stabilizing Lagoon (Quanz et al., 2021). In 1972, a dam was constructed at the mouth of the estuary, preventing the incursion of marine water into Boat Harbour (Province of Nova Scotia, 2022). Treated pulp mill effluent was released through the dam at the mouth of Boat Harbour into the marine environment of the Northumberland Strait (Figure 2.1) (GHD Limited, 2018). The dam converted the former estuary into two distinct waterbodies: a large freshwater lake upstream (referred to now as Boat Harbour), and a smaller estuarine inlet downstream of the dam; the opening to the Northumberland Strait (Province of Nova Scotia, 2022). The dam altered the hydrological characteristics of Boat Harbour, raising water levels by 2-3 m and flooding an area of 12 ha, increasing the footprint of the original estuary upstream of the dam by approximately 8% (AECOM, 2015).

In 1972 upgrades were made to the effluent treatment process at the BHETF, including the addition of lined discharge ditches which emptied into two twin settling basins, one operational while the other could be cleaned of settled material (JWEL and Beak Consultants, 1992). Each 50,000 m^3 settling basin permitted the settling of suspended solids from the effluent prior to its entry into an aerated stabilization basin (567,750 m^3; Appendix A) to permit secondary effluent treatment (Hoffman et al., 2017). Secondary treatment used aerators and aerobic microbes to break down biodegradable material (Cabrera, 2017). Effluent was then

released into the stabilizing lagoon (Boat Harbour; 3,400,000 m^3; Appendix A) where it remained for 20-30 d before release into the Northumberland Strait (GHD Limited, 2018). In 1991, an automated nutrition addition system was installed that supplied bulk urea and diammonium phosphate to the effluent prior to secondary treatment, with the aim of enhancing microbial growth and reducing effluent BOD levels (Ecometrix, 2016). However, this initiative ceased in 1992 when toxicity related to the ammonia addition was detected in the final effluent (Ecometrix, 2016). Automated nutrition addition resumed in 1997, with modifications that reportedly eliminated the toxic by-products that had previously occurred (Ecometrix, 2016). Despite the treatment stages employed at the BHETF being typical of secondary pulp mill effluent treatment facilities and meeting federal requirements for effluent quality parameters (Ecometrix, 2007, 2016), considerable contamination of the aquatic environment exists in Boat Harbour (Hoffman et al., 2017, 2019).

2.2.3 Boat Harbour Act and Remediation

The history of Boat Harbour's degradation cannot be extricated from the environmental and social injustices felt by the members of the Mi'kmaq community of PLFN. After decades of PLFN requesting the facility's closure, a pipeline leak in 2014 released raw pulp effluent onto sacred burial grounds in PLFN (Hoffman et al., 2015) and triggered protests that culminated in the provincial government's promise to cease operations at the BHETF. The *Boat Harbour Act* (2015) mandated the cessation of effluent release into Boat Harbour by January 31, 2020, and subsequent remediation of the waterbody and surrounding lands. Presently, the pulp mill has terminated operations and effluent release into Boat Harbour no longer occurs. Remediation of the BHETF will soon commence with the ultimate goals of restoring Boat Harbour to its pre-industrial tidal estuarine conditions and restoring the recreational and cultural capacity of the

land (Province of Nova Scotia, 2022). Currently, the remediation of Boat Harbour is undergoing a Federal Impact Assessment (*Canadian Environmental Assessment Act* [CEAA], 2012) with Nova Scotia Lands as the proponent responsible for the project. The total cost of the remediation is expected to exceed $292 million CAD (Eichinger & Walker, 2020). The geographic study area for the remediation project spans from the pulp mill, along the effluent pipeline, through existing BHETF lands, across Boat Harbour, and into the Northumberland Strait (GHD Limited, 2018).

As part of the remediation, unconsolidated, contaminated sediment (approximately 1,244,000 m^3) will be hydraulically dredged, de-watered, and placed in an existing containment cell located on-site at the BHETF (Eichinger & Walker, 2020; GHD Limited, 2018). The sediment is impacted by organic and inorganic contaminants including metals and metalloids (As, Cd, Cr, Pb, Hg and Zn) and dioxins and furans (PCDD/Fs) (Hoffman et al., 2017). In addition to the management of sediments, approximately 4,000,000 m^3 of water will require treatment prior to discharge back into Boat Harbour (Eichinger & Walker, 2020). While the various components of the BHETF are heavily contaminated, it has been reported that the BHETF was successful at treating and removing contaminated solids as remediation of the Northumberland Strait is not required (Eichinger & Walker, 2020). Part of the remediation process will involve decommissioning the dam at the mouth of Boat Harbour and restoring tidal flow. As tidal connection with the Northumberland Strait will be re-established, if contaminants within Boat Harbour are not effectively removed, they may migrate into the marine environment with deleterious impacts on the ecosystem (Maltby, 2021). Accordingly, monitoring ecosystem conditions in Northumberland Strait will be essential once remediation is completed. As part of the remediation plan, a five-year monitoring program will be implemented to evaluate the effectiveness of natural attenuation at the project site (GHD Limited, 2018). Environmental

baseline characterization studies that include both Boat Harbour and Northumberland Strait are essential prior to remediation as they will facilitate evaluation of remediation success by contextualizing post remediation environmental studies.

2.2.4 Gaps in Baseline Studies of the Marine Environment

While numerous baseline studies have been completed within Boat Harbour, gaps exist in our understanding of the Northumberland Strait. One opportunity for improved understanding is regarding the biogeochemical conditions following decades of pulp mill effluent release into this coastal environment. Baseline characterization of impacted wetlands and sediments have been completed within the BHETF, surrounding lands, and the Boat Harbour freshwater impoundment (Fraser et al., 2021; Hoffman et al., 2017; Quanz et al., 2021). Recent baseline studies have been completed in the marine environment, providing contaminant concentrations for individual marine biota (Chaudhary et al., 2020; Maltby, 2021; Romo et al., 2019). However, ecosystems are complex assemblages of interconnected biological, physical, and chemical components (Limburg et al., 2002). Owing to their dependence on non-living ecosystem components, organisms are linked to such abiotic factors through mechanisms of nutrient cycles and energy flows (Bormann & Likens, 1970). Pulp mill effluent released from Boat Harbour into the Northumberland Strait may have altered these nutrient and energy exchange systems. Therefore, evaluating ecosystem health requires a holistic assessment of organisms in the environment, the trophic relationships between organisms, and the foundation of energy and nutrients that support the ecosystem. Adequate characterization of the coastal marine environment is essential in detecting potential historical impacts of decades of effluent exposure. Upon restoration of tidal flow into Boat Harbour through remediation, such characterizations will be crucial when

considering potential consequences of contaminant migration or the ecological effects of freshwater intrusion on the coastal environment.

2.3 The Pulp and Paper Industry in Canada

2.3.1 Overview of the Pulp and Paper Industry in Canada

The pulp and paper industry plays a substantial socioeconomic role in Canada, but is simultaneously a source of environmental concern with terrestrial timber harvesting and aquatic effluent discharges. Serving as the global leader in production of both newsprint and bleached softwood kraft pulp (Natural Resources Canada [NRCAN], 2020), Canada is one of the largest pulp and paper production centers in the world (Bogdanski, 2014). In 2019, Canada produced 2.7 million tons of newsprint and 15.3 million tons of wood pulp (NRCAN, 2020). However, due to recent changes in consumer trends including the movement from newsprint to electronic media, the pulp and paper industry has begun to decline (NRCAN, 2020). In fact, all aggregate measures of sector size such as the value of exports, employment, gross domestic product (GDP), asset value, and capital expenditure trended downward over the 2000s (Bogdanski, 2014). More recently, the real GDP of the pulp and paper industry decreased by 1.3% and 7.9% in 2018 and 2019, respectively (NRCAN, 2020). Analyzing industry and consumer trends in combination with increasingly stringent environmental regulations, logical conclusions can be drawn that remediation of aquatic ecosystems historically contaminated with pulp and paper effluent will become a reality faced more frequently in Canada.

2.3.2 Pulp and Paper Effluent Regulations

Following recognition of the toxicity and environmental impacts of pulp mill effluents in aquatic environments, stronger effluent quality regulations were introduced in Canada. In 1992, amendments were made to the *Fisheries Act* (1985) with the aim of instituting stricter limits on effluent discharge parameters (Roach & Walker, 2017). The *Pulp and Paper Effluent Regulations* (PPER; 1992) established maximum allowable levels for water quality parameters, including limits for BOD, TSS, non-acute lethality assessments, and limits for PCDD/Fs (McMaster et al., 2006). Many kraft pulp mills switched from using elemental chlorine, an agent used in the bleaching process, to chlorine dioxide to meet PPER requirements for dioxins and furans (Hoffman et al., 2019). The owners of the pulp mill in Abercrombie Point switched from elemental chlorine to chlorine dioxide in 1997 (Hoffman et al., 2019). Pursuant to the 1992 amendments of the *Fisheries Act* (1985), all Canadian pulp and paper mills are required to implement cyclical Environmental Effects Monitoring (EEM) programs (Munkittrick et al., 2002). The EEM program is aimed at determining the effectiveness of the regulations' ability to protect fish and fish habitat (Roach & Walker, 2017). A typical EEM for the pulp and paper industry consists of an adult fish population survey, a benthic invertebrate community survey, a study of PCDD/Fs in fish tissues, a tainting study, and effluent toxicity testing (Walker et al., 2002).

To meet the new regulatory requirements of the 1992 amendments to the *Fisheries Act* (1985), numerous changes were required of mill manufacturing and effluent treatment processes (Walker et al., 2002). Consequently, many Canadian pulp and paper mills installed secondary effluent treatment (McMaster et al., 2006). Secondary treatment involves bacterial degradation of the biodegradable material within the effluent (Cabrera, 2017). This method is an improvement

over primary treatment, which is limited to the settling of suspended solids in the effluent (Cabrera, 2017). Secondary treatment improves effluent quality by reducing both BOD levels and quantities of organic compounds, which subsequently improve the pulp mill's environmental performance (Bowron et al., 2009). Despite improvements in federal regulations in the early 1990's, studies continue to report negative impacts of pulp mill effluent on aquatic biota (McMaster et al., 2006). Additionally, pulp and paper mills frequently discharge effluent into waterbodies that also receive municipal wastewater (Wayland & Hobson, 2001). The established limits for each mill fail to consider receiving waterbody characteristics such as carrying capacity of native biota or compounding cumulative effects of multiple effluents being discharged into a single waterbody (Government of Canada, 2013; Mendoza, 2016). Therefore, establishing uniform limits for select effluent parameters may not fully protect the health of all aquatic receiving environments across Canada (Walker et al., 2002).

2.3.3 Pulping Process and Wastewater Generation

Understanding the manufacturing process involved in the production of pulp mill effluent is necessary to assess the potential adverse ecological effects of effluent on aquatic ecosystems. The objective of the pulping process is to extract and recover cellulose fibers from lignin and other wood constituents (Hewitt et al., 2006). The manufacturing of pulp consists of either a chemical or mechanical treatment process. The mechanical process uses force with minimal chemical additives to release usable wood fiber (Owens, 1991). Alternatively, chemical treatment involves the use of chemicals to separate the wood fibers and develop the pulp product (Bajpai, 2015). Chemical procedures involve either kraft or sulfite treatment; however, kraft treatment is the dominant technology of the pulp and paper industry (Cheremisinoff &

Rosenfeld, 2009). Ultimately, the pulping process consists of initial processing, washing, and bleaching (Costa et al., 2009). Kraft pulping involves cooking wood chips at elevated temperatures using a solution known as "white liquor," which consists of sodium sulfide and sodium hydroxide (Cabrera et al., 2017). This solution dissolves the lignin that binds the cellulose fibers together in the wood (Cabrera et al., 2017). After cooking, the pulp is depressurized in blow tanks, and the fibers are separated from the residual digesting liquor ("black liquor") by washing the pulp before bleaching it (Costa et al., 2009). Since the PPER (1992) regulations, many mills have transitioned from elemental chlorine for bleaching to chlorine dioxide (Solomon, 2007). The black liquor that is produced during the pulping process is rich in organic compounds, including degraded lignin and residual chemicals from the digesting process (Costa et al., 2009). Residual material from the black liquor is collected at the bottom of the recovery boiler as smelt. This collection diverts potential contaminants from the wastewater treatment system and re-utilizes them in future production (Costa et al., 2009). The raw effluent released by pulp mills to treatment facilities is a complex mixture of products generated in the debarking, pulp washing, bleaching, and regeneration of chemicals in the pulping process (Hewitt et al., 2006). The properties of the generated wastewater are dependent on various factors including the type of raw material, pulping process, the recirculation of effluent, and the amount of water used in production (Patel et al., 2021).

2.4 Coastal Ecosystem Structure and Vulnerability

2.4.1 Coastal Ecosystem Biodiversity

Biodiversity is the variability among living organisms including the diversity within species, between species, and of ecosystems (United Nations Convention on Biological

Diversity, 1992). Within marine contexts, biodiversity encompasses interconnected components of an ecosystem from all levels of biological organization (Cochrane et al., 2016). Each of these ecosystem components has a unique structural and functional role (Cochrane et al., 2016). Coastal ecosystems are zones of high biodiversity and biological productivity, serving important roles in the provisioning of various ecosystem services (Lu et al., 2018). Ecosystem services provided by coastal zones include nutrient cycling, detoxification of pollutants, food production, provision of raw materials, and refuge for a variety of biological organisms (Luisetti et al., 2014). Forming the interface between land and sea, coastal ecosystems encompass a variety of forms of community structure including estuaries, seagrass beds, salt marshes, tidal flats, mangroves, coral reefs, and beaches (Barbier et al., 2011). The habitats that encompass coastal ecosystems have distinct physical and biological boundaries, largely due to tidal action, saltwater inundation, and wave action (Luisetti et al., 2014). Coastal areas are influenced greatly by local topography, tides, currents, meteorological effects, adjacent terrestrial systems, and anthropogenic impacts (Itoh et al., 2018). Many of these influences result in coastal ecosystems experiencing hydrological pulses of freshwater from watershed runoff or river discharge. These pulses can influence salinity profiles, nutrient profiles, and the input of organic matter, which subsequently alter species composition (Macias, 2018).

The presence of benthic habitats is a distinguishable feature of coastal zones, making them distinct from pelagic ocean environments (Itoh et al., 2018). Within coastal habitats, there is often distinct zonation of dominant vegetation, hydrodynamic structure, and substrate composition that collectively form distinct habitat types (Allee et al., 2000). Results of these ecosystem influences are dynamic mosaics of habitat that support a variety of species (Henseler et al., 2019). Leveraging the coastal ecosystem's ecological value, many fish, invertebrates, and

migratory birds use coastal ecosystems as habitat, migratory routes, spawning grounds, or nursery areas (Seitz et al., 2013). Being transient, fish and decapods like lobsters and crabs are responsible for linking various coastal habitats (Swadling et al., 2019). Sessile species such as bivalves provide important ecosystem services within coastal environments owing to their ability to filter large volumes of water and remove suspended material from the water column (Dame, 1993). In addition to the ecological services that coastal ecosystems provide, they also serve valuable economic functions. Coastal zones are economically important as fishing grounds that provide both food sources for humans and stable livelihoods (Lau et al., 2019). The importance of coastal biodiversity has led to an increased focus on the need to implement more rigorous environmental regulations aimed at preserving these ecosystems and the numerous services they provide (Arthington et al., 2010).

2.4.2 Coastal Food Web Structure

Coastal food webs are dynamic and complex systems of connectivity between organisms and their environment (Lu et al., 2018). Primary producers such as phytoplankton, macroalgae, and macrophytes form the base of coastal food webs (Henseler et al., 2019). Phytoplankton are microscopic organisms that produce oxygen and organic matter from sunlight, carbon dioxide, and inorganic nutrients (Falkowski, 2012). Due to increased delivery of nutrients into aquatic environments during the spring, large increases in phytoplankton populations called phytoplankton blooms occur (Trombetta et al., 2019). Phytoplankton form the base of many marine food webs, serving as a food source for zooplankton, which in turn are food for many invertebrate and fish species (Fenchel, 1988). Invertebrates and fish also contribute to energy flow in coastal ecosystems by transferring energy and nutrients to organisms located higher in

the food web (Hobson et al., 1992). Each species within a coastal food web occupies a specific trophic niche, defined as the resources that a species requires and the role or function that it plays in an environment (Layman et al., 2007). Trophic niches are influenced by the physiology, foraging strategy, food availability of an organism, and competition among other species (Moyo et al., 2021). Within coastal ecosystems, some species function as opportunistic consumers, feeding on multiple levels of the food web and occupying a broader trophic niche (Cicala et al., 2020). For example, large decapods such as lobsters feed on a benthic invertebrate-rich diet that also includes algae and detritus (Boudreau & Worm, 2012). The diversity of species and habitat types in coastal ecosystems contributes to these complex food web interactions observed in coastal zones.

Organic matter is ubiquitous in aquatic environments and is a major contributor to ecosystem energy and nutrient budgets (Koziorowska et al., 2016). There are multiple biogeochemical processes that remove, produce, and transform organic matter in coastal environments (Asmala et al., 2018). Organic matter in aquatic ecosystems is produced either internally (autochthonous) or is deposited into the waterbody from the terrestrial environment (allochthonous) (Stedmon et al., 2007). Autochthonous sources of organic matter include plankton, macroalgae, and macrophytes (Stedmon et al., 2007). Allochthonous sources include atmospheric deposition, groundwater discharges, and discharges from rivers and streams (Lønborg et al., 2017). Nutrients that fuel primary productivity in coastal ecosystems are commonly introduced by runoff from terrestrial land or from point-source wastewater discharges (Stedmon et al., 2007). Organic matter that supports the foundation of coastal food web structure can originate from a variety of sources, and the origin and abundance of these materials significantly influence food web structure and function (Hansson et al., 1997).

2.4.3 Anthropogenic Impacts to Coastal Ecosystems

While coastal ecosystems provide a variety of ecosystem services, they are particularly vulnerable to mounting environmental change (Ray, 2021). Serving as a buffer between the land and open ocean, coastal zones occupy considerable worldwide spatial area (Psomiadis, 2022). These areas are often densely populated by humans, making them particularly sensitive to anthropogenic impacts in addition to natural impacts (Lu et al., 2018). As with many coastal regions globally, Canada's coastal ecosystems are experiencing ongoing loss and degradation (Ray, 2021). Alterations in marine biodiversity are caused directly by exploitation, pollution, and habitat destruction, and they are caused indirectly through climate change and related shifts in marine biogeochemistry (Worm & Lotze, 2006). Coastal marine ecosystems are under intense pressure from anthropogenic pollution such as agricultural runoff, municipal wastewater, and industrial wastewater (Dailer et al., 2010). These activities frequently result in an excess supply of nitrogen, the primary limiting nutrient in coastal marine waters (Nixon et al., 1986). Globally, approximately 24% of the anthropogenic nitrogen released in coastal watersheds is estimated to reach coastal ecosystems (Malone & Newton, 2020). Nearshore runoff from land assists in fueling coastal productivity, and an excess supply of nutrients overstimulates the internal production of organic matter (Deininger & Frigstad, 2019). Consequently, an abundance of nutrients reduces water quality and ecosystem health by causing increased macrophyte and algal growth; known as eutrophication, which depletes oxygen levels in the water (Malone & Newton, 2020). Ecological food webs may become altered by eutrophication and habitat loss, threatening the provisioning of ecosystem services by coastal habitats (Carlier et al., 2008).

2.5 Coastal Ecosystems: Atlantic Canada Context

2.5.1 Atlantic Canadian Coastal Ecosystems

Atlantic Canada is one of the most productive marine environments in the world (Bernier, 2018). The Canadian Atlantic Ocean is divided into three bioregions (Figure 2.2): the Newfoundland and Labrador Shelves, the Scotian Shelf, and the Gulf of St. Lawrence. Within the Atlantic Region of Canada, there is over 40,000 km of coastline (Bernier et al., 2018). Among the three bioregions, there is distinct geomorphologic diversity, which creates a range of habitats that support a variety of marine productivity (Bernier et al., 2018). For example, intertidal mudflats in Atlantic Canadian coastal ecosystems support a diverse combination of marine organisms including worms, molluscs, crustaceans, shorebirds, and fish (Gerwing et al., 2015). Rocky intertidal shores are frequently dominated by macroalgal growth, bivalve molluscs, gastropods, and larger predators such as lobsters and crabs (Scrosati & Heaven, 2007). Atlantic Canada's coastal ecosystems are habitat for many important commercial species such as lobster (*Homarus americanus*), snow crab (*Chionoecetes opilio*), herring (*Clupea harengus*), and mackerel (*Scomber scombrus*) (Fisheries and Oceans Canada [DFO], 2021). The total value of fisheries landings in Atlantic Canada in 2019 was $3.2 billion CAD (DFO, 2021). Therefore, the diverse coastal ecosystems of Atlantic Canada serve many functions, both ecologically and economically.

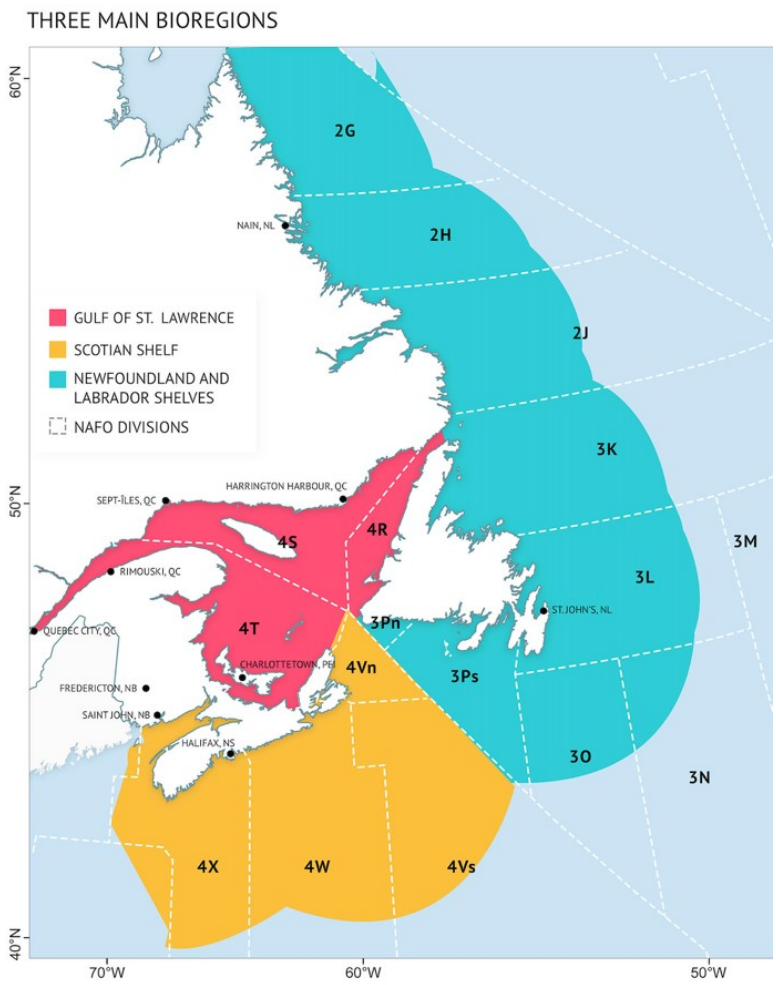

Figure 2.2 The three bioregions in Atlantic Canada (Bernier et al., 2018).

2.5.2 Nitrogen Loading in Atlantic Canadian Coastal Ecosystems

Many anthropogenic pressures influence the species composition and diversity that characterize coastal ecosystems in Atlantic Canada. Climate change is an unequivocally serious threat to Atlantic Canadian ecosystems and drives shifts in ecosystem composition (Bush & Flato, 2019). For example, ocean warming has resulted in populations of American lobster (*H. americanus*) moving further north and increasing in all bioregions in Atlantic Canada (Steneck & Wahle, 2013). Nutrient inputs from agricultural fertilizers and inputs from industrial and municipal wastewater also contribute to the alteration of ecosystems throughout the region

(Kelly et al., 2021). This excess nutrient loading threatens coastal ecosystems with potential reductions in diversity of biota and the subsequent loss of both ecosystem services and economic opportunities (Worm & Lotze, 2006).

Within the province of Nova Scotia, recent studies have attempted to quantify and identify sources of nitrogen loading in coastal ecosystems. Kelly et al. (2021) completed a study determining the regional distribution and intensity of nitrogen inputs from land-based sources to coastal marine environments within 109 watersheds in Atlantic Canada. Results determined that wastewater inputs contributed the most (>80%) to nitrogen loading rates. Additional sources that were identified as significant contributors to total nitrogen loading rates were atmospheric deposition and localized point-source pollution, such as seafood processing facilities (Kelly et al., 2021). Therefore, a diverse range of sources contribute to nitrogen loading in coastal ecosystems, with wastewater inputs playing the most influential role in Atlantic Canada. As expanding coastal industries and escalating climate change effects increase their pressure on aquatic ecosystems, quantifying nutrient loads affecting coastal watersheds will provide reliable baseline measurements of water quality (Rheuben et al., 2019). In addition to providing a foundational understanding of the health of aquatic resources, such quantifications provide a tool for detecting future environmental change.

2.5.3 Northumberland Strait

The Northumberland Strait (Figure 2.1) is in the southern portion of the Gulf of St. Lawrence bioregion and separates the Canadian province of Prince Edward Island (PEI) from New Brunswick (NB) and Nova Scotia (NS). The Northumberland Strait has an area of roughly 16,000 km^2 and is relatively shallow, with an average depth of 20 m throughout much of the

central region (Kranck, 1972). Due to its relatively shallow waters, the Northumberland Strait undergoes a wide annual range of temperature (Calder, 2003). Surface waters freeze over in winter and may exceed 20°C in the summer (Calder, 2003). The shallow waters create environments with strong tidal currents that promote elevated nutrient concentrations and favourable conditions for primary productivity (Kranck, 1972). Owens and Bowen (1977) describe current direction in the Northumberland Strait as traveling primarily from west to east. However, current direction is more complex locally among the many sheltered estuaries and bays, which may act independently from regional trends observed along the coastline. The substrate composition along the Northumberland Strait ranges from silt to mixed sediment (sand, shell hash, and gravel), with sandy and rocky intertidal environments (Stantec Consulting Ltd., 2019). The Northumberland Strait is inhabited by a range of biota including macrophytes, plankton, benthic species, reptiles, fish, marine mammals, and marine birds (DFO, 2007). The largest commercial fisheries in this region include lobster (*H. americanus*), rock crab (*Cancer irroratus*), scallops (*Argopecten irradians*), and herring (*C. harengus*) (DFO, 2007). For an environment that serves a large socioeconomic role in the region and has a diverse range of habitat and biota, the underlying biogeochemistry of the Northumberland Strait has not been well studied and remains a necessary avenue for future research. Further studies are required to delineate ecosystem changes throughout the region, such as characterizing nutrient inputs from surrounding watersheds that may be impacting these valuable habitats' environmental sustainability and viability.

Until January 2020, the Northumberland Strait was the receiving environment for treated pulp mill effluent from the BHETF (Chaudhary et al., 2020). Before operations ceased at the BHETF, approximately 70,000 m^3 of effluent was discharged each day into the Northumberland

Strait (Ecometrix, 2016). Discharge from Boat Harbour provided a significant influx of freshwater to the immediate receiving environment in the Northumberland Strait. Therefore, in addition to organic matter and inorganic nutrients contained in the effluent, the freshwater historical effluent additions may have locally altered ecosystem salinity conditions in the marine environment. Boat Harbour and the estuarine outflow region connecting to the Northumberland Strait have been previously classified as a mixohaline or brackish environment (Ecometrix, 2016). The most recent EEM report for Boat Harbour (Ecometrix, 2016) reported a salinity value of 3-5 ppt within the nearshore area of the discharge. Eastern and western portions of the Northumberland Strait are classified as polyhaline (Ecometrix, 2016), which typically has salinity values between 18-30 ppt (Rice et al., 2014). It is possible that former, frequent pulses of freshwater have influenced ecosystem dynamics within the former outflow region of Boat Harbour into the Northumberland Strait. Significant freshwater input can affect organisms and their habitat within coastal areas (Gillanders & Kingsford, 2002). These effects can include changes in species diversity and abundance and can alter feeding and developmental patterns (Gillanders & Kingsford, 2002). The effects of freshwater influence have been detected in previous studies near Boat Harbour. In 2000, DFO deployed an automated biological effects monitoring system called HABITRAP at the Boat Harbour outflow (Ecometrix, 2007). The device was deployed to provide a continuous record of mussel feeding rates, an indicator of contaminant sensitivity and overall population health (Ecometrix, 2007). Results of the HABITRAP experiment concluded that feeding rates of mussels were reduced at the outflow relative to reference locations, not resulting from effluent toxicity but rather, the influence of freshwater from the effluent (Ecometrix, 2016). Therefore, while it is likely that contaminants

and organic material in the effluent may have negatively impacted ecological activities in the coastal region, the impacts of historical freshwater intrusion cannot be ignored.

Modeling and delineation of the former effluent plume from Boat Harbour into the Northumberland Strait has reported that the buoyant nature of the freshwater effluent in a saline environment resulted in spatial variation in the plume (Ecometrix, 2016). The effluent was susceptible to slight weather changes and the direction it traveled was capable of being altered both daily and seasonally. Previous modelling has indicated that effluent permeated from the outflow location in the northeast direction within the Northumberland Strait, along a narrow path that extended at least 2 km (Ecometrix, 2016). Effluent concentrations decreased with distance from the shoreline and were reportedly less than 1% approximately 1 km from the shoreline (Ecometrix, 2016). Since January 2020, the BHETF has not been releasing effluent into the Northumberland Strait, and the impacts of historical effluent release within this region, such as the impacts on trophic dynamics between native biota, are largely unknown.

2.5.4 Previous Classification Studies in the Northumberland Strait

Prior to the remediation of Boat Harbour, baseline studies are necessary within the marine environment to both determine the historical impact of wastewater release and evaluate effectiveness of remediation efforts to restore the ecosystem. Previous baseline studies have been completed in the Northumberland Strait assessing the concentration of contaminants within the tissues of select marine biota (Figure 2.3). Specifically, these studies have evaluated the concentration of PCDD/Fs, metals, and metalloids usually attributable to pulp mill effluent (*e.g.* Al, As, Cd, Cr, Cu, Fe, Pb and Zn) in marine biota tissue (Chaudhary et al., 2020; Hoffman et al., 2017). Organisms that have been studied include blue mussels (*Mytilus edulis*), rock crab (*C.*

irroratus), and American lobster (*H. americanus*) (Chaudhary et al, 2020; Maltby, 2021; Romo et al., 2019). Figure 2.3 illustrates a comprehensive overview of the studies that have been completed on marine biota adjacent the former Boat Harbour outflow in the Northumberland Strait. Each of these studies have been unable to detect significant impacts on biota that could be directly linked to pulp mill effluent exposure. Romo et al. (2019) completed a review of historical documents, including government reports and peer-reviewed articles, to identify contaminants in marine biota in the Northumberland Strait. The study was conducted with the purpose of reviewing all existing studies in the region to inform future monitoring and establish a baseline for comparison in the Boat Harbour remediation project. The study found insufficient data to properly assess baseline conditions in the region due to inconsistencies in data reporting. Inconsistencies were identified in previous studies such as variation in the regulatory and technical requirements in each consecutive EEM cycle. Additionally, reference locations and the sentinel species analysed in each EEM study varied from cycle to cycle, providing an inconsistent basis by which to compare the results of studies over time.

Chaudhary et al. (2020) and Maltby (2021) completed studies on the environment adjacent the former outflow of the BHETF into the Northumberland Strait. These two contemporary studies assessed contaminant concentrations in marine biota tissues. Chaudhary et al. (2020) measured metal, metalloid, and PCDD/F concentrations in American lobster, rock crab, and blue mussels. Contaminant concentrations in tissues of each organism were compared to Canadian Food Inspection Agency (CFIA) guidelines (Canadian Food Inspection Agency, 2019). Results found limited contamination in biota and concluded that effluent posed a low risk to marine biota (Chaudhary et al., 2020). Similarly, Maltby (2021) collected American lobster adjacent the BHETF outflow and determined there were no significant differences in

contaminant concentrations (metals, metalloids, PCDD/Fs, and polycyclic aromatic hydrocarbons (PAHs) in lobster collected near the outflow relative to reference locations. The study concluded that the contamination level of lobsters adjacent the BHETF outflow was unlikely to be of concern moving forward with remediation efforts. To date, the studies that have been completed within this area have been unable to identify any significant biota effects resulting from pulp mill effluent exposure. However, each of these studies have detailed the impacts on individual marine biota, and there has been no ecosystem-wide assessment of the historical and potential legacy impacts of effluent within the marine environment. While no detectable effects on contaminant loadings in biota have been identified, the impacts of effluent on the energy and nutrient flow among multiple levels of the food web remain unknown. Designing baseline studies that evaluate trophic structure among biota across entire food webs is critical prior to remediation, and it may reveal impacts in the marine environment that traditional tissue contaminant analysis was unable to identify.

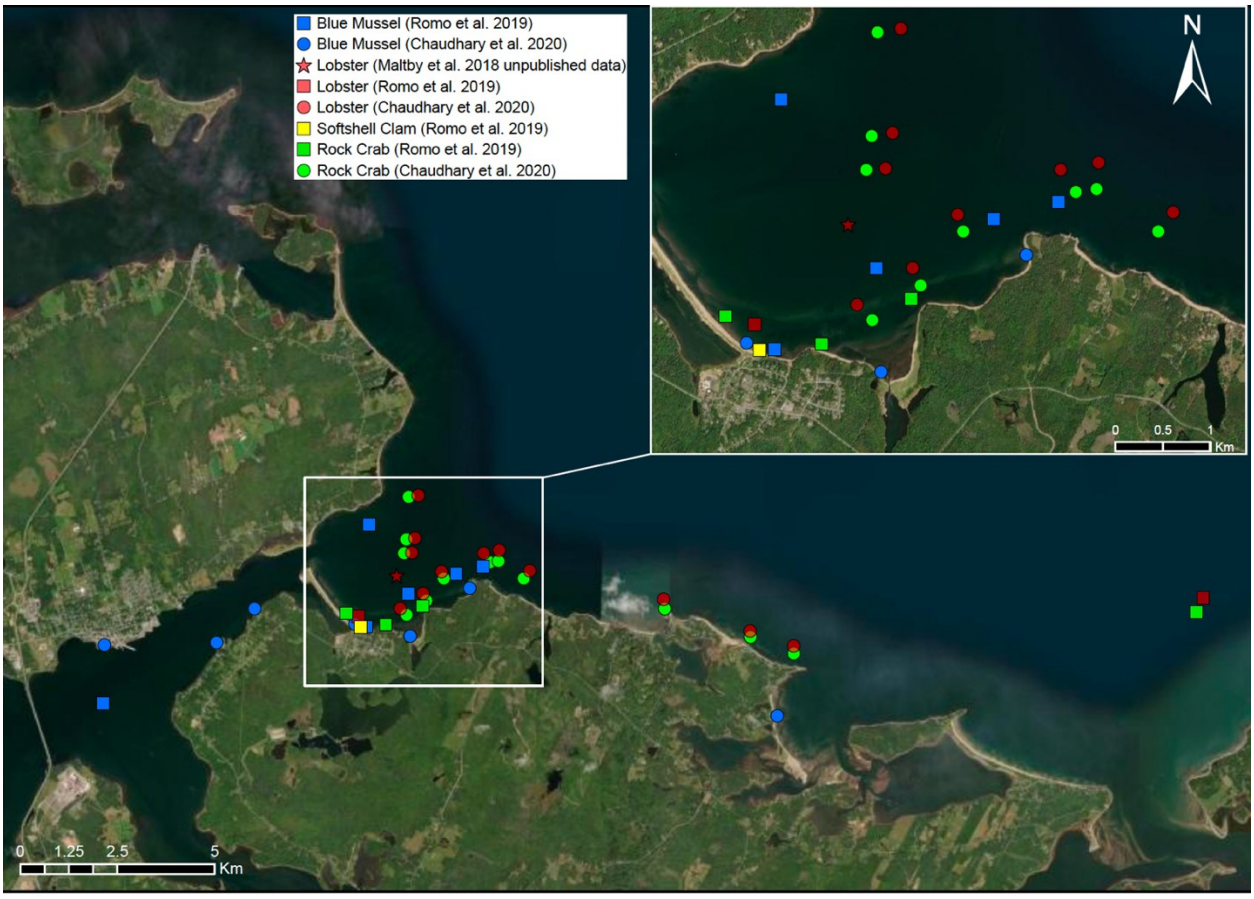

Figure 2.3 Comprehensive overview of previous studies on marine biota that have been completed in the region adjacent the former outflow of the BHETF into the Northumberland Strait.

2.5.5 Pictou Harbour

Many ecosystem stressors co-occur and can often be synergistic in their impacts on aquatic ecosystem structure (Weber et al., 2008). Pictou Harbour (Figure 2.1), located southwest of the BHETF, opens into the Northumberland Strait and is a source of many anthropogenic pollution inputs. Pictou Harbour is characterized by low flushing, and the mixing of this waterbody is ensured through wind and tidal action (Krauel, 1969). Pictou Harbour has a history of environmental contamination, including fecal coliforms, metals, nutrients, PCBs, and PAHs (Akaishi et al., 2007). In addition to pollution from commercial fisheries, a local shipyard, and agricultural runoff, there are multiple wastewater inputs that contribute to nutrient enrichment of

the harbour (Appendix A). Untreated municipal wastewater was released to Pictou Harbour until 2010 when a secondary treatment plant was installed in the town of Pictou (Infrastructure Canada, 2022). Secondary-treated municipal wastewater is collected from four neighbouring towns (population ~24, 000) and released from the town of Pictou, located to the west, into the harbour (Akaishi et al., 2007). To the northeast of the harbour, the PLFN wastewater treatment plant discharges primary-treated wastewater into the Northumberland Strait. The outflow of wastewater from PLFN (population ~485 (Statistics Canada, 2016) is located approximately 800 m west of the former BHETF outflow (Appendix A). Therefore, it is likely that aquatic biota may be experiencing negative impacts from exposure to these multiple, complex nutrient inputs from Pictou Harbour and the Northumberland Strait.

2.6 Marine Biogeochemical Cycling and Anthropogenic Disturbance

2.6.1 Marine Biogeochemical Cycles

Biogeochemical cycles are the transportation and cyclic transformation of elements within the Earths biosphere (Hedges, 1992). Carbon and nitrogen cycling is essential for the ecological integrity of marine ecosystems (Rabouille et al., 2001) with microbial communities being fundamental to the biogeochemical cycling of these elements (York, 2018). Within the marine carbon cycle, microorganisms serve as biological pumps by sequestering anthropogenic carbon dioxide from the atmosphere (Meyer et al., 2016). Additionally, microbial transformations of nitrogen in marine environments contribute to fluxes in the global nitrogen cycle (Hebert, 1999). Cycling of elements within the environment is influenced by a variety of physical, biological, chemical, and anthropogenic factors (Hedges, 1992). Human activity strongly influences marine biogeochemical cycling (Rabouille et al., 2001). For example, inputs from wastewater and agricultural runoff can contribute to coastal eutrophication, which disrupts

the ecological balance of nutrients and subsequent interactions among organisms in marine environments (Rabouille et al., 2001).

2.6.2 Carbon Cycle

The marine carbon cycle is a dynamic component of the global carbon budget (Ver et al., 1999). Carbon at the Earth's surface consists of both organic carbon derived from living organisms and soil and inorganic carbon derived from respiration and carbonate materials (Lacroix et al., 2021). The marine carbon cycle involves a series of pools and fluxes, from photosynthesis and primary production to decomposition and respiration (Bianucci & Denman, 2012). While the ocean receives carbon inputs from rivers and terrestrial run-off, most carbon exchange occurs with the atmosphere due to changes in the carbon content of surface water driven by physical, chemical, and biological processes (Ward et al., 2017). Within coastal zones, microbes rapidly decompose organic matter (Bauer et al., 2013). Primarily through the process of photosynthesis, organic matter production traps inorganic carbon into the organic molecules of aquatic biota that inhabit coastal regions (Ward et al., 2017). In contrast, both metabolic respiration and mineralization of organic detritus by heterotrophic organisms release carbon back into the environment (Kaiser et al., 2011). An excess of dissolved, particulate, or other non-living organic matter can result in oxygen depletion within a given environment; such excesses and subsequent depletions are becoming increasingly more frequent (Bauer et al., 2013).

Anthropogenic activities have progressively been modifying the marine carbon cycle. During the past three centuries, there has been a significant increase in the amount of organic carbon transported from land and stored in coastal zones (Ver et al., 1999). Activities such as burning fossil fuels, changes in land-use activities, increased dissolved and particulate loads,

organic matter transport, and feedback to biological productivity have disrupted the balance of carbon cycling in marine environments (Ver et al., 1999). The direction of future change in ecosystem production within coastal ecosystems is largely dependent on changes in the relative magnitude of organic matter inputs to coastal systems (Bauer et al., 2013). Likely, continued increases in organic matter from wastewater and other anthropogenic perpetuations will continue to affect the exchange and fate of carbon in coastal marine ecosystems.

2.6.3 Nitrogen Cycle

Nitrogen is central in marine biogeochemical cycling. The marine nitrogen cycle is a myriad of microbially-mediated reactions that control the distribution and speciation of nitrogen in marine environments (Casciotti, 2016). Nitrogen fixation typically refers to the processes that convert unreactive atmospheric nitrogen (N_2) into other forms of nitrogen (Gruber, 2008). Meanwhile, nitrogen assimilation generally refers to the biotic incorporation of nitrogen compounds (Herrero et al., 2001). Denitrification refers to the removal of bioavailable nitrogen, which is taken from the ocean and returned to fixed nitrogen (Casciotti, 2016). This removal is frequently a regulator of marine primary production since bioavailable nitrogen is often the limiting nutrient in marine primary production (Casciotti, 2016). Nitrogen transformations such as N_2 fixation and denitrification are mediated by specialized cyanobacteria (diazotrophs), which are responsible for the major source and sink processes of the nitrogen cycle (Falkowski, 1997). In coastal zones, new nitrogen in the form of nitrate (NO_3^-) is available to primary producers chiefly from N_2 fixation by diazotrophs and also from nutrient upwells (Richert et al., 2015).

Increasing nitrogen loads resulting from anthropogenic activities alter the marine nitrogen cycle and frequently cause changes in food web dynamics (Lotze & Milewski, 2004). Coastal

ecosystems face increasing pressure from pollution inputs, which modify the bioavailable supply of nitrogen in aquatic environments (Viana et al., 2013). Non-point pollution sources from agricultural fertilizers and point sources from municipal wastewater, seafood processing plants, and pulp and paper mills introduce excess nutrients like nitrogen into aquatic ecosystems (Nagel et al., 2018). Nutrient enrichment has a large impact on species composition, primary producer biomass, and subsequent coastal ecosystem productivity (Armitage & Fourqurean, 2009). For example, increased nutrient loading in waterbodies can trigger a chain of biogeochemical feedback, including shifts in phytoplankton biomass, formation of harmful algal blooms, and hypoxic conditions (Kelly et al., 2021). These oxygen-deficient waters stimulate the proliferation of hypoxia-tolerant species, which may replace native biota (Maúre et al., 2021). While it is known that anthropogenic enrichment can result in a loss of these ecosystem entities, gaps remain in our understanding of how this enrichment permeates throughout multiple trophic levels in an ecosystem, and subsequently, how it impacts food chain length.

2.7 Stable Isotopes

2.7.1 Stable Isotope Primer

Isotopes are forms of the same element that differ in the number of neutrons in their nuclei (Fry, 2006). Relative to radioactive isotopes, stable isotopes do not decay, thus providing a means to directly trace details of element cycling in ecosystems (Peterson & Fry, 1987). Carbon and nitrogen are two commonly applied stable isotopes in ecological studies (Fry, 2006). Carbon has two stable isotopes, ^{12}C and ^{13}C, which are differentiated by their respective atomic masses (12 and 13) and present in the environment as 98.89% and 1.11%, respectively (Nier, 1950). Similarly, nitrogen has two stable isotopes,^{14}N and ^{15}N, present in the environment as

99.64% and 0.36%, respectively (Fry, 2006). Stable isotope analysis compares the amount of a heavy isotope (an element with one additional neutron, *e.g.*, ^{13}C) to a light isotope (an element with one less neutron, *e.g.*, ^{12}C). Known as fractionation, stable isotope studies rely on an elements heavy and light isotopes having slightly different rates of reaction, which results in the differential loss or retention of one isotope of the element relative to the other (Peterson & Fry, 1987). Stable isotope ratios are represented using delta (δ) notation (Fry, 2006). For example, carbon and nitrogen stable isotopes are represented by $\delta^{13}C$ and $\delta^{15}N$, respectively. Stable isotope values are expressed in parts per thousand (‰) and are calculated relative to a universal standard. The standard for calculating $\delta^{13}C$ values is Pee Dee Belemnite (PDB), and the standard for calculating $\delta^{15}N$ values is atmospheric nitrogen (N_2). Stable isotope values are calculated according to the following equation (Eq. 1):

$$\delta X‰ = [(R_{sample} - R_{standard}) - 1] \times 1000 \qquad \text{[Eq. 1]}$$

Where X is the heavier isotope, R_{sample} is the ratio of the heavy to light isotope within the sample being analyzed and $R_{standard}$ is the ratio of the heavy to light isotope within the standard. An increase in the δ value indicates the presence of an increased amount of the heavier isotope relative to the lighter isotope; this sample is considered *enriched* (Risk et al., 2009). Alternatively, samples with a lower δ value are termed *depleted*.

2.7.2 Ecological Applications of Stable Isotopes

Stable isotope analysis is increasingly being applied in ecological studies. The ecological applications of stable isotopes have emerged in recent years for assessing aspects of organism

migration patterns, niche definition, resource use and diet composition, trophic-level estimates, and analysis of food web functioning (Layman et al., 2007). $\delta^{13}C$ and $\delta^{15}N$ are commonly used in ecological studies because they can collaboratively provide information on an organisms food source and trophic relationships (Peterson & Fry, 1987). $\delta^{15}N$ values are a useful measure of the specific sources of nitrogen supporting the base of food webs since different sources of nitrogen have distinct ranges of $\delta^{15}N$ values (McClelland et al., 1997). For example, sewage wastewater is typically isotopically enriched with a $\delta^{15}N$ value of 10+ ‰ (Heaton, 1986). Distinct $\delta^{15}N$ signatures in different sources of nitrogen are often used to assess nitrogen inputs to coastal ecosystems (Costanzo et al., 2001). Furthermore, nitrogen isotopes strongly fractionate, resulting in $\delta^{15}N$ enrichment of consumer organisms by a value of approximately 3.4‰ relative to their prey (DeNiro & Epstein, 1981) (Figure 2.4). Due to these predictable differences, $\delta^{15}N$ is also a useful indicator of an organisms trophic position within a food web (Post, 2002b). Figure 2.4 illustrates a food chain with three trophic levels: particulate organic matter (POM) as the primary producer, mussels as the primary consumer since they feed on POM (Rogers, 2003), and rock crab as the tertiary link because they prey on mussels (Boudreau & Worm, 2012). $\delta^{15}N$ measurements can elucidate an organism's trophic position and assist in gaining a greater understanding of the food web dynamics within a given region.

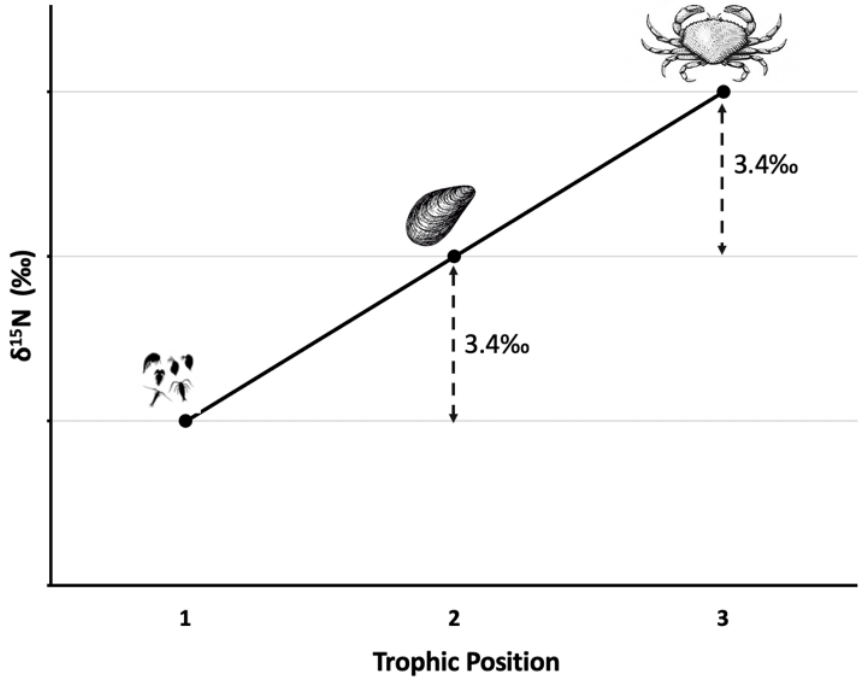

Figure 2.4 Example of a coastal food chain with the trophic position of each organism estimated from their $\delta^{15}N$ value, which fractionates by approximately 3.4‰ with each consecutive trophic level (Adapted from Bowes & Thorp, 2015).

Unlike nitrogen stable isotopes, $\delta^{13}C$ does not significantly fractionate between trophic levels in a food chain, averaging 0.4‰ at each consecutive level (DeNiro & Epstein, 1981). $\delta^{13}C$ values are indicators of an organisms diet because various sources of organic matter have different $\delta^{13}C$ signatures, which do not strongly fractionate between organisms in a food chain (Post, 2002a). In coastal ecosystem studies, $\delta^{13}C$ is frequently used to elucidate carbon sources since they can be interpreted as a proxy for terrestrial ($\delta^{13}C$ ~28‰) or marine ($\delta^{13}C$ ~20‰) sources of carbon (Fry, 2006). In ecological studies, a dual isotope analysis (measuring both $\delta^{13}C$ and $\delta^{15}N$) provides valuable information on a wide array of ecosystem processes, including identification of an organisms' nutrient sources and trophic position in a food web (Hobson et al., 2002). Attributed to their ability to provide this valuable ecosystem information, stable isotopes are used to delineate wastewater and other pollution inputs in aquatic ecosystems

(Arciszewski et al., 2014; Dubé et al., 2005). Ultimately, this delineation facilitates determination of how wastewater inputs affect food web structure.

Numerous studies have applied stable isotope analysis to quantify an organisms exposure to both municipal and industrial effluents (Arciszewski et al., 2014; Loomer et al., 2015). This technique is effective because effluents have stable isotope signatures distinct from the stable isotope signatures that naturally occur in receiving waterbodies (Savage, 2005). Therefore, stable isotope values of organisms within a food web exposed to wastewater reflect the food webs increased reliance on wastewater-derived nutrients (Loomer et al., 2015). Stable isotope analysis has been applied to investigate the environmental impacts of municipal sewage wastewater in aquatic environments (Costanzo et al., 2001; Morrissey et al., 2013). However, this technique has not been applied extensively in environments receiving pulp mill effluent. A considerable gap exists in measuring stable isotopes in receiving coastal waters, with most research being completed in freshwater environments (Arciszewski et al., 2014; Dubé et al., 2005; Galloway et al., 2003; Wayland & Hobson, 2001). However, it is important to assess the impacts of pulp mill effluent across varied habitats. Specifically, it is important to evaluate conditions in coastal marine contexts, where increased mixing, tidal effects, the role of salinity, and increased organic matter inputs may create conditions significantly different from their freshwater analogs. New marine baseline data may be required as results observed in freshwater environments might not be appropriate for comparison in coastal marine contexts. Additionally, many of the previous studies assess the impacts of pulp mill effluent on the ecosystem by measuring stable isotope values in tissues of a select number of species, which are often limited to one trophic position (Skinner et al., 2012). Therefore, the utility of using stable isotope analysis as a method for

assessing multi-trophic level impacts of pulp mill effluent in coastal marine environments has not been fully recognized.

2.7.3 Stable Isotope Analysis of Pulp Mill Effluent

The first foundational study applying stable isotope analysis in receiving environments for pulp mill effluent was by Wassenaar and Culp (1996) who characterized and traced the fate of pulp mill effluent in the Thompson River, British Columbia. Results from this study determined that stable isotopes were useful indicators to trace pulp mill effluent in the environment. Applied in receiving environments for pulp mill effluent, $\delta^{13}C$ values provide information on the contribution of terrestrial or exogenous carbon (Wayland & Hobson, 2001). Meanwhile, $\delta^{15}N$ values provide information on dietary preferences of organisms and quantitatively define their trophic position (McMahon et al., 2020). To date, stable isotope analyses in environments receiving pulp mill effluent have detected a terrestrial $\delta^{13}C$ signature (-30 to -25‰) in tissues of aquatic organisms (Farwell, 2000). These results are likely attributed to the intensive processing of trees in pulp manufacturing, with effluent incorporating the $\delta^{13}C$ value of terrestrial C_3 plants (Arciszewski et al., 2014). In contrast, $\delta^{15}N$ values tend to be more variable among samples exposed to pulp mill effluent. Depleted $\delta^{15}N$ values have been most commonly reported in previous studies and this result is attributed to the presence of chlorolignin, a by-product of wood pulp bleaching, and fertilizer additions in the wastewater treatment process (Wayland & Hobson, 2001). Galloway et al. (2003) reported depleted $\delta^{15}N$ values in slimy sculpin (*Catostomus cognatus*) exposed to pulp mill effluent, and Skinner et al. (2012) found depleted $\delta^{15}N$ in mummichog (*Fundulus heteroclitus*) in pulp mill effluent receiving environments. However, Dubé et al. (2005) sampled bone, gonad, liver, and white

muscle tissue of longnose sucker (*Catostomus catostomus*) exposed to pulp mill effluent and found enriched $\delta^{15}N$ values in all tissues. There are many factors that may influence the results of studies completed in environments receiving pulp mill effluent. Such influences could complicate interpretation of results and make the comparison of multiple studies challenging. These factors include the production processes employed at each pulp mill and the treatment procedures employed at wastewater treatment facilities (Oakes et al., 2010). Additionally, the species involved, the tissues sampled, and characteristics of the receiving waterbody make the comparison of stable isotope studies in environments exposed to pulp mill effluent challenging.

Analysis of stable isotope values following the closure of a pulp mill and cessation of effluent release into waterbodies may inform how pulp mill effluent impacts the biogeochemistry of aquatic ecosystems, both contemporarily and historically. Stable isotope studies completed after mill closures have become more prevalent in recent years. Mendoza (2016) completed a study to determine if $\delta^{13}C$ and $\delta^{15}N$ values of white sucker (*C. commersonii*) and macroinvertebrates (*Chironomidae, Hyalella, Caecidotea, Pisidium, Valvata*) reflected periods of mill operation and mill closure in Jackfish Bay, Ontario. Results found variation in stable isotope values among these periods with depleted $\delta^{15}N$ and enriched $\delta^{13}C$ of invertebrates exposed to effluent after a nine-month mill closure in 2011. These results reversed in 2013, 10 months after the mill re-opened. Additional research has been completed on the Mattagami River in Northern Ontario, where McMahon et al. (2020) evaluated the impacts of pulp mill effluent on $\delta^{13}C$ and $\delta^{15}N$ values in white sucker (*C. commersonii*) and mayflies (*Hexagenia* sp.), and Arciszewski et al. (2014) assessed historical $\delta^{13}C$ values in *C. commersonii* exposed to pulp mill effluent. These analyses were also coupled with the opportunity to assess changes in stable isotopes after the closure of a pulp mill. McMahon et al. (2020) detected elevated $\delta^{13}C$ values

and variable $\delta^{15}N$ values in white sucker and mayflies compared to reference locations five years after the closure of the mill. Results support the recovery of biota in Mattagami River and reduced reliance on wastewater-derived nutrients. Arciszewski et al. (2014) found age-related changes in white sucker captured downstream from the mill, with older fish having a residual pulp-derived $\delta^{13}C$ value in their tissues five years after the mills closure. However, fish that reached maturity after the closure of the mill did not display pulp-derived organic material in their $\delta^{13}C$ signatures. These studies were useful in demonstrating the utility of stable isotopes to document recovery after the closure of a pulp mill. Further studies applying stable isotope analysis to a wider suite of organisms may assist in documenting the impacts of pulp mill effluent on entire food webs. Additionally, future stable isotope studies may assist in assessing how entire food webs recover after ecosystem remediation.

Previous studies that have applied stable isotope analysis to quantify the impacts of pulp mill effluent on aquatic biota have detected overall distinguishable stable isotope signatures. Moreover, these studies have demonstrated how stable isotope analysis can be further leveraged to gain an ecosystem-wide understanding of environments impacted by anthropogenic pollution. Further exploration of this tool across both freshwater and marine habitats and among wider assemblages of species could demonstrate the utility of this method for monitoring ecosystem effects from wastewater inputs. Dubé et al. (2005) suggest that the incorporation of stable isotope analysis into routine EEM programs of receiving waters for pulp mill effluent may enable determination of the spatial extent of biotic exposure. Historically, evaluation of ecosystem conditions in receiving environments for pulp mill effluent has been completed using traditional contaminant concentrations in tissues of individual biota (Chaudhary et al., 2020). Stable isotope analysis may ameliorate traditional monitoring methods by evaluating the biogeochemical

conditions of the environment and linking various ecosystem components in a holistic ecosystem assessment (Layman et al., 2007). In addition to spatially quantifying the impacts of pulp mill effluent and assessing biotic interactions, stable isotope analysis may significantly improve the ability to quantify ecosystem recovery from exposure to anthropogenic pollution.

2.8 Food Web Dynamics

2.8.1 Food Chain Length

Food webs are depictions of the exchange of matter and energy among organisms within an ecosystem, visually displaying energy flow from basal primary producer resources to top predators (Post, 2002a). Food chains exist within complex food web networks and represent linear systems of feeding relationships within an ecosystem (Sabo et al., 2009). Food chain length is a fundamental ecosystem property that can be used to assess ecosystem health (Post, 2002a). The trophic position of an organism is defined by the hierarchical position it occupies within a food chain (Hussey et al., 2014). Elucidating trophic links in coastal marine ecosystems is a complex task; however, it is foundational to understanding how ecosystems function (Vizzini & Mazzola, 2006). As such, changes in food chain length and trophic position are becoming widely used as indicators of ecosystem degradation, and are useful in determining how ecosystems respond to environmental disturbance (Carscallen et al., 2012). Overall trophic structure within an ecosystem can provide a powerful means of monitoring anthropogenic disturbance in addition to quantifying the persistence and resilience of food webs (Hussey et al., 2014). Numerous methods have been applied to quantify trophic structure within food webs, and various theories have been proposed on which environmental factors influence the composition of these interactions.

2.8.2 Stable Isotope Applications of Food Chain Length

Traditional methods of reconstructing food webs have used stomach content analysis, fecal analysis, and behavioural observations (Post, 2002b). However, these methods are laborious and often provide only snapshots of an organisms overall diet (Polito et al., 2011). To overcome these limitations, $\delta^{15}N$ measurements have become a viable method for quantifying trophic position in food webs (Post, 2002b). Measuring stable isotope values to assess an organisms diet provides an integrated view of their assimilated diet over time, whereas previous methods provided an episodic representation of what the organism had most recently consumed (Nielsen et al., 2017). $\delta^{15}N$ values are used to estimate an organisms trophic position due to shifts in the proportion of heavy to light isotope values from one trophic level to the next (Post, 2002b). As previously mentioned, consumers are enriched in $\delta^{15}N$ by a value of approximately 3.4‰ relative to their food source (DeNiro & Epstein, 1981). Therefore, researchers can estimate an organisms trophic position based on their $\delta^{15}N$ values. However, the $\delta^{15}N$ of an organism alone provides minimal information about its absolute trophic position across ecosystems because there is considerable variation in $\delta^{15}N$ at the base of the food web where organisms derive their nitrogen (Post, 2002b; Vander Zanden et al., 1997). Therefore, an isotope baseline is critical in standardizing trophic position estimates for a given location (Vander Zanden et al., 1997). A baseline $\delta^{15}N$ value assists in interpreting if any observed variation in an organisms' $\delta^{15}N$ value reflects changes in food web structure or simply variation within a given location (Vander Zanden et al., 1997). Estimating trophic position of a consumer within a food web typically uses the following equation (Eq. 2) (Post, 2002b):

$$TP = \lambda + [(\delta^{15}N_{Consumer} - \delta^{15}N_{Base}) / \Delta_n] \qquad \text{[Eq. 2]}$$

Where λ is the trophic position of an organism used to estimate $\delta^{15}N_{Base}$. $\delta^{15}N_{Consumer}$ is the measured $\delta^{15}N$ in the consumer organism, and Δ_n is the enrichment in $\delta^{15}N$ per trophic level. By standardizing an organisms trophic position to an isotope baseline, the comparison of food chain length across multiple ecosystems or sampling locations is possible (Post, 2002b). Moreover, researchers can apply this technique to make comparisons among ecosystems and investigate the influence of anthropogenic factors on food chain length.

2.8.3 Food Chain Length and Wastewater Discharge

Stable isotope analysis has been conducted to estimate variation in an organisms' trophic position within receiving waters for municipal sewage wastewater and other environments impacted by anthropogenic disturbance (de Carvalho et al., 2021). The complex mixture of organic and inorganic nutrients in wastewater can influence ecosystem organization (Pinckney et al., 2001). These ecosystem responses may propagate across different levels of biological organization, from individual organisms to entire food webs (McClelland et al., 1997). For example, the enriched $\delta^{15}N$ value of sewage wastewater (+10 to +20‰ (Schubert et al., 2013) is typically reflected in the $\delta^{15}N$ values of primary producers and consumers that inhabit the receiving environments for wastewater (Freedman et al., 2012). Therefore, organisms inhabiting waterbodies receiving sewage wastewater typically have enriched $\delta^{15}N$ values and differences in their trophic position relative to unpolluted environments (Hadwen & Arthington, 2007). Organisms typically occupy higher relative trophic positions in aquatic environments receiving sewage wastewater (de Carvalho et al., 2021). Effluent-derived nutrient subsidies to primary producers at the base of the food web facilitate an increase in abundance and diversity of primary consumers, leading to shifts in higher trophic position (Freedman et al., 2012). However, the

degree of nutrient enrichment plays a role in determining food chain length in polluted ecosystems (Culp et al., 2000). At intermediate levels of nutrient enrichment, taxonomic diversity and overall abundance of benthic macroinvertebrates increase, but at high levels of enrichment, food chain length may decrease (Culp et al., 2000). Therefore, variable effects on food chain length may be observed in environments receiving wastewater. These effects may be particularly variable in environments such as the coastal Northumberland Strait where the degree of enrichment from historical effluent inputs is unknown.

2.8.4 Environmental Determinants of Food Chain Length

Many ecological hypotheses exist to explain differences in food chain length among ecosystems. While there has been considerable research completed, limited progress in determining the environmental factors responsible for variation in food chain length within ecological systems has been made (Post, 2002a). Three main hypotheses have been widely discussed as explanations for variation in food chain length: disturbance, resource availability, and ecosystem size (Pimm, 1982; Schoener, 1989). The '*dynamic stability hypothesis'* predicts that shorter food chains are more common in environments impacted by environmental disturbance (Pimm, 1982). This hypothesis is explained by longer food chains exhibiting greater instability and subsequently, a greater likelihood of being impacted by disturbance (Vander Zanden et al., 1999). According to this hypothesis, food chain length is likely to be shorter in ecosystems with frequent disturbance. The '*resource availability hypothesis'* posits that food chain length is constrained by basal energy supply due to energy losses that occur with each trophic transfer (Pimm 1982; Schoener 1989). This hypothesis predicts that longer food chains will occur in more productive ecosystems (McHugh et al., 2010). Finally, ecosystem size has

also been posited as an environmental determinant of food chain length (Schoener, 1989). The '*ecosystem size hypothesis*,' also referred to as the '*productive space hypothesis*,' is grounded in the idea that larger ecosystems have greater total resources available at the base of food webs and can sustain greater trophic diversity and spatial heterogeneity (Post, 2002a). Consequently, such ecosystems support longer food chains (Post, 2002a).

In addition to these three fundamental, widely cited theories of food chain length, ecosystem type (*e.g*., freshwater versus marine ecosystems) has also been suggested as a determinant of food chain length (Vander Zanden & Fetzer, 2007). Freshwater food webs rely on basal resources of terrestrial and aquatic origin, and the size structure of organisms is smaller relative to marine systems (Vander Zanden & Fetzer, 2007). Therefore, it has been suggested that freshwater food chains are shorter than marine food chains (Potapov et al., 2019). Despite wide applications of these theories in historical studies, uncertainty still exists with respect to the environmental determinants of food chain length and food web structure in ecosystems.

Despite each of these three hypotheses being grounded in theory, no individual hypothesis has received universal support within a single ecosystem type. There is considerable variation between studies that aim to determine which environmental factors are responsible for observed differences in food chain length across a spatial landscape (Takimoto & Post, 2013). It is likely that multiple environmental factors may be interacting to determine this important metric of community structure and ecological function within ecosystems. A review by Post (2002a) suggests that productivity was responsible for limiting food chain length in systems with low resource availability, and researchers found little evidence to suggest that disturbance contributed to differences in food chain length. Contributing to the discourse on environmental determinants of food chain length, McHugh et al. (2010) conducted a study that determined both

ecosystem size and the disturbance hypothesis were predictors of food chain length in stream ecosystems. Therefore, food chain length may not be simply explained by one individual theoretical framework. Ecosystems are complex systems of variation with multiple shaping influences (Limburg et al., 2002). As such, alterations in food chain length may be driven by multiple environmental factors (Post, 2002a). Former aquatic receiving environments for pulp mill effluent may experience multiple, iterative forces that determine food chain length in the local ecosystem. Receiving waters for pulp mill effluent can be classified as disturbed environments, and they also experience fluxes in organic matter input which can stimulate high productivity (Arciszewski et al., 2014). Hence, multiple hypotheses could be proposed to explain variation in food chain length within these environments. Furthermore, in a unique situation such as Boat Harbour, with pulses of freshwater into a coastal marine setting, there may also be variation in ecosystem type (*i.e.*, freshwater and marine distinctions) along a spatial gradient in the Northumberland Strait. Therefore, further research is required to assess how aquatic biota comprise the complex systems of trophic structure within receiving environments for pulp mill effluent.

Chapter 3: Assessing Spatial Impacts of Historical Pulp Mill Effluent on Trophic Dynamics in a Coastal Marine Ecosystem Using Stable Isotope ($\delta^{13}C$ and $\delta^{15}N$) Analysis

3.1 Introduction

Excess nutrient inputs from anthropogenic pollution frequently disrupt the structure and function of coastal marine ecosystems (Hadwen & Arthington, 2007). Municipal and industrial wastewater discharged into aquatic environments can impact the abundance and diversity of organisms, subsequently altering ecosystem services (Worm & Lotze, 2006). The Canadian pulp and paper industry generates large volumes of wastewater which have historically been linked to adverse ecological impacts (Munkittrick et al., 1994). Pulp mill effluent contains contaminants that result in both physiological impacts to aquatic organisms and shifts in community structure of native biota (Parrot et al., 2006). These adverse ecological impacts are frequently the result of organic and inorganic contaminants in the effluent, such as dioxins and furans (PCDD/Fs), metals and metalloids, and high nutrient content (Culp et al., 2000). An influx of nutrients such as ammonia and nitrate in pulp mill effluent, can result in the eutrophication of coastal waters and create oxygen-deficient (anoxic) conditions in aquatic environments (Culp et al., 2000). Anoxic conditions disrupt the biogeochemical balance that forms the foundation of energy and nutrient exchange between organisms in aquatic ecosystems (Medina-Galván et al., 2021).

In Canada, the *Pulp and Paper Effluent Regulations* (PPER, 1992) of the *Fisheries Act* (1985) established maximum allowable limits for specific effluent quality parameters including total suspended solids (TSS) and biological oxygen demand (BOD) (Munkittrick et al., 2002). The effectiveness of the PPER is monitored through the cyclical Environmental Effects Monitoring (EEM) program (Walker et al., 2002). A requirement for all Canadian pulp and paper

mills, the EEM program is a science-based tool that assesses the potential impacts of effluent on aquatic receiving environments (Roach & Walker, 2017). Despite the implementation of stricter regulations for pulp mill effluents and considerable research on the biological effects of effluent on individual organisms, gaps remain in our holistic understanding of the impacts of pulp mill effluents on entire ecosystem structure (Culp et al., 2003). Organisms are intimately linked to their surrounding environments through complex systems of nutrient and energy exchange (Bormann & Likens, 1970). As such, comprehensive assessments of the linkages among organisms and the nutrient and energy flow that support them is critical to properly evaluate the conditions in coastal environments receiving pulp mill effluent.

Prior to 1967, Boat Harbour, Nova Scotia, was a tidal estuary with cultural significance to the neighbouring Mi'kmaq community of Pictou Landing First Nation (PLFN) (Figure 3.1) (Bennet, 2013). Referred to as A'se'k by members of PLFN, the estuary and surrounding lands were used for spiritual, ceremonial, and recreational purposes (Castleden et al., 2016). The traditional and recreational uses of the land were compromised when a bleached kraft pulp mill began discharging wastewater into A'se'k, which had been converted into the Boat Harbour Effluent Treatment Facility (BHETF) (Hoffman et al., 2015). Effluent release into the BHETF resulted in near-complete mortality of aquatic biota and loss of sacred land for PLFN (Castleden et al., 2016). The natural estuarine conditions were further modified when Boat Harbour was cut off from the marine environment in 1972 by a dam built at the mouth of the estuary (Chaudhary et al., 2020). The dam drastically altered the hydrological conditions in Boat Harbour and effectively converted the former tidal estuary into two distinct waterbodies: a large freshwater lake upstream (still presently referred to as Boat Harbour), and a smaller estuarine inlet downstream of the dam. The dam caused water levels in Boat Harbour to rise by 2-3 m and

flooded an area of 12 ha, increasing the footprint of the original estuary upstream of the dam by approximately 8% (AECOM, 2015). The natural, biologically productive, and pre-industrial conditions of Boat Harbour have been severely impacted by both the dam and historical pulp mill effluent release into the environment (Hoffman et al., 2017).

Pulp mill effluent passed through a series of treatment stages in the BHETF (Appendix A) to improve effluent quality prior to discharge into the estuarine inlet and subsequently, the Northumberland Strait (Figure 3.1) (Hoffman et al., 2017). During early effluent treatment, wastewater from the pulp mill passed through a pipeline (~3 km) and raw effluent was discharged into surrounding ditches and wetlands (Quanz et al., 2021). Throughout the history of the BHETF, upgrades occurred such as the addition of lined discharge ditches that emptied into settling basins, the latter designed to permit the settling of suspended solids (Hoffman et al., 2017). The next stage in the effluent treatment process was secondary wastewater treatment within the aeration stabilization basin where pumps forced atmospheric air into the effluent to support aerobic microbes that broke down the effluents biodegradable material (Ecometrix, 2016; Hoffman et al., 2019). In 1997, an automated nutrition addition system was installed, supplying bulk urea and diammonium phosphate to the effluent to enhance microbial growth prior to effluent entering the aeration stabilization basin (Ecometrix, 2016). From the aeration stabilization basin, effluent was released into a stabilizing lagoon (Boat Harbour) where it remained for 20-30 d prior to discharge (~70,000 m^3/day) into the Northumberland Strait (Chaudhary et al., 2020). Appendix A illustrates wastewater treatment stages at the former BHETF.

The provincial *Boat Harbour Act* (2015) mandated the cessation of effluent release into the BHETF, effective January 2020, with subsequent remediation of Boat Harbour and

surrounding lands (Eichinger & Walker, 2020). The goal of the remediation is to restore Boat Harbour to a tidal estuary and return possession of the waterbody and surrounding lands to PLFN (Province of Nova Scotia, 2022). Decommissioning of the dam will occur as part of the remediation process, restoring tidal conditions to Boat Harbour (GHD Limited, 2018). The remediation plan for Boat Harbour is currently undergoing a Federal Environmental Assessment (*Canadian Environmental Assessment Act [CEAA]*, 2012) with the central focus of removing approximately 1,244,000 m^3 of unconsolidated, contaminated sediment from Boat Harbour (Eichinger & Walker, 2020). Baseline studies of Boat Harbour and the adjacent marine environment of the Northumberland Strait are a critical component of remediation to both assess the impacts of historical effluent exposure on the ecosystem and determine the effectiveness of remediation on restoring natural ecosystem processes. Baseline studies assessing the conditions within the impounded Boat Harbour waterbody and the surrounding wetlands have been completed (Fraser et al., 2021; Quanz et al., 2021). There have also been baseline studies completed which assess contaminant (metal, metalloid, and PCDD/F) concentrations in individual marine biota from the Northumberland Strait. Marine biota previously studied include blue mussels (*Mytilus edulis*), rock crab (*Cancer irrorartus*), and American lobster (*Homarus americanus*) (Chaudhary et al., 2020; Maltby, 2021). Previous studies that assessed contaminant concentrations in marine biota have been unable to detect significant impacts attributable to historical pulp mill effluent exposure (Chaudhary et al., 2020; Maltby, 2021). However, these studies have evaluated conditions of individual biota and have not addressed impacts of historical effluent exposure on food web structure, including the nutrient and energy contributions that underlie critical ecosystem functions. While previous baseline studies in the region have served an essential role in informing remediation measures, further research must be

conducted to provide an ecosystem-wide analysis of biogeochemical conditions that support trophic dynamics among organisms within the marine environment. An assessment that details the spatial impacts of pulp mill effluent on the coastal ecosystem, including the ecological impacts among biota within the food web, would provide a more holistic and comprehensive evaluation of ecosystem conditions prior to remediation.

Stable isotope analysis is a tool which has been employed in numerous ecological impact assessments, including tracing influences of wastewater inputs on aquatic receiving environments (Savage, 2005). Stable isotopes are forms of the same element possessing different numbers of neutrons in their nuclei (Fry, 2006). Stable isotope ratios compare the amount of a heavy isotope (an element with one additional neutron, *e.g.*,^{13}C) to a light isotope (an element with one less neutron, *e.g.*, ^{12}C). Heavy and light isotopes of an element have slightly different rates of reaction in biological and environmental matrices, resulting in differential loss or retention of one isotope relative to the other in a process known as isotopic fractionation (Fry, 2006). Stable isotope values are represented using delta (δ) notation and are expressed in parts per thousand (‰). Two isotopes frequently measured in ecological studies are carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), each having two naturally occurring stable isotopes (^{12}C,^{13}C and ^{14}N,^{15}N; Fry, 2006). Stable isotope values are calculated according to the following equation (Eq. 1):

$$\delta X‰ = [(R_{sample} - R_{standard}) - 1] \times 1000 \qquad \text{[Eq. 1]}$$

Where X is the heavier isotope, R_{sample} is the ratio of the heavy isotope to the light isotope within the sample being analyzed and $R_{standard}$ is the ratio of the heavy isotope to the light isotope within the standard. An increase in the δ value indicates an increased amount of the heavier isotope

relative to the lighter isotope and this isotope value is termed *enriched*. Alternatively, lower δ values are termed *depleted*.

As different $\delta^{13}C$ signatures exist among various sources of organic matter in ecosystems, stable isotopes of carbon are often used in ecological studies to elucidate an organisms dietary source (Post, 2002b). Within coastal ecosystems, $\delta^{13}C$ values are used to distinguish between terrestrial versus marine sources of carbon, since terrestrial carbon often has a relatively depleted $\delta^{13}C$ signature ($\delta^{13}C \sim -28‰$) (Fry, 2006). In contrast, the $\delta^{13}C$ value of marine algae is typically around -22‰ (Duarte et al., 2018). $\delta^{15}N$ values are often used in ecological studies to delineate the specific sources of nitrogen supporting the base of food webs since different sources of nitrogen have distinct ranges of $\delta^{15}N$ values (McClelland et al., 1997). For example, global mean nitrate from seawater typically has a $\delta^{15}N$ value of 3-5‰ (Sigman et al., 2000), synthetic fertilizer typically 0‰, and animal manure typically 8‰ (Kendall, 1998). Sewage wastewater is typically isotopically enriched with a $\delta^{15}N$ value of 10+‰ (Heaton, 1986). These distinct $\delta^{15}N$ signatures can be used to assess relative nitrogen inputs to coastal ecosystems (Costanzo et al., 2001). Additionally, $\delta^{15}N$ values are used in ecological studies to estimate an organism's trophic position in a food web since $\delta^{15}N$ values increase by approximately 3.4‰ at each consecutive trophic level (Minagawa & Wada, 1984). A dual stable isotope analysis, evaluating both $\delta^{13}C$ and $\delta^{15}N$, can provide valuable ecological information regarding the source of organic matter within ecosystems and help elucidate trophic relationships in a biological community (Peterson et al., 1985).

Stable isotope analysis has been successfully applied to trace nutrient inputs from wastewater in aquatic ecosystems and evaluate ecosystem structure in wastewater receiving environments (Spies et al., 1989). This method of tracing wastewater in aquatic environments

relies on distinct differences in stable isotope signatures between effluent and the receiving waterbody (Babaranti et al., 2019). Based on these isotopic differences, stable isotope analysis can quantitatively assess organisms' reliance on wastewater-derived nutrients (Loomer et al., 2015) and determine how exposure to wastewater influences the trophic dynamics within an ecosystem (Freedman et al., 2012). Stable isotope analysis has been applied in aquatic environments to identify patterns of exposure to pulp mill effluent in species of fish (Arciszewski et al., 2014), invertebrates (Wassenaar & Culp, 1996), and macroalgae (Wayland & Hobson, 2001). Stable isotope studies of pulp mill effluent have detected a terrestrially derived carbon signature (-30‰ to -25‰) from trees released to the effluent during intensive processing of the pulp manufacturing process (Arciszewski et al., 2014). Many studies also report depleted $\delta^{15}N$ signatures in biota within receiving environments for pulp mill effluent due to the presence of chlorolignin, a by-product of wood pulp bleaching, contained in the effluent (Wayland & Hobson, 2001). While these previous studies have demonstrated the utility of stable isotope analysis for identifying and mapping pollution sources, this technique has yet to be applied across multiple trophic levels in a coastal ecosystem. Additionally, most of the studies that evaluate stable isotope values of biota exposed to pulp mill effluent have been completed in freshwater environments (Arciszewski et al., 2014; Galloway et al., 2003; McMahon et al., 2020), with considerably less research in marine environments. Within coastal environments, factors such as increased hydrologic mixing, organic matter inputs, tidal effects, and salinity may render the stable isotope values detected in freshwater biota incomparable. Application of this method across multiple trophic levels and in marine contexts will further our understanding of the spatial impacts of historical pulp mill effluent on the flow of energy and nutrients among organisms in a coastal food web.

Elucidating trophic links in coastal marine environments is a complex task; however, it is necessary for understanding of how ecosystems function (Vizzini & Mazzola, 2006). Food chain length is a fundamental ecological attribute representing the linear sequence of energy flow among organisms within an ecosystem (Vander Zanden & Fetzer, 2007). Alterations in food chain length are increasingly being used to evaluate the effects of wastewater pollution in aquatic environments (Carscallen et al., 2012). Measurements of $\delta^{15}N$ values in biota tissues have become a widely applied method for quantifying trophic position within food webs (Post, 2002b). Furthermore, trophic position estimates according to the $\delta^{15}N$ values of an organism can be used to assess the permeating effects of wastewater throughout multiple levels of a food web (Kwak & Zedler, 1997). To standardize an estimate of trophic position for individual organisms within a given location, $\delta^{15}N$ values must be compared to an isotope baseline at each sampling location (Post, 2002b). Estimating the trophic position of a consumer within a food web typically uses the following equation (Eq. 2) (Post, 2002b):

$$TP = \lambda + [(\delta^{15}N_{Consumer} - \delta^{15}N_{Base}) / \Delta_n] \quad \text{[Eq. 2]}$$

Where λ is the trophic position of an organism used to estimate $\delta^{15}N_{Base}$, $\delta^{15}N_{Consumer}$ is the measured nitrogen isotope ratio in the consumer organism, and Δ_n is the enrichment in $\delta^{15}N$ per trophic level. Standardizing the estimate of trophic position for a given organism to an isotope baseline facilitates the comparison of food chain length across various sampling locations (Post, 2002b). Therefore, this technique can be applied to evaluate the influence of environmental factors, such as wastewater exposure, on food chain length (Vander Zanden et al., 1997).

Exposure to wastewater can influence an ecosystem's biological organization and disrupt the trophic interactions between native aquatic biota (Freedman et al., 2012). Previous studies assessing variation in trophic position of organisms exposed to municipal sewage wastewater have determined that organisms typically occupy relatively higher trophic positions within polluted waters (de Carvalho et al., 2021). However, these effects may vary depending on the degree of nutrient enrichment within the aquatic environment (Freedman et al., 2012). At intermediate levels of nutrient enrichment, taxonomic diversity and overall abundance of benthic macroinvertebrates increase; however, as eutrophication increases and effects become toxic, food chain length decreases (Culp, 2000). While studies assessing food chain length in environments exposed to sewage wastewater have identified alterations in food web dynamics among organisms, there has been considerably less research assessing the impacts of pulp mill effluent on food chain length and trophic structure. Assessing the potential alterations of energy and matter flow among organisms exposed to pulp mill effluent is important to evaluate overall ecosystem health and fill this critical knowledge gap.

Traditional contaminant analyses of biota tissue are frequently used as a method for evaluating ecosystem conditions in aquatic receiving environments for pulp mill effluent (Chaudhary et al., 2020). Stable isotope analysis may augment traditional monitoring methods in receiving environments for pulp mill effluent by both evaluating the biogeochemical foundation of the environment and linking various ecosystem components in a holistic ecosystem assessment. Historical release of organic-rich effluent combined with significant discharge of freshwater from Boat Harbour into the marine environment of the Northumberland Strait has likely impacted the ecosystem dynamics in the coastal environment. Prior to remediation of Boat Harbour, it is important to understand the spatial impacts of historical effluent release on the

marine environment by evaluating food web structure and the nutrient sources that support the local food web. This study assesses the variability in $\delta^{15}N$ and $\delta^{13}C$ in the coastal food web along a spatial gradient of historical pulp mill effluent exposure from Boat Harbour and evaluates impacts of pulp mill effluent on trophic dynamics along the Northumberland Strait coastline.

3.2 Methods

3.2.1 Study Area and Sampling Locations

The Northumberland Strait (Figure 3.1) is in the southern portion of the Gulf of St. Lawrence and separates the provinces of Prince Edward Island (PEI) from New Brunswick (NB) and Nova Scotia (NS). The Northumberland Strait extends approximately 225 km and is relatively shallow, with an average depth of 20 m throughout most of the central region (Kranck, 1972). Ocean currents in this region travel west to east; however, flow patterns are more complex locally between the many sheltered estuaries and bays along the coast (Owens & Bowen, 1977).

While variable depending on weather and tides, the effluent plume from Boat Harbour into the Northumberland Strait extended along a narrow path to the northeast, with a 1% effluent envelope extending 2 km northeast (Ecometrix, 2016). In addition to organic and inorganic contaminants in the pulp mill effluent, release of effluent from Boat Harbour resulted in significant discharge of freshwater into the Northumberland Strait. Before cessation of effluent release from Boat Harbour in January 2020, salinity at the former outflow point into the Northumberland Strait varied considerably from marine conditions, with surface values between 3-5 ppt (Ecometrix, 2016).

In addition to pulp mill effluent, this region of the Northumberland Strait receives additional wastewater inputs from the surrounding communities. Untreated municipal wastewater was released to Pictou Harbour until 2010 when a secondary treatment plant was installed in the town of Pictou (Infrastructure Canada, 2022). Secondary-treated municipal wastewater from neighbouring towns (population ~24,000 (Statistics Canada, 2016) is released into Pictou Harbour (Figure 3.1), which ultimately flows northeast into the Northumberland Strait. Additionally, primary-treated wastewater from the community of Pictou Landing First Nation (PLFN) (population ~485 (Statistics Canada, 2016) is discharged into the Northumberland Strait, approximately 800 m northwest of the former Boat Harbour outflow (Figure 3.1). Appendix A illustrates the multiple wastewater discharge locations surrounding the study area.

For this study, 11 sampling locations were selected (Table 1, Figure 3.1) spanning approximately 30 km of coastline adjacent to Boat Harbour in the Northumberland Strait. Sampling locations represent a spatial gradient of historical pulp mill effluent exposure, originating at the former Boat Harbour outflow and traveling in the northeast (15 km) and northwest (15 km). Sampling locations for marine biota were selected based on prevailing ocean currents in the region and delineation of the former pulp mill effluent plume in the Northumberland Strait. Figure 3.1 and Table 3.2 summarize species collected at each sampling location, which comprised areas up to 2 km^2. Among sampling locations, certain species were only present in estuarine locations while others were collected offshore. These distinctions within given sampling locations are provided in Table 3.2. Collection of all organisms across all sampling locations was not possible as some organisms were not found at certain locations despite sampling effort.

Table 3.1 Sampling locations with abbreviations showing distance and direction from the former Boat Harbour outflow.

Sampling Location	Abbreviated Name	Latitude (°N)	Longitude (°W)	Distance from Boat Harbour (km)	Direction from Boat Harbour
Caribou Island	CRI	45.4551	-62.4048	10.8	NW
Seaview Cemetery	SVC	45.4110	-62.4051	3.63	NW
Harbour Lights	HRL	45.4133	-62.3945	3.09	NW
Lighthouse Beach	LHB	45.4040	-62.3906	1.28	NW
Boat Harbour Outflow	BHO	45.4016	-62.3822	0	-
Halfway Point	HWP	45.4033	-62.3820	0.57	N
Mackenzie Head	MKH	45.4050	-62.3812	1.26	NE
Chance Harbour	CNH	45.4037	-62.3500	4.04	NE
Black Point	BLP	45.4017	-62.3302	6.98	NE
Melmerby Beach	MLB	45.3943	-62.3051	9.85	NE
Big Island	BGI	45.3849	-62.2719	14.48	NE

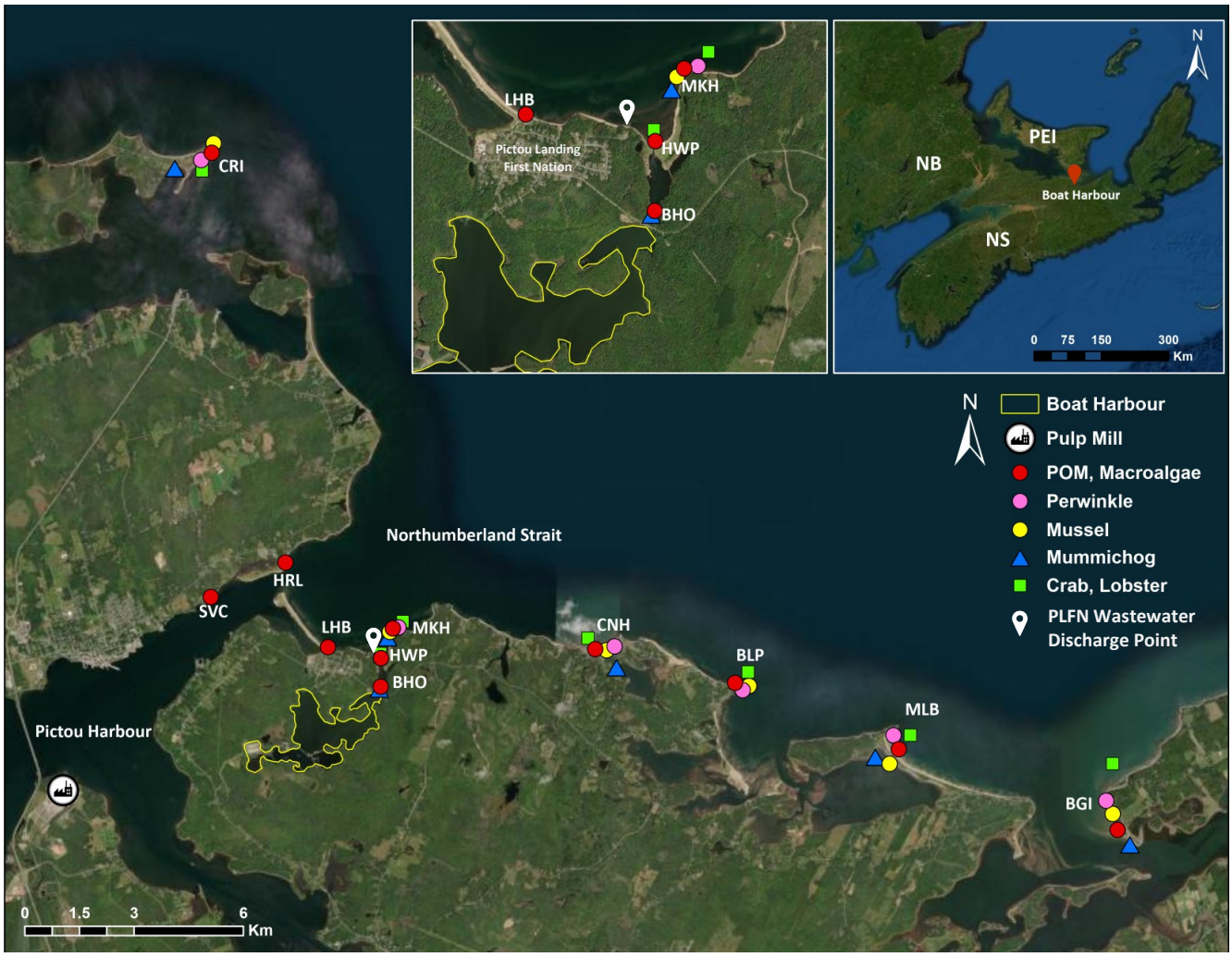

Figure 3.1 Map of the Northumberland Strait coastline illustrating the former location of the pulp mill, Pictou Landing First Nation (PLFN), PLFN wastewater discharge point, Boat Harbour, and the sampling locations for all biota and their abbreviated name.

Table 3.2 Sampling locations; X's represent offshore sampling locations and circles estuarine locations.

Organism	CRI	SVC	HRL	LHB	BHO	HWP	MKH	CNH	BLP	MLB	BGI
POM	X	X	X	X	X	X	X	X	X	X	X
Macroalgae	X	X	X	X	X	X	X	X	X	X	X
Periwinkle	X						X	X	X	X	X
Mussel	X						X	X	X	•	X
Mummichog	•				X		X	•		•	X
Crab	X					X	X	X	X	X	X
Lobster	X					X	X	X	X	X	X

The intertidal area adjacent the former Boat Harbour outflow (BHO) provides a diversity of habitat types encompassing cobble or gravel to exposed boulders to sandy substrates with algal growth. The shoreline to the northeast and northwest of the former outflow has sandy bottom substrates, isolated pockets of rock and cobble, and abundant macroalgal growth in nearshore coastal areas (Figure 3.2). Substrate composition at the former Boat Harbour outflow consists of a layer of unconsolidated organic matter deposition from historical pulp mill effluent (GHD Limited, 2018).

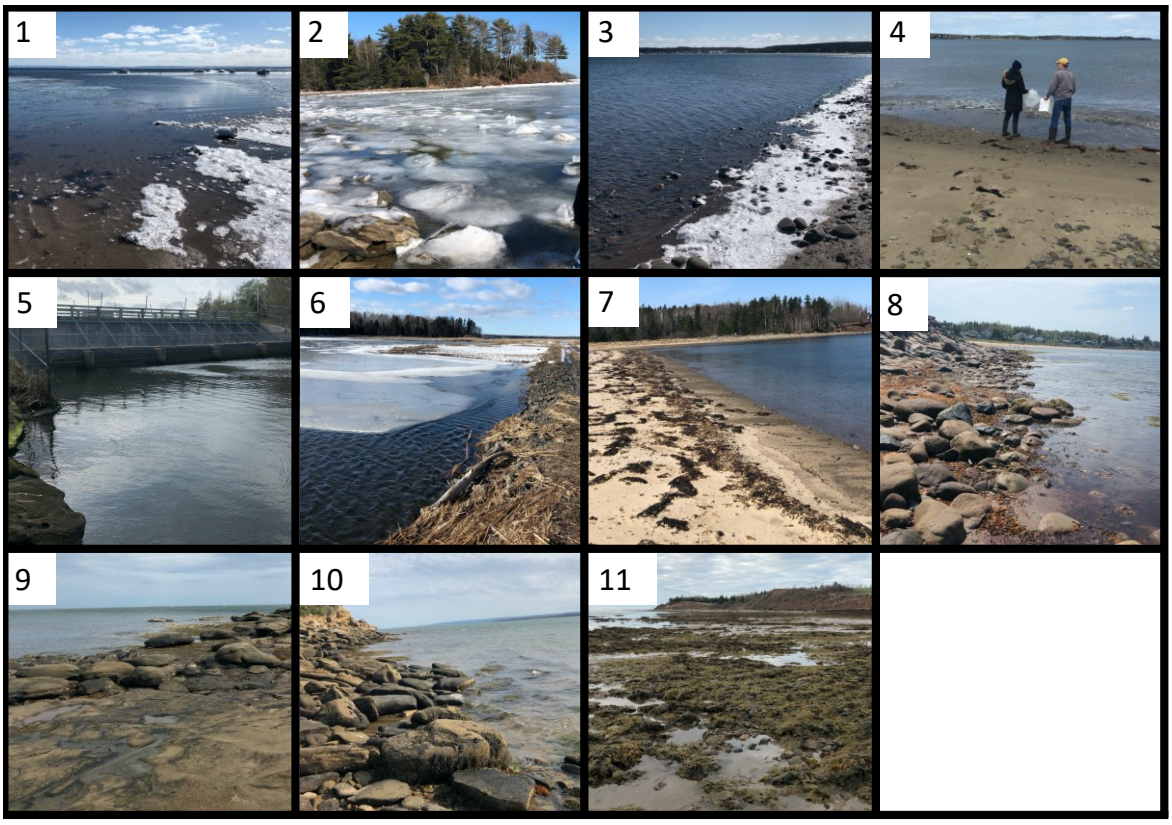

Figure 3.2 Photos of sampling locations 1) Caribou Island 2) Seaview Cemetery 3) Harbour Lights 4) Lighthouse Beach 5) Boat Harbour Outflow 6) Halfway Point 7) Mackenzie Head 8) Chance Harbour 9) Black Point 10) Melmerby Beach 11) Big Island.

3.2.2 Sample Collection

Particulate organic matter (POM) and macroalgae (*Fucus veisculosus*) were collected as representatives of the food web base. POM was collected during three seasons (Winter; March 5-7, 2021, Spring; April 25-28, 2021, and summer; July 28-31, 2021) to assess temporal baseline shifts in $\delta^{15}N$ and $\delta^{13}C$ values. For POM collection, triplicate water grabs were retrieved at each sampling location using plastic carboys at a depth of approximately 30 cm below the water surface. Macroalgae was collected April 25-28, 2021. To ensure sample integrity, all macroalgae samples were collected from mature specimens attached to rock substrates, rather than free

floating. At each location, macroalgae samples (*n*=3) of approximately 20 g each were rinsed of epibionts using deionized water before storage at -20°C.

Consumer species, including both invertebrates and fish, were selected based on their abundance along the Northumberland Strait coast and their ease of collection. The following consumers were collected: periwinkles (*Littorina littorea*), blue mussels (*Mytilus edulis*), mummichogs (*Fundulus heteroclitus*), rock crab (*Cancer irroratus*), and American lobster (*Homarus americanus*). Prior to collection, a scientific collection permit (Section 52 License No. SG-RHQ-21-007) was obtained from DFO, Gulf Region. Additionally, all methods of collection and euthanasia for invertebrates and fish were approved prior to collection by Dalhousie University Committee on Laboratory Animals (Protocol No. 20-132).

Corresponding to the sampling locations for each organism (Table 3.2; Figure 3.1), periwinkles (*n*=24) and blue mussels (*n*=15) were collected opportunistically by hand at low tide May 25-29, 2021. Periwinkle shell height (length of shell from apex to aperture) and mussel shell length were measured using a Vernier caliper to the nearest 0.1 mm and ranged from 15.0-20.0 mm and 40.0-60.0 mm, respectively. Periwinkles and mussels were immediately placed on ice in the field and were frozen upon return to the laboratory at -20°C.

Mummichog were collected July 13-14, 2021, using a 20 m beach seine (6 mm mesh size) trawled over predominantly sandy substrate near littoral vegetation. At each location, mummichogs (*n*=3) were captured, identified, and euthanized in the field following the Canadian Council on Animal Care (CCAC)'s two-step euthanasia procedure (CCAC, 2010). Mummichogs were dosed in a 0.01% solution of MS-222, followed by cervical dislocation. Mummichog total length ranged from 70.0-110.0 mm. Mummichogs were then placed on ice and frozen (-20°C) upon return to the laboratory.

Rock crab and American lobster were collected June 7-15, 2021, by coordinating with local commercial harvesters during the lobster fishing season (Fishing District 26A). Triplicate samples of both rock crab and American lobster were collected from each location and immediately placed on ice. Carapace length of rock crab and American lobster ranged from 95.0-130.0 mm and 80.0-99.0 mm, respectively. Both rock crab and American lobster were frozen (-20°C) after returning to the laboratory. Follow-up measurements of water temperature and salinity (n=3) were conducted at each sampling location with a YSI 650 MDS Sonde on March 26 and 27, 2022. It was not apparent that these parameters were influencing the stable isotope results until the data was analyzed, explaining the temporal differences in sampling of biota and water quality parameters.

3.2.3 Sample Processing

In the laboratory, POM was filtered onto pre-combusted (450°C, 4 h) glass microfibre filters (Whatman GF/F, 47 mm diameter) using a vacuum pump. Filter papers were frozen until the time of processing, at which point, they were freeze-dried (-60°C, 24 h). A quarter of each freeze-dried filter paper was sent to the Stable Isotopes in Nature Laboratory (SINLAB) at the University of New Brunswick, Fredericton, NB for bulk stable isotope analysis of carbon and nitrogen. Macroalgae samples were freeze-dried at -60°C for 48 h. Dried individual samples were ground into a fine, homogenous powder using a mortar and pestle. While *F. vesiculosus* exhibits apical growth, meaning that new tissues grow only at the tips of the plant's fronds, whole frond tissue of each sample was homogenized and used for the analysis. Determining isotope variation in different regions of the plant was beyond the scope of this study; however, isotope variability has been shown in different morphological regions of macroalgae (Howarth et

al., 2019; Viana et al., 2014). Aliquots of ground samples were weighed to an average of 3.2 ±0.1 mg in tin capsules (Isomass 8 x 5 mm pressed capsules). Samples were sent to the SINLAB for bulk stable isotope analysis of carbon and nitrogen. For periwinkles, foot muscle tissue was used for stable isotope analysis due to its high protein and low lipid content (Warnakulasooriya et al., 2017). After thawing, snail visceral mass was removed from the shell, the foot muscle tissue was isolated from the remaining visceral mass, and the operculum was removed. The foot tissue was rinsed with deionized water after isolation. Foot muscle tissue from eight individuals was pooled to create one sample of sufficient mass to enable stable isotope analyses. For mussel sample preparation, adductor muscle tissue was selected for analysis due to its high protein content and low tissue turnover rate (Gorokhova & Hansson, 1999). Muscle tissue from three individuals were pooled to create one composite sample with sufficient mass to enable stable isotope analyses. Sample preparation of mummichogs involved isolation of dorsal white muscle tissue for stable isotope analyses (Skinner et al., 2012). For both rock crab and American lobster, the crusher claw muscle tissue was selected for stable isotope analyses. The claw was isolated, split apart, and muscle tissue was removed. Muscle tissue samples from the periwinkles, blue mussels, mummichogs, rock crab, and American lobster were rinsed with deionized water following isolation. Muscle tissue of all species were freeze-dried (-60°C, 48 h) before grinding each sample into fine, homogenous individual powders using a mortar and pestle. Aliquots of each sample (1.5 ±0.3 mg) were weighed into tin capsules and were sent to the SINLAB for bulk stable isotope analysis of carbon and nitrogen.

3.2.4 Laboratory Analyses

Dried, ground, and homogenized individual samples were analyzed for carbon and nitrogen stable isotope ratios using an Elemental Analyzer (Carlo Erba NC2500, Italy) coupled to a Continuous Flow-Isotope Ratio Mass Spectrometer (Finnigan Mat Delta Plus, Thermo Finnigan, Bremen, Germany) at the SINLAB. Stable isotope measurements are reported in delta (δ) notation in parts per thousand (‰) according to the equation (Eq. 1):

$$\delta^{13}C \text{ or } \delta^{15}N=[(R_{sample}/R_{standard}) - 1] \text{ X } 1000 \qquad \text{[Eq. 1]}$$

Where R_{sample} is the isotope ratio for the sample and $R_{standard}$ is the isotope ratio of the standard (PeeDee Belemnite (PDB) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$). Measured isotope ratios were corrected on a 3- or 4-point scale using in-house working standards (for animal tissues: caffeine, bovine liver and muskellunge muscle; for plant materials: corn meal, seaweed plant, Spiruline, and Ephedra) that were calibrated against International Atomic Energy Agency (IAEA) standards and were tested for verification as part of the SINLAB quality assurance and quality control (QA/QC) protocols. Analytical precision, measured as the standard deviation of repeat analyses of standards, averaged 0.04‰ and 0.08‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Approximately 20% of plant and animal tissue samples (*n*=28) were submitted in duplicate for quality control and the difference (mean ±standard error, SE) between replicates for $\delta^{13}C$ and $\delta^{15}N$ was 0.20 ±0.09 ‰ and 0.18 ±0.07‰, respectively.

Correction of $\delta^{13}C$ data for lipid content was performed on animal tissues with C:N ratios >3.5 following recommendations in Post et al. (2007). Periwinkles were the only animal tissue

that exceeded this value (mean C:N ratio of 3.89 ±0.12). The following equation (Eq. 3) (Post et al., 2007) was used to correct periwinkle $\delta^{13}C$ values:

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + (0.99)\,(C:N) \quad \text{[Eq. 3]}$$

Additionally, all macroalgae $\delta^{13}C$ data were corrected for lipids, regardless of lipid content, again following recommendations in Post et al. (2007). The following equation (Eq. 4) (Post et al., 2007) was suggested for plant species when mean %C in samples is <40% (mean %C in macroalgae samples was 33.41 ±5.07%):

$$\Delta\delta^{13}C = -3.02 + 0.09\,(\%Carbon) \quad \text{[Eq. 4]}$$

3.2.5 Trophic Position Calculations

To calculate the trophic position of consumers (periwinkles, blue mussels, mummichogs, rock crab, and American lobster), a $\delta^{15}N$ baseline for each location was calculated by pooling POM $\delta^{15}N$ values for the three different sampling seasons. After establishing a mean $\delta^{15}N$ baseline value for each location, the following equation (Eq. 2) from Post (2002b) was used to calculate trophic position of each organism at each sampling location:

$$TP = \lambda + [(\delta^{15}N_{Consumer} - \delta^{15}N_{Base}) / \Delta_n] \quad \text{[Eq. 2]}$$

Where λ is the trophic position of the baseline indicator used to estimate the $\delta^{15}N_{Base.}$ λ was given a value of 1.2 (Vinagre et al., 2012) since POM constitutes a wide range of not just

primary producers but also microbial heterotrophs, detritus, and microzooplankton (Lorrain et al., 2015). $\delta^{15}N_{Consumer}$ is the measured value in each individual sample for each organism, $\delta^{15}N_{Base}$ is the calculated isotope baseline for each location, and Δ_n is the enrichment in $\delta^{15}N$ per trophic level (3.4‰ (Minagawa & Wada, 1984; Post, 2002b).

3.2.6 Statistical Analyses

All statistical analyses were completed using SPSS statistical package (version 27). Prior to completing statistical analyses, model residuals were assessed for normality and homogeneity of variance using Shapiro-Wilk and Levene's Test, respectively. To test for significant seasonal and/or spatial differences in POM $\delta^{15}N$ and $\delta^{13}C$, separate one-way analyses of variance (ANOVA) were conducted. Where significant differences were detected among seasons for a given location or among locations within each individual season, pairwise comparisons were evaluated using a Bonferonni correction.

To evaluate spatial differences in stable isotope values (both $\delta^{15}N$ and $\delta^{13}C$) for macroalgae and higher-trophic-level organisms, individual one-way ANOVA analyses were performed among sampling locations for each organism. Where significant differences among locations were detected, pairwise comparisons were evaluated using a Bonferonni correction. Statistical analyses were also performed on trophic position data to evaluate variation in trophic position for each organism among sampling locations. Individual one-way ANOVA analyses were completed for trophic position of each organism with sampling location as the factor. Where significant differences in trophic position among locations was detected for an organism, pairwise comparisons were evaluated using a Bonferonni correction. To determine if the size of

each organism impacted its trophic position, a linear regression between individual trophic position and organism size was performed for each organism.

For all statistical tests performed, if the model residuals did not meet the assumptions for parametric testing, data was first log-transformed to attempt to correct the heterogeneity among the data. Where log-transforming the data failed to correct the heterogeneity, a non-parametric Kruskall-Wallis H test was completed. For *post-hoc* analyses when data did not meet the assumptions of parametric data, a Mann-Whitney U test using a Bonferonni-adjusted significance level was performed. For certain analyses with non-parametric data sets, despite a Kruskall-Wallis test indicating significant differences among seasons, a pairwise Mann-Whitney test was unable to identify significant interaction effects. This is likely the result of a small sampling size and variance in the results being spread across many pairwise treatments in the analysis. Finally, linear regression was used to investigate the relationship between salinity values at each location and both $\delta^{15}N$ and $\delta^{13}C$ values for each organism. For organisms collected in estuarine locations, those salinity values were used in the analysis rather than the offshore salinity value.

3.3 Results

3.3.1 Salinity Analysis

Surface salinity values were measured at each location and a discernible trend was identified with lower salinity values at locations closest to Boat Harbour (Figure 3.3). Namely, the two locations closest to Boat Harbour (BHO and HWP) each had salinity values <10 ppt (mean salinity value (n=3): 6.1 ±0 ppt and 9.1 ±0 ppt for BHO and HWP, respectively). These values contrast with locations with the highest salinity: MLB and CRI which had mean salinity

values (n=3) of 30.9 ±0 ppt and 30.8 ±0.01 ppt, respectively. As there were sampling locations where organisms were collected at both offshore and estuarine sites, salinity values were measured at both locations. Salinity in the estuarine locations was expectedly lower than the offshore locations for each respective sampling location (CRI, CNH, MLB; Figure 3.3).

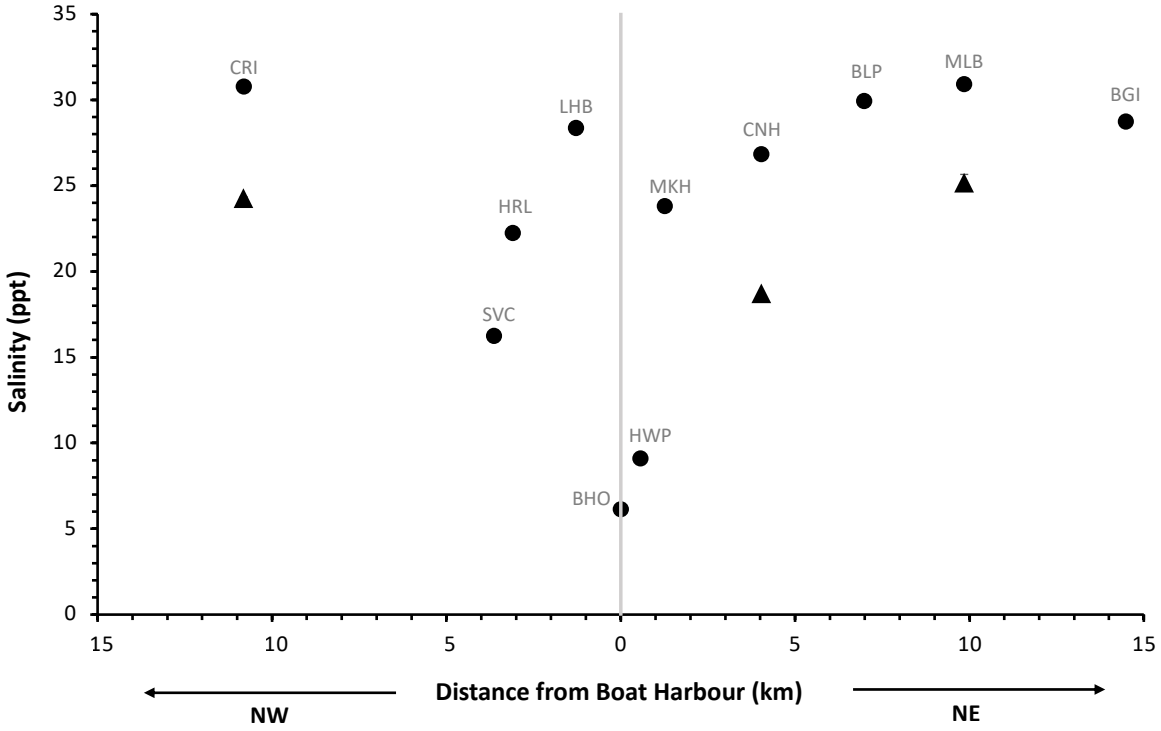

Figure 3.3 Mean (±SE) salinity values for each sampling location as a function of their distance from the former Boat Harbour outflow (BHO). Circles indicate offshore sampling locations and triangles represent estuarine sampling locations. Letters denote abbreviated sampling location names.

Linear regression analyses completed between salinity values at each sampling location and the stable isotope values for each organism determined a positive relationship between both $\delta^{13}C$ and salinity and $\delta^{15}N$ and salinity for most organisms. A trend of increasing $\delta^{13}C$ and $\delta^{15}N$ with increasing salinity was observed (Figures 3.4 and 3.5). $\delta^{13}C$ values in organisms had a

stronger relationship with salinity than did $\delta^{15}N$ values. The strongest relationship identified was both $\delta^{13}C$ and $\delta^{15}N$ values for lobsters ($\delta^{13}C$: $R^{2=}0.756$, p=0.01, and $\delta^{15}N$: $R^{2=}0.647$, p=0.029). A strong positive relationship was also identified for mussel $\delta^{13}C$ ($R^{2=}0.579$, p=0.079). Appendix C provides salinity and temperature data for each sampling location and results of the linear regression analyses for salinity and stable isotope values ($\delta^{13}C$ and $\delta^{15}N$).

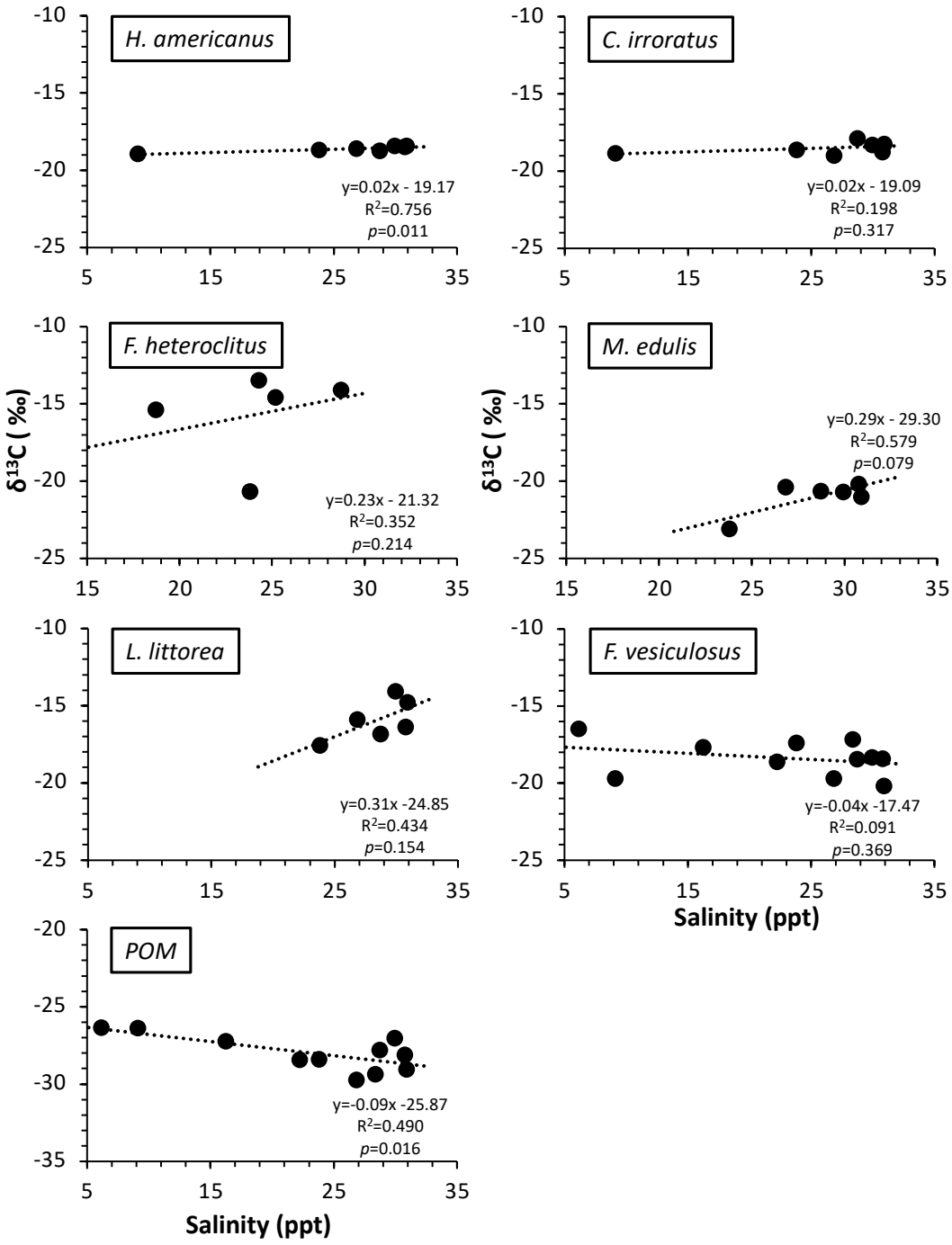

Figure 3.4 Linear regression between $\delta^{13}C$ (‰) values for each organism and salinity (ppt) at all sampling locations.

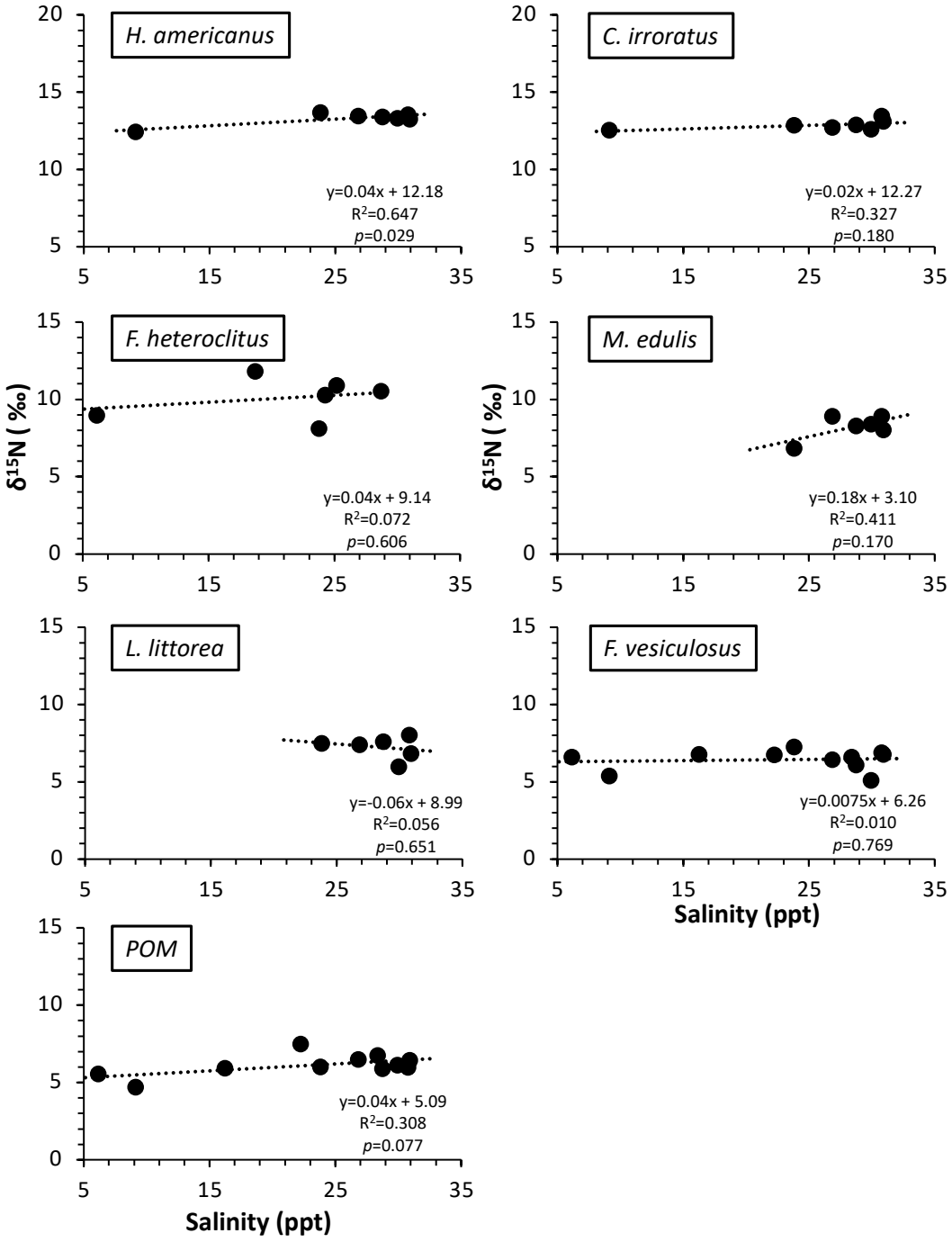

Figure 3.5 Linear regression between $\delta^{15}N$ (‰) values for each organism and salinity (ppt) at all sampling locations.

3.3.2 Variation in Stable Isotope Baseline

Temporal variation in POM stable isotope values among locations was greatest for $\delta^{13}C$, with nine out of eleven sampling locations having significantly different $\delta^{13}C$ values between the three sampling seasons (winter, spring, and summer). Overall, $\delta^{13}C$ values among sampling locations were significantly more depleted in winter (-29.71 to -26.34‰) compared to summer (-26.21 to -21.42‰) (Figure 3.6). Temporal variation in $\delta^{13}C$ was greatest at locations furthest northeast (BGI: -27.42 to -21.42‰) and northwest (CRI: -28.11 to -21.69‰) from the Boat Harbour Outflow (BHO), with the range of $\delta^{13}C$ values among seasons converging at BHO (-27.38 to -26.21‰) (Figure 3.6). In contrast, POM $\delta^{15}N$ values did not demonstrate a strong seasonal effect among locations, with only three (HRL, BHO, and MKH) out of eleven locations having significant differences in $\delta^{15}N$ values among seasons. The greatest seasonal variation in $\delta^{15}N$ was observed at the BHO location with significantly depleted $\delta^{15}N$ values in summer (winter, spring; $p<0.001$) (Figure 3.6).

Spatial variability in POM stable isotope values among locations was observed for $\delta^{13}C$ in all three sampling seasons (winter; $p<0.001$, spring; $p=0.006$, summer; $p=0.001$). High variation in $\delta^{13}C$ values among locations was observed during the winter, with no discernible spatial trend observed along the Northumberland Strait coastline (Figure 3.6). However, a slight spatial trend in POM $\delta^{13}C$ was observed during both spring and summer with depleted $\delta^{13}C$ values at the BHO location and locations directly northwest (LHB) and northeast (HWP, MKH) from the former outflow point (Figure 3.6). Within these two sampling seasons, $\delta^{13}C$ values became enriched with increasing distance in both the northeast and northwest direction (Figure 3.6). For POM $\delta^{15}N$ values, there were no significant differences in $\delta^{15}N$ values among locations for winter ($p=0.05$) or spring ($p=0.101$). A significant spatial difference in POM $\delta^{15}N$ values was

detected for the summer sampling period ($p<0.001$). A well pronounced spatial trend in $\delta^{15}N$ values in the summer sampling period resulted in significantly depleted $\delta^{15}N$ at the BHO location ($n=3$; mean 1.58 ±0.14‰). Mean $\delta^{15}N$ values ($n=3$) became increasingly enriched as distance increased from BHO in both the northeast (CRI; 6.15 ±0.22‰) and northwest (BGI; 4.96 ±0.29‰) direction (Figure 3.6). POM $\delta^{15}N$ at the BHO location was significantly depleted relative to all other sampling locations ($p<0.001$ for all 10 interaction effects). Appendix C provides the mean $\delta^{13}C$ and $\delta^{15}N$ values of POM for each sampling season among locations and results of seasonal and spatial statistical analyses for POM.

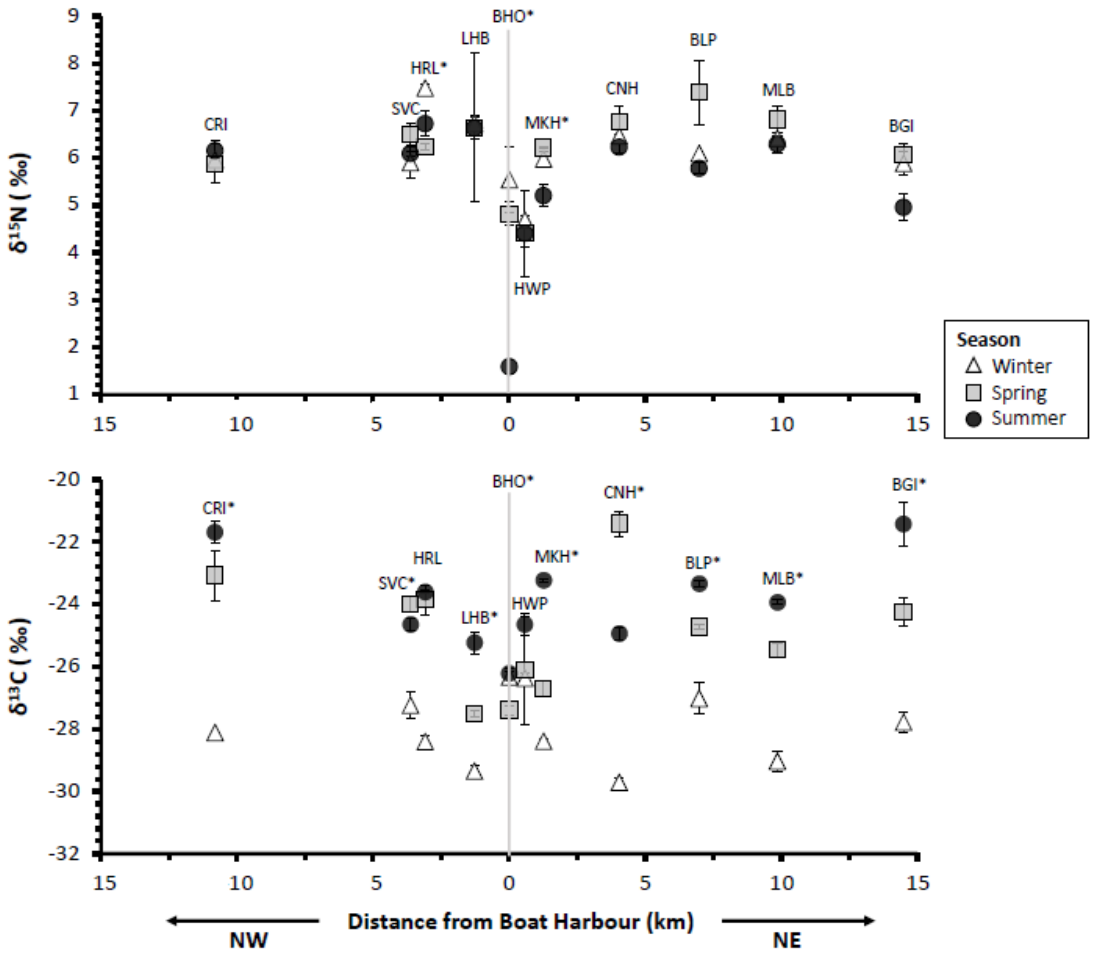

Figure 3.6 Mean (±SE) $\delta^{15}N$ (top) and $\delta^{13}C$ (bottom) values for POM among three sampling seasons (winter, spring, and summer) for each sampling location. * Indicates significant differences in stable isotope values among seasons within each given location. Letters denote the sampling locations.

3.3.3 Spatial Variation in $\delta^{13}C$ and $\delta^{15}N$ values

Spatial variation in $\delta^{13}C$ and $\delta^{15}N$ values was identified along the Northumberland Strait coastline, with certain organisms exhibiting more pronounced spatial trends in stable isotope values than others. Macroalgae (*F. vesiculosus*) tissue had significant spatial differences in both $\delta^{13}C$ (p=0.001) and $\delta^{15}N$ (p=0.001) values among locations. However, high spatial variability in the two stable isotope values was observed along the coastline, with no discernible spatial trends

along the gradient of historical pulp mill effluent exposure (Figures 3.7 and 3.8). Significant spatial differences were also observed for periwinkles (*L. littorea*). Both periwinkle $\delta^{13}C$ ($p<0.001$) and $\delta^{15}N$ ($p<0.001$) values were significantly different among sampling locations. Like macroalgae, there was high spatial variability in both stable isotope values along the coastline, with no discernible spatial trends relative to the former Boat Harbour outflow (Figures 3.7 and 3.8). While no linear spatial pattern was detected along the gradient in the northeast and northwest direction from the former Boat Harbour outflow, periwinkles collected at the BHO location exhibited the lowest $\delta^{13}C$ values (Figure 3.7). Additionally, periwinkles collected at the BLP location had noticeably enriched $\delta^{13}C$ and depleted $\delta^{15}N$ (Figures 3.7 and 3.8, respectively) relative to all other sampling locations. American lobster (*H. americanus*) did not demonstrate any significant spatial differences in $\delta^{13}C$ ($p=0.178$) nor $\delta^{15}N$ ($p=0.116$) values among locations. Additionally, high variability in stable isotope values of lobsters within each individual location was observed (Figures 3.7 and 3.8). Therefore, while mean stable isotope values (both $\delta^{15}N$ and $\delta^{13}C$) of lobsters were lowest at the sampling location nearest the outflow (HWP), any statistical differences by location were obscured by high variability in stable isotope values among individual lobsters. Significant spatial variation was observed for rock crab (*C. irroratus*) $\delta^{13}C$ values ($p=0.04$) although no statistically significant differences in rock crab $\delta^{15}N$ values ($p=0.113$) were identified. Crab $\delta^{13}C$ values had a high degree of spatial variation along the coastline (Figure 3.7). Like lobster stable isotope values, crab $\delta^{13}C$ values were low at the location nearest Boat Harbour (HWP ($n=3$): mean -18.84 ±0.42‰). However, a high degree of variation in $\delta^{13}C$ values among individual crabs collected at the HWP location was observed (Figure 3.7). $\delta^{13}C$ values for crabs showed a spatial trend, with increasing $\delta^{13}C$ values to the northeast of the former Boat Harbour outflow (Figure 3.7). Crab $\delta^{15}N$ values fell within a narrow

range (12.53 to 13.42‰), with no discernible spatial variation in these values along the coastline (Figure 3.8).

The two organisms that demonstrated the highest degree of spatial variation and an observable spatial trend in both $\delta^{13}C$ and $\delta^{15}N$ values from the former Boat Harbour outflow were blue mussels (*M. edulis*) and mummichogs (*F. heteroclitus*). Mussels had significant spatial differences in both $\delta^{13}C$ ($p<0.001$) and $\delta^{15}N$ ($p<0.001$) among locations. Mummichogs also showed significant differences among sampling locations for both $\delta^{13}C$ ($p<0.001$) and $\delta^{15}N$ ($p<0.001$). Overall, a trend for both mussels and mummichogs was depleted $\delta^{13}C$ and depleted $\delta^{15}N$ at locations nearest the former Boat Harbour outflow (Figures 3.7 and 3.8). Since mussels were not present at the BHO location, the closest sampling location for mussels to the Boat Harbour outflow was Mackenzie Head (MKH). Mussels collected from the MKH location had $\delta^{13}C$ values that were significantly depleted ($n=5$; mean -23.09 ±0.16‰) relative to all other sampling locations ($p<0.001$ for all interaction effects). Mussel $\delta^{13}C$ values at MKH were 2.88‰ lower than the furthest sampling location to the northwest, CRI. Mussels from all other sampling locations occupied a narrow $\delta^{13}C$ range (-21.02 to -20.21‰) (Figure 3.7) with similar results observed for mussel $\delta^{15}N$ values. Mussels collected at the MKH location had depleted $\delta^{15}N$ values (mean ($n=5$): 6.82 ±0.06‰), significantly different from values at four of the five other sampling locations. Mussel $\delta^{15}N$ values from all other sampling locations occupied a narrow range (8.01 to 8.92‰) (Figure 3.8) although mussel $\delta^{15}N$ values at MKH were 2.10‰ lower than the furthest sampling location to the northwest, CRI. Within a given location, there was very low variation among individual mussel samples (Figures 3.7 and 3.8).

Mummichogs were sampled at both the BHO and MKH locations and these two locations were significantly different from all other locations with respect to both their $\delta^{13}C$ ($p<0.001$ for

all interaction effects) and $\delta^{15}N$ values ($p<0.001$ for all interaction effects). Mummichog $\delta^{13}C$ values were depleted at the BHO and MKH locations (mean (n=3): -20.08 ±0.06‰ and -20.67 ±0.15‰, respectively). For mummichogs collected at all other sampling locations along the coastline, $\delta^{13}C$ values fell within a narrow range (-15.38 to -13.47‰) (Figure 3.7) although mummichog $\delta^{13}C$ values at MKH were 7.20‰ lower than the furthest sampling location to the northwest, CRI. Additionally, mummichog $\delta^{15}N$ values were depleted at both the BHO and MKH locations (mean (n=3): 8.97 ±0.35‰ and 8.11 ±0.20‰, respectively) (Figure 3.8). Mummichog $\delta^{15}N$ values at MKH were 2.16‰ lower than the furthest sampling location to the northwest, CRI. Like $\delta^{13}C$, the $\delta^{15}N$ values of mummichogs collected at all locations (excluding BHO and MKH) fell within a narrow range (10.27 to 11.80‰) (Figure 3.8). Like mussels, there was little variation in both stable isotope values among individual samples at each given sampling location (Figures 3.7 and 3.8).

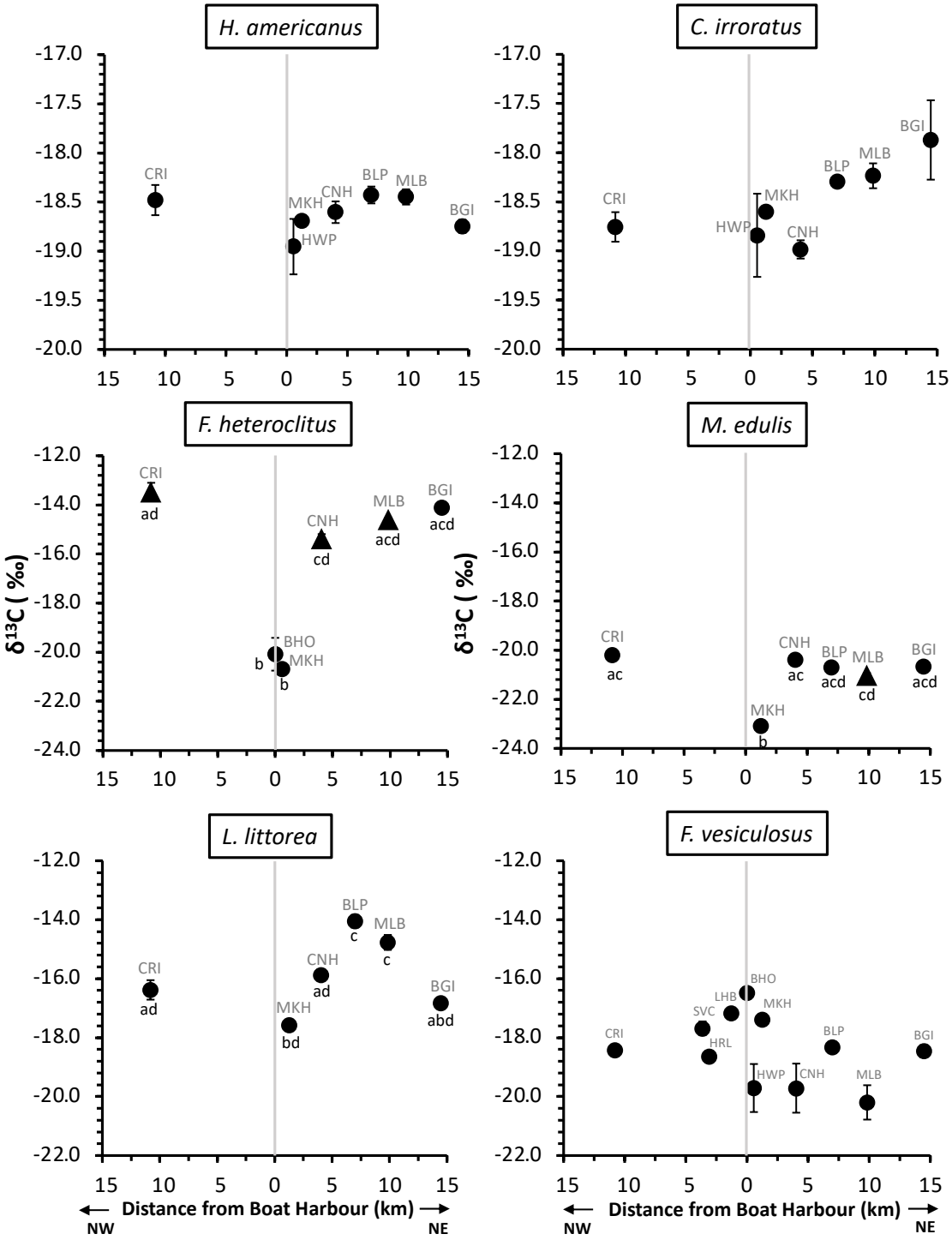

Figure 3.7 Mean (±SE) $\delta^{13}C$ values for macroalgae and consumers sampled April-July 2021 at each location. Solid circles represent organisms collected in offshore sampling locations and triangles represent organisms collected in estuarine locations. Uppercase letters indicate sampling locations and lowercase letters indicate similarities between locations.

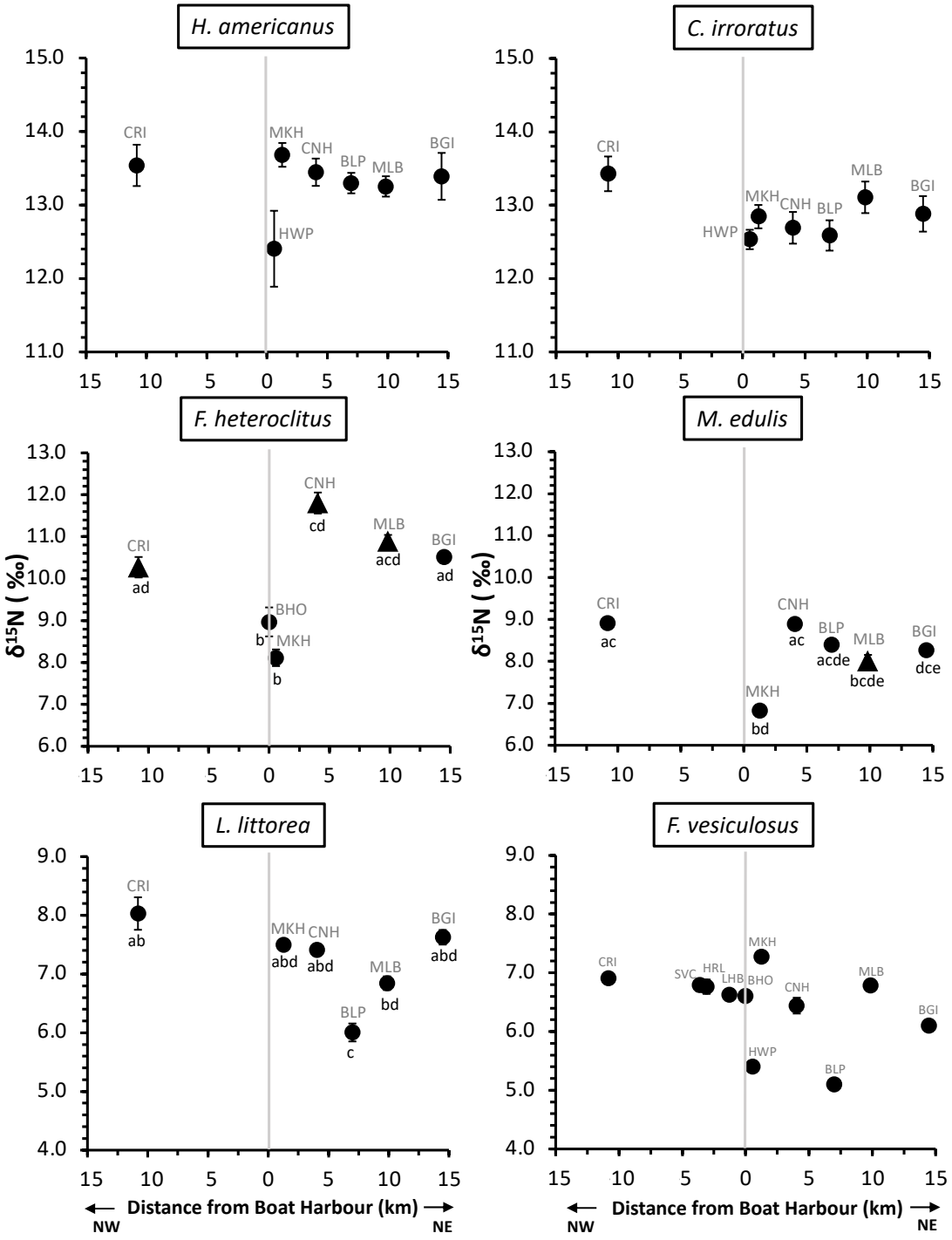

Figure 3.8 Mean (±SE) $\delta^{15}N$ values for macroalgae and consumers sampled April-July 2021 at each location. Solid circles represent organisms collected in offshore sampling locations and triangles represent organisms collected in estuarine locations. Uppercase letters indicate sampling locations and lowercase letters indicate similarities between locations.

Appendix D provides the mean $\delta^{13}C$ and $\delta^{15}N$ values of macroalgae and consumer organisms for each sampling season among locations and results of the one-way ANOVA and pairwise comparisons analyses.

3.3.4 Trophic Position and Food Chain Length

Significant differences in mean trophic position among sampling locations were identified for all species: periwinkle ($p<0.001$), mussel ($p<0.001$), mummichog ($p<0.001$), crab ($p<0.001$), and lobster ($p=0.033$). Linear regression analysis indicated that organism size did not affect the trophic position of any organism included in the study: periwinkle ($p=0.252$; $R^{2=}0.065$), mussel ($p=0.135$; $R^{2=}0.065$), mummichog ($p=0.175$; $R^{2=}0.090$), crab ($p=0.057$; $R^{2=}0.161$), and lobster ($p=0.221$; $R^{2=}0.062$). This was likely a result of only similar-sized organisms being selectively collected in the field.

Periwinkles occupied a low trophic position at all sampling locations (range 1.06 to 1.79). Trophic position of periwinkles was the lowest at the BLP location (mean ($n=3$): 1.06 ±0.04) (Figure 3.9), which was significantly different from all other sampling locations for periwinkles. Crab and lobster both followed a very similar spatial trend in their mean trophic position among sampling locations (Figure 3.9). There was high spatial variation among the two organisms' trophic positions; however, mean trophic position of both species was highest at the closest sampling location to Boat Harbour (HWP) (mean trophic position of crab and lobster at HWP ($n=3$): 3.56 ±0.04 and 3.53 ±0.15, respectively). For crab, the trophic position at the HWP location was significantly different from three of the five other sampling locations. For lobster, significant interaction effects were unable to be detected in a Mann-Whitney pairwise analysis, despite a Kruskall-Wallis test detecting significant differences in trophic position among

locations. The two organisms that exhibited the greatest spatial differences in trophic position among sampling locations were mussels and mummichogs. Mussel trophic position was the lowest at the location nearest the former Boat Harbour outflow (MKH) (mean (n=5) 1.50 ±0.02) (Figure 3.9). Mussel trophic position at this location was significantly different from four out of the five other sampling locations for mussels (CRI, CNH, BLP, BGI; p=0.02). The trophic position of mussels at the MKH location was 0.50 positions less than the furthest sampling location to the northwest, CRI (Figure 3.9). Like mussels, mummichogs also occupied a lower trophic position at the MKH location (mean (n=3) 1.88 ±0.06) (Figure 3.9). Trophic position at this location was significantly different from all other sampling locations for mummichogs (p<0.001 for all five interaction effects), including mummichogs collected at the BHO location. Interestingly, mummichog trophic position at the MKH location was lower than the BHO location by a value of 0.79 (Figure 3.9). Except for the MKH location, there was little spatial variation in mummichog trophic position along the coastline, with trophic position at all other locations occupying a narrow range (2.45 to 2.76) (Figure 3.9). Appendix E provides the mean trophic position of all organisms across sampling locations, results of the one-way ANOVA and pairwise comparisons analyses, and the linear regression analyses.

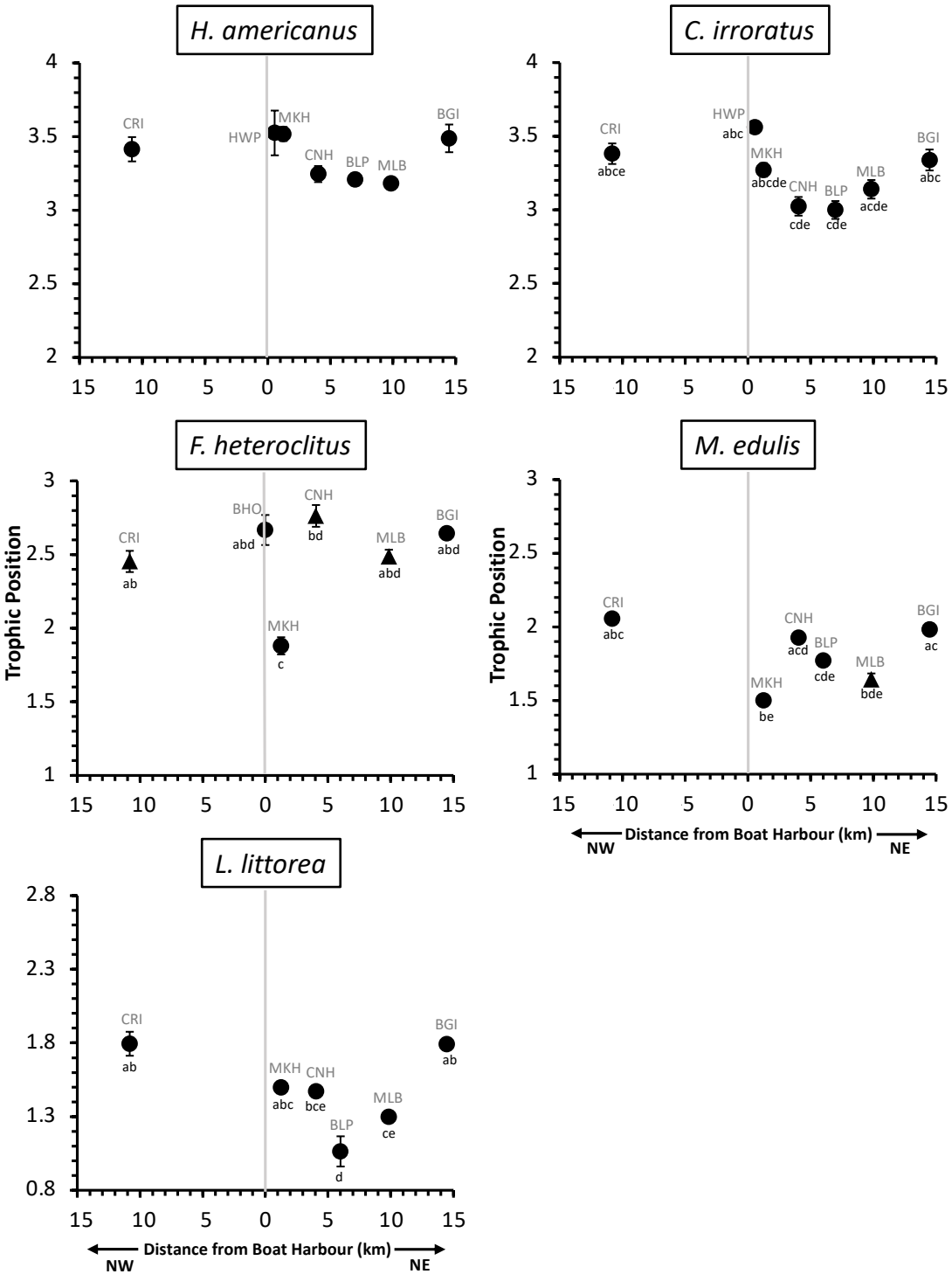

Figure 3.9 Mean (±SE) trophic position of consumer species. Shaded circles represent species collected in offshore locations and triangles indicate species collected in estuarine locations. Uppercase letters indicate sampling locations and lowercase letters indicate similarities between locations.

3.3.5 Food Web Dynamics

Biplots of the $\delta^{13}C$ and $\delta^{15}N$ values for all consumer species suggest that food web structure was different at locations nearest the former Boat Harbour outflow (Figure 3.10, Figure 3.11). These differences in food web structure were primarily driven by mussel and mummichog $\delta^{13}C$ and $\delta^{15}N$ values. Mussels from the MKH location were depleted in $\delta^{13}C$ and $\delta^{15}N$. Mummichogs from both MKH and BHO were also depleted in $\delta^{13}C$ and $\delta^{15}N$, occupying a distinct trophic niche from mummichogs collected at other sampling locations along the coastline. Based on the stable isotope biplots (Figures 3.10 and 3.11), mummichogs from both MKH and BHO appear to be feeding on similar sources of carbon to mussels. At the MKH location and the BHO location for mummichogs, these two species are feeding at lower overall trophic positions. Periwinkles occupied a similar trophic niche among locations, except for the BLP location which exhibited depleted $\delta^{15}N$ and enriched $\delta^{13}C$ values (Figure 3.10). Mussels from the MKH location appear to be sharing similar dietary sources of nitrogen as periwinkles (Figure 3.10). Additionally, mussels from MKH appear to be feeding on more terrestrially-derived POM relative to mussels collected at other sampling locations along the coastline (Figure 3.10). Little variation existed in $\delta^{13}C$ and $\delta^{15}N$ values for both lobsters and crabs, with these two species occupying the highest trophic positions in the coastal food web (Figure 3.10).

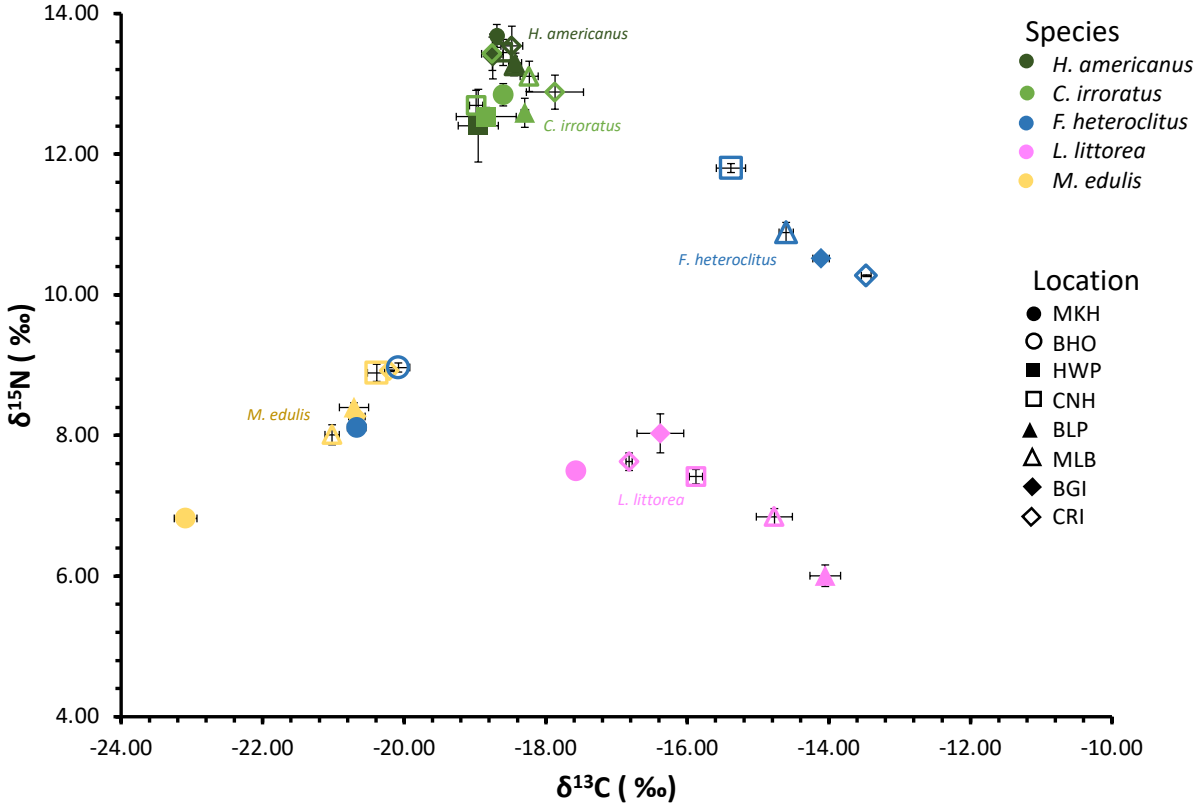

Figure 3.10 Stable isotope biplot displaying mean (±SE) $\delta^{13}C$ and $\delta^{15}N$ values for consumer species at all sampling locations.

Grouping sampling locations into sites near Boat Harbour (BHO, HWP, and MKH) and reference locations (all other sampling locations in the study) illustrates differences in trophic niche space between species from these two aggregated locations (Figure 3.11). Most consumer organisms have lower $\delta^{13}C$ and lower $\delta^{15}N$ adjacent Boat Harbour relative to reference locations, especially in mussels and mummichogs.

Mummichogs from locations near Boat Harbour have a very similar stable isotope signature to mussels from reference locations (Figure 3.11). Lobsters and crabs have similar stable isotope signatures and occupy a similar trophic niche within the food web, with both species having lower $\delta^{15}N$ and $\delta^{13}C$ at locations near Boat Harbour (Figure 3.11). The exception to the trend of

depleted $\delta^{15}N$ and depleted $\delta^{13}C$ was observed with macroalgae and periwinkles (Figure 3.11). Macroalgae at locations near Boat Harbour were lower in $\delta^{15}N$ and higher in $\delta^{13}C$ relative to reference locations (Figure 3.11). Periwinkles at locations near Boat Harbour were lower in $\delta^{13}C$ and higher in $\delta^{15}N$ relative to reference locations (Figure 3.11).

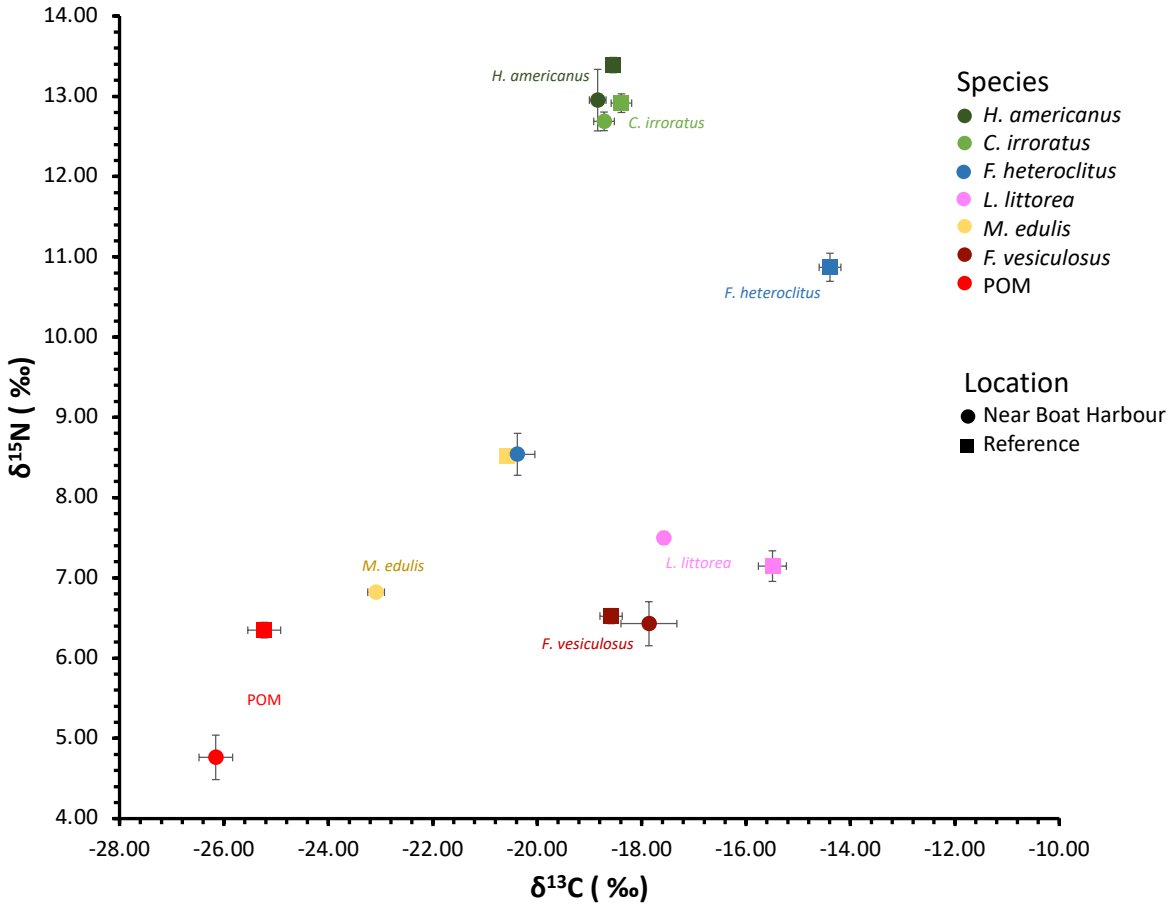

Figure 3.11 Stable isotope biplot displaying mean (±SE) $\delta^{13}C$ and $\delta^{15}N$ values of all organisms included in the study aggregated to relatively pulp mill effluent influenced locations adjacent Boat Harbour and all other sampling locations (Reference).

3.4 Discussion

3.4.1 Variability in POM $\delta^{13}C$ and $\delta^{15}N$

Stable isotope analysis assessed spatial variability in sources of carbon and nitrogen along a gradient in historical pulp mill effluent exposure from Boat Harbour along the Northumberland Strait coastline. Spatial variation was observed in stable isotope values of POM with depleted $\delta^{13}C$ and $\delta^{15}N$ values at locations nearest the former Boat Harbour outflow. There are two main factors that may be responsible for the observed isotopic trends: 1) historical pulp mill effluent exposure and 2) distinct spatial heterogeneity in hydrogeological characteristics along the Northumberland Strait coastline. Anthropogenic pollution can be a distinguishable source of spatial isotope variability (Dailer et al., 2010) with many studies reporting depleted $\delta^{15}N$ and depleted $\delta^{13}C$ values with pulp mill effluent exposure (Freedman et al., 2012). Consequently, the depleted $\delta^{13}C$ and $\delta^{15}N$ values in POM adjacent the former Boat Harbour outflow may be a result of historical exposure and incorporation of pulp mill effluent-derived nutrients. Depleted $\delta^{15}N$ is often indicative of high nutrient environments, such as environments receiving wastewater inputs (Ma et al., 2012). As part of Rayleigh isotope fractionation, primary producers discriminate against the heavier nitrogen stable isotope (^{15}N) during assimilation of nitrate and ammonium (Mariotti et al., 1984). Environments with high nutrient concentrations result in less discrimination against ^{15}N and consequently, have depleted $\delta^{15}N$ signatures (Ryabenko et al., 2012). For carbon stable isotopes, the $\delta^{13}C$ value of pulp mill effluent resembles terrestrial C_3 plants (near -28‰ (Farwell, 2000), reflecting the wood products utilized in the pulping process (Arcisewski et al., 2014). Both depleted $\delta^{13}C$ and $\delta^{15}N$ values of POM near the former Boat Harbour outflow likely reflect a stable isotope signature of pulp mill effluent exposure.

While historical pulp mill effluent exposure may have contributed to the observed variation in stable isotope values adjacent Boat Harbour, another explanation is the spatial heterogeneity and complex coastal environment of the Northumberland Strait. Specifically, the main drivers of spatial variation in POM isotopic values may be differences in nutrient sources among sampling locations, with distinctions between terrestrial and marine nutrient sources. Coastal environments are influenced by both terrestrial and marine domains, with several organic matter sources (Vizzini et al., 2005). The pronounced differences in salinity gradient observed along the coastline, with significantly lower salinity values near Boat Harbour, suggests distinct differences in terrestrial versus marine organic matter sources among sampling locations. The area near Boat Harbour is likely receiving more terrestrial inputs from Boat Harbour and its watershed, subsequently lowering salinity values in this area. Lower salinity values at locations nearest Boat Harbour corresponds to depleted $\delta^{13}C$ and depleted $\delta^{15}N$ values in POM. In contrast, locations with the highest salinity (MLB and CRI) had the most enriched $\delta^{13}C$ and $\delta^{15}N$ values. Variation in stable isotope values along a salinity gradient has been reported in the literature (Davias et al., 2014; Kristensen et al., 2019). Fry (2002) suggests that significant changes in stable isotopes commonly occur across salinity gradients, reflecting differences in the geochemistry and mixing of freshwater and seawater. Stable isotope values typically vary across salinity gradients because of differences in carbon isotope signatures of dissolved inorganic carbon (DIC) and POM, with $\delta^{13}C$ values becoming more depleted as the relative contribution of DIC from freshwater origins increase (Hoeinghaus et al., 2011). Additionally, terrestrial inputs of organic matter into coastal waterbodies are frequently depleted in $\delta^{15}N$ (<5‰; Kendall et al., 1988; Mayer et al., 2002), compared with marine nitrate (NO_3^-) (~5‰; Sigman et al., 2009).

Therefore, it is likely that differences in nutrient sources are partially driving isotopic variability along the Northumberland Strait coastline.

Stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ was completed for POM during winter, spring, and summer to assess seasonal variability in the stable isotope baseline along the Northumberland Strait coastline. Results reinforced the potential drivers of spatial variability are also acting seasonally with $\delta^{13}C$ values demonstrating the most seasonal variability among all sampling locations. Previous studies assessing POM stable isotope values have also reported high seasonal variation in $\delta^{13}C$ values (Vizzini & Mazzola, 2003). This seasonal variability is typically the result of environmental factors that have been shown to influence POM $\delta^{13}C$ such as water temperature, pH, CO_2 concentrations, and phytoplankton growth rates (Laws et al., 1995). Typically, $\delta^{13}C$ values are depleted during winter and spring due to higher terrestrial runoff and subsequent dominance of terrestrial and freshwater POM, relative to marine POM sources (Ye et al., 2017). Additionally, low phytoplankton growth rates during winter result in lower POM $\delta^{13}C$ values attributable to isotopic fractionation associated with carbon fixation (Laws et al., 1995), consistent with the observed results. Temperature thus has a large indirect effect on isotopic fractionation and effects POM $\delta^{13}C$ values. During summer, elevated temperatures lower CO_2 solubility, leading to a subsequent decrease in isotopic fractionation and increases in POM $\delta^{13}C$ values (Savoye et al., 2003). Depleted POM $\delta^{13}C$ values were identified during winter, likely because of increased precipitation and significant snow melt during sampling (March 2021). POM $\delta^{13}C$ values became seasonally enriched during summer, reflecting elevated surface water temperatures and less organic matter decomposition with preferential use of ^{12}C and kinetic fractionation of the heavier ^{13}C isotope in decomposition

(Volk et al., 2018). The seasonal enrichment of POM $\delta^{13}C$ is therefore consistent with seasonal patterns of temperature and precipitation in Atlantic Canada.

In contrast to $\delta^{13}C$ values, POM $\delta^{15}N$ exhibited little seasonal variability across most sampling locations. However, three locations did exhibit significant POM $\delta^{15}N$ seasonal differences, two of which were nearest the former Boat Harbour outflow (BHO and MKH) and were significantly depleted during the summer sampling period. There are four main factors that could potentially drive observed variability in POM $\delta^{15}N$ values: 1) nutrient sources (terrestrial vs marine); 2) nutrient concentrations; 3) dissolved inorganic nitrogen (DIN) speciation; and 4) primary producer community composition.

Depleted POM $\delta^{15}N$ values near Boat Harbour may result from increased terrestrial nutrient inputs into coastal waterbodies as these inputs are frequently depleted in $\delta^{15}N$ (Kendall, 1998). Specifically, during the warmer summer period, nutrient rich terrestrial run-off is retained in the less dense surface layer of seasonally stratified coastal waters, providing an effective physical barrier retaining increased nutrient concentrations in the euphotic zone (McGovern et al., 2020). Water samples for POM filtration were collected near the surface in coastal waters, which may have been highly nutrient-rich with terrestrial carbon during the summer sampling period. Excess nutrients contribute to greater nitrogen isotopic fractionation and lower POM $\delta^{15}N$ values (Savoye et al., 2003). Therefore, an increase in terrestrial nutrients near Boat Harbour may be driving the lower $\delta^{15}N$ values, with a pronounced spatial trend in summer associated with warmer temperatures and increased nutrient concentrations.

Another potential explanation for the depleted $\delta^{15}N$ values of POM near Boat Harbour is the seasonal variation in nitrogen species present within coastal ecosystems. Marine primary producers can use different nitrogen sources (nitrate and ammonium; NO_3^- and NH_4^+), each of

which exhibit different $\delta^{15}N$ signatures (Craine et al., 2015). The $\delta^{15}N$ of ammonium is generally more depleted than the $\delta^{15}N$ of nitrate (Granger & Wankel, 2016). In winter and early spring, primary producers rely on "new" nitrogen in the form of nitrate. During the summer months, most of the nitrate present in the aquatic environment has been used and primary producers rely on recycled ammonia, depleted in $\delta^{15}N$ (Granger & Wankel, 2016). Therefore, depleted $\delta^{15}N$ values are likely to be observed during the summer period if ammonium were the dominant nitrogen species (Hou et al., 2013). Locations nearest the former Boat Harbour outflow may be experiencing this variation in nitrogen speciation to a greater degree than other sampling locations along the coastline due to high ammonium concentrations from the wastewater treatment process. The primary-treated municipal wastewater treatment plant in PLFN, located in close proximity to the former Boat Harbour outflow, is likely also contributing to higher ammonium concentrations in the study area. Additionally, other locations along the coastline may have higher nitrate concentrations during this seasonal period relative to the BHO and MKH locations. These other sampling locations may have increased hydrologic mixing and tidal action making nitrate more readily available to primary producers.

Finally, another potential explanation for depleted POM $\delta^{15}N$ near Boat Harbour is primary producer community composition. Specifically, growth of nitrogen-fixing cyanobacteria near the former outflow point, may explain the lower POM $\delta^{15}N$ in summer. Planktonic cyanobacteria have been observed to fix nitrogen at ecologically significant rates in coastal ecosystems and largely influence their biogeochemistry (Chamberlain et al., 2014). Nitrogen-fixing cyanobacteria counteract the losses of bioavailable nitrogen to the atmosphere through processes of denitrification and anaerobic ammonium oxidation (Bauersachs et al., 2009). Previous studies have identified a strong relationship between nitrogen-fixing cyanobacteria and

depleted $\delta^{15}N$ values of organic matter in aquatic environments (Bauersachs et al., 2009; Lehmann et al., 2004). Fixation of N_2 results in the formation of organic matter with a $\delta^{15}N$ value close to 0‰ (Bauersachs et al., 2009), a value close to what was identified at the BHO location during summer POM sampling (1.58 ±0.14‰, *n*=3). Prolific algal growth was observed in regions near the Boat Harbour outflow during POM summer sampling. While it is unknown if this prolific growth was indeed cyanobacteria, this observation may serve as a potential explanation for the seasonal variation in POM $\delta^{15}N$ values.

3.4.2 Consumer Spatial Variability in $\delta^{13}C$ and $\delta^{15}N$

The trends in stable isotope values for POM along a spatial gradient in historical pulp mill effluent exposure were consistent with the stable isotope values of select consumer species. Distinguishable $\delta^{13}C$ and $\delta^{15}N$ values were identified for certain species at locations near the former outflow of pulp mill effluent from Boat Harbour. The two species that exhibited spatial variation in stable isotope values the most were mussels and mummichogs. Both of these species exhibited depleted $\delta^{13}C$ and depleted $\delta^{15}N$ values at locations nearest Boat Harbour. Other species included in the study (American lobster, rock crab, macroalgae, and periwinkles) did not reveal these pronounced stable isotope differences along a gradient of historical effluent exposure from Boat Harbour.

The main drivers of isotopic differences observed in consumer organisms are likely consistent with the potential explanations for differences in POM stable isotope values. Historical pulp mill effluent exposure and/or spatial heterogeneity along the coastline with varying terrestrial and marine nutrient sources are potential explanations for distinct stable isotope values near Boat Harbour. Depleted $\delta^{13}C$ and $\delta^{15}N$ in mussels and mummichogs

collected near Boat Harbour is consistent with previous studies measuring stable isotope values in biota exposed to pulp mill effluent (Freedman et al., 2012; Galloway et al., 2003). However, release of pulp mill effluent into the Northumberland Strait ceased seventeen months prior to sampling, making it impossible to measure the stable isotope signature of the effluent itself. The absence of a stable isotope signature for effluent presents challenges in the elucidation of a direct relationship between the spatial variation in stable isotope values of biota and their exposure to pulp mill effluent. While exposure to pulp mill effluent may explain these results, it is challenging to draw this direct relationship in the absence of stable isotope values for effluent from Boat Harbour. Dubé et al. (2005) suggest that large natural ranges in stable isotope values, complex food web interactions, and variable biogeochemical conditions are factors that may make it difficult to detect effluent contributions to stable isotope values in biota. These confounding variables and their impacts on the interpretation of results in effluent-receiving environments were evident. To determine if the observed results are indeed attributable to pulp mill effluent, replication of this study following remediation of Boat Harbour is recommended. Following remediation efforts, stable isotope values in the region near Boat Harbour may be altered as a function of time, indicating biota recovery from effluent (Arcisewski et al., 2014). If such alterations in stable isotope values were obtained following Boat Harbour remediation, it may be suggested that historical pulp mill effluent was likely the contributing factor for stable isotope values in biota collected near the former effluent outflow.

An additional factor that may be contributing to the observed variation in stable isotope values along the coastline is the wastewater discharge from PLFN, located 800 m from the former Boat Harbour outflow (Figure 3.1). Sewage wastewater is typically enriched in $\delta^{15}N$ (+10 to +20‰ (Schubert et al., 2013) due to the volatilization of ^{14}N-rich ammonia during the

processes of nitrification and denitrification (Aravena et al., 1993). Progressive enrichment of $\delta^{15}N$ occurs as nitrate is removed during the wastewater treatment process (Kendall, 1998). However, enrichment is dependent on the type of wastewater treatment employed and if the released nitrogen occurs as organic nitrogen, ammonia, or nitrate (Archana et al., 2016). Therefore, while enriched $\delta^{15}N$ is commonly reported in advanced wastewater treatment processes, depleted $\delta^{15}N$ is often reported in primary-treated wastewater (Schmidt et al., 2016). Primary-treated wastewater is released from PLFN and because of northeast prevailing ocean currents, may be incorporated into the stable isotope signatures of biota tissues near the former Boat Harbour outflow, specifically those at Mackenzie Head. Biota from Mackenzie Head were depleted in $\delta^{15}N$, indicating that wastewater release from the PLFN facility may be a persistent source of local nutrients in the region. Future studies may collect samples of the wastewater from the PLFN facility and assess its stable isotope signature to confirm if the depleted values in biota near Boat Harbour may be partially attributed to wastewater release from PLFN.

Hydrological differences among sampling locations are another potential explanation for the isotopic trends observed in consumers. Specifically, the pronounced salinity gradient because of varying sources of organic matter (terrestrial versus marine) among locations is a likely explanation for stable isotope variation along the coastal Northumberland Strait. Watershed-level inputs of freshwater and nutrients from the narrow channel near the former Boat Harbour outflow appear to be exerting a strong influence on the stable isotope values of local organisms. Additionally, runoff from local creeks, springs, wetlands, and ponds may be responsible for the large variation in salinity near Boat Harbour and the subsequent stable isotope signatures detected in biota from this area. Discharge of municipal wastewater from PLFN may also be contributing to the observed variation in salinity. Accompanying lower salinity values due to

terrestrial runoff is greater allochthonous inputs of freshwater phytoplankton, sloughed-off periphyton, terrestrial POM from vascular plant detritus, and soil POM (Ito et al., 2013). These inputs frequently result in depleted $\delta^{13}C$ values in aquatic environments (Ito et al., 2013), consistent with results of this study. In addition to local geomorphology and surface water runoff, it may also be possible that freshwater intrusion from Boat Harbour into the Northumberland Strait is still occurring. A modelling report by AECOM (2015) determined that groundwater discharge to Boat Harbour was approximately 11,569 m^3/day (AECOM, 2015). Therefore, significant recharge into the Boat Harbour waterbody results in release into the Northumberland Strait, contributing freshwater inflows to the immediate estuarine environment adjacent the existing dam. A pronounced salinity gradient contributing to stable isotope variation would be consistent with previous studies that have identified physical drivers, such as freshwater inputs, as major controls on the spatial dynamics in coastal ecosystems (Nelson et al., 2015).

Organism mobility is an important factor to consider and explains why certain species were more suitable bioindicators than others. Organism mobility can confound the interpretation of stable isotope results since migrating organisms attenuate stable isotope signatures from different biogeochemical zones, making it challenging to link isotope values to a given location (Mendoza, 2016). Mussels are frequently considered suitable bioindicators in pollution studies, largely due their sessile nature and ability to accurately reflect environmental conditions within a defined area (Azizi et al., 2018). Therefore, the pronounced spatial trend in stable isotope values in mussels along the coast may be due to their lack of mobility. Alternatively, decapod crustaceans such as the American lobster are relatively mobile creatures. Comeau & Savoie (2002) conducted a mark-recapture analysis of American lobsters in the southwestern Gulf of St.

Lawrence between 1980 and 1997 and determined that lobsters traveled an average distance of 19 km in the central Northumberland Strait. Additionally, rock crab has also been shown to travel relatively far distances. Comeau et al. (2012) tagged rock crab in Prince Edward Island and monitored their movement for one year, determining that crabs traveled an average of 1.6 km. The mobility of these two species may explain the high variability in stable isotope values and may render these species unsuitable as bioindicators for detecting spatial variation in coastal environments. Fish are also rather mobile organisms and certain species of fish have been found to represent only intermittent exposure to wastewater due to their movement patterns (Loomer et al., 2015; Mendoza, 2016). However, previous studies have determined that mummichogs exhibit high site fidelity and serve as adequate bioindicators in stable isotope studies (Nelson et al., 2015). Skinner et al. (2012) applied stable isotope analysis to determine that mummichogs in the Miramichi (NB) River Estuary exhibited a site fidelity of approximately 200 m. Therefore, these species occupy a narrow geographic region, and this trait likely explains why mummichogs were suitable bioindicators.

While the mobility of mummichogs among sampling locations was not likely due to the proximity of sampling locations, it may explain results obtained for mummichog $\delta^{15}N$ values at both the BHO and MKH locations. Mummichogs collected at the MKH and BHO locations had depleted $\delta^{15}N$ values (significantly different from all other locations; however, not significantly different from one another). Nonetheless, an unusual spatial trend was observed with MKH mummichogs exhibiting a lower $\delta^{15}N$ signature than mummichogs collected at BHO. Mummichogs from these two locations may represent one population, or two populations from these locations may be mixing. A dense mat of organic deposition from historical pulp mill effluent release at the BHO location likely limits the diversity of taxa, and consequently, food

availability in the immediate Boat Harbour outflow region. Perhaps due to limited food availability at the BHO location, mummichogs travel north to feed at the MKH location.

To draw a causal link between the stable isotope values of consumer organisms near the former Boat Harbour outflow and variables responsible for the observed trends in certain biota (*e.g.*, pulp mill effluent and/or spatial heterogeneity), further investigation is required. Additional studies could measure stable isotope values and complement these with additional methods of biomonitoring. For example, mixed function oxygenase (MFO) induction has been found as a method of identifying pulp mill effluent exposure (Mendoza, 2016). Mendoza (2016) used fish population endpoints, MFO activity, and stable isotope analysis to distinguish fish at locations exposed to pulp mill effluent from reference locations. While stable isotope analysis was a useful technique for identifying spatial patterns of carbon and nitrogen sources along the Northumberland Strait coastline, other sensitive physiological measurements in biota may complement stable isotope analysis. Measurements of MFO activity, gonad size, or liver somatic index (LSI) in organisms may enhance the resolution of stable isotope analysis and permit determination of the factors responsible for the observed trends. Together, stable isotope analysis and a physiological indicator of health for an organism may provide a more precise means of identifying and tracing effluent exposure.

3.4.3 Coastal Food Web Structure

This study also assessed the impacts of historical exposure to pulp mill effluent on coastal food web structure along the Northumberland Strait. Results show that bulk stable isotope analysis was a useful method for elucidating trophic dynamics and food web structure along the coastal Northumberland Strait. The stable isotope biplots of each organism's $\delta^{13}C$ and $\delta^{15}N$

values revealed shifts in carbon and nitrogen sources for most consumer species. Species with similar modes of feeding, namely lobsters and crabs, clustered closely and exhibited trophic niche overlap. The wide range of $\delta^{15}N$ values illustrated on the stable isotope biplots indicate the existence of diverse trophic levels in the structured food web along the Northumberland Strait coastline. $\delta^{15}N$ values increased with expected trophic position, which supports the consensus that higher $\delta^{15}N$ values are found in organisms with higher trophic positions (DeNiro & Epstein, 1981). Additionally, the stable isotope biplots revealed a wide range in carbon sources along the coastline, suggesting that organisms feed on different and perhaps diverse ranges of carbon sources along the coastal Northumberland Strait.

Regarding variation in food web structure, pronounced differences in the trophic position of both mussels and mummichogs from locations near Boat Harbour were observed. Mummichogs collected from the two locations nearest Boat Harbour (BHO and MKH) fell outside the defined range of carbon and nitrogen sources for mummichogs from all other sampling locations, with lower $\delta^{13}C$ and $\delta^{15}N$ values. This result suggests that mummichogs are exploiting different organic matter sources near Boat Harbour. Results also suggest that mummichogs from BHO and MKH are feeding on similar carbon sources to one another and are likely mixing populations. Mummichogs from sampling locations further from Boat Harbour appear to be feeding on a wider assemblage of food items due to their larger range in both $\delta^{13}C$ and $\delta^{15}N$ values. Although mummichogs are more generalist feeders and occupy a wide trophic niche (Thompson, 2015), mummichogs collected near Boat Harbour appear to be feeding more exclusively on POM or a food source feeding on considerable quantities of organic material.

Like mummichogs, mussels from sites nearest the former outflow are feeding on sources depleted in carbon relative to their respective populations along the coastline. While mussels

form a defined cluster in the stable isotope biplots, MKH mussels fell outside the defined niche breadth of mussels from all other sampling locations. Mussels from the MKH location occupy a lower overall trophic position, with different sources of dissolved inorganic nitrogen (DIN) and a different carbon source, most likely from PLFN sewage wastewater. Therefore, there are distinct differences in food web dynamics for both mummichogs and mussels near the former Boat Harbour outflow, with stable isotope values suggesting both species are feeding at lower overall trophic positions. This result may be indicative of enrichment from pulp mill effluent or from terrestrial sources such as runoff or freshwater inputs. Consistent with impacts from pulp mill effluent, food chains are reportedly shorter in environments that are frequently disturbed (Pimm 1982). However, coastal complexities such as terrestrial runoff and PLFN wastewater discharge are likely contributing to alterations in the hydrodynamic regime in the area, creating a defined salinity gradient. Observed differences in salinity may be responsible for alterations in food web dynamics. Shorter food webs have been reported in environments with lower salinity relative to longer food chains in higher salinity environments (Vander Zanden & Fetzer, 2007). Therefore, variation in the observed food web structure may be a result or a combination of historical pulp mill effluent and terrestrial inputs that shift biogeochemical conditions among coastal locations.

The highest trophic level consumers (lobsters and crabs) occupied slightly higher trophic positions at locations nearest Boat Harbour. These results support differences in food web structure along the Northumberland Strait with species from areas near Boat Harbour feeding on different dietary sources. Stable isotope values of mussels and mummichogs suggest they are feeding at lower relative trophic positions near Boat Harbour, meanwhile stable isotope values of higher-trophic level consumers; lobsters and crabs, indicate they are feeding at higher relative trophic positions. This is consistent with previous studies completed in anthropogenically-

impacted environments that report higher trophic positions of predators in a food web (Barst et al., 2020). Stable isotope values of lobsters and crabs near Boat Harbour suggest that these species are potentially feeding on less diverse food sources relative to other locations. Lobsters and crabs near the former outflow may be feeding on a less diverse diet due to lower species diversity in this area. For example, lobsters and crabs near Boat Harbour may be feeding more exclusively on mussels relative to other locations along the coastline. In contrast, lobsters and crabs may be feeding on more diverse food items along the coastline such as polychaetes, crustaceans, and echinoderms (Hudon & Lamarche, 1989), lowering their overall $\delta^{15}N$ value. This potential explanation would be consistent with the theory of simpler, less diverse food webs adjacent Boat Harbour, relative to locations further along the coastline. Historical literature has reported similar findings, with less diverse communities of organisms in polluted aquatic environments (Kwak & Zedler, 1997; Read et al., 1978). Overall, the lower trophic position of primary consumers and higher trophic position of predatory species suggests potentially less complex food webs near Boat Harbour. This may be explained by the persistence of pulp mill effluent-derived nutrients or increased terrestrial inputs, including freshwater inputs, causing variation in salinity and subsequent alteration of food web dynamics.

A wide range of $\delta^{13}C$ values, indicative of a diverse range of carbon sources for organisms, may be explained by variation in the dominant primary production sources along the coastline. Specifically, dominant primary production sources may align with the observed variation in salinity along the coastline, potentially accounting for broad differences in $\delta^{13}C$ values of consumers. Typically, in more freshwater environments, dominant primary production occurs through C_3 photosynthetic pathways (Hoeinghaus et al., 2011). Examples of these primary producers are floating aquatic macrophytes which frequently exhibit depleted $\delta^{13}C$

values (-30 to -23‰ (Smith & Epstein, 1971). Alternatively, in coastal marine environments with higher salinity, C_4 production is the dominant primary production pathway (Hoeinghaus et al., 2011). Examples of these C_4 primary producers are estuarine seagrasses (*e.g.*, *Spartina* sp.) which frequently exhibit enriched $\delta^{13}C$ values (-13 to -10‰ (Hoeinghaus et al., 2011). Therefore, observed differences in $\delta^{13}C$ values among sampling locations may be due to differences in the dominant carbon source or mode of primary production. Organisms collected near Boat Harbour exhibited lower $\delta^{13}C$ values, potentially explained by dominating C_3 primary production in this area. In contrast, organisms collected from marine locations such as CRI, had enriched $\delta^{13}C$ values, potentially due to a greater reliance on C_4 primary production. For example, mummichogs were collected in reference locations by trawling a beach seine near dense stands of *Spartina* sp. The enriched $\delta^{13}C$ values of mummichogs from these locations may indicate their reliance on C_4 macrophytes. This contrasts with locations near Boat Harbour (BHO and MKH), where mummichogs had depleted $\delta^{13}C$ signatures. Mummichog $\delta^{13}C$ values near Boat Harbour were similar to the $\delta^{13}C$ value of mussels, indicating a diet largely influenced by organic material. These results appear to be consistent with the dominating vegetation that was observed at each sampling location. Seagrass, including *Spartina* sp., was dominant at locations with higher salinity. In contrast, the dominant vegetation near Boat Harbour was floating algae, with an absence of C_4 primary production sources such as seagrass.

In addition to mummichogs, periwinkles are significant grazers of *Spartina* sp. (Silliman & Zieman, 2001); therefore, their enriched $\delta^{13}C$ (like that of mummichogs in reference locations), may be indicative of a diet rich in C_4 macrophytes. A shift from C_3 dependent foods webs to more C_4 based food webs along a salinity gradient has been observed in previous studies (Hoeinghaus et al., 2011; Winemiller et al., 2011). Garcia et al. (2007) observed a similar stable

isotope trend along a salinity gradient. Consumer species in marine regions assimilated greater amounts of carbon from C_4 production sources, reflected by enriched $\delta^{13}C$ in consumer tissue (Garcia et al., 2007). In more freshwater zones, C_3 production sources dominated, resulting in depleted $\delta^{13}C$ in consumer tissues (Garcia et al., 2007), like those observed in the present study. An important consideration in the interpretation of results is that the organisms sampled may not be fully representative of the entire food web structure along the coastline. Therefore, they likely do not encompass all primary production sources and all food sources for consumers. A greater number of species is required, specifically a greater number of primary production sources, to fully elucidate the food web interactions along this complex coastal ecosystem of the Northumberland Strait.

Variation in the amount and origin of nutrients are common along salinity gradients which is often reflected in the composition and functioning of food webs (Hoeinghaus et al., 2011). The combination of biogeochemical dissimilarities and potential historical persistence of organic matter from pulp mill effluent likely also explains variation in species abundance at locations near Boat Harbour. Certain species were unable to be collected at the BHO location including mussels, snails, crab, and lobster. Beyond the stable isotope results of this study, the absence of these species near the former outflow is likely indicative of varying environmental conditions relative to other sampling locations along the Northumberland Strait. Differences in species abundance and diversity has been identified in numerous studies where anthropogenic and terrestrial inputs contribute to a loss of biological diversity (Kolasinski et al., 2016). Perhaps the community composition near the former outflow has shifted towards species that are more pollution-tolerant or more tolerant of varying salinity. Mummichogs, which were indeed present at the outflow, are tolerant to a wide range of conditions, including varying salinity and exposure

to pollutants (Weis, 2002). It would be beneficial in future studies to supplement stable isotope analysis in organisms that are present at impacted sites with community measures of diversity and abundance. For example, the Simpson's diversity index and the Shannon Wiener diversity index measure the concentration of individuals of the same species and the distribution of all species. These ecological measures would provide further information on the conditions of the ecosystem at each sampling location. While stable isotope analysis was useful to distinguish spatial patterns in nutrient sources and provide important information on food web structure, it does not provide information on the diversity or abundance of species at each location, another important indicator of ecosystem health. These multiple lines of evidence, applying both stable isotope analysis and ecological diversity measures, would further assist in gaining a more holistic understanding of the coastal ecosystem and its spatial variation.

An important consideration when interpreting the results of this study is the baseline $\delta^{15}N$ used to estimate each organism's trophic position. Overall, this study found that POM was not the most suitable indicator for baseline $\delta^{15}N$ estimates of trophic position. POM as a baseline indicator likely resulted in an underestimation of the trophic position of organisms. This determination was based on the trophic position estimates for mussels and periwinkles. Mussels and periwinkles are primary consumers and, thus, typically occupy a trophic position of 2 (Hoeunghaus & Davis, 2007; Post, 2002b). However, periwinkle trophic position ranged from 1.06 to 1.79 and mussel trophic position ranges from 1.5 to 2.05 among sampling locations. Post (2002b) determined the baseline stable isotope signature of an ecosystem is best accomplished using long-lived primary consumers. Primary producer stable isotope values are highly variable, which can complicate their use as a baseline for consumers that integrate $\delta^{15}N$ for longer periods of time (Post, 2002b). Long-lived primary consumers should preferentially be selected as

baseline indicators since they typically exhibit less variation in their stable isotope values (Post, 2002). Future studies completing an ecosystem-wide study of food web structure in this region should collect a wider assemblage of species, specifically long-lived primary consumers, to provide a more accurate baseline for estimating trophic position.

Another common discrepancy associated with stable isotopes studies that estimate trophic position is the use of a fixed $\delta^{15}N$ enrichment factor. For consistency and comparability with other stable isotope food web studies, this study used the enrichment factor most commonly reported in the literature, 3.4‰ (Minagawa & Wada, 1984). However, the stable isotope enrichment factor for individual trophic transfers is demonstrably variable with studies reporting a range in $\delta^{15}N$ enrichment from 2 to 5‰ (Post, 2002b). Applying a fixed average enrichment factor across all trophic levels of a food web could lead to errors in trophic position estimates. Therefore, despite its wide application and being deemed a robust and widely applicable value (Post, 2002b), it is important to consider the ecological uncertainty associated with applying this mean trophic fractionation factor across all trophic positions in a food web.

3.5 Limitations and Future Studies

As with many ecological research studies, this study had limitations which are important to consider in the design of future experimental studies. Time and financial constraints limited the number of species that were included in the study and the number of samples of each species collected at each location. A wider assemblage of species would assist in filling gaps in trophic niche space in the food web along the coastline, potentially drawing more direct links between an organism and its potential food source. Specifically, this study would have been strengthened by a greater number of long-lived primary consumer species to serve as a baseline estimate of $\delta^{15}N$

at each sampling location. Further, while the aim during the collection of organisms at each location was to obtain triplicate samples of each species, more samples of each species would have strengthened the statistical analyses.

Another limitation was that the study was confined to a two-year graduate-level project and, thus, sampling only occurred over one year. It would be beneficial to measure stable isotope values in organisms on an annual basis to determine temporal variation and potential recovery of the marine environment following exposure to pulp mill effluent. Additionally, as this study was limited with respect to time and financial resources, a full analysis of the nutrient concentrations along the coastal Northumberland Strait was not completed. A useful step in future studies would be to evaluate the nutrient conditions (*e.g.*, nitrate, nitrite, ammonium, phosphate concentrations) at each sampling location. Since it was not possible to measure pulp mill effluent stable isotope values, nutrient analysis would have been beneficial to serve as a proxy for potential nutrient enrichment from effluent and provide a baseline of nutrient concentrations prior to the remediation of Boat Harbour.

Many ecological studies would benefit from both laboratory-based and field-based research. Therefore, future studies may complement this research with laboratory-based analyses of organisms exposed to pulp mill effluent. A controlled, laboratory-based analysis may address gaps that were identified within the current study. It may explain if the spatial differences observed were indeed a function of historical exposure to pulp mill effluent. Additionally, a laboratory-based study would eliminate confounding factors such as organism mobility and diet-switching among organisms, providing further clarity on the stable isotope signatures of each organism and their direct food web links. However, the drawback of laboratory-based research is omitting the natural variation observed in the field. Therefore, it is suggested that laboratory-

based experiments serve as a complement to field-based research, to provide clarity on which variables are responsible for the observed stable isotope variability.

Finally, future avenues of research may apply compound specific stable isotope analysis (CSIA) in the Northumberland Strait coastal region to provide greater resolution of food web structure. CSIA of individual amino acids is a method that estimates trophic position based upon differences in the $\delta^{15}N$ of trophic and source amino acids within an organism's tissues (McClelland & Montoya, 2002). This method has been shown to greatly refine information regarding the trophic interactions among organisms (Chikaraishi et al., 2009). CSIA may greatly complement or enhance the accuracy of trophic position estimates in ecological studies, such as those obtained in the present study. Ongoing advances in CSIA may assist in distinguishing between different nitrogen sources in aquatic ecosystems, thus, providing a more robust evaluation of trophic structure.

3.6 Conclusion

This study assessed spatial variation in $\delta^{13}C$ and $\delta^{15}N$ of organisms occupying a coastal food web formerly exposed to pulp mill effluent. Additionally, it assessed variation in trophic dynamics and dietary sources of carbon and nitrogen along a spatial gradient in historical exposure to pulp mill effluent prior to remediation. Results identified spatial variation in stable isotope values at locations near Boat Harbour and along the Northumberland Strait coastline. Mussels and mummichogs were identified as suitable bioindicator taxa since they revealed pronounced spatial differences in their stable isotope values along the coastline. Mussels and mummichogs had distinct stable isotope signatures near Boat Harbour, with depleted tissue $\delta^{13}C$ and $\delta^{15}N$ values. Consequently, they also occupied lower overall trophic positions at these

locations, indicating variability in food web structure potentially due to historical pulp mill effluent exposure. However, observed differences in stable isotope values and subsequent food web dynamics may not be exclusively attributable to pulp mill effluent exposure. It is likely that stable isotope values of organisms are related to both historical pulp mill effluent and spatial heterogeneity along the coastline. A pronounced salinity gradient was measured among sampling locations, with low salinity values reported near Boat Harbour. While the stable isotope signatures identified in organisms near Boat Harbour aligned with previous stable isotope signatures of organisms exposed to pulp mill effluent, variation in salinity is likely also a contributing factor. Differences in salinity and terrestrial inputs along the coastline likely contribute to differences in dominant species, including dominant primary production sources. Further, discharge from the adjacent outflow of PLFN wastewater treatment facility is likely also contributing to varying nutrient conditions along the coastal environment. Primary-treated wastewater from PLFN may be causing increased ammonium concentrations, resulting in the observed depleted $\delta^{15}N$ near Boat Harbour. This spatial heterogeneity and biogeochemical dissimilarities among coastal sampling locations likely contributed to variation in nutrient sources along the coastline and subsequent variation in trophic dynamics between organisms.

This study confirmed that stable isotope analysis can assist in the elucidation of the origin and fate of organic matter in coastal ecosystems and serve to delineate food web interactions. Further, this study reaffirmed that coastal ecosystems are characterized by high spatial variability and complexity. Determining variables responsible for differences in $\delta^{13}C$ and $\delta^{15}N$ in organisms at locations near the former outflow of pulp mill effluent from Boat Harbour can be assessed by using this method for monitoring ecosystem health following remediation. Applying this method as a monitoring technique following remediation may indicate potential alterations in

biogeochemical conditions in the coastal ecosystem and subsequently, how these conditions affect native biota. This study serves as a baseline of biogeochemical conditions and food web structure of the coastal ecosystem in the Northumberland Strait near Boat Harbour, which has not previously been assessed. This baseline can be used to evaluate ecosystem conditions once remediation is complete and gain a more holistic understanding of the complex nutrient inputs and food web interactions along the Northumberland Strait coastal environment.

Chapter 4: Bioindicators in Stable Isotope Studies: A Review and Evaluation of their Suitability to Assess Wastewater Pollution

4.1 Summary of Chapter III Bioindicator Suitability

Chapter III provided an assessment of the sources of nitrogen and carbon using stable isotope analysis within a coastal marine environment formerly receiving pulp mill effluent. A suite of marine taxa was included in the study and results indicated that certain taxa were more sensitive bioindicators of ecosystem conditions within the coastal region. Stable isotope values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were measured in the following species: macroalgae (*Fucus vesiculosus*), periwinkles (*Littorina littorea*), blue mussels (*Mytilus edulis*), mummichogs (*Fundulus heteroclitus),* rock crab (*Cancer irroratus*), and American lobster (*Homarus americanus*). Both blue mussels and mummichogs demonstrated the most pronounced spatial trend in stable isotope values relative to Boat Harbour, a former wastewater treatment facility for bleached kraft pulp mill effluent. Both species exhibited depleted $\delta^{13}C$ and $\delta^{15}N$ values near the former outflow from Boat Harbour, reflecting potential stable isotope signatures from pulp mill effluent-derived nutrients. However, it must be noted that while stable isotope signatures near Boar Harbour reflected values consistent with pulp mill effluent exposure, pulp mill effluent may not be exclusively responsible for the stable isotope values reported within the study. Stable isotope values of both mussels and mummichogs reflected incorporation of terrestrial-derived nutrients while also revealing substantial spatial heterogeneity along the coastline. Consequently, while stable isotope values adjacent a former pulp mill effluent outflow are described, a temporal assessment following remediation of the contaminated ecosystem will be required to contextualize the stable isotope patterns identified in chapter III and confirm their connection to historical pulp mill effluent exposure.

F. vesiculosus, a brown perennial macroalgae abundant along the Northumberland Strait coastline was selected as a primary producer organism within the chapter III analysis. Stable isotope analysis revealed spatial patterns in $\delta^{13}C$ and $\delta^{15}N$ of *F. vesiculosus* that were not consistent with the expected gradients to reflect varying degrees of pulp mill effluent exposure. Consequently, it was determined within this study that this species, with whole plant tissues sampled, was not a suitable bioindicator for revealing enrichment effects of historical pulp mill effluent. Within the study, whole plant tissue was freeze-dried, homogenized, and used in the stable isotope analysis. However, *F. vesiculosus* exhibits apical growth, meaning the tips of the fronds are the region of new growth within the plant (Viana et al., 2015). In fact, stable isotope variability has been identified in different morphological regions of macroalgae (Howarth et al., 2019). Therefore, sampling whole plant tissue may have contributed to the observed stable isotope variability. Isolating older regions of the plant fronds may have produced a signal indicative of historical pulp mill effluent. Within chapter III, stable isotope signatures in the regions of older growth may have become diluted by new growth in the tips of the algal blades, reflecting contemporary conditions. Nonetheless, within the design and methodology of this particular study, the long-lived brown macroalgae, *F. vesiculosus*, did not reveal effects of historical pulp mill effluent exposure.

The gastropod periwinkle snail, *L. littorea*, did not prove to be a useful bioindicator within the chapter III stable isotope analysis. Like *F. vesiculosus*, both the $\delta^{13}C$ and $\delta^{15}N$ signatures of *L. littorea* were highly variable along the Northumberland Strait coastline and did not reveal effects of pulp mill effluent exposure adjacent to the former Boat Harbour outflow. *L. littorea* has many characteristics of a good bioindicator: they are abundant along the coastline, easy to collect, they are not a highly mobile species (Vermeulen et al., 2011), and they have a

relatively long tissue turnover rate (McIntyre & Flecker, 2006). Nonetheless, the stable isotope signatures of this species were highly variable, which has also been reported in many gastropod species (Warnakulasooriya et al., 2017). An explanation for the observed variability may be the diverse diet of *L. littorea* and potential diet-switching throughout their life history (Imrie et al., 1990), which can obscure stable isotope signatures among locations. Therefore, while *L. littorea* has characteristics of a good bioindicator species, other primary consumer species may be more suitable as indicators of environmental pollution in coastal environments.

Mussels (*M. edulis*) were considered one of two suitable bioindicator species within the chapter III stable isotope analysis. Many studies report that bivalves are good bioindicator species and provide an accurate reflection of environmental conditions within a geographic region (Fry & Allen, 2003; Riera et al., 1999). Within chapter III, *M. edulis* had distinct stable isotope signatures of both $\delta^{13}C$ and $\delta^{15}N$ (both depleted) at the former outflow of pulp mill effluent from Boat Harbour. There are many characteristics of *M. edulis* that may explain its suitability as a bioindicator within the chapter III study. First, they reflect local environmental conditions due to their sedentary nature (Cossa, 1989). Additionally, they have a relatively low tissue turnover rate (Fry & Allen, 2003), making them capable of reflecting environmental conditions over a long period of time. Mussels also have a relatively simple diet; filter feeding suspended organic matter in the water column (Puccinelli et al., 2019). Therefore, their stable isotope signature does not become confounded by a variety of food items throughout their life history (Wen et al., 2010). Within chapter III, a distinguishable trend in the effectiveness of filter feeding bioindicator species relative to those that feed on benthic sources was observed. As filter feeding organisms, mussels may have been more likely to incorporate a stable isotope signature from the buoyant pulp mill effluent (Ecometrix, 2016), relative to benthic-feeding organisms

such as periwinkles. The results from chapter III suggest that *M. edulis* should be considered in future stable isotope studies designed to identify and delineate wastewater inputs in coastal ecosystems.

Mummichogs (*F. heteroclitus*) were also identified as a suitable bioindicator species within the chapter III stable isotope analysis. *F. heteroclitus* had distinct $\delta^{13}C$ and $\delta^{15}N$ (both depleted) at the former outflow from Boat Harbour, reflecting potential enrichment from historical pulp mill effluent. Many fish species have been criticized for their use in stable isotope analysis due to their movement patterns, which may confound the interpretation of their stable isotope results (Hicks et al., 2017). However, mummichogs within the chapter III analysis did not appear to have high mobility among sampling locations. Indeed, mummichogs have been identified as a coastal species exhibiting high site fidelity (Skinner et al., 2012). High site fidelity in mummichogs likely explains why this species had similar stable isotope signatures to mussels since they were not amortizing stable isotope signatures over multiple spatially separated locations. Results from chapter III suggest mummichogs may be a good bioindicator species in future stable isotope studies attempting to identify and trace wastewater in aquatic environments.

The two decapod crustaceans utilized in chapter III: rock crab (*C. irroratus*) and American lobster (*H. americanus*), were not identified as good bioindicators for assessing historical pulp mill effluent. Within individual samples at each location (n=3) both of these species exhibited high variation in their stable isotope values for both carbon and nitrogen. This high variation among individuals is likely the result of their complex diets and mobility. Both *C. irroratus* and *H. americanus* feed on multiple trophic levels with diets ranging from macroalgae, detritus, polychaetes, molluscs, small crustaceans, and echinoderms (Boudreau & Worm, 2012). Diverse feeding strategies, changing with age, can confound stable isotope interpretation of

opportunistic organisms consuming slightly differing diets and thereby incorporating stable isotope values reflecting those different dietary items. Another potential explanation for why these two species were not suitable bioindicators within chapter III is their relatively high mobility, which has been reported in both species (Comeau et al., 2011; Comeau & Savoie, 2002), resulting in a stable isotope signature that is not fully representative of a given geographic region. Therefore, the results from chapter III suggest that both crabs and lobsters may not be suitable bioindicators in stable isotope studies aimed at detecting and tracing wastewater due to their complex diet and relatively high mobility.

4.2 Bioindicators to Detect Wastewater Pollution

Bioindicators are used to evaluate impacts of anthropogenic stressors on aquatic ecosystems (Walker et al., 2013) and must accurately reflect conditions of their environment of capture (Holt & Miller, 2011). Many bioindicators have the capacity to accumulate unique biogeochemical signatures that integrate and reflect local ambient conditions, providing an early detection of anthropogenic impacts to ecosystems (Parmar et al., 2016). Bioindicators are commonly used as proxies in aquatic ecosystems for detecting the ecological effects of wastewater pollution, such as sewage wastewater or pulp mill effluent (Jirova et al., 2016). A suitable bioindicator should be abundant in the environment and capable of providing measurable responses to varying environmental conditions (Holt & Miller, 2011). Additionally, a suitable bioindicator should be well studied, yielding a strong understanding of the organism's ecology and life history (Holt & Miller, 2011). Numerous bioindicator taxa have been suggested for use in aquatic ecosystems, with each possessing their own unique characteristics, making each taxa suitable for detecting a suite of environmental stressors. For example, macroalgae are considered good bioindicators of metal pollution in waterbodies due to their wide distribution,

rapid response to pollutants, and ability to accumulate and concentrate metals in their tissues over prolonged periods (Khalil et al., 2021). Numerous taxa have proven useful for detecting and mapping the spatial distribution of wastewater in aquatic environments such as macroalgae, benthic invertebrates, and fish species (Freedman et al., 2012; Loomer et al., 2015).

Environmental Effects Monitoring (EEM) is a regulatory requirement for all Canadian pulp and paper mills that discharge effluent into waterbodies under the *Pulp and Paper Effluent Regulations* (PPER, 1992) of the *Fisheries Act* (1985). The EEM program is designed to assess impacts of effluent discharge on aquatic organisms and their habitat (Munkittrick et al., 2002). The EEM program uses a variety of aquatic organisms to assess environmental conditions in receiving waterbodies for pulp mill effluent (Courtenay et al., 2002). Aquatic macroinvertebrates possess many traits of good bioindicators for detecting wastewater and are commonly employed in environmental monitoring and in the EEM program (Muralidharan et al., 2010). Additionally, a variety of fish taxa have been used to evaluate environmental conditions in aquatic receiving environments. For example, the Canadian regulated PPER EEM program used over 60 species of fish in the first four cycles to evaluate effects of pulp mill effluent on Canadian aquatic habitats (Mendoza, 2016). Likewise, a variety of taxa have been used to assess conditions in environments impacted by sewage wastewater. Taxa that have been employed as bioindicators of sewage wastewater include macrophytes (Costanzo et al., 2001), macroalgae (Rogers, 2003), fish (Bergfur et al., 2009), and invertebrates ranging from insects (Morrissey et al., 2013) to crustaceans (di Lascio et al., 2013).

Despite numerous taxa being employed to evaluate wastewater impacts on aquatic ecosystems, there is no consensus on which bioindicators are most effective at adequately characterizing ecosystem health. Developing a standardized suite of bioindicator organisms that

can be used for assessing pollution sources may assist in identifying adverse ecological effects and deteriorating conditions in aquatic environments. Additionally, applying similar taxa across multiple studies permits temporal evaluations within a given waterbody to assess shifts in environmental conditions. Using similar taxa as bioindicators of wastewater exposure effects also permits comparison of effects of a given wastewater source across aquatic environments in multiple studies or monitoring programs. While there have been numerous studies evaluating wastewater impacts on biota (Lachs et al., 2019; Lozano-Bilbao et al., 2018; Vermeulen et al., 2011), there is no comprehensive review of the efficacy of each bioindicator taxa to detect wastewater inputs.

4.3 Bioindicators in Stable Isotope Analysis

Bioindicators provide a valuable and practical means by which to measure biogeochemical variation in aquatic environments (Munroe et al., 2018). The study of stable isotope signatures in certain taxa have proven to be useful tools for detecting and mapping wastewater in aquatic environments (Loomer et al., 2015). Stable isotope analysis compares the amount of a heavy isotope (an element with one additional neutron in the nucleus, *e.g.* C^{13}) to a light isotope (an element with one less neutron in the nucleus, *e.g.* C^{12}) in a sample relative to international standards. Stable isotope values are represented using delta notation (δ) and are expressed in parts per thousand (‰) (Fry, 2006). Wastewater often has unique stable isotope signatures, distinct from that of the aquatic receiving environment (Loomer et al., 2015). Therefore, measuring stable isotope values in tissues from organisms inhabiting wastewater receiving environments can quantitatively determine an organisms' reliance on wastewater-derived nutrients (Fry, 2006). For example, nitrogen stable isotope ($\delta^{15}N$) values of biota have

proven to be an effective method for detecting anthropogenic nitrogen inputs, specifically sewage wastewater (McClelland et al., 1997). Sewage wastewater is typically enriched in $\delta^{15}N$ (+10‰ (Schubert et al., 2013) due to the volatilization of ^{14}N-rich ammonia during the processes of nitrification and denitrification (Aravena et al., 1993). In contrast, stable isotope values of pulp mill effluent are typically depleted in both $\delta^{15}N$ and $\delta^{13}C$ relative to ambient conditions in aquatic ecosystems (Wayland & Hobson, 2001). Many studies report stable isotope signatures in pulp mill effluent that reflect terrestrially derived organic matter (-30 to -25‰) due to intensive processing of trees during the pulp manufacturing process (Arciszewski et al., 2014). Several studies have successfully employed stable isotope analysis to detect pulp mill effluent in several taxa including macroalgae (Wayland & Hobson, 2001), invertebrates (McMahon et al., 2020), and fish (Skinner et al., 2012). Therefore, stable isotope analysis is an effective method for detecting and tracing terrestrial nutrient sources entering aquatic ecosystems and may have high utility in future environmental monitoring programs.

Incorporating stable isotope analysis into routine biomonitoring programs has been suggested as a method for enhancing the detection of adverse ecological impacts and providing greater resolution relative to conventional water quality monitoring practices (Dubé et al., 2005). Within aquatic environments receiving wastewater, early detection of nutrient enrichment is required to prevent further degradation of the ecosystem and subsequent loss of biodiversity (Vermeulen et al., 2011). In hydrodynamically complex environments, conventional water quality parameters may not adequately quantify wastewater impacts. Vermeulen et al. (2011) suggest that initial effects of wastewater pollution are likely to be missed if evaluation of ecosystem conditions is restricted to species community composition and physiochemical water properties such as nutrient concentrations, salinity, and effluent plume dispersion. These

parameters are known to vary greatly over time and space, and often require longer periods of time to reflect any adverse ecological effects (Carballo et al., 1996). Additionally, these parameters do not provide an indication of the uptake of wastewater-derived nutrients by aquatic biota that inhabit receiving environments.

Such difficulties in assessing long-term water quality can be overcome by assessing bioindicator taxa that assimilate pollution signatures into their tissues, reflecting their habitat quality (Wayland & Hobson, 2001). Stable isotope values in aquatic organism tissues can provide a time-integrated measure of wastewater and an alternative monitoring tool for assessing water quality (Dubé et al., 2005). For example, bioindicators such as macroalgae and bivalves provide a potential means of assessing the spatial extent and subsequent biological effects of wastewater discharge in aquatic environments (Dudley & Shima, 2010). The application of bioindicators that integrate stable isotope signatures from wastewater into their tissues may greatly assist in detection of long-term water quality conditions. Despite a wide variety of bioindicator taxa having been used in stable isotope analysis, there is no consensus on the most suitable organisms for assessing the impacts of wastewater. Identification of appropriate bioindicator taxa can be problematic given the diversity of aquatic organisms, their specific life histories, and habitat/species distribution differences among studies. Therefore, a comprehensive review of previous stable isotope studies in aquatic receiving environments for wastewater is required to properly assess which taxa are the most suitable bioindicators of ambient nutrient concentrations from wastewater. Furthermore, a standardized set of criteria for the selection of appropriate bioindicators that properly characterize ecosystem health is fundamental. The following study aims to: 1) review and evaluate the suitability of bioindicator species that have been used in previous stable isotope studies in environments impacted by pulp mill effluent

and/or sewage wastewater, and 2) develop criteria for the selection of appropriate bioindicators to ensure environmental conditions in receiving environments for wastewater are properly characterized.

4.4 Methods

To evaluate suitability of bioindicators used in previous stable isotope studies to assess wastewater inputs, a systematic literature review was conducted. In this study, wastewater was defined as either pulp mill effluent or municipal sewage wastewater. The systematic literature review was conducted through Scopus and Google Scholar using the following keywords: "stable isotope" AND "wastewater", "stable isotope" AND "effluent", "stable isotope" AND "sewage", "stable isotope" AND "pulp mill effluent." A total of 94 papers (Appendix D) were compiled that met the criteria of measuring stable isotope values of biota in aquatic receiving environments for wastewater. Of the 94 papers included, only 8 studies were completed in environments exclusively impacted by pulp mill effluent. The remaining papers were completed in environments impacted by sewage wastewater or both sewage wastewater and pulp mill effluent. All papers were reviewed for taxa employed in the stable isotope analysis and authors comments regarding their perceived suitability as bioindicators of wastewater exposure. Biota included within the review were divided into four large taxonomic groups: vascular plants, macroalgae, invertebrates, and fish. Each taxonomic group was then further classified into higher taxonomic resolution such as the class and order of taxa. The frequency of each taxonomic group was identified, and the suitability of each taxonomic group was evaluated by compiling and summarizing comments regarding their effectiveness in each study.

4.5 Results and Discussion

4.5.1 Bioindicator Taxa Occurrence

Dividing biota into four large taxonomic groups, invertebrates were the most widely employed bioindicator in stable isotope studies to assess wastewater inputs (Figure 4.1). Of the 94 papers reviewed, 62 included one or more invertebrate taxa. For the additional taxonomic groups: 34 studies included fish, 33 included macroalgae, and 23 included vascular plants (Figure 4.1). Further dividing the invertebrate group into higher taxonomic resolution, class Malacostraca (including shrimp, prawns, crayfish, amphipods, isopods, lobsters, and crabs) were the most frequently employed bioindicators (Figure 4.2). Of the 94 studies reviewed, 26 included one or more bioindicator taxa in the class Malacostraca. The second most frequently employed invertebrate class was Bivalvia (including mussels, oysters, and scallops), used in 23 studies. Additional invertebrate groups frequently used as bioindicators include class Gastropoda (17), class Insecta (16), class Anthozoa (10), and class Polychaeta (7) (Figure 4.2). Tables 4.1-4.4 divide the four large taxonomic groups into higher taxonomic resolution and provide the number of studies including each taxon.

For vascular plants, the most frequently applied bioindicator was *Zostera* sp. (eelgrass) and *Avicennia marina* (mangrove) (Table 4.1). For macroalgae, the dominant taxa was *Ulva* sp. (sea lettuce) (Table 4.2). For invertebrates, there was a diverse assemblage of arthropods, dominated by two major classes, Insecta and Malacostraca (Table 4.3). The most dominant insect taxonomic groups were order Diptera (including chironomids), and order Ephemeroptera (mayflies) (Table 4.3). The most dominant taxonomic groups in class Malacostraca were order Decapoda (including shrimp, prawns, lobster, crab, and crayfish) and order Amphipoda (amphipods) (Table 4.3). Another frequently applied bioindicator taxa in stable isotope studies

conducted in aquatic receiving environments for wastewater is class Anthozoa (including sea anemones, stony corals, and soft corals) (Figure 4.2; Table 4.3). Fish taxa included in stable isotope studies in receiving environments for wastewater spanned 32 taxonomic families (Table 4.4). The most frequent families of fish in the reviewed studies were Catostomidae (suckers), Percidae (perch), and Salmonidae (salmon, trout, char, and whitefish) (Table 4.4).

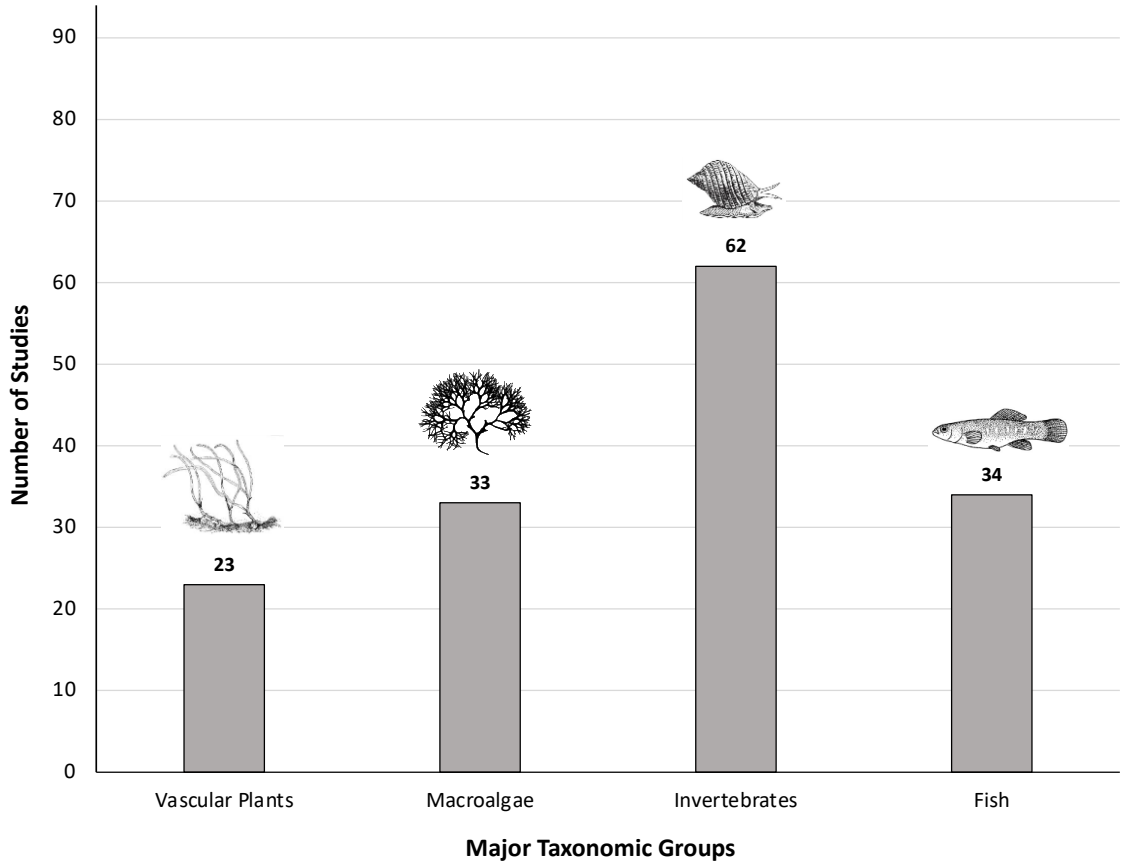

Figure 4.1 Frequency of bioindicator taxonomic groups used in the reviewed studies to assess wastewater inputs in aquatic ecosystems using stable isotope analysis.

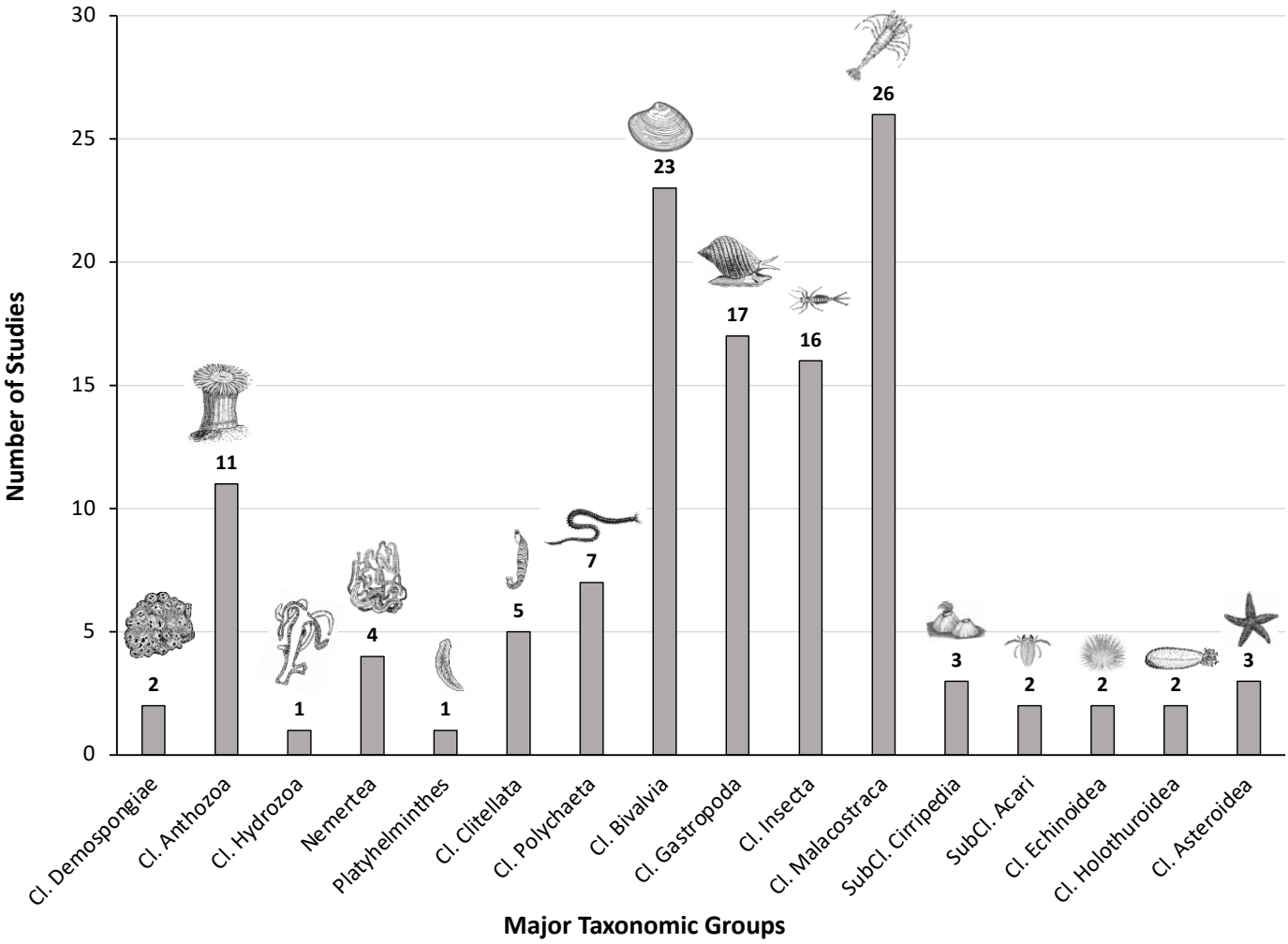

Figure 4.2 Breakdown of invertebrate bioindicator taxa used the reviewed studies to assess wastewater inputs in aquatic ecosystems using stable isotope analysis

Table 4.1 Review of vascular plant taxa used as bioindicators in stable isotope studies to assess wastewater inputs in aquatic environments, divided into their respective taxonomic groups and the number of studies that included each taxa.

Order	Family	Genus/ Species	Common Name	Number of Studies	References
Najadales	Zosteraceae	*Zostera* sp.	Eelgrass	9	Connolly et al. (2013), Costanzo et al. (2001), Fourqurean et al. (1997), Jones et al. (2001), McClelland et al. (1997), Piola et al. (2006), Román et al. (2019), Schubert et al. (2013), Suzzi et al. (2022)
Alismatales	Araceae Posidoniaceae Hydrocharitaceae Cymodoceaceae	*Lemna* sp.*,* *Posidonia* sp.*, Thalassia* sp.*, Thalassodendron* sp.*,* *Syringodium filiforme,* *Cymodocea* sp.	Duckweed, Mediterranean tapeweed, Turtle grass, Manatee grass	8	Carruthers et al. (2005), Connolly et al. (2013), González-De Zayas et al. (2020), Lassauque et al. (2010), Morris et al. (2009), Mwaura et al. (2017), Vizzini & Mazzola (2006), Wassenaar & Culp (1996)
Lamiales	Verbenaceae	*Avicennia marina*	Mangrove	7	Costanzo et al. (2001), Hadwen & Arthington (2007), Jones et al., (2001), Piola et al. (2006), Pitt et al. (2009), Smith et al. (2016), Warnakulasooriya et al. (2017)
Poales	Poaceae	*Spartina alterniflora,* *Phragmites australis*	Smooth cordgrass, Common reed	4	Cole et al. (2004), McClelland et al. (1997), Oakes et al. (2010), Pruell et al. (2006)

Table 4.2 Review of macroalgae taxa used as bioindicators in stable isotope studies to assess wastewater inputs in aquatic environments, divided into their respective taxonomic groups and the number of studies that included each taxa.

Division	Class	Order	Genus/ Species	Common Name	Number of Studies	References
Phaeophyta	Phyaeophyceae	Fucales	*Fucus vesiculosus*, *Carpophyllum maschalocarpum*, *Sargassum* sp.	Fucoids (bladder wrack, common flapjack, gulf weed)	6	Dudley & Shima (2010), González-De Zayas et al. (2020), Hobbie et al. (1990), Riera et al. (2000), Savage (2005), Savage & Elmgren (2004)
		Dictyotales	*Lobophora* sp., *Padina* sp., *Dictyota* sp.	Thalloid brown seaweed, Peacocks tail	3	Lachs et al. (2019), Mwaura et al. (2017), Umezawa et al. (2002)
		Scytosiphonales	*Colpomenia sinuosa, Endarachne binghamiae, Asteronema breviarticulatum*	Sinuous ballweed	3	Barile (2004), Dailer et al. (2010), Oakes & Eyre (2015)
		Laminariales	*Ecklonia radiata*	Kelp	2	Fernandes et al. (2012), Gartner et al. (2002)
Chlorophyta	Ulvophyceae	Ulvales	*Ulva* sp.	Sea lettuce	15	Babaranti et al. (2019), Barr et al. (2020), Barile (2004), Connolly et al. (2013), Dailer et al. (2010), Fernandes et al. (2012), Gartner et al. (2002), McClelland et al. (1997), Mwaura et al. (2017), Oakes & Eyre (2015), Pruell et al. (2006), Riera et al. (2000), Rogers (1999), Rogers (2003), Tucker et al. (1999)
		Cladophorales	*Cladophora sp. Chaetomorpha linum*	River weed, Spaghetti algae	6	Barile (2004), Dailer et al. (2010), Fernandes et al. (2012), Lapointe et al. (2005), McClelland et al. (1997), Wassenaar & Culp (1996)
		Bryopsidales	*Caulpera* sp., *Penicillus* sp., *Halimeda* sp., *Codium isthmocladum, Avrainvillea* sp., *Udotea petiolata*	Sea grapes, Shaving brush, Fan green seaweed, Mermaid fan	4	Barile (2004), González-De Zayas et al. (2020), Morris et al. (2009), Vizzini & Mazzola (2006)
	Mixed species				2	Kaminski et al. (2018), Pitt et al. (2009)
	Conjugatophyceae	Zygnematales	Spirogyra sp., Mougeotia sp.	Water silk, Mougeotia	1	Wassenaar & Culp (1996)
Rhodophyta	Florideophyceae	Gigartinales	*Catenella nipae, Hypnea musciformis, Ahnfeltiopsis concinna, Rissoella verruculosa*	Red macroalgae	5	Costanzo et al. (2001), Costanzo et al. (2005), Dailer et al. (2010), Jones et al. (2001), Vermeulen et al. (2011)
		Ceramiales	Vidalia sp., *Acanthophora* sp., *Bryothamnion triquetrum, Laurencia* sp.	Red macroalgae	4	Barile (2004), Dailer et al. (2010), Gartner et al. (2002), Lapointe et al. (2005)
		Gracilariales	*Gracilaria* sp.	Red macroalgae	3	Barile (2004), Costanzo et al. (2001), McClelland et al. (1997)
		Rhodymeniales	*Botryocladia spinulifera*	Red macroalgae	1	Barile (2004)

Table 4.3 Review of invertebrate taxa used as bioindicators in stable isotope studies to assess wastewater inputs in aquatic environments, divided into their respective taxonomic groups and the number of studies that included each taxa.

Phylum	Subphylum	Class	Order	Common Name	Number of Studies	References
Porifera		Demospongiae		Sponge	2	Dolenec et al. (2007), Gartner et al. (2002)
Cnidaria		Anthozoa		Anemone	11	Conlan et al. (2006), Dolenec et al. (2005), Dolenec et al. (2007), Gearing et al. (1991), González-De Zayas et al. (2020), Heikoop et al. (2000), Lachs et al. (2019), Lozano-Bilbao et al. (2018), Risk et al. (2009), Tucker et al. (1999), Dover et al. (1992)
		Hydrozoa		Hydroid	1	González-De Zayas et al. (2020)
Nemertea				Ribbon worm	4	Conlan et al. (2006), Gearing et al. (1991), Spies et al. (1989), Tucker et al. (1999)
Platyhelminthes		Turbellaria		Planarian	1	di Lascio et al. (2013)
Annelida		Polychaeta		Bristle worm	7	Gearing et al. (1991), Hadwen & Arthington (2007), Riera et al. (2000), Sampaio et al. (2010), Spies et al. (1989), Tucker et al. (1999), Waldron et al. (2001)
		Clitellata		Worm, Leech	5	Bergfur et al. (2009), di Lascio et al. (2013), Riera et al. (2000), Vander Zanden et al. (2005), Xu & Zhang (2012)
Mollusca		Bivalvia		Bivalve	23	Babaranti et al. (2018), Bergfur et al. (2009), Carmichael et al. (2008), Conlan et al. (2006), Daskin et al. (2008), Fertig et al. (2009), Fertig et al. (2014), Forrest et al. (2007), Freedman et al. (2012), Gearing et al. (1991), Hicks et al. (2017), Lassauque et al. (2010), Loomer et al. (2015), Mendoza (2016), Piola et al. (2006), Pruell et al. (2006), Riera et al. (2000), Rogers (1999), Rogers (2003), Sampaio et al. (2010), Thibault et al. (2020), Tucker et al. (1999), Waldron et al. (2001)
		Gastropoda		Gastropod	17	di Lascio et al. (2013), Freedman et al. (2012), Gearing et al. (1991), Kaminski et al. (2018), Lachs et al. (2019), Loomer et al. (2015), Mendoza (2016), Oakes & Eyre (2015), Pruell et al. (2006), Riera et al. (2000), Rogers (1999), Rogers (2003), Sampaio et al. (2010), Vander Zanden et al. (2005), Vermeulen et al. (2011), Warnakulasooriya et al. (2017), Rožič et al. (2014)
Arthropoda	Hexapoda		Ephemeroptera	Mayfly	11	Bergfur et al. (2009), Cormier et al. (2021), Farwell (2000), Hicks et al. (2017), Loomer et al. (2015), McMahon et al. (2020), Morrissey et al. (2013), Robinson et al. (2016), Smucker et al. (2018), Vander Zanden et al. (2005), Wassenaar & Culp (1996)
			Diptera	True flies, non-biting midges (chironomids)	10	deBruyn et al. (2002), di Lascio et al. (2013), Hicks et al. (2017), Loomer et al. (2015), Mendoza (2016), Robinson et al. (2016), Smucker et al. (2018), Vander Zanden et al. (2005), Wassenaar & Culp (1996), Xu & Zhang (2012)
			Trichoptera	Caddisfly	8	Bergfur et al. (2009), deBruyn et al. (2002), Hicks et al. (2017), Loomer et al. (2015), Morrissey et al. (2013), Smucker et al. (2018), Vander Zanden et al. (2005), Wassenaar & Culp (1996)
			Plecoptera	Stonefly	4	Bergfur et al. (2009), Robinson et al. (2016), Smucker et al. (2018), Wassenaar & Culp (1996)
			Coleoptera	Beetle	4	Hicks et al. (2017), Loomer et al. (2014), Smucker et al. (2018), Vander Zanden et al. (2005)
			Odonata	Dragonfly	4	deBruyn et al. (2002), Loomer et al. (2015), Smucker et al. (2018), Vander Zanden et al. (2005)
			Hemiptera	True bugs (*i.e.* back swimmer	2	deBruyn et al. (2002), Vander Zanden et al. (2005)
			Megaloptera	Dobsonfly	2	Smucker et al. (2018), Vander Zanden et al. (2005)
						Connolly et al. (2013), di Lascio et al. (2013), Dudley & Shima (2010), Hadwen & Arthington (2007), Munroe et al. (2018), Pitt et al. (2009), Pruell et al. (2006), Riera et al. (2000), Robinson et al. (2016), Smith et al. (2016),

	Crustacea	Malacostraca	Decapoda	Decapod (lobster, crab, prawn, shrimp, crayfish)	16	Steffy & Kilham (2004), Suzzi et al. (2022), Tucker et al. (1999), van de Merwe et al. (2016), Dover et al. (1992), Vander Zanden et al. (2005)
			Amphipoda	Amphipod	11	Bergfur et al. (2009), Cormier et al. (2021), deBruyn et al. (2002), di Lascio et al. (2013), Mendoza (2016), Morrissey et al. (2013), Riera et al. (2000), Robinson et al. (2016), Sampaio et al. (2010), Tucker et al. (1999), Vander Zanden et al. (2005)
			Isopoda	Isopod	9	Bergfur et al. (2009), di Lascio et al. (2013), Dudley & Shima (2010), Hansson et al. (1997), Hicks et al. (2017), Loomer et al. (2015), Mendoza (2016), Robinson et al. (2016), Vander Zanden et al. (2005)
			Stomatopoda	Stomatopod	1	Risk & Erdmann (2000)
		Theocostraca (SubCl. Cirripedia)		Barnacle	3	Dolenec et al. (2006), Dolenec et al. (2007), Kaminski et al. (2018)
	Chelicerata	Arachnida (SubCl. Acari)	Trombidiformes	Water mite	2	deBruyn et al. (2003), Robinson et al. (2016)
Echinodermata		Asteroidea		Sea star	3	Conlan et al. (2006), Tucker et al. (1999), Dover et al. (1992)
		Echinoidea		Sea urchin	2	Conlan et al. (2006), Dover et al. (1992)
		Holothuroidea		Sea cucumber	2	Tucker et al. (1999), Dover et al. (1992)

Table 4.4 Review of fish taxa used as bioindicators in stable isotope studies to assess wastewater inputs in aquatic environments, divided into their respective taxonomic groups and the number of studies that included each taxa.

Family	Common Name	Number of Studies	Author/ Date
Catostomidae	Suckers	10	Arciszewski et al. (2014), deBruyn et al. (2002), Dubé et al. (2005), Farwell (2000), Freedman et al. (2012), Galloway et al. (2003), Hoffman et al. (2012), McMahon et al. (2020), Mendoza (2016), Steffy & Kilham (2004)
Percidae	Perch	8	Bergfur et al. (2009), deBruyn et al. (2002), Freedman et al. (2012), Hansson et al. (1997), Hicks et al. (2017), Hoffman et al. (2012), Loomer et al. (2015), Robinson et al. (2016),
Salmonidae	Salmon, Trout, Char, and Whitefish	6	Bergfur et al. (2009), Dubé et al. (2005), Freedman et al. (2012), Hoffman et al. (2012), Steffy & Kilham (2004), Wassenaar & Culp (1996)
Cyprinidae	Minnows and Carps	4	Bergfur et al. (2009), di Lascio et al. (2013), Freedman et al. (2012), Steffy & Kilham (2004)
Mugillidae	Mullet	4	di Lascio et al. (2013), Hadwen & Arthington (2007), Schlacher et al. (2007), Smith et al. (2016)
Leuciscidae	Nooksack Dace, Northern Redbelly Dace	4	deBruyn et al. (2003), Freedman et al. (2012), Steffy & Kilham (2004), Wassenaar & Culp (1996)
Fundulidae	Mummichogs and Killifish	3	Freedman et al. (2012), Pruell et al. (2006), Skinner et al. (2012)
Ambassidae	Glassfish	3	Hadwen & Arthington (2007), Schlacher et al. (2007), Smith et al. (2016)
Anguillidae	Eel	3	Bergfur et al. (2009), Freedman et al. (2012), di Lascio et al. (2013)
Centrarchidae	Sunfish	3	di Lascio et al. (2013), Freedman et al. (2012), Hoffman et al. (2012)
Sillaginidae	Whiting	3	Conlan et al. (2006), Hadwen & Arthington (2007), Smith et al. (2016)
Esocidae	Pike	3	Bergfur et al. (2009), deBruyn et al. (2002), Freedman et al. (2012)
Sparidae	Sea Bream	3	Hadwen & Arthington (2007), Smith et al. (2016), Vizzini & Mazzola (2006)
Clupeidae	Herring and Bony Bream	3	Freedman et al. (2012), Hansson et al. (1997), van de Merwe et al. (2016)
Osmeridae	Smelt	2	Hansson et al. (1997), Hoffman et al. (2012)
Cottidae	Sculpin	2	Galloway et al. (2003), Wassenaar & Culp (1996)
Ictaluridae	Brown Bullhead	2	deBruyn et al. (2002), Freedman et al. (2012)
Gasterosteidae	Stickleback	2	Freedman et al. (2012), Hoffman et al. (2012)
Pleuronectidae	Winter Flounder and Pacific Dover	2	Tucker et al. (1999), Spies et al. (1989)
Gerreidae	Common Silver Belly Roach	2	Hadwen & Arthington (2007), Schlacher et al. (2007)
Serranidae	Comber	1	Vizzini & Mazzola (2006)
Scorpaenidae	Black Scorpion Fish	1	Vizzini & Mazzola (2006)
Lotidae	Burbot	1	Bergfur et al. (2009)
Petromyzontidae	Brook Lamprey	1	Bergfur et al. (2009)
Eleotridae	Common Bully	1	van den Heuvel et al. (2007)
Moridae	Blue Antimora	1	Dover et al. (1992)
Hexagrammidae	Longspine Combfish	1	Spies et al. (1989)
Kyphosidae	Mado	1	Gaston & Suthers (2004)
Paralichthyidae	Pacific Sanddab	1	Spies et al. (1989)
Centrarchidae	Smallmouth Bass	1	Freedman et al. (2012)
Moronidae	Striped Bass	1	Freedman et al. (2012)
Atherininae	Smallmouth Hardyhead	1	Connolly et al. (2013)

4.5.2 Bioindicator Suitability

4.5.2.1 Vascular Plants

Table 4.1 summarizes vascular plant bioindicators most frequently used in stable isotope analyses to detect wastewater inputs. In coastal and estuarine environments, elevated $\delta^{15}N$ has been detected in many marine vascular plants and macroalgae found near sewage wastewater outfalls (Warnakulasooriya et al., 2017). These $\delta^{15}N$ signatures have been shown to decrease to relatively low levels along spatial gradients of wastewater exposure (Costanzo et al., 2001). As a result of nitrogen availability typically limiting plant growth in marine systems, primary producers assimilate wastewater-derived nitrogen into their tissues rapidly (Warnakulasooriya et al., 2017). Therefore, aquatic vascular plants can absorb and assimilate wastewater-derived nutrients and their tissue $\delta^{15}N$ reflects exposure over a given timeframe (Costanzo et al., 2001; Oakes et al., 2010).

Multiple aquatic macrophytes have been used to detect and delineate wastewater inputs in aquatic environments. In tropical estuaries, the mangrove *Avenicennia marina* is commonly used as a bioindicator and has shown success in the detection of complex nutrient inputs from wastewater (Hadwen & Arthington, 2007; Pitt et al., 2009). *A. marina* strips nutrients efficiently from the water column and sediment via their well-developed root system (Warnakulasooriya et al., 2017). Warnakulasooriya et al. (2017) conducted a stable isotope study in a tropical Australian estuary and concluded that *A. marina* was a suitable bioindicator for sewage wastewater due to high repeatability among samples, the ease of collection, and the ubiquitous presence of the species in tropical estuaries.

Another macrophyte frequently used in previous stable isotope studies in receiving environments for wastewater is eelgrass, *Zostera* sp. Eelgrass leaf nitrogen content has been

suggested as an alternative time-integrated, quantitative measure of nutrient pollution in aquatic environments (Schubert et al., 2013). Numerous studies have employed *Zostera* sp. to successfully map the spatial pattern of sewage wastewater in aquatic environments (Forqurean et al., 1997; Jones et al., 2001). Therefore, *Zostera* sp. has high potential as a proxy of wastewater-derived nitrogen. The turnover rates of nitrogen within aquatic macrophytes are relatively slow, ranging from weeks to months (Jones et al., 2001). As such, the use of macrophytes in stable isotope studies assessing wastewater inputs can offer temporal integration of the ^{15}N signal source (Pitt et al., 2009). While aquatic vascular plants have been identified as suitable bioindicators for wastewater, numerous studies have deemed them less efficient relative to macroalgae (Cole et al., 2004). Rooted plants uptake nutrients from the water column in addition to their root system, relying less heavily on direct uptake of nitrogen from the water column relative to macroalgae (McClelland et al., 1997). The more complex nutrient budgets of vascular plants relative to macroalgae makes them less likely to reflect the signature of dissolved inorganic nitrogen (DIN) in the water column (McClelland et al., 1997). Therefore, while macrophytes may be adequate bioindicators that have demonstrated some degree of suitability in the detection and spatial mapping of wastewater inputs, macroalgae may be more suitable to reflect water quality conditions.

4.5.2.2 Macroalgae

While vascular plants obtain nutrients from the water column and sediment via their roots, macroalgae depend solely on DIN from the water column by assimilating stable isotope signatures of biologically active nitrogen into their thalli (Connolly et al., 2013). Macroalgae have been suggested as more suitable bioindicators than vascular plants, reflecting a more

representative stable isotope signature of water quality (Cole et al., 2004). Oakes & Eyres (2015) conducted a stable isotope analysis on macroalgae and numerous invertebrate organisms and determined that macroalgae was the most appropriate bioindicator of sewage wastewater in the estuarine region since it demonstrated the greatest assimilation and was relatively easy to collect. Therefore, macroalgae may be a suitable bioindicator in many studies due their sedentary nature, ease of collection, ability to continuously incorporate DIN from the water column, and their frequent occurrence in a variety of habitats.

Macroalgae have relatively rapid growth rates and subsequent tissue turnover, making them good bioindicators of short-term pulses in water column DIN (Babaranti et al., 2019). However, within this review a distinction in tissue turnover rates was observed among classes of macroalgae. Wallentinus (1984) identified varying nutrient uptake characteristics in different functional groups of macroalgae. Foliose and filamentous macroalgal species have high nutrient uptake rates and reflect this enrichment in their tissues over a relatively short period of time (Wallentinus, 1984). In contrast, species with slow nitrogen uptake rates, such as brown macroalgae, only exhibit a shift in their stable isotope signature after prolonged exposure (Dudley & Shima, 2010). Gartner et al. (2002) conducted a stable isotope study and confirmed this distinction, showing that filamentous and foliose algae assimilated sewage-derived DIN within days. In contrast, slower-growing kelp species had lower nutrient uptake capacities and did not show significant shifts in stable isotope values during the same time period (Gartner et al., 2002). Therefore, careful consideration must be given to the functional group of macroalgae selected as a bioindicator within a given study since nitrogen assimilation varies among groups (Gartner et al., 2002). The brown macroalgal species used in chapter III and also commonly applied in many stable isotope studies in receiving environments for wastewater (Table 4.2) is

Fucus vesiculosus (Riera et al., 2000; Savage, 2005). This species has a relatively long tissue turnover time and was considered a good indicator of ambient water quality conditions over timescales of several years (Savage & Elmgren, 2004). Alternatively, *Ulva* sp. is a filamentous green macroalgae that is a frequently applied bioindicator in stable isotope studies (Table 4.2) (Babaranti et al., 2019; Barile, 2004). This species has a rapid growth rate and assimilates nutrients into its tissues over periods of days to weeks (Kaminski et al., 2018). Therefore, *Ulva* sp. may be a suitable bioindicator when immediate effects of wastewater are desired since it provides a stable isotope signal over a relatively short time frame. Such species can be useful in the early detection of nutrient enrichment or transient nutrient inputs, both of which are important to prevent further degradation of aquatic habitats (Dudley & Shima, 2010). Macroalgae with short tissue turnover times can also be used to evaluate improvements in the quality of wastewater treatment. For example, Rogers (2003) detected changes in the $\delta^{15}N$ value of *Ulva* sp. following upgrades to a sewage treatment plant. These alterations were not detectable in longer-lived organisms at the same timescale (Rogers, 2003).

Macroalgae have proven to be a suitable bioindicator in stable isotope studies designed to detect and trace wastewater inputs. Stable isotope values in macroalgae have been suggested as an alternative to water quality nutrient analysis since they record nutrient flows and transient pulses, which are difficult to measure and may be missed in direct grab samples assessing water quality (Lin & Fong, 2008). DIN in the water column is continuously incorporated into macroalgal tissues, making them suitable indicators to effectively trace the movement of wastewater-derived nutrients (Fernandes et al., 2012). Application of macroalgal bioindicators must be accompanied by an understanding of the life history of the macroalgae and selection of the appropriate species should support the goals of the study. If observations of long-term trends

are desired, a brown macroalgae may be most suitable, whereas green filamentous algae have been suggested as appropriate bioindicators for detecting short-term alterations in nutrient concentrations.

4.5.2.3 Invertebrates: Class Malacostraca

While invertebrates were the most frequent bioindicator group identified among the 94 stable isotope studies in receiving environments for wastewater, there was high variability in their perceived suitability to assess wastewater inputs. The most frequently studied invertebrate bioindicator group was class Malacostraca (*n*=26), which exhibited the highest variation in suitability as a bioindicator due to mobility of various species and diversity of feeding modes (Dover et al., 1992). Within this class, order Decapoda was the most frequent taxonomic group used as bioindicators, with lobsters, crab, prawns, shrimp, and crayfish the most commonly utilized. Some taxa within Decapoda were perceived more suitable as bioindicators of sewage wastewater than others (Connolly et al., 2013). Pitt et al. (2009) determined stable isotope values of two crab species (*Australoplax australis* and *Parasesarma erythrogramma*) were each capable of reflecting upgrades to a sewage wastewater treatment plant by measuring their stable isotope values. Similarly, Connolly et al. (2013) measured stable isotope values in the blue swimmer crab (*Portunus armatus*) exposed to sewage wastewater and determined they exhibited a spatial pattern of $\delta^{15}N$, indicating exposure to wastewater. However, the complex diets and mobility of other decapod species have hampered their successful utilization as adequate bioindicators of wastewater. Riera et al. (2000) used green crab (*Carcinus maenas*) to detect discharge from a sewage treatment plant and while they indicated some degree of exposure, this species was not considered as well-suited to detect wastewater as macroalgae also included in the study. Dover et al. (1992) completed a stable isotope study in deep sea environments receiving sewage

wastewater and determined that the squat lobster (*Munidopsis* sp.) was not suitable for reflecting sewage wastewater exposure due to its mobility. Additional decapod species: shrimp and prawn, are commonly employed in stable isotope studies to detect wastewater exposure and have generally shown success in this role (Smith et al., 2016). Munroe et al. (2018) employed the Eastern king prawn (*Melicertus plebejus*) and determined it was an effective bioindicator of sewage wastewater. However, this study also emphasized that the migratory behaviour of *M. plebejus* to inshore nursery areas must be considered in future studies since it may confound the interpretation of stable isotope results (Munroe et al., 2018). Smith et al. (2016) used a wide variety of primary consumer species and determined that the greentail prawn (*Metapenaeus bennettae*) was the most suitable bioindicator of sewage wastewater exposure relative to various fish species and polychaetes included in the study. However, like most bioindicator taxa in class Malacostraca, the success of various species to serve as indicators of wastewater was inconsistent.

Suzzi et al. (2022) and Pruell et al. (2006) used different shrimp species to detect sewage wastewater and found high variation among sampling locations, concluding they were unsuitable bioindicators. Another group of organisms within class Malacostraca is order Amphipoda and order Isopoda. Overall, amphipods and isopods employed in stable isotope studies to detect wastewater were successful as bioindicators (di Lascio et al., 2013). Dudley & Shima (2010) attributed the success of isopods to detect sewage wastewater to their movement patterns and feeding ranges, which extend over sufficiently short distances, permitting detection of sewage dispersal patterns. Similarly, di Lascio et al. (2013) studied a wide array of primary consumers and determined that the isopod (*Proasellus coxalis*) was a good indicator of sewage wastewater exposure. Amphipods and isopods are small organisms with relatively fast nitrogen turnover

rates, making them easily affected by environmental changes (Dudley & Shima, 2010). The applicability of both amphipods and isopods $\delta^{15}N$ as indicators of short-term changes in water quality conditions is promising. Benthic amphipods and isopods are ecologically important organisms, numerically dominant in aquatic ecosystems and easily collected, and are tolerant to wide ranges of physiochemical conditions (de-la-Ossa-Carretero et al., 2012). These characteristics make them suitable bioindicators in water quality monitoring programs and as indicators of wastewater exposure. According to previous studies employing malacostracan organisms, amphipods and isopods have shown the highest degree of suitability as bioindicators of wastewater exposure.

4.5.2.4 Invertebrates: Class Bivalvia

Overall, benthic filter feeding bivalves appear to be good bioindicators of wastewater exposure. Bivalves are ubiquitous in many freshwater and marine habitats and serve as ecologically important bioindicators in many stable isotope studies assessing wastewater inputs (Freedman et al., 2012; Rogers, 2003). Bivalves are relatively long-lived species, reflecting changes in environmental conditions that occur over extended time periods (Rogers, 2003). Contributing to their widespread success as bioindicators, adult bivalves are sessile and therefore accurately represent environmental conditions within a given region (Riera et al., 2000). The feeding mode of bivalves has also contributed to their success as suitable bioindicators (Piola et al., 2006). Bivalves are filter feeders that directly ingest and assimilate material from the water column including phytoplankton, zooplankton, bacteria, and detritus (Arapov et al., 2010). Therefore, bivalves can assimilate the distinct $\delta^{13}C$ and $\delta^{15}N$ signatures of wastewater organic matter (Dudley & Shima, 2010).

The most widely applied bivalve indicator taxa was *Mytilus* sp. (Babaranti et al., 2019; Rogers, 1999; Rogers, 2003). Studies employing *Mytilus* sp. to detect and trace wastewater inputs revealed that this taxon has high utility in delineating wastewater inputs (Freedman et al., 2012; Lassauque et al., 2010). Another bivalve taxon commonly applied as a bioindicator in stable isotope studies for assessing wastewater was oysters (most commonly *Crassostrea* sp.). Fertig et al. (2009) suggest that oysters are ideal bioindicators for detecting wastewater since they assimilate nitrogen from wastewater, are sessile, hardy, and do not substantially change diets post-metamorphosis or with developmental stages. Therefore, their usefulness as a bioindicator species is not confounded by life cycle patterns or feeding modes (Fertig et al., 2009). Additional studies that used oysters to detect wastewater exposure identified this taxon as an ideal bioindicator (Daskin et al., 2008; Riera et al., 2000). Organisms within the class Bivalvia were effective at detecting wastewater exposure. These results combined with the success of *Mytilus* sp. to detect potential enrichment from historical pulp mill effluent in chapter III, suggest that bivalves should be considered as bioindicators in future stable isotope studies in aquatic receiving environments for wastewater.

4.5.2.5 Invertebrates: Class Gastropoda

Gastropods are another frequently employed bioindicator in stable isotope studies in aquatic environments impacted by wastewater (Rožič et al. 2014; Vermeulen et al., 2011). The most common gastropod taxa used in previous stable isotope studies has been marine snails: *Narassius* sp. (Gearing et al., 1991), *Littorina* sp. (Oakes & Eyre, 2015), and limpets: *Patella* sp. (Vermeulen et al., 2011) and *Cellana* sp. (Kaminski et al., 2018). The use of benthic deposit feeders such as gastropods have proven useful in the elucidation of wastewater origins and

mapping wastewater permeation in aquatic ecosystems (Warnakulasooriya et al., 2017). Like bivalves, gastropods are relatively long-lived species that provide a time-integrated picture of exposure to anthropogenic impacts (Dudley & Shima, 2010). Many previous stable isotope studies have identified gastropods as suitable bioindicators of wastewater (di Lascio et al., 2013; Pruell et al., 2006). Oakes and Eyre (2015) measured stable isotope values in a variety of snail species and determined they were all good bioindicators of sewage wastewater exposure due to their low mobility and long-life history. Additionally, many studies have identified the limpet, *Patella* sp., as a good tracer of anthropogenically-derived organic matter in coastal zones (Rogers, 2003). However, there appears to be a common theme reported in the literature of high intra and inter specific variation in gastropod stable isotope values in wastewater receiving environments (Vermeulen et al., 2011). These findings are similar to what was observed in chapter III with high variability in $\delta^{13}C$ and $\delta^{15}N$ values of *Littorina littorea* among sampling locations. Warnakulasooriya et al. (2017) determined stable isotope values of gastropods showed promise as a monitoring tool for wastewater; however, high variability due to spatial differences in food source availability may reduce their reliability as tracers of wastewater in aquatic environments. Similarly, Vermeulen et al. (2011) detected spatially variable stable isotope signatures of wastewater-derived nitrogen in gastropods, attributed to confounding effects of habitat heterogeneity (Dudley & Shima, 2010; Lachs et al., 2009). Moyo et al. (2021) concluded that further analysis of gastropod stable isotope values in polluted environments is required to facilitate their use in the context of future monitoring programs for wastewater. Since variability in stable isotope values has been observed, gastropods may be unsuitable bioindicators for detecting spatial patterns in wastewater exposure, results consistent with those reported in chapter III. Since bivalves and gastropods are both primary consumers and exhibit similar life

histories and tissue turnover, in heterogeneous habitats with varying food sources, bivalves may serve as more suitable bioindicators due to their simplified feeding modes and lack of mobility.

4.5.2.6 Invertebrates: Class Insecta

Class Insecta was well represented in many stable isotope studies conducted in aquatic receiving environments for wastewater. Many studies conducted in freshwater environments have employed organisms from this taxonomic class (Morrissey et al., 2013). In most studies, organisms in class Insecta were identified as suitable bioindicators for wastewater (Vander Zanden et al., 2005). Morrissey et al. (2013) conducted a study that aimed to determine if sewage wastewater could be detected in riverine environments by measuring $\delta^{13}C$ and $\delta^{15}N$ values in a variety of larval stage insects including caddisflies and mayflies. The study concluded insect $\delta^{15}N$ values may provide an effective addition to classical biomonitoring surveys to detect the impacts of sewage wastewater (Morrissey et al., 2013). While stable isotope studies in environments impacted by pulp mill effluent were severely underrepresented in the literature relative to sewage wastewater, many of the studies conducted in environments receiving pulp mill effluent utilized various insect taxa as bioindicators (McMahon et al., 2020; Wassenaar & Culp, 1996). McMahon et al. (2020) studied the larval-stage mayfly (*Hexagenia* sp.) and detected variation in $\delta^{13}C$ values after the closure of a pulp mill, concluding that mayflies were ideal bioindicators within the given study. Mendoza (2016) also measured stable isotope values in *Hexagenia* sp. in environments impacted by pulp mill effluent and likewise concluded that larval stage mayflies were suitable bioindicators. Larval stage mayflies are sedentary, abundant, and short-lived; therefore, sensitive to short term changes in ecosystem conditions (McMahon et al., 2020). These characteristics resulted in mayflies being good bioindicators in the reviewed

studies (McMahon et al., 2020; Mendoza, 2016). Many additional studies identified insects as suitable bioindicators of wastewater (Cormier et al., 2021; deBruyn et al., 2002; Hicks et al., 2017). Robinson et al. (2016) conducted stable isotope analysis on larval mayflies, stoneflies, and chironomids and identified enriching trends in their stable isotope values immediately downstream of a municipal wastewater treatment plant. These results, along with similar studies, have validated the ability of wastewater-derived nutrients to be incorporated into the tissues of various insect taxa (deBruyn et al., 2002; Xu & Zhang, 2012). Of the numerous studies that included insects as bioindicators, only one study explicitly stated that the selected taxa were unsuitable as bioindicators. Wayland and Hobson (2001) conducted a stable isotope study on caddisflies and mayflies and determined that adult aquatic insects did not reflect stable isotope signatures in wastewater to the same extent as algae, also used within the study. The potential explanation for these observations was the upstream migration of insect taxa following their emergence, which introduced spatial variability in their stable isotope signatures (Wayland & Hobson, 2001). Overall, larval stage insects have proven to be adequate bioindicators in freshwater receiving environments for wastewater; however, careful consideration must be given to the potential mobility of each insect species throughout their life history.

4.5.2.7 Invertebrates: Class Polychaeta

Another frequently employed group of taxa within stable isotope studies conducted in wastewater receiving environments is class Polychatea. Polychaetes exhibit a wide array of feeding modes from suspension feeders, deposit feeders, and predatory feeders (Gearing et al., 1991). Therefore, while polychaetes are typically ubiquitous in polluted aquatic environments (Dean, 2008), variation in feeding modes among this taxonomic class confounded their

suitability as bioindicators in many studies (Tucker et al., 1999). For example, Gearing et al. (1991) completed a stable isotope study of over 20 polychaete species in an environment impacted by sewage wastewater and observed variability in stable isotope values, attributed to their feeding modes. The generalist, mobile predator *Nephtys incisa,* was highly enriched in $\delta^{15}N$, reflecting sewage wastewater exposure while the suspension feeder, *Steblospio benedicti*, showed less enriched stable isotope signatures (Gearing et al., 1991). This variation among individual species of polychaetes has been observed in numerous other studies. Sampaio et al. (2010) and Tucker et al. (1999) conducted a stable isotope study on multiple species of polychaetes and determined that some species reflected the presence of sewage particulate matter while others did not indicate exposure effects. Therefore, to adequately assess their utility as bioindicator organisms, further research aimed at assessing individual feeding modes among polychaete species is required to determine their efficacy to detect wastewater in aquatic ecosystems.

4.5.2.8 Invertebrates: Class Anthozoa

Organisms within the class Anthozoa were relatively well represented in the literature as bioindicators of wastewater in stable isotope studies. Generally, most studies indicated that anthozoan taxa were suitable bioindicators for detecting wastewater (Heikoop et al., 2000; Lozano-Bilbao et al., 2018; Tucker et al., 1999). Heikoop et al. (2000) determined that $\delta^{15}N$ values of the lobe coral (*Porites lobata*) indicated enrichment from sewage wastewater. Within this study, Hekioop et al. (2000) determined that shallow water corals are particularly sensitive to sewage nutrient inputs and are good potential bioindicators of ambient DIN in coastal ecosystems. Corals are also suitable bioindicators in many studies due to their generally slow

growth rate, providing a time-integrated assessment of environmental conditions (Risk et al., 2009), although the growth rate of corals can vary among individual species (Redding et al., 2013). Tucker et al. (1999) identified suitable bioindicators in coral species across varying growth rates. Both the fast-growing scleractinian coral, *Acropora* sp., and the slow-growing leather coral, *Sinularia* sp., were capable of wastewater detection, each capturing changes in nutrient dynamics in a coastal ecosystem over different time periods (Tucker et al., 1999).

In addition to corals, sea anemones have been identified as useful bioindicators of wastewater in many stable isotope studies (Lozano-Bilbao et al., 2018). Anemones are filter feeding organisms that capture food with thoracic appendages known as cirri (Dolenec et al., 2005). Like bivalves, their sessile nature renders them capable of spatially elucidating primary sources of nutrients in aquatic environments (Lozano-Bilbao et al., 2018). Dolenec et al. (2005) suggest that the anemone Mediterranean snakelocks (*Anemonia sulcata*) is a suitable bioindicator that can be used to assess and monitor the conservation status of coastal ecosystems. This study was reinforced by Lozano-Bilbao et al. (2018) who observed a gradient in $\delta^{15}N$ values in *A. sulcata* with increasing distance from a sewage outfall, indicating that this species is indeed able to reflect wastewater exposure through its stable isotope values. Therefore, taxa within class Anthozoa have proven to be sensitive organisms whose $\delta^{15}N$ signatures can be used to indicate wastewater inputs in coastal ecosystems.

An important consideration in the evaluation of biota suitability for the detection of wastewater sources to aquatic environments is the underlying biogeochemistry and background $\delta^{15}N$ of the surrounding environment. This is particularly relevant within class Anthozoa since high variation exists in the geographic study areas of this taxa relative to other taxa included in the literature review. Anthozoan taxa were primarily located in tropical locations while other

taxonomic groups were primarily from temperate regions. Environments with background $\delta^{15}N$ values that deviate significantly from the wastewater $\delta^{15}N$ signature may permit the identification of bioindicators that may otherwise be unsuitable in different environmental conditions. For example, reef corals are frequently used as bioindicators of sewage wastewater in oligotrophic environments where the baseline $\delta^{15}N$ is low (2-4‰ (Meador et al., 2007). Therefore, detection of nitrogen-enriched sewage wastewater in contrast to background conditions is more easily accomplished. However, in temperate environments the $\delta^{15}N$ value is higher relative to oligotrophic tropical waters. For example, offshore Nova Scotia background $\delta^{15}N$ values are approximately 5-7‰ (Sherwood et al., 2011). Therefore, the ability for taxa to reveal biogeochemical differences between wastewater and the background environment is reduced relative to tropical latitudes. Ambient biogeochemical conditions of the environment is therefore an important consideration in the evaluation and selection of taxa to serve as effective bioindicators.

4.5.2.9 Invertebrates: Additional Taxa

Additional invertebrate taxa that have been less extensively studied in stable isotope studies conducted in wastewater receiving environments are ribbon worms (phylum Nemertea), worms (subclass Oligochaeta), leeches (subclass Hirudinea), barnacles (subclass Cirripedia), and echinoderms (phylum Echinodermata). Many of these taxa were assessed among a suite of other taxa and comments regarding their perceived suitability as bioindicators were excluded in many studies. Ribbon worms were included in four studies and appear to be adequate bioindicators of sewage wastewater (Conlan et al., 2006; Gearing et al., 1991). Within the class Clitellata, both di Lascio et al. (2013) and Vander Zanden et al. (2005) measured stable isotope values of leeches

and identified enrichment in environments impacted by sewage wastewater. Similarly, stable isotope studies have been completed on aquatic oligochaetes, with organisms indicating some degree of exposure to sewage-derived nutrients (Bergufur et al., 2009; Riera et al., 2000). Barnacles were used as bioindicators in two studies and their suitability was variable (Dolenec et al., 2010; Kaminski et al., 2018). Dolenec et al. (2010) concluded that $\delta^{13}C$ and $\delta^{15}N$ values in the acorn barnacle (*Balanus perforatus*) suggest that this species could serve as a potential bioindicator for sewage-derived nutrients. However, Kaminski et al. (2018) was unable to detect exposure to wastewater in the stable isotope values of the rose barnacle (*Tesseropora rosea)*. Therefore, while barnacles possess many features of good bioindicators, further studies are required to fully evaluate their suitability to detect wastewater inputs. Additionally, echinoderms (including sea urchins, sea cucumbers, and sea stars) have been employed as bioindicators for wastewater in stable isotope studies. Generally, most studies identified these taxa as suitable bioindicators (Conlan et al., 2006; Tucker et al., 1999). Tucker et al. (1999) assessed stable isotope values of both a sea cucumber (*Caudina* sp.) and mud star (*Ctenodicsus* sp.) in an environment receiving sewage wastewater and determined that both species were suitable bioindicators (Tucker et al., 1999). Likewise, Conlan et al. (2006) measured stable isotope values in a sea urchin (*Sterechinus neumayeri*) and sea star (*Odontaster validus*) and determined that both species showed evidence of sewage assimilation, with authors recommending these species as bioindicators for future wastewater detection. Assessment of the suitability of these additional taxonomic groups as bioindicators is challenging due to the limited number of studies using these organisms to detect wastewater exposure. Therefore, further research is required to evaluate the utility of stable isotope values in these under-represented taxonomic groups to detect and delineate wastewater exposure.

4.5.2.10 Fish

Many fish species have been used in stable isotope studies to detect wastewater in both freshwater and marine environments. Results regarding their suitability as bioindicators were variable. Some studies have identified fish as suitable bioindicators of wastewater pollution (Connolly et al., 2013; Wassenaar & Culp, 1996). Steffy & Kilham (2004) detected sewage wastewater nutrients in a variety of species including creek chub (*Semotilus atramaculatus*), brown trout (*Salmo trutta*), and blacknose dace (*Rhinichthys atratulus*). Additionally, both Schlacher et al. (2007) and Smith et al. (2016) were able to identify exposure to sewage wastewater reflected in the stable isotope values of a variety of coastal fish species. However, there are many characteristics of fish that vary among individual species and were identified as confounding factors in several stable isotope studies in receiving environments for wastewater. Namely, fish as a phylogenetic group are extremely diverse, inhabiting a range of habitats, and exhibiting a variety of foraging behaviours and dietary preferences which change throughout their life histories to significantly complicate interpretation of stable isotope signatures (Dubé et al., 2005). For example, Dubé et al. (2005) suggests that omnivory in the benthic-feeding longnose sucker (*Catostomus catostomus*) likely contributed to variation in its $\delta^{15}N$ values.

While diverse and complex feeding strategies may complicate interpretation of stable isotope results, organism mobility was the confounding factor most discussed in studies involving fish (Freedman et al., 2012). The most frequent fish species used as a bioindicator was white sucker (*Catostomus commersonii*) (Arciszewski et al., 2014; McMahon et al., 2020) which exhibit many traits making them suitable as bioindicators of wastewater in stable isotope studies: widespread abundance, tolerance to a range of environmental conditions, and a lifespan of up to 20 years (Mendoza, 2016). However, most studies that used this species referred to its mobility,

which may result in a stable isotope signature not truly representative of the stable isotope signatures of local wastewater. White sucker has been reported to migrate over 40 km during spring migrations (Mendoza, 2016) and were consequently considered unsuitable bioindicators in many stable isotope studies (Farwell, 2000; Mendoza, 2016). To mitigate challenges with fish mobility, Munkittrick et al. (2002) recommends the use of small-bodied fish which typically have smaller, well-defined home ranges relative to larger-bodied fish. For example, small-bodied mummichog fish (*Fundulus heteroclitus*) (Skinner et al., 2012) and slimy sculpin (*Cottus cognatus*) have been identified as suitable bioindicators due to their high site fidelity and narrow home range (Galloway et al., 2003). In the present thesis, these attributes of small-bodied fish contributed to the conclusion mummichog were considered suitable bioindicator species in chapter III, with their stable isotope values indicating a gradient in potential historical exposure to pulp mill effluent.

While select studies have determined stable isotope values in fish were adequate for assessing wastewater exposure, mobility-induced variation in stable isotope incorporation suggests large-bodied fish may not be the most suitable for assessing wastewater inputs in aquatic ecosystems. Many studies that assess stable isotope values on a wide suite of organisms have determined that invertebrates are better indicators of wastewater exposure than fish (di Lascio et al., 2013). Therefore, understanding the movement and migration patterns of the selected bioindicator species is essential for accurate interpretation of stable isotope results. Before selecting a fish species to use in stable isotope studies for detecting wastewater, a comprehensive understanding of their movement patterns is recommended.

Table 4.5 Summary of taxa most frequently used in stable isotope studies to assess wastewater inputs in aquatic ecosystems and their suitability as bioindicators. (+) indicate positive qualities regarding the taxa's suitability and (-) indicate negative qualities.

Taxa Group	Class/ Subclass of Organisms	Suitability Comments
Vascular Plants		(+)Have successfully detected and delineated wastewater inputs in aquatic environments (+)Slow growth makes them a suitable time-integrated measure of wastewater inputs (-)Acquire nutrients from both roots and leaves, making them less likely to reflect water column DIN relative to macroalgae
Macroalgae		(+)Good bioindicators to detect wastewater and reflect DIN in water column Careful consideration of macroalgal species is required due to differences in tissue turnover among macroalgal taxonomic groups
Invertebrate	Cl. Malacostraca (Order Amphipoda, Order Isopoda, Order Decapoda)	(+)Amphipods and isopods appear to be among the most successful bioindicators for wastewater due to their abundance and high site fidelity (-)Range of results regarding their suitability among different taxonomic orders (-)Mobility and complex feeding modes of decapods has rendered them less successful as bioindicators
Invertebrate	Cl. Bivalvia	(+)Have been frequently used and considered ideal bioindicators in stable isotope studies detecting wastewater (+)Filter feeders that incorporate wastewater-derived POM (+)Sedentary (+)Easy to collect (+)Long lived organisms that permit time-integrated measure of ecosystem conditions
Invertebrate	Cl. Gastropoda	(+)Low mobility organisms that reflect local conditions (-)Feed on an occasionally-diverse diet and prone to diet switching (-)High variability observed in heterogenous habitats across numerous studies (-)Further studies required to validate their suitability as bioindicators for wastewater
Invertebrate	Cl. Insecta	(+)Low mobility (+)Larvae abundant in freshwater environments (+)Short lived and sensitive to short term changes in environmental conditions (-)One study found confounding effects of mobility in adult insects
Invertebrate	Cl. Polychaeta	(+)Limited mobility (+)Ubiquitous in polluted aquatic environments (-)Variety of feeding modes has resulted in variation of stable isotope patterns among individual species (-)Further research required on different feeding modes to identify suitable species as bioindicators
Invertebrate	Cl. Anthozoa	(+)Sedentary filter feeders that reflect local environmental conditions (+)Long- lived organisms that permit time-integrated measure of environmental conditions
Fish		(+)Long lived organisms that permit time-integrated measure of ecosystem conditions (-)Many studies report mobility of larger-bodied fish as a confounding factor for interpretation of stable isotope results. However, diet, mobility, and site fidelity can vary markedly by species and age class.

4.3.4 Recommendations and Criteria

This study identified the use of numerous biological taxa as bioindicators of municipal sewage and/or pulp mill effluent wastewater in stable isotope analyses. The number of studies which successfully determined enrichment effects of wastewater in biota demonstrates the utility of stable isotopes in identifying and tracing wastewater-derived nutrients in aquatic ecosystems. Employing stable isotope analysis in aquatic environments receiving wastewater could be used to target and prioritize mitigation efforts in polluted environments. Additionally, this study demonstrated that stable isotope measurements in aquatic biota may be a useful tool in future biomonitoring programs aimed at assessing impacts from sewage wastewater or pulp mill effluent. Many studies would benefit from using multiple bioindicators that integrate ambient nutrient conditions over different time scales. Multiple bioindicators offer a selection of complementary vantage points for evaluating wastewater impacts on aquatic ecosystems at various spatial and temporal scales. Many studies discussed the value in applying multiple taxa as bioindicators for detecting wastewater dispersal patterns (Dudley & Shima, 2010). Taxa have varying feeding modes, home ranges, and life histories which make some organisms better suited to detect certain environmental conditions relative to others.

Based on this review, through knowledge of an organism's life history, growth rate, and feeding ecology, taxa can be selected to optimize the information obtained from stable isotope analysis of aquatic biota in wastewater receiving environments. However, goals of an individual study should guide the selection of appropriate bioindicators. Studies seeking to identify long-term historical effects of enrichment or studies occurring in environments where wastewater release no longer occurs, may opt for bioindicators that do not show rapid fluctuations in their stable isotope signatures. Studies that are designed to evaluate short-term alterations in

ecosystem health due to wastewater discharges should employ taxa which rapidly respond to environmental conditions through changes in their stable isotope values. Stable isotope values in biota are a useful tool for detecting changes in the environment in response to sewage wastewater and pulp mill effluent. However, careful consideration of multiple ecological criteria must be considered in the selection of appropriate bioindicator species. Table 4.6 outlines six criteria designed to guide the selection of suitable bioindicator taxa for stable isotope studies in aquatic receiving environments for wastewater. Careful consideration must be given to these criteria, while ensuring the goals of the study and the characteristics of the aquatic receiving environment are regarded.

Table 4.6 Criteria for the selection of suitable bioindicator taxa in stable isotope studies conducted in receiving environments for wastewater to ensure adequate characterization of aquatic ecosystem conditions.

Criteria	Comment
1) *Mobility*	Understanding an organisms' site fidelity is crucial in the interpretation of stable isotope results. High mobility in a bioindicator is often not a true reflection of ecosystem health within the impacted region. Sessile organisms are recommended.
2) *Tissue turnover and life history*	Understanding an organisms' tissue turnover and life history is essential and should align with the goals of the study.
3) *Limited diet complexity*	A complex diet can confound interpretation of stable isotope results. Limited diet complexity is recommended.
4) *Ease of collection*	Taxa must be relatively easy to collect for use in stable isotope studies and their distribution broad enough to permit comparison across multiple studies.
5) *Directly exposed to wastewater*	Taxa must be directly exposed to wastewater and reflect environmental conditions within the impacted region.
6) *Ubiquitous*	Taxa must be ubiquitous in the environment to permit collection of statistically significant sample sizes.

Organism mobility is the first factor that must be considered in the selection of appropriate bioindicator taxa. After reviewing numerous studies that assess stable isotope values in biota within receiving environments for effluent, the confounding factor that impacted the interpretation of the results the most was mobility of taxa. An understanding of the site fidelity and movement or migratory patterns of the selected species is crucial to assess the stable isotope values within a particular region and adequately evaluate the impacts of wastewater inputs.

Sessile species such as macroalgae, bivalves, and sea anemones, or organisms that exhibit high site fidelity, such as small-bodied fish, appear to be the most suitable bioindicators to elucidate primary sources of nutrients in aquatic ecosystems.

The utility of taxa as bioindicators is highly dependent on understanding their life history. Careful consideration must be given to an organism's tissue turnover rate and life history since these factors will determine the duration of exposure that is detected in the stable isotope signatures. Smaller organisms with faster tissue turnover rates can be easily affected by environmental and physical changes. In contrast, long-lived organisms with long tissue turnover rates better reflect environmental conditions over longer periods of time. Therefore, selection of an organism that has been well-studied and has a well-defined life history is recommended in stable isotope studies. Furthermore, the selection of appropriate bioindicators should align with the goals of the specific study. If the goal is to provide early detection of wastewater inputs or improvements in wastewater treatment processes, taxa with a short tissue turnover rate, such as *Ulva* sp., taxa in class Insecta, or amphipods and isopods are recommended. In contrast, if long-term effects of exposure or detection of a historical stable isotope signature from wastewater is desired, taxa with long tissue turnover rates are recommended such as brown macroalgae, bivalves, or sea anemones.

An organism with a complex diet can complicate interpretation of stable isotope results. A species feeding ecology must be well defined for bioindicator approaches to be effective. For example, omnivorous species such as decapod crustaceans can confound interpretation of results since a variety of prey items increase variability of stable isotope values. Additionally, taxonomic groups where variation in feeding modes has been observed among individual species may require additional research to properly elucidate which species are better suited

bioindicators. Taxa with a well-defined feeding ecology and that prey on a limited number of dietary items, such as filter feeding bivalves, provide less ambiguity in their stable isotope results and a more representative reflection of local environmental conditions.

The ease of collection must also be considered in the selection of appropriate bioindicator taxa for detecting wastewater inputs. Taxa that are easily collected along the shoreline or at low tide are suggested for use in stable isotope studies evaluating wastewater impacts in marine ecosystems. Additionally, ease of collection often results in an organism being used in numerous studies, permitting comparison of stable isotope values across multiple studies. The selected bioindicator must also be directly exposed to wastewater. Direct exposure ensures adequate characterization of the impacts of wastewater release on the aquatic ecosystem. Studies designed to characterize spatial variation of wastewater inputs should select a species that is resident at the outflow location and directly exposed to wastewater inputs. In chapter III, mummichog fish and macroalgae were sampled directly at the outflow to ensure direct effluent exposure. The final criteria for selection of appropriate bioindicators is that the selected taxa must be ubiquitous or highly abundant in the studied environment. The selected bioindicator must be sufficiently abundant to permit the collection of a statistically significant sample size that does not impact the population through inclusion in the study. These six criteria are aimed at the selection of suitable bioindicator taxa in stable isotope studies aimed at assessing wastewater inputs in aquatic receiving environments. Careful consideration of these six criteria will permit the selection of effective bioindicators that accurately characterize environmental conditions in an anthropogenically-impacted region.

4.3.5 Conclusion

This study was aimed at reviewing bioindicator taxa used in previous stable isotope studies to detect wastewater inputs in aquatic ecosystems and evaluate the suitability of various bioindicators. Results determined that a diverse assemblage of taxa from four large taxonomic groups: vascular plants, macroalgae, invertebrates, and fish, have been used in stable isotope studies to detect wastewater inputs. Many taxa were effective at identifying enrichment effects of wastewater, which was reflected in their stable isotope signatures. However, certain characteristics of various taxa resulted in variation in their suitability as effective bioindicators. It was evident that the goals of a particular study are important considerations in the selection of bioindicator species. Therefore, one taxon may not be universally effective at achieving the goals of numerous studies in wastewater-affected environments. Whether the aim of the study is simply to detect and delineate wastewater inputs, identify improvements in wastewater treatment procedures, or evaluate ecosystem recovery following cessation of effluent release; these various goals should influence the taxa selected as bioindicators. Selection of adequate bioindicator species to address the goals of the study is important to ensure ecosystem health is properly characterized. Studies designed to measure short-term changes in environmental quality should include species which rapidly respond to changing water quality conditions, such as *Ulva* sp, larval-stage insect taxa, or amphipods and isopods. These species quickly incorporate changes in stable isotope signatures from their surrounding aquatic environments. Studies seeking to determine long-term changes in water quality conditions or recovery following the closure of a wastewater treatment plant may opt for longer-lived species such as brown macroalgae or bivalves. Mussels are one of the most suitable bioindicators used in both freshwater and marine environments to detect wastewater inputs. Due to their sedentary nature, relatively long-life

histories, ability to filter particulate organic matter, and reflect wastewater stable isotope signatures, mussels and other bivalves are effective bioindicators.

Within stable isotope studies utilizing bioindicators of wastewater, it is important to consider characteristics of the receiving environment. Understanding the nature of the study site such as current direction, mixing effects, and the diversity and abundance of native species is important in the selection of adequate bioindicators for wastewater in any stable isotope study. With consideration for both the goals of the study and characteristics of the receiving environment, a set of six criteria were developed for selection of bioindicator taxa in stable isotope studies designed to assess pulp mill effluent and/or sewage wastewater in aquatic receiving environments. The recommended criteria are as follows: 1) organism mobility, 2) organism tissue turnover and life history, 3) limited diet complexity, 4) ease of collection, 5) direct exposure to wastewater, and 6) ubiquity in the environment. A thorough consideration and understanding of these six criteria is recommended to permit the selection of suitable bioindicators in ecological studies measuring stable isotope values in taxa exposed to wastewater.

Chapter 5: Future Environmental Monitoring Recommendations and Conclusions

5.1 Summary of Research

This study aimed to evaluate spatial variability of nutrient sources and assess trophic structure along a coastal marine environment, Northumberland Strait, adjacent to a former wastewater treatment facility for pulp mill effluent prior to remediation. The three objectives for this study were as follows:

1. Assess variability in sources of carbon and nitrogen along a spatial gradient historically exposed to pulp mill effluent along the coastal Northumberland Strait prior to ecosystem remediation,
2. Evaluate potential impacts of pulp mill effluent exposure on trophic dynamics along the coastal Northumberland Strait prior to ecosystem remediation, and
3. Evaluate the suitability of bioindicator taxa for assessing wastewater inputs in aquatic receiving environments using stable isotope analysis.

To achieve the objectives of this research, bulk stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) was conducted on a suite of coastal marine biota along a spatial gradient of historical pulp mill effluent exposure from Boat Harbour, Nova Scotia. Stable isotopes values of the following biota were measured: particulate organic matter (POM), macroalgae (*Fucus vesiculosus*), periwinkles (*Littorina littorea*), blue mussels (*Mytilus edulis*), rock crab (*Cancer irroratus*), and American lobster (*Homarus americanus*). To identify spatial variation in sources of carbon and nitrogen, biota samples were collected at sampling locations along a 30 km stretch of coastline from the former outflow of pulp mill effluent into the Northumberland Strait. Estimates of trophic position for each organism using their $\delta^{15}N$ values permitted an analysis of variation in trophic dynamics along the Northumberland Strait coastline. Following the

completion of the stable isotope study, a systematic literature review was conducted to investigate previous bioindicator taxa that have been used in stable isotope studies to assess wastewater (pulp mill effluent and/or municipal sewage wastewater) inputs in aquatic ecosystems. The suitability of each taxon was examined, and a list of criteria was developed to guide the selection of appropriate bioindicator taxa for assessing wastewater inputs in future stable isotope studies.

5.2 Research Findings

This study reaffirmed that stable isotopes are valuable ecological tools for monitoring and assessing ecosystem conditions and elucidating trophic dynamics in environments previously exposed to wastewater. Results identified variation in stable isotope values of biota along a gradient of historical pulp mill effluent exposure. Depleted $\delta^{13}C$ and $\delta^{15}N$ values were identified in biota from areas adjacent the former Boat Harbour outflow into the Northumberland Strait. Results also suggested variation in trophic dynamics among sampling locations with certain taxa (mussels and mummichogs) occupying lower overall trophic positions near Boat Harbour. The two main explanations for the observed variation in both stable isotope values and trophic dynamics along the Northumberland Strait coastline is: 1) the persistence of nutrient-rich conditions due to historical pulp mill effluent exposure from Boat Harbour, and 2) varying hydrological characteristics among sampling locations, with significantly lower surface salinity values measured near Boat Harbour.

The stable isotope values measured in biota near Boat Harbour are consistent with previous stable isotope values measured in biota from regions exposed to pulp mill effluent (Galloway et al., 2003; Wayland & Hobson, 2001). This finding suggests that some degree of nutrient enrichment may persist in the coastal Northumberland Strait due to historical pulp mill

effluent release and this enrichment is reflected in stable isotope values of biota tissues. However, the complex coastal ecosystem and spatial heterogeneity of the Northumberland Strait may also be contributing to variation in stable isotope values. The primary-treated wastewater outflow from PLFN, located in close proximity to the former Boat Harbour outflow, is likely a local source of nutrients to the region and may contribute to the observed variation in stable isotope values. $\delta^{15}N$ values of primary-treated sewage wastewater have been shown to be isotopically depleted, such as those observed in the present study, in contrast to more advanced wastewater treatment processes which result in enriched $\delta^{15}N$ signatures (Archana et al., 2016). The depleted stable isotope values in biota tissues near Boat Harbour, specifically those measured at the Mackenzie Head sampling location, may be indicative of high ammonium concentrations as a result of wastewater release from PLFN.

In addition to varying biogeochemical conditions in the study area, a pronounced salinity gradient was detected among sampling locations, with low salinity measured near Boat Harbour relative to sites located northeast and northwest along the coastline. This finding suggests that increased terrestrial inputs near Boat Harbour are contributing to distinct organic matter sources, driving spatial variation in stable isotope values along the coastline. Stable isotope variation along a salinity gradient has been well reported in the literature, with frequently depleted $\delta^{13}C$ and $\delta^{15}N$ values in more freshwater regions (Hoeinghaus et al., 2011), like those measured in this study. $\delta^{13}C$ values become more depleted as the relative contribution of DIC from freshwater origins increase and terrestrial inputs of organic matter into coastal waterbodies are frequently depleted in $\delta^{15}N$ (<5‰; Kendall et al., 1988; Mayer et al., 2002), compared with marine nitrate (NO_3^-) (~5‰; Sigman et al., 2009). Therefore, differences in the geochemistry and mixing of

freshwater and seawater may serve as a potential explanation for the observed variation in stable isotope values.

Furthermore, variation in salinity frequently accompanies alterations in food web dynamics because of differences in dominant primary production pathways among freshwater and marine environments. Therefore, differences in trophic dynamics among sampling locations may be a result of differences in salinity among sampling locations. A shift from C_3 dependent foods webs to more C_4 based food webs along a salinity gradient has been observed in previous studies (Hoeinghaus et al., 2011; Winemiller et al., 2011). In environments with lower salinity, dominant primary production occurs through C_3 photosynthetic pathways which are depleted in $\delta^{13}C$ values (Hoeinghaus et al., 2011). In contrast, in marine environments with higher salinity, C_4 production is the dominant primary production pathway which typically has a more enriched $\delta^{13}C$ value (Hoeinghaus et al., 2011). Therefore, pulp mill effluent may not be exclusively contributing to variation in both stable isotope values and trophic dynamics along the coastal Northumberland Strait. Pronounced differences in the hydrological characteristics may be driving the stable isotope variation or causing differences in species diversity among sampling locations. Future monitoring of stable isotope values in biota near Boat Harbour will clarify the main drivers of spatial variation since ecosystem recovery from effluent can be documented through shifting stable isotope signatures (Arciszewski et al., 2014). Changing stable isotope signatures in biota tissues near Boat Harbour would be expected following remediation if the depleted $\delta^{13}C$ and $\delta^{15}N$ values were indeed reflecting historical exposure to pulp mill effluent.

Certain organisms demonstrated a more pronounced spatial trend in stable isotope values along a gradient of historical pulp mill effluent. Mussels and mummichogs were identified as suitable bioindicator taxa for revealing spatial heterogeneity in varying nutrient sources along the

Northumberland Strait coastline. Their narrow home range was likely the primary factor contributing to their ability to identify spatial trends in stable isotope values within the study. One of the key findings from chapter III was that different taxa exhibited pronounced variation in their suitability for detecting potential wastewater inputs in the Northumberland Strait. This finding initiated a systematic literature review of previous stable isotope studies conducted in receiving environments for municipal and industrial wastewaters with the aim of identifying suitable bioindicator taxa for detecting and tracing wastewater (pulp mill effluent and/or municipal sewage wastewater) inputs in aquatic ecosystems. While a variety of taxonomic groups have been used in previous studies, macroalgae, bivalves, and small-bodied fish appear to have the highest suitability for evaluating environmental conditions in stable isotope studies conducted in wastewater receiving environments. However, the objectives of a specific study should be considered in the selection of appropriate bioindicators. For example, to detect stable isotope signatures of effluent in a waterbody that received historical wastewater discharges, preferential selection of long-lived bioindicator taxa with a narrow home range is recommended, such as bivalves. Alternatively, short-lived taxa such as filamentous green algae can be used to reveal more frequent or pulsatile shifts in nutrient conditions within wastewater receiving environments. The selection of bioindicator taxa within a given study will reflect the goals of the study, the characteristics of the receiving environment, and consider the following criteria:

1) Mobility of taxa: understanding site fidelity of an organism is crucial in the interpretation of stable isotope results since highly mobile organisms may not provide an accurate reflection of ecosystem conditions in a particular area.

2) Tissue turnover and life history: Taxa have varying tissue turnover rates and differ in life history complexity, both of which must be well understood and selected for according to the goals of the study.
3) Limited diet complexity: A complex diet, feeding on multiple dietary sources with numerous trophic interactions, can confound the interpretation of stable isotope results.
4) Ease of collection: Taxa must be relatively easy to collect for use in stable isotope studies and to permit temporal comparisons.
5) Directly exposed to wastewater: Taxa must be directly exposed to wastewater to reflect environmental conditions within the impacted region.
6) Ubiquitous: Taxa must be ubiquitous in the environment to permit collection of statistically significant sample sizes.

The results of this study provide a holistic baseline characterization of the coastal region of the Northumberland Strait, adjacent to the former outflow of pulp mill effluent from Boat Harbour, prior to remediation. Prior to this study, the biogeochemistry along the Northumberland Strait coastline had not been previously assessed. Therefore, while this study characterized anthropogenic impacts in a former coastal receiving environment for effluent, it also provides a baseline of nutrient dynamics in a region that was not previously characterized. The findings of this study can serve as a baseline for comparing nutrient conditions in the Northumberland Strait and provide a baseline for assessing the effectiveness of remediation at restoring natural ecosystem processes.

5.3 Recommendations for Future Environmental Monitoring

A goal of the Boat Harbour remediation is to restore Boat Harbour to its pre-industrial state of a tidal estuary. To ensure successful completion of this goal, effective characterization of

the ecosystem in Boat Harbour and the Northumberland Strait is required before and after remediation. Stable isotope analysis provides an effective method for quantifying ecosystem conditions by assessing underlying nutrient sources available to biota and elucidating trophic dynamics among biota in an entire food web (Freedman et al., 2012; Loomer et al., 2015). This method provides valuable information on aquatic ecosystem functioning and permits an effective ecosystem characterization for guiding management and conservation approaches. The use of stable isotope analysis in future environmental monitoring programs in the coastal Northumberland Strait is recommended to evaluate the effectiveness of remediation at restoring natural ecosystem processes. Previous studies have shown that stable isotopes in long-lived biota can document ecosystem recovery following historical exposure to pulp mill effluent (Arciszewski et al., 2014; McMahon et al., 2020). Therefore, future monitoring can use the current study to assess temporal shifts in stable isotope values, which may indicate shifting environmental conditions and potential ecosystem recovery. Additionally, with the decline of the Canadian pulp and paper industry (Bogdanski, 2014), increased remedial action within aquatic receiving environments is highly likely. Stable isotope analysis is recommended as a monitoring method for assessing spatial impacts of nutrient enrichment in aquatic receiving environments for pulp mill effluent and for quantifying ecosystem recovery following the cessation of effluent flow.

The selection of regionally appropriate bioindicator taxa to quantify and assess ecosystem conditions is also suggested. This study identified blue mussels and mummichogs as appropriate bioindicator species among a suite of coastal biota. These species meet many of the criteria for suitable bioindicators in stable isotope studies in aquatic receiving environments for wastewater. Both mussels and mummichogs occupy a narrow home range, providing an accurate reflection of

environmental conditions in the region. Further, they are both relatively long-lived, are relatively easy to collect, and are ubiquitous along the coastal Northumberland Strait. Based on the results of the study, blue mussels and mummichogs are recommended for future environmental monitoring in this region to ensure adequate characterization of aquatic ecosystem conditions.

5.4 Conclusions

This study provided an ecosystem-wide assessment of the underlying biogeochemistry in an impacted coastal ecosystem previously exposed to historical pulp mill effluent. Stable isotope analysis was effective at assessing sources of carbon and nitrogen and examining trophic dynamics along the Northumberland Strait coastline, adjacent to Boat Harbour. Results identified spatial variation in sources of carbon and nitrogen along the coastline, with depleted $\delta^{13}C$ and $\delta^{15}N$ values in biota near the former outflow of pulp mill effluent from Boat Harbour. However, it is likely that spatial heterogeneity and natural ecosystem processes, such as increased delivery of terrestrial nutrients and confounding local nutrient sources from municipal wastewater discharge are the main drivers of variation in $\delta^{13}C$ and $\delta^{15}N$ values of biota along the coastline. Future environmental monitoring is recommended, using suitable bioindicator taxa such as blue mussels and mummichogs, to assess temporal shifts in the underlying biogeochemistry following the remediation of Boat Harbour. Remediation activities include restoring tidal flow to Boat Harbour, which may have profound effects on the coastal biogeochemistry in the region. Stable isotope analysis is suggested as an effective method for detecting and tracing nutrient sources and its application is recommended to assess both spatial and temporal changes as part of environmental monitoring for the remediation of Boat Harbour.

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Appendix A: Supplemental Maps and Images

Figure A1: Boat Harbour Effluent Treatment Facility wastewater treatment stages

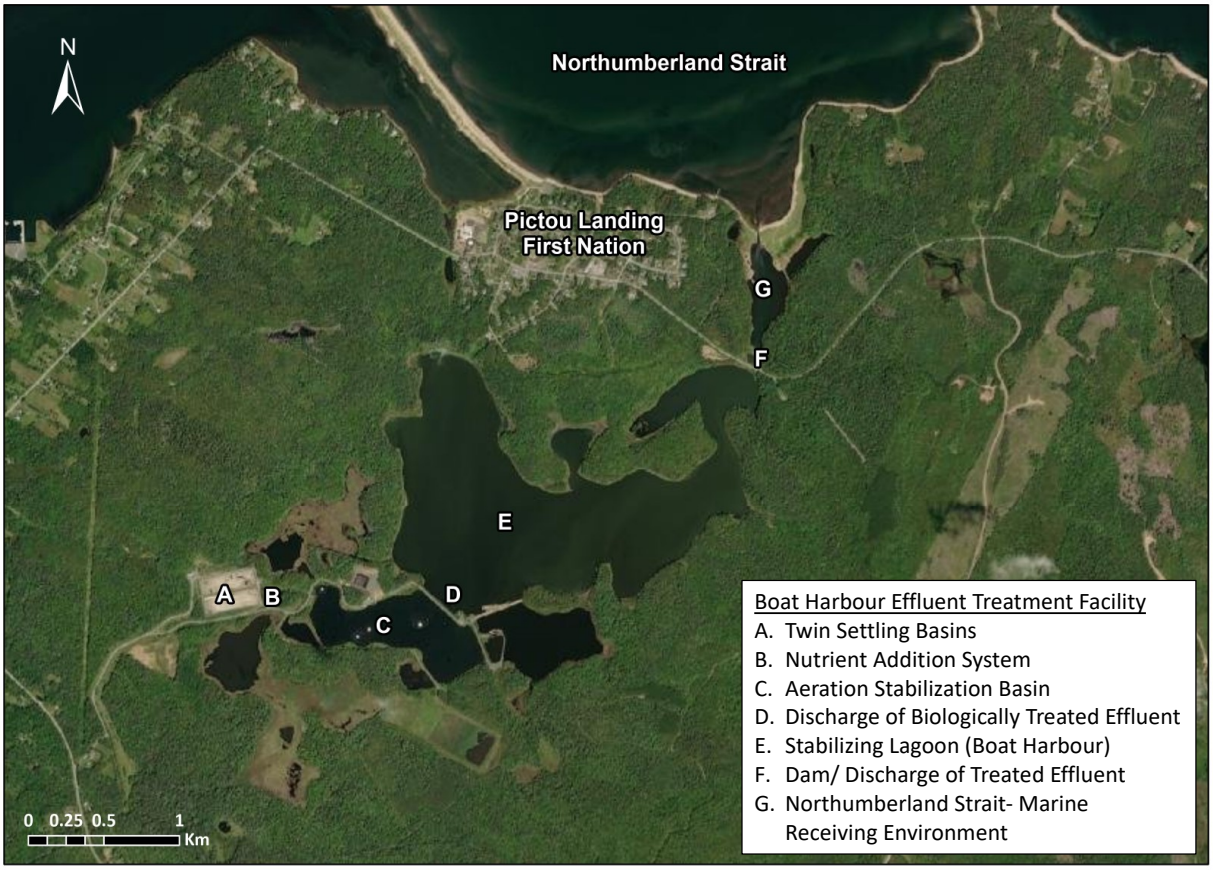
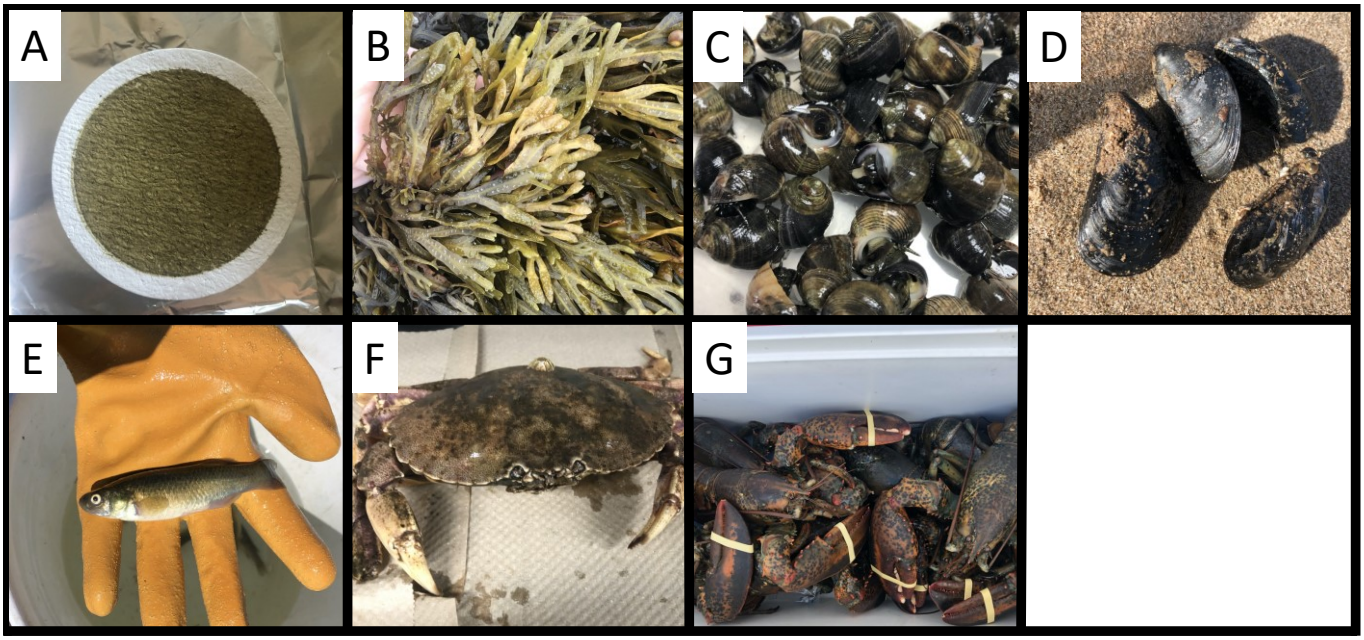


Figure A2: Wastewater discharge locations in Pictou, Nova Scotia

Figure A3: Coastal marine taxa used within the study: A) particulate organic matter (POM), B) macroalgae; *Fucus vesiculosus*, C) periwinkles; *Littorina littorea*, D) blue mussels; *Mytilus edulis*, E) mummichog; *Fundulus heteroclitus*, F) rock crab; *Cancer irroratus,* and G) American lobster; *Homarus americanus*.



Appendix B: Salinity Data and Analysis

Table B1: Salinity values and water temperature at each sampling location within the study. Measurements were taken March 26, 27 2022.

Sampling Location	Water Temperature (°C)	Mean Salinity (ppt) (*n*=3)	Standard Error
CRI (Offshore)	0.05	30.8	0
CRI (Estuarine)	0.11	24.3	0.01
SVC	5.82	16.2	0
HRL	3.88	22.2	0.2
LHB	0.75	28.4	0.2
BHO	4.97	6.1	0
HWP	4.86	9.1	0
MKH	4.30	23.8	0
CNH (Offshore)	0.34	26.8	0.1
CNH (Estuarine)	3.42	18.7	0
BLP	-0.15	29.9	0.1
MLB (Offshore)	0.61	30.9	0.1
MLB (Estuarine)	0.75	25.2	0.5
BGI	-0.50	28.7	0.1

Table B2: Results of linear regression for relationship between organism stable isotope value (both $\delta^{13}C$ and $\delta^{15}N$) and salinity values at the sampling locations.

	$\delta^{13}C$			$\delta^{15}N$		
Organism	r	R^2	*p-value*	r	R^2	*p-value*
POM_{Winter}	-0.700	0.490	0.016	0.555	0.308	0.077
Macroalgae	-0.301	0.091	0.369	0.100	0.010	0.769
Periwinkle	0.659	0.434	0.154	-0.237	0.056	0.651
Mussel	0.761	0.579	0.079	0.641	0.411	0.170
Mummichog	0.593	0.352	0.214	0.269	0.072	0.606
Crab	0.445	0.198	0.317	0.571	0.327	0.180
Lobster	0.870	0.756	0.011	0.804	0.647	0.029

Appendix C: POM $\delta^{15}N$ and $\delta^{13}C$ Analysis

Table C1: Mean $\delta^{15}N$ and $\delta^{13}C$ (‰) values for particulate organic matter (POM) within each sampling season (winter, spring, summer) by sampling location.

	Winter					*Spring*					*Summer*				
		$\delta^{15}N$		$\delta^{13}C$			$\delta^{15}N$		$\delta^{13}C$			$\delta^{15}N$		$\delta^{13}C$	
Location	*n*	Mean	SE	Mean	SE	*n*	Mean	SE	Mean	SE	*n*	Mean	SE	Mean	SE
CRI	2	7.88	1.91	-28.45	0.34	3	5.89	0.41	-23.07	0.81	3	6.15	0.22	-21.69	0.35
SVC	3	5.91	0.34	-27.24	0.43	3	6.49	0.24	-24.00	0.25	2	6.10	0.06	-24.65	0.21
HRL	2	7.49	0.07	-28.41	0.19	3	6.23	0.07	-23.85	0.48	3	6.73	0.27	-23.60	0.06
LHB	3	6.74	0.18	-29.35	0.19	3	6.64	1.57	-27.50	0.08	3	6.64	0.23	-25.24	0.34
BHO	3	5.54	0.70	-26.34	0.17	3	4.82	0.25	-27.39	0.15	3	1.58	0.14	-26.21	0.16
HWP	3	4.69	0.09	-26.37	0.17	3	4.41	0.91	-26.13	1.73	3	4.41	0.30	-24.65	0.36
MKH	3	5.99	0.15	-28.38	0.09	3	6.22	0.01	-26.69	0.25	3	5.20	0.23	-23.23	0.06
CNH	3	6.47	0.02	-29.71	0.17	3	6.76	0.34	-21.41	0.40	3	6.23	0.11	-24.93	0.20
BLP	2	6.10	0	-27.02	0.50	3	7.39	0.68	-24.73	0.08	3	5.79	0.12	-23.35	0.11
MLB	3	6.43	0.04	-29.03	0.34	3	6.81	0.27	-25.48	0.24	3	6.29	0.19	-23.93	0.08
BGI	2	5.89	0.24	-27.78	0.33	3	6.08	0.22	-24.23	0.46	3	4.96	0.29	-21.42	0.72

Table C2: Results of one-way ANOVA for seasonal differences in POM $\delta^{15}N$ and $\delta^{13}C$ by location across the three sampling seasons. Bolded values indicate significant differences in stable isotope values among seasons.

		$\delta^{15}N$	$\delta^{13}C$
Location	Season	*p-value*	*p-value*
CRI	Winter Spring Summer	0.574*	**0.002**
SVC	Winter Spring Summer	0.357	**0.002***
HRL	Winter Spring Summer	**0.018**	0.119
LHB	Winter Spring Summer	0.996	**<0.001**
BHO	Winter Spring Summer	**<0.001***	**0.004**
HWP	Winter Spring Summer	0.67*	0.301*
MKH	Winter Spring Summer	**0.027***	**0.027***
CNH	Winter Spring Summer	0.265	**<0.001**
BLP	Winter Spring Summer	0.102	**0.044***
MLB	Winter Spring Summer	0.218	**<0.001**
BGI	Winter Spring Summer	0.053	**0.002**

* Kruskall-Wallace Test conducted on non-parametric data set

Table C3: Pairwise comparison analysis using a Bonferroni correction indicating significant differences in POM $\delta^{15}N$ (shaded) and $\delta^{13}C$ among seasons for each sampling location.

Isotope	Location	Interaction	*p-value*
$\delta^{13}C$	CRI	Winter-Spring	0.006
		Winter-Summer	0.002
$\delta^{13}C$	SVC	Winter-Spring	0.003
		Winter-Summer	0.012
$\delta^{15}N$	HRL	Winter-Spring	0.02
$\delta^{13}C$	LHB	Winter-Spring	0.004
		Spring-Summer	0.001
		Winter-Summer	0.001
$\delta^{13}C$	BHO	Spring-Winter	0.060
		Spring-Summer	0.005
$\delta^{15}N$	BHO	Summer-Spring	<0.001
		Summer-Winter	<0.001
$\delta^{13}C$	MKH	*	
$\delta^{15}N$	MKH	*	
$\delta^{13}C$	CNH	Winter-Spring	<0.001
		Summer-Spring	<0.001
		Winter-Summer	<0.001
$\delta^{13}C$	BLP	*	
$\delta^{13}C$	MLB	Spring-Winter	<0.001
		Spring-Summer	0.013
		Winter-Summer	<0.001
$\delta^{13}C$	BGI	Spring-Winter	0.029
		Spring-Summer	0.047
		Winter-Summer	0.002

*Mann-Whitney test was unable to detect significant interaction effects

Table C4: Results of one-way ANOVA for spatial differences in POM $\delta^{15}N$ and $\delta^{13}C$ between sampling locations. Bolded values indicate significant differences in stable isotope values between sampling locations.

	$\delta^{15}N$	$\delta^{13}C$
Season	*p-value*	*p-value*
Winter	0.050*	**<0.001**
Spring	0.101*	**0.006***
Summer	**<0.001**	**0.001***

* Kruskall-Wallace Test conducted on non-parametric data set

Table C5: Pairwise comparison analysis using a Bonferroni correction for POM $\delta^{15}N$ (shaded) and $\delta^{13}C$ between locations by sampling season

Isotope	Season	Interaction	*p-value*
$\delta^{13}C$	Winter	CRI-BHO	0.004
		CRI-HWP	0.004
		SVC-LHB	<0.001
		SVC-CNH	<0.001
		SVC-MLB	0.006
		HRL-BHO	0.004
		HRL-HWP	0.005
		LHB-BHO	<0.001
		LHB-HWP	<0.001
		LHB-BLP	0.001
		BHO-MKH	0.001
		BHO-CNH	<0.001
		BHO-MLB	<0.001
		HWP-MKH	0.002
		HWP-CNH	<0.001
		HWP-MLB	<0.001
		CNH-BLP	<0.001
		CNH-BGI	0.009
		BLP-MLB	0.006
$\delta^{13}C$	Spring	*	
$\delta^{13}C$	Summer	*	
$\delta^{15}N$	Summer	CRI-BHO	<0.001
		CRI-HWP	<0.001
		CRI-BGI	0.046
		SVC-BHO	<0.001
		SVC-HRL	0.004
		HRL-BHO	<0.001
		HRL-HWP	<0.001
		HRL-MKH	0.003
		HRL-BGI	<0.001
		LHB-BHO	<0.001
		LHB-HWP	<0.001
		LHB-MKH	0.007
		BHO-HWP	<0.001
		BHO-MKH	<0.001
		BHO-CNH	<0.001
		BHO-BLP	<0.001
		BHO-MLB	<0.001
		BHO-BGI	<0.001
		HWP-CNH	<0.001
		HWP-BLP	0.011
		HWP-MLB	<0.001
		CNH-BGI	0.025
		MLB-BGI	0.015

*Mann-Whitney test was unable to detect significant interaction effects

Appendix D: Macroalgae and Consumer $\delta^{15}N$ and $\delta^{13}C$ Analysis

Table D1: Mean $\delta^{15}N$ and $\delta^{13}C$ (‰) for macroalgae and higher trophic level organisms included in the study by sampling location.

			$\delta^{15}N$		$\delta^{13}C$	
Species	Location	*n*	Mean	SE	Mean	SE
Fucus vesiculosus	CRI	4	6.91	0.04	-18.42	0.19
	SVC	3	6.80	0.10	-17.68	0.24
	HRL	4	6.77	0.12	-18.64	0.05
	LHB	4	6.63	0.07	-17.17	0.09
	BHO	3	6.61	0.08	-16.48	0.07
	HWP	3	5.40	0.01	-19.71	0.81
	MKH	3	7.28	0.01	-17.39	0.06
	CNH	3	6.44	0.13	-19.71	0.83
	BLP	2	5.10	0.01	-18.33	0.12
	MLB	4	6.79	0.09	-20.20	0.58
	BGI	4	6.10	0.09	-18.46	0.21
Littorina littorea	CRI	4	8.03	0.28	-16.38	0.33
	MKH	4	7.50	0.08	-17.58	0.09
	CNH	3	7.41	0.10	-15.88	0.09
	BLP	4	6.01	0.15	-14.05	0.22
	MLB	4	6.85	0.12	-14.77	0.26
	BGI	3	7.63	0.13	-16.82	0.04
Mytilus edulis	CRI	6	8.92	0.01	-20.21	0.69
	MKH	6	6.82	0.06	-23.09	0.16
	CNH	6	8.89	0.12	-20.39	0.13
	BLP	6	8.40	0.06	-20.71	0.21
	MLB	6	8.01	0.14	-21.02	0.10
	BGI	6	8.27	0.05	-20.67	0.12
Fundulus heteroclitus	CRI	4	10.27	0.25	-13.47	0.37
	BHO	3	8.97	0.35	-20.08	0.67
	MKH	3	8.11	0.20	-20.67	0.15
	CNH	4	11.80	0.25	-15.38	0.19
	MLB	4	10.89	0.15	-14.60	0.08
	BGI	4	10.52	0.14	-14.11	0.19
Cancer irroratus	CRI	3	13.43	0.24	-18.76	0.15
	HWP	3	12.53	0.13	-18.84	0.42
	MKH	3	12.84	0.21	-18.60	0.03
	CNH	3	12.69	0.22	-18.98	0.09
	BLP	4	12.59	0.21	-18.30	0.05
	MLB	3	13.11	0.22	-18.24	0.13
	BGI	4	12.88	0.24	-17.87	0.40
Homarus americanus	CRI	4	13.54	0.28	-18.48	0.15
	HWP	4	12.40	0.52	-18.96	0.28
	MKH	3	13.68	0.16	-18.69	0.02
	CNH	4	13.45	0.18	-18.60	0.11
	BLP	3	13.30	0.14	-18.43	0.09
	MLB	4	13.26	0.14	-18.45	0.08
	BGI	4	13.39	0.32	-18.75	0.07

Table D2: Results from one-way ANOVA for $\delta^{15}N$ and $\delta^{13}C$ values of macroalgae and higher-trophic level organisms among sampling locations in the study. Bolded values indicate significant differences in stable isotope values between sampling locations.

	$\delta^{15}N$	$\delta^{13}C$
Species	*p value*	*p value*
F. vesiculosus	**0.001***	**0.001***
L. littorea	**<0.001**	**<0.001**
M. edulis	**<0.001***	**<0.001**
F. heteroclitus	**<0.001**	**<0.001**
C. irroratus	0.113	**0.040***
H. americanus	0.116	0.178

* Kruskall Wallace Test conducted on non-parametric data set

Table D3: Pairwise comparison analysis using a Bonferroni correction of $\delta^{13}C$ values between sampling locations for macroalgae and higher-trophic level organisms.

Organism	Interaction	*p-value*
F. vesiculosus	*	
L. littorea	CRI-MKH	0.015
	CRI-BLP	<0.001
	CRI-MLB	<0.001
	MKH-CNH	0.001
	MKH-BLP	<0.001
	MKH-MLB	<0.001
	CNH-BLP	<0.001
	CNH-MLB	0.048
	BLP-BGI	<0.001
	MLB-BGI	<0.001
M. edulis	CRI-MKH	<0.001
	CRI-MLB	0.002
	MKH-CNH	<0.001
	MKH-BLP	<0.001
	MKH-MLB	<0.001
	MKH-BGI	<0.001
	CNH-MLB	0.024
F. heteroclitus	CRI-BHO	<0.001
	CRI-MKH	<0.001
	CRI-CNH	0.004
	BHO-CNH	<0.001
	BHO-MLB	<0.001
	BHO-BGI	<0.001
	MKH-CNH	<0.001
	MKH-MLB	<0.001
	MKH-BGI	<0.001
C. irroratus	*	

*Mann-Whitney test was unable to detect significant interaction effects

Table D4: Pairwise comparison analysis using a Bonferroni correction of $\delta^{15}N$ values between sampling locations for macroalgae and higher-trophic level organisms.

Organism	Interaction	*p-value*
F. vesiculosus	*	
L. littorea	CRI-BLP	<0.001
	CRI-MLB	0.001
	MKH-BLP	<0.001
	CNH-BLP	<0.001
	BLP-BGI	<0.001
	BLP-MLB	0.007
M. edulis	CRI-MKH	0.002
	CRI-MLB	0.002
	CRI-BGI	0.002
	MKH-CNH	0.001
	MKH-BLP	0.002
	MKH-BGI	0.002
	CNH-MLB	0.002
	CNH-BGI	0.002
F. heteroclitus	CRI-BHO	0.016
	CRI-MKH	<0.001
	CRI-CNH	0.002
	BHO-CNH	<0.001
	BHO-MLB	<0.001
	BHO-BGI	0.003
	MKH-CNH	<0.001
	MKH-MLB	<0.001
	MKH-BGI	<0.001
	CNH-BGI	0.009

*Mann-Whitney test was unable to detect significant interaction effects

Appendix E: Trophic Position Analysis

Table E1: Mean trophic position of each higher trophic level organism by sampling location using POM $\delta^{15}N$ as a baseline value for each location.

Species	Location	*n*	Mean Trophic Position	SE
L. littorea	CRI	4	1.79	0.08
	MKH	4	1.70	0.02
	CNH	3	1.47	0.03
	BLP	4	1.06	0.04
	MLB	4	1.30	0.03
	BGI	3	1.79	0.04
M. edulis	CRI	6	2.05	0
	MKH	6	1.50	0.02
	CNH	6	1.93	0.03
	BLP	6	1.77	0.02
	MLB	6	1.64	0.04
	BGI	6	1.98	0.02
F. heteroclitus	CRI	4	2.45	0.07
	BHO	3	2.67	0.10
	MKH	3	1.88	0.06
	CNH	4	2.76	0.07
	BLP	4	2.49	0.04
	BGI	4	2.64	0.04
C. irroratus	CRI	3	3.38	0.07
	HWP	3	3.56	0.04
	MKH	3	3.27	0.05
	CNH	3	3.02	0.06
	BLP	4	3.00	0.06
	MLB	3	3.14	0.06
	BGI	4	3.34	0.07
H. americanus	CRI	4	3.41	0.08
	HWP	4	3.53	0.15
	MKH	3	3.52	0.05
	CNH	4	3.25	0.05
	BLP	3	3.21	0.04
	MLB	4	3.18	0.04
	BGI	4	3.49	0.09

Table E2: Results of linear regression for relationship between organism trophic position and body size.

Organism	r	R^2	*p-value*
Periwinkle	0.255	0.090	0.252
Mussel	0.254	0.065	0.135
Mummichog	0.300	0.090	0.175
Crab	0.402	0.161	0.057
Lobster	0.248	0.062	0.221

Table E3: Results of one-way ANOVA analyses for spatial differences in trophic position by location among organisms. Bolded values indicate significant differences in trophic position among sampling locations.

Species	*p-value*
L. littorea	**<0.001***
M. edulis	**<0.001***
F. heteroclitus	**<0.001**
C. irroratus	**<0.001**
H. americanus	**0.033**

* Kruskall-Wallace Test conducted on non-parametric data set

Table E4: Pairwise comparison analysis using a Bonferroni correction indicating significant differences in trophic position among sampling locations for each consumer organism.

Species	Interaction	*p-value*
L. littorea	CRI-CNH	0.011
	CRI-BLP	<0.001
	CRI-MLB	<0.001
	MKH-BLP	<0.001
	MKH-MLB	<0.001
	CNH-BLP	<0.001
	CNH-BGI	0.018
	BLP-MLB	0.004
	BLP-BGI	<0.001
	MLB-BGI	<0.001
M. edulis	CRI-MKH	0.002
	CRI-BLP	0.002
	CRI-MLB	0.002
	MKH-CNH	0.002
	MKH-BLP	0.002
	MKH-BGI	0.002
	CNH-MLB	0.002
	BLP-BGI	0.002
	MLB-BGI	0.002
F. heteroclitus	CRI-MKH	<0.001
	CRI-CNH	0.048
	BHO-MKH	<0.001
	MKH-CNH	<0.001
	MKH-MLB	<0.001
	MKH-BGI	<0.001
C. irroratus	CRI-CNH	0.028
	CRI-BLP	0.009
	HWP-CNH	<0.001
	HWP-BLP	<0.001
	HWP-MLB	0.007
	CNH-BGI	0.046
	BLP-BGI	0.013
H. americanus	*	

*Mann-Whitney test was unable to detect significant interaction effects

Appendix F: Bioindicator Taxa Literature Review- Included Studies

Table F1: Chronological list of studies included in Chapter 4: Bioindicators in stable isotope studies: a review and evaluation of their suitability to assess wastewater pollution.

Author(s)	Year	Sewage Wastewater (SWW)/ Pulp Mill Effluent (PME)	Bioindicator Taxa
Spies et al.	1989	SWW	*Microstomus pacificus*, *Citharichthys sordidus*, *Zaniolepis latipinnis*, Glyceridae, *Tharyx tesselata*, Lumbirineridae, *Pectinaria californiensis*, Nemerteans
Hobbie et al.	1990	SWW	*Fucus vesiculosus*
Gearing et al.	1991	SWW	*Mercenaria mercenaria*, *Ensis directus*, *Mya arenaria*, *Mulinia lateralis*, *Polycirrus eximius*, *Pandora gouldiana*, *Crepidula fornicata*, *Crepidula plana*, *Ceriantheopsis americanus*, *Petricola phalodiformis*, *Anadara transversa*, *Chaetozone* sp., *Polydora ligni*, *Streblospio benedicti*, *Mediomastus ambiseta*, *Pitar morrhuana*, *Nucula annulata*, *Ninoe nigripes*, *Yoldia limatula*, Maldanidae, *Pherusa affinis*, *Eupleura caudata*, *Lumbrineris fragilis*, *Cerebratulus* sp., *Nassarius trivittatus*, *Nereis virens*, *Ophioglycera gigantea*, *Nephtys incisa*, *Turbonilla* sp.
Dover et al.	1992	SWW	*Echinus affinis*, *Benthodytes sanguinolenta*, *Flabellum angular*, *Brissopsis mediterranea*, *Pectinaster filholi*, *Hedingia albicans*, *Glyphocrangon sculpta*, *Munidopsis* sp.
Wassenaar & Culp	1996	PME	Chironomidae, *Ephemerella* sp., *Baetis* sp., Perlodidae, Hydropsyche, *Ameletus* sp., Heptageniidae, Cottidae, *Oncorhynchus mykiss*, *Rhinichthys cataractae*
Fourqurean et al.	1997	SWW	*Zostera marina*
Hansson et al.	1997	SWW	*Mysis mixta*, *Mysis relicta*, *Sprattus sprattus*, *Osmerus eperlanus*, *Clupea harengus*, *Stizostedion lucioperca*
McClelland et al.	1997	SWW	*Zostera marina*, *Gracilaria tikvahiae*, *Cladophora vagabunda*, *Enteromorpha* sp., *Spartina alterniflora*
Rogers	1999	SWW	*Ulva lactuca*, *Mytilus galloprovincialis*, *Cellana tramoserica*
Tucker et al.	1999	SWW	*Ulva lactuca*, *Mytilus edulis*, *Ampelisca* sp., *Artica islandica*, *Thracia conradi*, *Leptocheirus* sp., *Caudina* sp., Phyllodicidae, Amphaertidae, Maldanidae, Arabellidae, *Actinodiscus* sp., *Homarus americanus,* Nemerteans, *Ceriantheopsis* sp., Goniadidae, *Nephtys* sp., *Pleuronectes americanus*
Farwell	2000	PME	*Hexagenia* sp., *Catostomus commersonii*
Heikoop et al.	2000	SWW	*Porites lobata*
Riera et al.	2000	SWW	*Fucus vesiculosus*, *Enteromorpha* sp., *Cerastoderma edule*, *Nereis* sp., *Corophium volutator*, *Carcinus maenas*, Oligochaeta, Enchytraeidae, *Lumbricillus* sp., *Tubificoides* sp., *Crassostrea gigas*, *Mytilus edulis*, *Crepidula fornicata*, *Littorina littorea*, *Littorina saxatilis*, *Gammarus locusta*, *Eulimnogammarus obtusatus*
Risk & Erdmann	2000	SWW	*Gonodactylinus viridis*
Costanzo et al.	2001	SWW	*Zostera capricorni*, *Gracilaria edulis*, *Catenella nipae*, *Avicennia marina*
Jones et al.	2001	SWW	*Zostera capricorni*, *Avicennia marina*, *Catenella nipae*
Wayland & Hobson	2001	SWW, PME	*Cladophora* sp., *Spirogyra* sp., *Mougeotia* sp., *Lemna* sp., Trichoptera, Ephemeroptera
Waldron et al.	2001	SWW	*Nereis virens*, *Scoloplos armiger*, *Nephtys incisa*, *Abra nitida*
Gartner et al.	2002	SWW	*Ulva australis*, *Vidalia* sp., *Ecklonia radiata*, *Clathria* sp., *Pyura australis*
Umezawa et al.	2002	SWW	*Padina* sp., *Dictyota* sp.
deBruyn et al.	2003	SWW	Chironomidae, Amphipoda, Trichoptera, Odonata, Hydracarina, Notonectidae, *Esox lucius*, *Sander vitreus*, *Catostomus commersonii*, *Etheostoma nigrum*, *Notropis hudsonius*, *Ameiurus nebulosus*, *Notropis atherinoides*
Galloway et al.	2003	SWW, PME	*Catostomus commersonii*, *Cottus cognatus*
Rogers	2003	SWW	*Ulva lactuca*, *Mytilus galloprovincialis*, *Cellana tramoserica*

Author(s)	Year	Sewage Wastewater (SWW)/ Pulp Mill Effluent (PME)	Bioindicator Taxa
Barile	2004	SWW	*Ulva lactuca*, *Chaetomorpha linum*, *Enteromorpha intestinalis*, *Codium isthmocladum*, *Caulerpa prolifera*, *Caulerpa racemosa*, *Caulerpa sertularioides*, *Bryothamnion triquetrum*, *Gracilaria tikvahiae*, *Botryocladia spinulifera*, *Laurencia poiteaui*, *Colpomenia sinuosa*
Cole et al.	2004	SWW	*Spartina alterniflora*
Gaston & Suthers	2004	SWW	*Atypichthys strigatus*
LaPointe et al.	2004	SWW	*Cladophora catanata*, *Laurencia intricata*
Savage & Elmgren	2004	SWW	*Fucus vesiculosus*
Steffy & Kilham	2004	SWW	*Salmo trutta*, *Catostomus commersonii*, *Semotilus atromaculatus*, *Rhinichthys atratulus*, Cambaridae
Carruthers et al.	2005	SWW	*Thalassia testudinum*
Costanzo et al.	2005	SWW	*Catenella nipae*
Dolenec et al.	2005	SWW	*Anemonia sulcata*
Dubé et al.	2005	SWW, PME	*Catostomus catostomus*
Savage	2005	SWW	*Fucus vesiculosus*
Vander Zanden et al.	2005	SWW	Bivalvia, Gastropoda, Amphipoda, Isopoda, Ephemeroptera, Trichoptera, Chironomidae, Corixidae, Tipulidae, Oligochaeta, Decapoda, Dytiscidae, Odonata, Tanypodinae, Hirudinea, Megaloptera
Conlan et al.	2006	SWW	*Trematomus bernacchii*, *Alcyonium antarcticum*, *Cnemidocarpa verrucosa*, *Laternula elliptica*, *Sterechinus neumayeri*, *Odontaster validus*
Dolenec et al.	2006	SWW	*Balanus perforatus*
Dubé et al.	2006	PME	*Oncorhynchus mykiss*
Piola et al.	2006	SWW	*Avicennia marina*, *Zostera capricorni*, *Saccostrea glomerata*
Pruell et al.	2006	SWW	*Ulva lactuca*, *Spartina alterniflora*, *Nassarius obsoletus*, *Geukensia demissa*, *Palaemonetes pugio*, *Fundulus heteroclitus*
Vizzini & Mazzola	2006	SWW	*Posidonia oceanica*, *Caulerpa prolifera*, *Udotea petiolata*, *Diplodus sargus*, *Scorpaena porcus*, *Serranus cabrilla*, *Diplodus vulgaris*, *Sarpa salpa*, *Sepia officinalis*, *Parborlasia corrugatus*
Dolenec et al.	2007	SWW	*Balanus perforatus*, *Aplysina aerophoba*, *Anemonia sulcata*
Forrest et al.	2007	SWW	*Atrina zelandica*, *Pecten novaezelandiae*, *Perna canaliculus*
Hadwen & Arthington	2007	SWW	*Avicennia marina*, *Marphysa* sp., *Metapenaeus bennettae*, *Gerres subfasciatus*, *Ambassis marianus*, *Rhabdosargus sarba*, *Sillago ciliata*, *Mugil cephalus*
Schlacher et al.	2007	SWW	*Ambassis jacksoniensis*, *Gerres subfasciatus*, *Sillago ciliata*, *Valamugil georgii*
van den Heuvel et al.	2007	PME	*Gobiomorphus cotidianus*
Carmichael et al.	2008	SWW	*Mercenaria mercenaria*
Daskin et al.	2008	SWW	*Crassostrea virginica*
Bergfur et al.	2009	SWW	*Asellus aquaticus*, *Baetis* sp., Heptageniidae, Oligochaeta, Sphaeriidae, *Taeniopteryx nebulosa*, *Gammarus pulex*, *Hydropsyche angustipennis*, *Hydropsyche pellucidula*, *Hydropsyche alitalia*, *Alburnus alburnus*, *Phoxinus phoxinus*, *Lampetra planeri*, *Rhyacophila nubila*, *Gymnocephalus cernua*, *Perca fluviatilis*, *Salmo trutta*, *Lota lota*, *Anguilla anguilla*, *Esox lucius*
Fertig et al.	2009	SWW	*Crassostrea virginica*
Morris et al.	2009	SWW	*Caulerpa prolifera*, *Cymodocea nodosa*
Pitt et al.	2009	SWW	*Avicennia marina*, Filamentous algae, *Australoplax australis*, *Parasesarma erythrodactyla*
Risk et al.	2009	SWW	Antipatharians
Dailer et al.	2010	SWW	*Hypnea musciformis*, *Ulva fasciata*, *Acanthophora spicifera*, *Ahnfeltiopsis concinna*, *Asteronema breviarticulatum*, *Cladophora sericea*
Dudley & Shima	2010	SWW	*Carpophyllum maschalocarpum*, *Amphoroidea media*, *Petrolisthes elongatus*
Lassauque et al.	2010	SWW	*Posidonia oceanica*, *Mytilus galloprovincialis*
Oakes et al.	2010	PME	*Phragmites australis*
Sampaio et al.	2010	SWW	*Abra alba*, *Nephtys* sp., *Pectinaria koreni*, *Ampelisca* sp., *Glycera* sp., *Sternaspis scutata*, *Tellina fabula*, *Hyalinoecia bilineata*, *Diopatra macrocensis*, *Lumbrineris gracilis*, *Nassarius* sp.
Vermeulen et al.	2011	SWW	*Rissoella verruculosa*, *Patella caerulea*, *Monodonta turbinata*

Author(s)	Year	Sewage Wastewater (SWW)/ Pulp Mill Effluent (PME)	Bioindicator Taxa
Carmichael et al.	2012	SWW	*Crassostrea virginica*
Fernandes et al.	2012	SWW	*Cladophora valonioides*, *Ecklonia radiata*, *Ulva lactuca*
Freedman et al.	2012	SWW, PME	*Ameiurus nebulosus*, *Catostomus commersonii*, *Fundulus diaphanus*, *Morone americana*, *Perca flavescens*, *Gobiomorphus cotidianus*, *Anguilla rostrata*, *Alosa aestivalis*, *Alosa pseudoharengus*, *Salmo salar*, *Salvelinus fontinalis*, *Esox niger*, *Esox masquinongy*, *Luxilus cornutus*, *Margariscus margarita*, *Notemigonus crysoleucas*, *Phoxinus eos*, *Semotilus corporalis*, *Apeltes q* *Morone saxatilis*, *Lepomis gibbosus*, *Micropterus dolomieu*, Unionidae, *Amnicola* sp.
Hoffman et al.	2012	SWW	*Pomoxis nigro* *s*, *Percina caprodes*, *Osmerus mordax*, *Sander vitreus*, *Coregonus clupeaformis*, *Perca flavescens*, *Culaea inconstans*, *Etheostoma nigrum*, *Moxostoma* sp., *Catostomus* sp.
Skinner et al.	2012	SWW, PME	*Fundulus heteroclitus*
Xu & Zhang	2012	SWW	Chironomidae, Oligochaeta
Connolly et al.	2013	SWW	*Ulva lactuca*, *Zostera* sp., *Posidonia* sp., *Portunus armatus*, *Atherinosoma microstoma*
di Lascio et al.	2013	SWW	*Echinogammarus veneris*, *Proasellus coxalis*, *Asellus aquaticus*, *Procambarus clarkii*, Oligochaeta, *Bithynia tentaculata*, Chironomidae, Turbellaria, Hirudinea, *Anguilla anguilla*, *Abramis brama*, *Barbus plebejus*, *Carassius auratus*, *Cyprinus carpio*, *Leuciscus cephalus*, *Rutilus rubilio*, *Gymnocephalus cernuus*, *Lepomis gibbosus*, *Mugil cephalus*
Morrissey et al.	2013	SWW	Baetidae, Heptageniidae, Hydropsychidae, Gammaridae
Schubert	2013	SWW	*Zostera marina*
Arciszewski et al.	2014	PME	*Catostomus commersonii*
Fertig et al.	2014	SWW	*Crassostrea virginica*
Rožič et al.	2014	SWW	*Patella caerulea*
Loomer et al.	2015	SWW	Ephemerellidae, *Stenonema* sp., *Asellus* sp., Chironomidae, Elmidae, Hydropsychidae, Sphaeriidae, *Physella* sp., Simuliidae, *Argia* sp., *Enallagma* sp., *Hetaerina* sp., *Calopteryx* sp., *Etheostoma caeruleum*, *Etheostoma blennioides*
Oakes & Eyre	2015	SWW	*Ulva lactuca*, *Endarachne binghamiae*, *Bembicium nanum*, *Nerita atramentosa*, *Morula marginalba*
Mendoza	2016	PME	Chironomidae, *Hyalella* sp., *Caecidotea* sp., *Pisidium* sp., *Valvata* sp., *Catostomus commersonii*
Robinson et al.	2016	SWW	Decapoda, Gammaridae, Hydracarina, Isopoda, Ephemeroptera, Plecoptera, Diptera, *Etheostoma caeruleum*, *Etheostoma blennioides*
Smith et al.	2016	SWW	*Avicennia marina*, *Scylla serrata*, *Glycera* sp., *Trypaea australiensis*, *Metapenaeus bennettae*, *Silago ciliata*, *Mugil cephalus*, *Ambassis marianus*, *Rhabdosargus sarba*
van de Merwe et al.	2016	SWW	*Macrobrachium australiense*, *Metapenaeus* sp., *Fenneropenaeus merguiensis*, *Nematalosa erebi*
Hicks et al.	2017	SWW	*Etheostoma caeruleum*, Ephemerellidae, *Stenonema* sp., *Asellus* sp., Chironomidae, Elmidae, Hydropsychidae, Simuliidae
Mwaura et al.	2017	SWW	*Ulva* sp., *Padina* sp., *Cymodocea rotundata*, *Thalassia hemprichii*, *Thalassodendron ciliatum*
Warnakulasooriya et al	2017	SWW	*Avicennia marina*, *Telescopium telescopium*, *Terebralia semistriata*
Kaminski et al.	2018	SWW	*Tesseropora rosea*, *Cellana tramoserica*, Filamentous turf algae
Lozano-Bilbao et al.	2018	SWW	*Anemonia sulcata*
Munroe et al.	2018	SWW	*Melicertus plebejus*
Smucker et al.	2018	SWW	Aeshnidae, Corydalidae, Perlidae, Tipulidae, Limnephilidae, Hydropsychidae, Philopotamidae, Heptageniidae, Psephenidae
Babaranti et al.	2019	SWW	*Ulva lactuca*, *Mytilus galloprovincialis*
Lachs et al.	2019	SWW	*Lobophora* sp., *Drupella* sp., *Acropora* sp., *Sinularia* sp.
Barr et al.	2020	SWW	*Ulva pertusa*
González-De Zayas et al.	2020	SWW	*Avrainvillea* sp., *Halimeda* sp., *Penicillus* sp., *Sargassum* sp., *Thalassia testudinum*, *Syringodium filiforme*, *Plexaurella nutans*, *Pterogorgia anceps*, *Gorgonia flabellum*, *Millepora alcicornis*
McMahon et al.	2020	PME	*Hexagenia* sp., *Catostomus commersonii*
Roman et al.	2020	SWW	*Zostera noltei*
Thibault et al.	2020	SWW	Arcidae, Pectinidae, Pteriidae, Spondylidae
Cormier et al.	2021	SWW	*Hyalella* sp., *Caenis* sp., Baetidae
Suzzi et al.	2022	SWW	*Palaemon intermedius*

Appendix G: Additional Supplementary Material

Figure G1: Fisheries and Oceans Canada (DFO) scientific license.

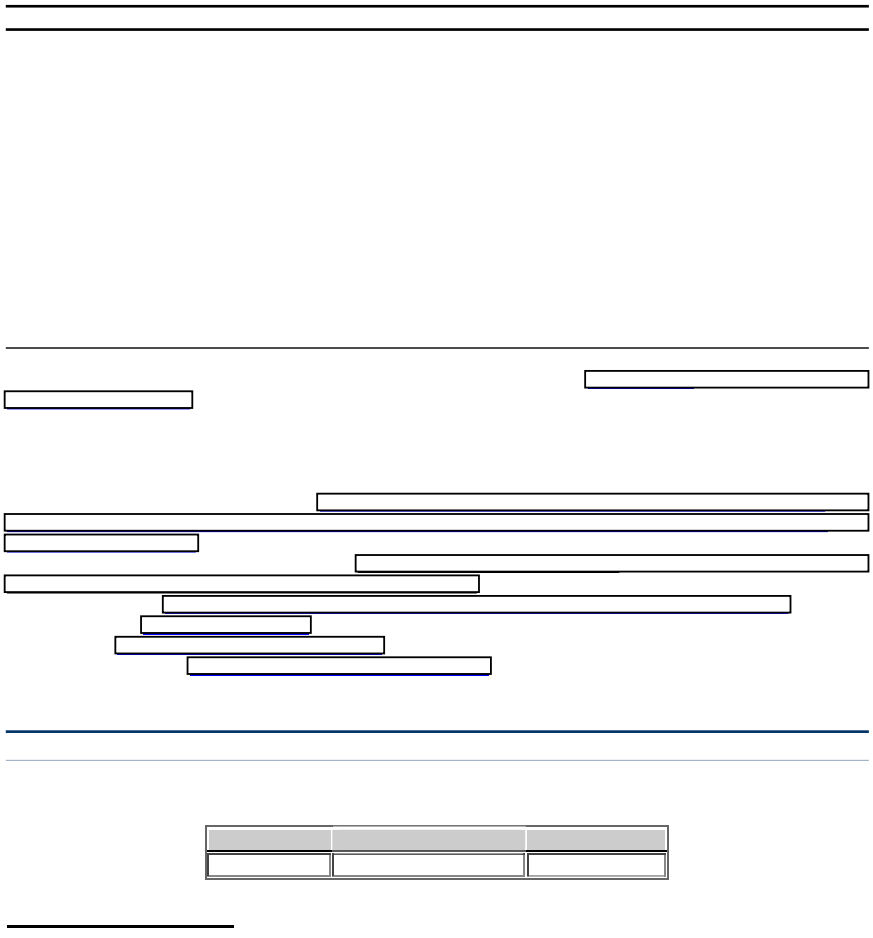


Figure G2: Dalhousie University Committee on Laboratory Animals (UCLA) ethics approval

Workflow Message	Workflow Message
DALHOUSIE UNIVERSITY, UNIVERSITY COMMITTEE ON LABORATORY ANIMALS NOTICE OF PROTOCOL APPROVAL PROTOCOL NUMBER: I21-04 EXPIRY DATE: April 01, 2023 INVESTIGATOR: Dr Tony Walker CATEGORY/LEVEL: A - (experiments on most invertebrates or on live isolates) TITLE OF STUDY: (I21-04) Assessing spatial gradients of d15N and differences in food web length within a coastal ecosystem exposed to industrial pulp mill effluent. Protocol is approved, please note the expiry date for your records. Thank you, Jennifer Wipp, Coordinator University Committee on Laboratory Animals Dalhousie University 1355 Oxford Street, Room 1336 902.494.1270 UCLA@dal.ca WEBSITE: https://www.dal.ca/dept/animal-ethics.html	DALHOUSIE UNIVERSITY, UNIVERSITY COMMITTEE ON LABORATORY ANIMALS NOTICE OF PROTOCOL APPROVAL PROTOCOL NUMBER: 20-132 EXPIRY DATE: April 01, 2022 INVESTIGATOR: Dr Tony Walker CATEGORY/LEVEL: D - (experiments which cause moderate to severe distress or discomfort) TITLE OF STUDY: (20-132) Assessing spatial gradients of d15N and differences in food web length within a coastal ecosystem exposed to industrial pulp mill effluent. Protocol is approved, please note the expiry date for your records. Thank you, Jennifer Wipp, Coordinator University Committee on Laboratory Animals Dalhousie University 1355 Oxford Street, Room 1336 902.494.1270 UCLA@dal.ca WEBSITE: https://www.dal.ca/dept/animal-ethics.html

Figure G3: Stable Isotopes in Nature Laboratory (SINLAB) Interpretation Guide

SINLAB
Stable Isotopes in Nature Laboratory

UNB
UNIVERSITY OF NEW BRUNSWICK

SINLAB INTERPRETATION GUIDE

For further information please visit our website:
https://www.isotopeecology.com/

Instrumentation

Continuous Flow-Isotope Ratio Mass Spectrometry (CF-IRMS) is used for stable isotope analysis of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$. The SINLAB currently operates the following mass spectrometer/conflo combinations:

- $Delta^{Plus}$ XP – Conflo III
- Delta V Plus – Conflo IV

(All manufactured by Thermo Finnigan; Bremen, Germany)

Carbon & Nitrogen Methodology

Dried, ground and homogeneous samples are weighed into tin capsules and analyzed for $\delta^{13}C$ and $\delta^{15}N$ by an Elemental Analyzer (EA) coupled to one of the IRMS/Conflo combinations listed above. Samples are introduced into the EA by an autosampler where complete combustion occurs in the presence of oxygen to generate CO_2 and nitrogen oxide (N_xO_x) gases. Combustion occurs in a quartz tube filled with chromium oxide and silvered cobaltous oxide. A second quartz tube filled with fine copper wire is used for the reduction of nitrogen oxides (N_xO_x) to N_2 gas. Gas Chromatography (GC) is used to separate CO2 and N2 peaks with helium as a carrier gas. A water trap of magnesium perchlorate & silica chips is located before the GC column to remove water.

The SINLAB currently utilizes two elemental analyzers for $\delta^{13}C$ and $\delta^{15}N$ analyses.

Elemental Analyzer	Autosampler	Combustion Temperature	Reduction Temperature	GC Length	GC Temperature
CE NC2500 (Carlo Erba; Milan, Italy)	PN150	1050ºC	650ºC	4m	50ºC
Costech 4010 (Costech; California, USA)	Zero Blank	1000ºC	650ºC	3m	40ºC

Stable isotope measurements are reported as isotope delta δ in parts per thousand (‰) relative to the international standard: Vienna Pee Dee Belemnite (VPDB) for carbon, and atmospheric air (AIR) for nitrogen. Isotope values are normalized using secondary standards: USGS61, BLS, and MLS for animal tissues; and CMS, SPS, SPL and EPS for sediments and plant material. All of these standards were calibrated against IAEA standards. See below for standard descriptions.

Table G1: Stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) for each individual taxon received from the Stable Isotopes in Nature Laboratory (SINLAB).

Winter POM Sampling										
Location	Tissue Type	Date	Amount	CO2 Ampl	N2 Ampl	d13C	d15N	%C	%N	C/N
BLP	POM	04-Jun-21	1/4TH	1.145	0.700	-27.51	6.10	NA	NA	NA
BLP	POM	04-Jun-21	1/4TH	0.940	0.590	-26.52	6.10	NA	NA	NA
BGI	POM	04-Jun-21	1/4TH	1.049	0.585	-28.11	6.13	NA	NA	NA
BGI	POM	04-Jun-21	1/4TH	0.864	0.496	-27.45	5.65	NA	NA	NA
MLB	POM	04-Jun-21	1/4TH	1.155	0.842	-29.69	6.37	NA	NA	NA
MLB	POM	04-Jun-21	1/4TH	1.217	0.846	-28.87	6.49	NA	NA	NA
MLB	POM	04-Jun-21	1/4TH	1.369	1.008	-28.54	6.42	NA	NA	NA
CNH	POM	04-Jun-21	1/4TH	1.586	1.193	-29.92	6.50	NA	NA	NA
CNH	POM	04-Jun-21	1/4TH	1.488	1.147	-29.84	6.47	NA	NA	NA
CNH	POM	04-Jun-21	1/4TH	1.581	1.029	-29.38	6.44	NA	NA	NA
MKH	POM	08-Jun-21	1/4TH	2.235	1.068	-28.21	6.11	NA	NA	NA
MKH	POM	08-Jun-21	1/4TH	2.751	1.131	-28.51	5.68	NA	NA	NA
HWP	POM	08-Jun-21	1/4TH	1.963	0.914	-26.09	4.51	NA	NA	NA
HWP	POM	08-Jun-21	1/4TH	2.018	0.953	-26.35	4.74	NA	NA	NA
HWP	POM	08-Jun-21	1/4TH	2.025	0.949	-26.67	4.82	NA	NA	NA
BHO	POM	08-Jun-21	1/4TH	2.057	1.198	-26.30	4.95	NA	NA	NA
BHO	POM	08-Jun-21	1/4TH	2.139	1.121	-26.65	4.74	NA	NA	NA
SVC	POM	08-Jun-21	1/4TH	1.689	0.395	-28.04	5.38	NA	NA	NA
SVC	POM	09-Jun-21	1/4TH	0.968	0.393	-27.09	5.81	NA	NA	NA
SVC	POM	09-Jun-21	1/4TH	1.156	0.563	-26.58	6.54	NA	NA	NA
LHB	POM	09-Jun-21	1/4TH	1.997	1.212	-29.56	6.55	NA	NA	NA
LHB	POM	09-Jun-21	1/4TH	2.378	1.681	-29.52	7.09	NA	NA	NA
LHB	POM	09-Jun-21	1/4TH	2.948	2.445	-28.96	6.57	NA	NA	NA
CRI	POM	09-Jun-21	1/4TH	2.061	1.062	-28.11	5.97	NA	NA	NA
CRI	POM	09-Jun-21	1/4TH	3.306	1.077	-28.78	9.80	NA	NA	NA
HRL	POM	09-Jun-21	1/4TH	2.537	2.267	-28.60	7.42	NA	NA	NA
HRL	POM	09-Jun-21	1/4TH	1.088	0.902	-28.22	7.55	NA	NA	NA
Spring POM Sampling										
Location	Tissue Type	Date	Amount	CO2 Ampl	N2 Ampl	d13C	d15N	%C	%N	C/N
BGI	POM	12-Jul-21	1/8	0.622	0.347	-23.32	6.20	NA	NA	NA
BGI	POM	12-Jul-21	1/8	0.437	0.216	-24.74	5.65	NA	NA	NA
BGI	POM	12-Jul-21	1/8	0.485	0.249	-24.64	6.38	NA	NA	NA
SVC	POM	12-Jul-21	1/8	0.689	0.334	-24.17	6.70	NA	NA	NA
SVC	POM	12-Jul-21	1/8	0.792	0.371	-24.32	6.02	NA	NA	NA
SVC	POM	12-Jul-21	1/8	1.326	0.753	-23.50	6.76	NA	NA	NA
MLB	POM	12-Jul-21	1/8	0.635	0.340	-25.85	6.32	NA	NA	NA
MLB	POM	12-Jul-21	1/8	0.775	0.451	-25.56	6.86	NA	NA	NA
MLB	POM	12-Jul-21	1/8	0.597	0.358	-25.03	7.26	NA	NA	NA
CNH	POM	12-Jul-21	1/8	0.627	0.273	-21.39	7.32	NA	NA	NA
CNH	POM	12-Jul-21	1/8	0.579	0.271	-22.12	6.80	NA	NA	NA
CNH	POM	13-Jul-21	1/4	1.197	0.530	-20.73	6.16	NA	NA	NA

CRI	POM	13-Jul-21	1/4	1.541	0.786	-22.15	6.57	NA	NA	NA
CRI	POM	13-Jul-21	1/4	1.853	0.929	-22.38	5.94	NA	NA	NA
CRI	POM	13-Jul-21	1/4	2.189	1.046	-24.69	5.15	NA	NA	NA
LHB	POM	13-Jul-21	1/4	1.843	1.233	-27.47	3.72	NA	NA	NA
LHB	POM	13-Jul-21	1/4	3.463	2.827	-27.66	7.11	NA	NA	NA
LHB	POM	13-Jul-21	1/4	2.735	2.289	-27.38	9.10	NA	NA	NA
HWP	POM	13-Jul-21	1/4	1.520	0.802	-22.67	6.22	NA	NA	NA
HWP	POM	13-Jul-21	1/4	2.717	1.280	-27.77	3.61	NA	NA	NA
HWP	POM	13-Jul-21	1/4	2.004	1.115	-27.96	3.39	NA	NA	NA
BHO	POM	13-Jul-21	1/4	2.978	2.235	-27.10	4.58	NA	NA	NA
BHO	POM	14-Jul-21	1/4	2.637	1.952	-27.46	4.56	NA	NA	NA
BHO	POM	14-Jul-21	1/4	2.447	1.955	-27.60	5.33	NA	NA	NA
BLP	POM	14-Jul-21	1/4	1.804	1.246	-24.60	7.39	NA	NA	NA
BLP	POM	14-Jul-21	1/4	2.052	1.348	-24.70	8.56	NA	NA	NA
BLP	POM	14-Jul-21	1/4	1.632	1.1	-24.88	6.22	NA	NA	NA
MKH	POM	14-Jul-21	1/4	1.543	1.025	-26.92	6.22	NA	NA	NA
MKH	POM	14-Jul-21	1/4	2.426	1.491	-26.20	6.21	NA	NA	NA
MKH	POM	14-Jul-21	1/4	2.343	1.462	-26.96	6.23	NA	NA	NA
HRL	POM	14-Jul-21	1/4	2.826	1.853	-24.80	6.09	NA	NA	NA
HRL	POM	14-Jul-21	1/4	1.946	1.481	-23.28	6.28	NA	NA	NA
HRL	POM	14-Jul-21	1/4	2.078	1.602	-23.46	6.31	NA	NA	NA
Summer POM Sampling										
Location	Tissue Type	Date	Amount	CO2 Ampl	N2 Ampl	d13C	d15N	%C	%N	C/N
BGI	POM	27-Sep-21	1/4TH	4.963	2.261	-20.05	5.37	NA	NA	NA
BGI	POM	27-Sep-21	1/4TH	4.798	1.977	-21.75	4.39	NA	NA	NA
BGI	POM	27-Sep-21	1/4TH	4.975	2.103	-22.47	5.13	NA	NA	NA
BLP	POM	27-Sep-21	1/4TH	4.346	1.490	-23.56	5.79	NA	NA	NA
BLP	POM	27-Sep-21	1/4TH	5.132	1.825	-23.25	5.99	NA	NA	NA
BLP	POM	27-Sep-21	1/4TH	5.286	1.949	-23.23	5.58	NA	NA	NA
CRI	POM	27-Sep-21	1/4TH	6.164	2.001	-21.17	6.44	NA	NA	NA
CRI	POM	27-Sep-21	1/4TH	6.126	2.129	-21.54	6.31	NA	NA	NA
CRI	POM	27-Sep-21	1/4TH	3.860	1.313	-22.34	5.71	NA	NA	NA
CNH	POM	27-Sep-21	1/4TH	4.014	1.472	-24.71	6.32	NA	NA	NA
CNH	POM	27-Sep-21	1/4TH	4.014	1.391	-24.76	6.01	NA	NA	NA
CNH	POM	28-Sep-21	1/4TH	4.484	1.555	-25.33	6.37	NA	NA	NA
HWP	POM	28-Sep-21	1/4TH	3.970	1.689	-24.48	4.07	NA	NA	NA
HWP	POM	28-Sep-21	1/4TH	4.981	2.104	-25.34	5.02	NA	NA	NA
HWP	POM	28-Sep-21	1/4TH	4.020	1.832	-24.15	4.14	NA	NA	NA
HRL	POM	28-Sep-21	1/4TH	4.194	1.785	-23.67	6.37	NA	NA	NA
HRL	POM	28-Sep-21	1/4TH	3.035	1.320	-23.64	6.55	NA	NA	NA
HRL	POM	28-Sep-21	1/4TH	3.594	1.609	-23.49	7.26	NA	NA	NA
LHB	POM	28-Sep-21	1/4TH	4.052	1.889	-24.60	6.62	NA	NA	NA
LHB	POM	28-Sep-21	1/4TH	4.018	1.916	-25.33	7.04	NA	NA	NA
LHB	POM	28-Sep-21	1/4TH	4.269	1.893	-25.77	6.25	NA	NA	NA
MKH	POM	28-Sep-21	1/4TH	4.213	1.766	-23.35	5.66	NA	NA	NA
MKH	POM	29-Sep-21	1/4TH	4.551	1.945	-23.12	5.01	NA	NA	NA
MKH	POM	29-Sep-21	1/4TH	3.607	1.547	-23.23	4.94	NA	NA	NA
MLB	POM	29-Sep-21	1/4TH	6.129	2.259	-23.83	6.04	NA	NA	NA

MLB	POM	29-Sep-21	1/4TH	5.310	2.132	-24.09	6.66	NA	NA	NA
MLB	POM	29-Sep-21	1/4TH	5.020	2.129	-23.87	6.18	NA	NA	NA
BHO	POM	29-Sep-21	1/4TH	5.566	3.126	-26.07	1.31	NA	NA	NA
BHO	POM	29-Sep-21	1/4TH	7.316	4.109	-26.04	1.75	NA	NA	NA
BHO	POM	29-Sep-21	1/4TH	6.181	3.440	-26.53	1.69	NA	NA	NA
SVC	POM	29-Sep-21	1/4TH	5.350	2.410	-24.44	6.15	NA	NA	NA
SVC	POM	29-Sep-21	1/4TH	5.177	2.265	-24.86	6.04	NA	NA	NA
Macroalgae										
Location	Tissue Type	Date	Weight (mg)	CO2 Ampl	N2 Ampl	d13C	d15N	%C	%N	C/N
SVC	Macroalgae	23-Sep-21	3.156	16.067	2.438	-17.4	6.8	35.24	1.75	20.15
SVC	Macroalgae	23-Sep-21	3.386	15.183	2.177	-17.7	7.0	30.93	1.49	20.74
SVC	Macroalgae	23-Sep-21	3.269	16.064	2.302	-17.9	6.6	33.77	1.59	21.21
HRL	Macroalgae	23-Sep-21	3.131	16.425	1.261	-18.9	7.0	37.60	1.00	37.66
HRL	Macroalgae	23-Sep-21	3.189	16.396	1.361	-18.9	6.9	36.90	1.06	34.84
HRL	Macroalgae	23-Sep-21	3.171	15.849	1.219	-18.7	6.5	35.06	0.93	37.77
HRL	Macroalgae	23-Sep-21	3.202	16.419	1.058	-19.1	6.7	36.54	0.82	44.42
MKH	Macroalgae	23-Sep-21	3.099	16.720	1.911	-17.7	7.3	38.37	1.50	25.55
MKH	Macroalgae	23-Sep-21	3.066	16.789	1.937	-17.9	7.3	38.88	1.53	25.38
MKH	Macroalgae	23-Sep-21	3.363	17.727	2.270	-17.9	7.3	38.31	1.64	23.35
CRI	Macroalgae	23-Sep-21	3.163	16.040	1.225	-18.8	7.0	36.03	0.95	37.87
CRI	Macroalgae	23-Sep-21	3.061	15.346	1.047	-18.2	6.8	36.02	0.89	40.39
CRI	Macroalgae	23-Sep-21	3.296	16.005	1.227	-18.3	7.0	34.92	0.94	37.13
CRI	Macroalgae	23-Sep-21	3.103	14.740	1.185	-18.8	6.9	33.29	0.96	34.84
CNH	Macroalgae	23-Sep-21	3.174	16.276	1.533	-20.6	6.5	36.63	1.18	30.99
CNH	Macroalgae	23-Sep-21	3.070	15.662	1.684	-20.9	6.6	35.48	1.28	27.68
CNH	Macroalgae	23-Sep-21	3.080	15.079	1.796	-18.1	6.2	33.86	1.38	24.58
BGI	Macroalgae	23-Sep-21	3.303	15.793	1.659	-18.4	5.9	33.54	1.20	27.97
BGI	Macroalgae	23-Sep-21	3.213	13.741	1.669	-18.6	6.2	28.90	1.22	23.74
BGI	Macroalgae	23-Sep-21	2.822	13.223	1.336	-18.1	6.1	31.76	1.13	28.06
BGI	Macroalgae	23-Sep-21	3.286	15.853	1.840	-18.2	6.3	34.07	1.34	25.36
BLP	Macroalgae	23-Sep-21	2.953	14.955	1.805	-18.6	5.1	35.19	1.46	24.06
BLP	Macroalgae	23-Sep-21	3.199	16.150	2.060	-18.4	5.1	35.86	1.55	23.11
LHB	Macroalgae	23-Sep-21	3.218	17.128	1.869	-17.8	6.7	38.34	1.42	26.97
LHB	Macroalgae	23-Sep-21	3.325	17.682	2.032	-17.5	6.5	38.29	1.45	26.39
LHB	Macroalgae	23-Sep-21	3.230	17.408	1.964	-17.4	6.5	38.49	1.45	26.64
LHB	Macroalgae	23-Sep-21	3.256	16.233	1.824	-17.4	6.8	35.28	1.34	26.25
HWP	Macroalgae	23-Sep-21	3.477	12.983	3.539	-17.4	5.4	25.86	2.33	11.12
HWP	Macroalgae	23-Sep-21	3.340	14.755	4.220	-20.4	5.4	31.34	2.87	10.91
HWP	Macroalgae	23-Sep-21	3.195	13.642	3.873	-20.3	5.4	31.94	2.86	11.16
MLB	Macroalgae	23-Sep-21	3.069	9.315	1.004	-19.7	7.0	19.44	0.75	25.89
MLB	Macroalgae	23-Sep-21	3.300	11.453	1.196	-19.6	6.7	22.95	0.86	26.77
MLB	Macroalgae	23-Sep-21	2.960	9.860	0.924	-17.4	6.9	21.43	0.72	29.80
MLB	Macroalgae	23-Sep-21	3.240	10.598	1.229	-19.7	6.6	21.23	0.87	24.46
BHO	Macroalgae	23-Sep-21	2.925	15.784	2.498	-16.9	6.6	38.10	2.01	18.93
BHO	Macroalgae	23-Sep-21	2.885	15.699	2.450	-17.0	6.7	38.23	1.98	19.32
BHO	Macroalgae	23-Sep-21	3.168	17.010	2.480	-16.8	6.5	38.74	1.88	20.66
Consumers										
Location	Tissue Type	Date	Weight (mg)	CO2 Ampl	N2 Ampl	d13C	d15N	%C	%N	C/N

MLB	Mussel	21-Sep-21	1.244	8.351	6.571	-21.21	7.78	39.83	11.23	3.55
MLB	Mussel	21-Sep-21	1.730	11.190	9.834	-20.53	8.33	39.79	12.05	3.30
MLB	Mussel	21-Sep-21	1.477	9.864	8.483	-21.05	7.64	40.44	12.18	3.32
MLB	Mussel	21-Sep-21	1.356	8.925	7.254	-21.07	8.53	39.44	11.36	3.47
MLB	Mussel	21-Sep-21	1.769	11.090	9.037	-21.17	7.81	38.71	10.89	3.55
MLB	Mussel	21-Sep-21	1.569	9.933	8.228	-21.09	7.98	38.67	11.19	3.46
BGI	Mussel	21-Sep-21	1.990	13.007	12.011	-20.42	8.17	40.89	12.79	3.20
BGI	Mussel	21-Sep-21	1.845	12.048	10.531	-21.08	8.19	40.53	12.15	3.34
BGI	Mussel	21-Sep-21	1.790	11.218	10.044	-20.28	8.22	38.70	11.97	3.23
BGI	Mussel	21-Sep-21	1.693	10.458	9.157	-20.71	8.28	37.69	11.51	3.28
BGI	Mussel	21-Sep-21	1.645	10.858	9.546	-20.73	8.52	40.53	12.35	3.28
BGI	Mussel	21-Sep-21	1.377	9.160	7.827	-20.78	8.24	40.02	12.17	3.29
BLP	Mussel	21-Sep-21	1.562	10.685	9.502	-20.69	8.62	41.54	12.89	3.22
BLP	Mussel	21-Sep-21	1.326	9.067	7.885	-20.61	8.24	40.91	12.67	3.23
BLP	Mussel	21-Sep-21	1.806	11.973	10.849	-21.28	8.46	41.10	12.75	3.22
BLP	Mussel	21-Sep-21	2.008	13.120	12.140	-20.95	8.37	41.44	12.88	3.22
BLP	Mussel	21-Sep-21	1.900	12.679	11.599	-20.03	8.32	41.62	12.94	3.22
CNH	Mussel	21-Sep-21	1.434	9.806	8.648	-19.86	8.48	41.37	12.87	3.22
CNH	Mussel	21-Sep-21	1.403	9.430	8.239	-20.12	9.40	40.27	12.44	3.24
CNH	Mussel	21-Sep-21	2.062	13.309	12.229	-20.52	8.84	41.00	12.63	3.25
CNH	Mussel	21-Sep-21	2.007	13.333	12.450	-20.41	8.63	41.92	13.18	3.18
CNH	Mussel	21-Sep-21	1.496	10.076	8.684	-20.27	9.24	41.00	12.41	3.30
CNH	Mussel	21-Sep-21	1.551	10.832	9.462	-20.83	8.80	42.72	12.98	3.29
CNH	Mussel	21-Sep-21	1.305	9.188	7.896	-20.75	8.86	42.12	12.81	3.29
CRI	Mussel	21-Sep-21	1.454	9.832	8.703	-20.38	8.93	40.77	12.70	3.21
CRI	Mussel	21-Sep-21	1.524	10.359	9.018	-20.22	8.86	41.28	12.57	3.28
CRI	Mussel	21-Sep-21	1.824	11.033	9.591	-19.93	8.92	37.30	11.20	3.33
CRI	Mussel	21-Sep-21	1.355	9.150	8.133	-20.38	8.93	40.54	12.74	3.18
CRI	Mussel	21-Sep-21	1.326	8.953	7.789	-20.20	8.91	40.44	12.49	3.24
CRI	Mussel	21-Sep-21	1.435	9.729	8.526	-20.17	8.94	40.76	12.59	3.24
MKH	Mussel	21-Sep-21	2.142	14.180	12.915	-22.94	6.94	42.03	12.76	3.29
MKH	Mussel	21-Sep-21	1.608	11.166	9.649	-23.78	6.86	42.43	12.69	3.34
MKH	Mussel	21-Sep-21	1.900	12.856	11.645	-22.99	6.98	42.33	13.03	3.25
MKH	Mussel	21-Sep-21	1.422	9.749	8.525	-23.28	6.59	41.24	12.74	3.24
MKH	Mussel	21-Sep-21	2.057	12.812	11.597	-22.67	6.89	38.86	11.96	3.25
MKH	Mussel	21-Sep-21	2.086	14.014	12.767	-22.86	6.67	42.61	13.00	3.28
BGI	Snail	21-Sep-21	1.904	12.009	8.877	-17.50	7.86	39.23	9.94	3.95
BGI	Snail	21-Sep-21	1.809	11.180	8.115	-17.40	7.57	37.83	9.53	3.97
BGI	Snail	21-Sep-21	1.654	9.912	7.390	-17.22	7.44	36.17	9.52	3.80
MKH	Snail	21-Sep-21	1.726	11.087	8.049	-18.27	7.27	39.10	9.83	3.98
MKH	Snail	21-Sep-21	1.315	8.666	6.081	-18.16	7.51	39.32	9.86	3.99
MKH	Snail	21-Sep-21	1.409	9.221	6.152	-18.20	7.58	39.13	9.26	4.22
MKH	Snail	21-Sep-21	1.346	8.920	6.108	-18.53	7.62	39.54	9.63	4.11
CNH	Snail	21-Sep-21	1.597	10.152	7.526	-16.48	7.56	38.44	10.00	3.84
CNH	Snail	21-Sep-21	1.422	8.662	6.271	-16.46	7.22	36.48	9.42	3.87
CNH	Snail	21-Sep-21	1.381	8.717	6.329	-16.20	7.46	37.82	9.79	3.86
MLB	Snail	21-Sep-21	1.592	9.873	7.382	-15.47	6.94	37.45	9.86	3.80
MLB	Snail	21-Sep-21	1.773	11.088	8.634	-15.44	7.06	38.44	10.38	3.70

MLB	Snail	21-Sep-21	1.592	10.230	7.906	-14.35	6.52	39.01	10.56	3.70
MLB	Snail	21-Sep-21	1.567	9.421	7.003	-15.41	6.86	36.59	9.59	3.82
CRI	Snail	21-Sep-21	1.518	9.600	6.743	-16.26	8.25	38.14	9.47	4.03
CRI	Snail	21-Sep-21	1.657	10.559	7.880	-17.67	8.71	38.69	10.12	3.83
CRI	Snail	21-Sep-21	1.231	7.759	5.550	-16.97	7.58	37.29	9.63	3.87
CRI	Snail	21-Sep-21	1.711	10.765	8.052	-16.74	7.58	38.32	10.02	3.82
BLP	Snail	21-Sep-21	1.868	11.689	8.713	-14.51	5.92	39.00	9.97	3.91
BLP	Snail	21-Sep-21	1.355	8.739	6.376	-15.18	6.46	38.71	10.06	3.85
BLP	Snail	21-Sep-21	1.420	9.110	6.649	-14.25	5.80	38.53	9.99	3.86
BLP	Snail	21-Sep-21	1.695	10.749	7.982	-14.32	5.83	38.97	10.07	3.87
BHO	Fish	21-Sep-21	1.524	10.799	9.166	-21.18	8.29	42.92	12.70	3.38
BHO	Fish	21-Sep-21	1.287	9.488	7.718	-18.88	9.42	44.43	12.81	3.47
BHO	Fish	21-Sep-21	1.582	11.710	9.885	-20.19	9.19	45.40	13.24	3.43
CNH	Fish	21-Sep-21	1.651	12.033	10.645	-14.98	12.43	44.86	13.68	3.28
CNH	Fish	21-Sep-21	1.309	9.774	8.474	-15.34	11.43	44.99	13.78	3.27
CNH	Fish	21-Sep-21	1.719	12.685	11.401	-15.30	11.36	46.15	14.15	3.26
CNH	Fish	21-Sep-21	1.948	13.901	12.545	-15.91	11.99	45.40	13.74	3.31
MKH	Fish	21-Sep-21	1.683	12.252	10.692	-20.78	7.73	45.12	13.54	3.33
MKH	Fish	21-Sep-21	1.744	13.021	11.704	-20.86	8.20	46.57	14.28	3.26
MKH	Fish	21-Sep-21	1.648	11.957	10.312	-20.38	8.40	44.91	13.36	3.36
CRI	Fish	21-Sep-21	2.063	13.814	12.768	-12.95	10.29	42.36	13.21	3.21
CRI	Fish	21-Sep-21	1.981	12.609	11.570	-12.90	10.35	39.57	12.43	3.18
CRI	Fish	21-Sep-21	1.421	10.495	9.199	-14.50	10.82	44.90	13.81	3.25
CRI	Fish	21-Sep-21	1.697	12.529	11.201	-13.53	9.63	45.75	14.05	3.26
BGI	Fish	21-Sep-21	1.575	11.667	10.038	-13.55	10.55	45.79	13.59	3.37
BGI	Fish	21-Sep-21	1.343	10.186	8.698	-14.26	10.72	45.84	13.78	3.33
BGI	Fish	21-Sep-21	1.522	11.281	9.753	-14.23	10.68	45.62	13.67	3.34
BGI	Fish	21-Sep-21	1.704	12.528	11.147	-14.40	10.12	45.79	13.99	3.27
MLB	Fish	21-Sep-21	1.941	13.865	12.747	-14.38	10.86	45.38	14.06	3.23
MLB	Fish	20-Sep-21	1.462	10.596	1.550	-14.58	10.47	44.14	13.37	3.30
MLB	Fish	20-Sep-21	1.853	11.496	1.717	-14.73	11.02	38.16	11.71	3.26
MLB	Fish	20-Sep-21	2.032	14.313	2.239	-14.72	11.19	45.08	13.84	3.26
CRI	Lobster	20-Sep-21	1.437	10.724	1.536	-18.43	14.37	45.68	13.59	3.36
CRI	Lobster	20-Sep-21	1.522	10.968	1.630	-18.93	13.41	44.02	13.49	3.26
CRI	Lobster	20-Sep-21	1.538	10.979	1.520	-18.28	13.18	43.71	12.52	3.49
CRI	Lobster	20-Sep-21	1.347	9.814	1.355	-18.28	13.20	43.94	12.66	3.47
MLB	Lobster	20-Sep-21	2.122	13.941	1.946	-18.29	13.62	41.85	11.56	3.62
MLB	Lobster	20-Sep-21	1.617	10.871	1.609	-18.57	13.04	41.01	12.50	3.28
MLB	Lobster	20-Sep-21	1.464	10.670	1.553	-18.60	13.04	44.56	13.49	3.30
MLB	Lobster	20-Sep-21	2.206	15.344	2.329	-18.34	13.31	45.17	13.24	3.41
CNH	Lobster	20-Sep-21	1.758	12.338	1.843	-18.44	13.56	43.59	13.19	3.30
CNH	Lobster	20-Sep-21	1.970	14.129	2.180	-18.40	13.49	45.81	13.89	3.30
CNH	Lobster	20-Sep-21	1.473	10.497	1.539	-18.86	13.81	43.47	13.21	3.29
CNH	Lobster	20-Sep-21	1.513	10.795	1.546	-18.72	12.93	43.43	12.80	3.39
BLP	Lobster	20-Sep-21	1.779	12.081	1.629	-18.31	13.51	41.87	11.46	3.65
BLP	Lobster	20-Sep-21	1.335	9.768	1.412	-18.60	13.04	44.05	13.37	3.29
BLP	Lobster	20-Sep-21	1.987	14.078	2.088	-18.37	13.34	45.14	13.18	3.42
BGI	Lobster	20-Sep-21	1.923	13.660	2.067	-18.69		45.06	13.51	3.33

BGI	Lobster	20-Sep-21	2.003	14.080	2.123	-18.85	13.07	45.04	13.37	3.37
BGI	Lobster	20-Sep-21	1.638	10.268	1.470	-18.87	13.00	38.34	11.43	3.35
BGI	Lobster	20-Sep-21	1.903	13.721	1.986	-18.58	13.15	45.87	13.18	3.48
BHO	Lobster	20-Sep-21	1.817	12.902	1.934	-18.41	13.23	44.73	13.50	3.31
BHO	Lobster	20-Sep-21	2.307	15.215	2.334	-18.79	12.56	43.10	12.77	3.37
BHO	Lobster	20-Sep-21	1.272	14.203	1.991	-19.75	10.91	71.47	19.72	3.62
BHO	Lobster	20-Sep-21	1.285	9.449	1.387	-18.87	12.92	44.57	13.66	3.26
MKH	Lobster	20-Sep-21	1.585	11.308	1.662	-18.73	13.37	43.95	13.25	3.32
MKH	Lobster	20-Sep-21	1.481	10.976	1.594	-18.70	13.77	45.66	13.66	3.34
MKH	Lobster	20-Sep-21	1.768	12.583	1.856	-18.65	13.91	44.56	13.19	3.38
MLB	Crab	20-Sep-21	1.858	12.757	1.990	-18.00	12.80	43.34	13.56	3.20
MLB	Crab	20-Sep-21	1.717	12.209	1.936	-18.27	12.99	44.15	14.20	3.11
MLB	Crab	20-Sep-21	1.451	10.458	1.639	-18.44	13.52	43.87	14.21	3.09
BLP	Crab	20-Sep-21	1.767	12.092	1.891	-18.25	13.20	42.61	13.52	3.15
BLP	Crab	20-Sep-21	1.311	9.379	1.411	-18.25	12.32	43.13	13.62	3.17
BLP	Crab	20-Sep-21	1.276	9.413	1.416	-18.23	12.34	44.12	13.92	3.17
BLP	Crab	20-Sep-21	1.735	12.516	1.959	-18.45	12.50	45.15	14.26	3.17
CNH	Crab	20-Sep-21	1.665	11.040	1.671	-18.80	13.12	40.47	12.61	3.21
CNH	Crab	20-Sep-21	1.350	9.068	1.368	-19.12	12.45	40.38	12.81	3.15
CNH	Crab	20-Sep-21	2.187	13.730	2.192	-19.03	12.51	39.99	12.66	3.16
MKH	Crab	20-Sep-21	1.675	11.367	1.784	-18.57	12.99	41.69	13.41	3.11
MKH	Crab	20-Sep-21	1.435	9.865	1.514	-18.57	13.01	41.52	13.31	3.12
MKH	Crab	20-Sep-21	1.760	11.344	1.669	-18.67	12.53	39.63	11.93	3.32
BHO	Crab	20-Sep-21	1.556	10.949	1.659	-18.53	12.36	43.01	13.43	3.20
BHO	Crab	20-Sep-21	1.231	9.135	1.369	-18.31	12.79	44.55	14.05	3.17
BHO	Crab	20-Sep-21	1.813	12.587	1.965	-19.68	12.45	43.50	13.69	3.18
CRI	Crab	20-Sep-21	1.346	9.502	1.410	-18.61	13.90	42.64	13.26	3.22
CRI	Crab	20-Sep-21	1.821	11.600	1.791	-18.60	13.16	39.54	12.44	3.18
CRI	Crab	20-Sep-21	1.644	11.142	1.741	-19.06	13.23	41.63	13.31	3.13
BGI	Crab	20-Sep-21	1.667	11.130	1.700	-17.12	12.37	41.01	12.85	3.19
BGI	Crab	20-Sep-21	2.098	13.988	2.312	-17.64	13.29	42.50	13.86	3.07
BGI	Crab	20-Sep-21	1.433	9.865	1.536	-17.70	13.30	41.78	13.57	3.08
BGI	Crab	20-Sep-21	1.389	9.621	1.492	-19.02	12.57	41.72	13.51	3.09