# COMPARING HISTORICAL AND CONTEMPORARY MACROFAUNAL COMMUNITIES AND FUNCTIONAL TRAITS IN A SUBARCTIC EMBAYMENT IN NEWFOUNDLAND, CANADA

**By**

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## Thesis abstract

Studies examining biological communities in relation to environmental factors over time are essential for understanding natural sources of community variation (i.e., structure and function), as well as their response to stressors. Benthic macrofauna are important biological indicators used to assess environmental quality in marine ecosystems. Placentia Bay is a large subarctic embayment on the southeast coast of Newfoundland (NL) that has been identified as an Ecologically and Biologically Significant Area (EBSA) that is exposed to anthropogenic stressors (e.g., fisheries and hazardous substances). The overarching aim of my thesis research was to assess the influence of various environmental factors on the community structure and functional traits of macrofauna in Placentia Bay using contemporary and historical biological and environmental data. Comparison of macrofauna at 8 stations sampled in 1998 and 2019–2020 ($n$=77) showed significant temporal changes in community structure and function, without loss of function (i.e., 36 morphological/behavioral modalities). The historical community had significantly higher densities, but lower evenness and diversity compared to contemporary years. Macrofauna were dominated by highly tolerant (82.3%) subsurface deposit feeders (62.1%) having small body sizes, whereas contemporary communities had a higher proportion of medium tolerant species/taxa (7.2 vs. 36.7%) and nearly equal proportions of subsurface and surface deposit feeders (26.7 and 29.1%) with small-medium body sizes. These changes are likely a reflection of the large reduction in the relative proportion of polychaetes (91 vs. 58%). Community patterns were related to the sedimentary habit. Sediments have become coarser with higher levels of sedimentary total organic matter in the contemporary compared to the historical years. Interestingly, while grain size has also become coarser between 2019 and 2020 communities were similar. Moreover, even though contaminants (examined in 2020 only) were not above probable biological effect levels, depth and heavy metal concentrations were most correlated to macrofaunal community structure among different areas in the bay. This research indicates the need for studies specifically designed to better quantify temporal changes in total organic carbon and heavy metals in relation to potential anthropogenic sources and effects on macrofauna in the bay. It is recommended that total organic carbon and C/N ratios (indicator of food quality/source) are continuously monitored to examine how their levels change over time and the possible source(s) of enrichment. Additionally, as development continues in coastal regions such as Placentia Bay future studies examining acceptable contaminant levels and possible biological outcomes are needed.

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## Thesis Format

This manuscript is formatted as a grouped manuscript composed of two independent chapters containing their own introduction, methods, results, discussion, and conclusion. A general introduction and conclusion are included.

## Thesis Introduction

Canada is home to the longest coastline in the world spanning more than 240 000 km (Ganter et al. 2021; Warren & Lulham 2021; Taylor et al. 2014). Coastal ecosystems are one of the most productive areas of the ocean and with human populations localized around them, they are subject to multiple anthropogenic disturbances (Dobson & Frid 2009; Diaz et al. 2004) including eutrophication, fisheries, hazardous substances, physical disturbance, shipping traffic, and climate change (Nestlerode et al. 2020; Byers & Grabowski 2013). One of today's largest scientific challenges is to understand how natural and anthropogenic disturbances affect the structure and functioning of marine ecosystems and the services they provide with the aim for sustainable future development (Heip & McDonough 2012). Biodiversity (e.g., measured as species richness) has been recognized as an important factor in maintaining ecosystem functioning and the many services associated with the coastal environment (Muntadas et al. 2016; Balvanera et al. 2006). A meta-analysis conducted by Cardinale et al. (2006) found that species loss affects ecosystem functioning (i.e., reduced productivity/biomass), however, the magnitude of the effect depends on which species are lost. The viewpoint that ecosystem function has greater stability when a community is species rich (Covich et al. 2004; Loreau 2004) was initially proposed by Darwin (1859), echoed by Macarthur (1955), and modeled by May (1973) (Peterson et al. 1998).

This idea is further supported when examining communities with high diversity and functionally redundant traits. The species redundancy hypothesis (Walker 1992) proposes that multiple species may fulfill the same functional role (i.e., functional redundancy) which can contribute to community stability (Kang et al. 2015; Walker 1992). Functional redundancy is thought to increase temporal ecosystem service stability by dampening the effect of species loss via function retention (Biggs et al. 2020; Walker 1992). For example, even when the number of species are reduced, functional roles may remain fulfilled by surviving taxa, supporting ecosystem recovery from disturbance (resilience) (Kun et al. 2019; Loreau 2004). A meta-analysis by Biggs et al. (2020) suggested that both community structure and ecosystem function are important and should be assessed when considering ecosystem stability and provisions since particular services relate to ecosystem function and others correspond to the presence of specific species. This is exemplified in a study by Frid and Caswell (2015) who found that redundant functional traits (implying ecological functioning) could be maintained under changing species composition as taxa being eliminated are replaced by others with similar traits (McLean et al. 2019; Frid & Caswell 2015). Alternatively, when they examined communities in which functional traits were fulfilled by a few species (i.e., unique traits by rare species), functional traits had greater variation with the removal of a few rare species even if there was not a significant change in species composition within the community (McLean et al. 2019; Frid & Caswell 2015). Thus, with global climate change altering ecosystem conditions, information pertaining to both community structure and ecosystem function are necessary to understand and determine important drivers of environmental change and predict the resilience of communities ( Vause et al. 2019; Biggs et al. 2015). Studying which ecosystem properties support resilience can aid in management efforts through identifying ecologically important regions as well as those susceptible to disturbance (Miatta et al. 2021; Biggs et al. 2015).

Improving marine ecosystem management in light of environmental change and anthropogenic influences, requires understanding how habitats and communities will be affected by such disturbances (Drejou et al. 2021). Presently, these efforts depend upon ecological monitoring and assessments to evaluate the condition (e.g., pollutant/contaminant level, sedimentary organic content, biological indicators) of the environment, serving as an early-warning signal of potential negative impacts (i.e., species loss or ecosystem function) (Drejou et al. 2021; Niemi & McDonald 2004). A common biological indicator used in ecological assessments of the coastal environments are soft-sediment benthic macrofauna (herein referred to as organisms retained on a 500 µm screen) (Drejou et al. 2021; Snelgrove & Butman 1994). These organisms exhibit high diversity in seafloor sediments and contribute to a variety of essential ecosystem functions including acceleration of organic matter decomposition, bioturbation, and filtration (Chen 2021; Covich et al. 1999). They are an important food source for many species and play a vital role in nutrient cycling and energy flow (Drejou et al. 2021; Covich et al. 2004). Their fundamental position in marine food webs affects the success of many commercially important fish (Henkel & Gilbert 2020). Loss and alterations of benthic communities would consequently not only affect higher trophic levels which rely on them for food, but also alter the many ecosystem services that they provide.

Using benthic macrofauna as bioindicators is an essential component of national and international monitoring and assessment programs (e.g., Ramey et al. 2011; Van Hoey et al. 2010; Borja & Dauer 2008; CABIN, CAFF, Directive WFD: 2000/60/EC; Directive MSFD: 2008/56/EC). Several characteristics of these organisms have contributed to their establishment as bioindicators (Lenihan & Micheli 2001). For instance, benthic macrofauna are primarily sedentary exposing them to prolonged disturbance. They are trophically diverse and exhibit species-specific tolerances or sensitivity to organic enrichment (Borja & Dauer 2008; Pearson & Rosenberg 1978). As such they can exhibit marked changes related to environmental conditions as they rapidly respond to stressors (Fuchs et al. 2020; Taghon et al. 2017b; Villnas & Norkko, 2011; Borja & Dauer, 2008). Studies characterizing community structure (e.g., species composition, richness, and diversity) of macrofauna in relation to natural and anthropogenic environmental factors form the foundation for conducting mechanistic research aimed at determining specific processes responsible for creating observed community patterns, detecting anthropogenic impacts, and predicting future longer-term community changes resulting from environmental change (Dreujou et al. 2021; Grassle et al. 2009; Lenihan & Mecheli 2001).

Abiotic and biotic processes influencing the distribution and community structure of macrofauna include small-scale (cm to m's) sedimentary habitat features (e.g., sediment grain size, organic carbon, oxygen, contaminants) and relatively larger-scale (kms) oceanographic conditions (e.g., depth, temperature, salinity, surface production, circulation, disturbance) (Olafasson et al. 1994; Morrisey et al. 1992; Barry & Dayton 1991). Species interactions also play a role in shaping community patterns at smaller scales (mm to m's) (Snelgrove 1999; Olafasson et al. 1994). Given the intimate association macrofauna have with their sedimentary habitat, differences in sediment grain size, organic content, stability, and porosity all contribute to the well-known, characteristically patchy distributions of benthic communities (Snelgrove & Butman 1994; Rhoads & Young 1970). Hydrodynamic conditions or water velocity near the seafloor can influence sediment type. Fine sediments (silty-muddy; 0.06–31 µm grain size) generally occur in areas of weak flow and allow for greater deposition of organic material, whereas coarser sediments (sand; 63–500 µm grain size) are present in fast flowing waters with large amounts of sediment resuspension (Dyer 1986). Fine sediments consequently tend to be richer in organic content resulting from both decreased water velocity, as well as their enhanced ability to bind organic carbon (Snelgrove & Butman 1994). This implies not only an animal-sediment relationship but also that of animal and organic carbon distributions (Snelgrove & Butman 1994; Sanders et al. 1962). Indeed, species abundance and diversity are generally positively correlated with sedimentary organic carbon (Ricciardi & Bouget 1999; Davies & Payne 1984). Organic loading through both natural and anthropogenic activities (e.g., sewage discharge, excess phosphorus and nitrogen, natural terrestrial debris), however, can lead to anoxic conditions via decomposition of organic material driving oxygen depletion (Pearson & Rosenberg 1978). Benthic communities are considered stable when there is high diversity, high richness, and intermediate abundances of species present (Pearson & Rosenberg 1978). In contrast, polluted sediments have been linked to high species abundances with low diversity and species richness (Pearson & Rosenberg 1978). Feeding mode of macrofauna is also influenced by hydrodynamics. Generally, deposit feeders have greater abundances in silty-muddy sediments whereas suspension feeders are associated with coarse sands (Snelgrove & Butman 1994). Some species are able to switch from deposit to suspension feeding under different water velocities near the seafloor and this behavior is suggested to be motivated by food acquisition (Riisgard & Kamermans 2000).

Water temperature and photoperiod influence community structure through surface production, which affects the supply of food to the benthos, and life cycle changes (Bêche et al. 2006). Specifically, these factors play an integral role in modulating food resources through phytoplankton blooms in spring and summer months which in turn affect reproduction, larval development, and growth of benthic species (Fuchs et al. 2020; Bêche et al. 2006; Coma et al. 2000). Globally ~71% of coastal areas have already experienced an increase in sea surface temperature (SST) from 1982–2019 at a rate of 0.25 °C per decade (Alexander et al. 2018; Lima & Wethey 2012). A recent study examining warming based on sea surface temperature (SST) anomalies, in the north Atlantic (i.e., at St. John’s, Newfoundland [NL] Canada) projected an increase of 0.4–2.2 °C over the next 50 years (Han et al. 2015). Spatio-temporal SST gradients are important in influencing wind patterns, storm development, and wave activity through ocean-atmosphere interactions (Reguero et al. 2019; Bengtsson et al. 2006). Attributed to rising SST (C2ES 2020), the North Atlantic Basin has seen an increase in the intensity of tropical storms including hurricanes (Méndez-Tejeda & Hernández-Ayla 2023). Moreover, wave activity in terms of wave height has also increased at high latitudes in recent decades (Reguero et al. 2019). Physical disturbance to the benthic habitat by storms and wave action have the potential to influence seafloor substates (Herkul et al. 2016) through erosion and deposition of sediments. Disturbance from storms can also affect circulation patterns (Ma et al. 2017), salinity, and bottom flow.

Disturbance may decrease diversity on a small scale; however, it can also generate spatial heterogeneity and contribute to patchy distributions observed in the benthos (Roxburg et al. 2004; Grassle & Maciolek 1992). Connell (1978), who brought forth the intermediate disturbance hypothesis, proposed that species diversity would be greatest at intermediate levels of disturbance, and reduced under low and high levels of disturbance. It is suggested that under intermediate disturbances (accounting for frequency and intensity) competition and mortality would be suppressed or equal to the disturbance, allowing for maximum species diversity (Huston 1979; Connell 1978). This scenario implies that near competitive equilibrium there could be multiple competing species all increasing at a gradual rate (Huston 1979). Post disturbance conditions might therefore be ideal for colonization, creating the potential to sustain multiple taxa. Additionally, species possessing the ability to recover quickly post-disturbance contribute to overall resilience of the ecosystem (Davis & Moritz 2013). For instance, species able to survive natural disturbance likely possess traits allowing them to withstand harsh conditions (Bêche et al. 2006). Evaluating spatial and temporal community structure and traits in response to disturbance can provide insight into how communities may cope with environmental change and the degree to which ecosystem functioning might be affected (Miatta et al. 2021; McLean et al. 2019). There is a need for short and long-term studies examining community structure and function of coastal ecosystems in the face of environmental change and anthropogenic stressors (Bêche et al. 2006).

### *Study Site - Placentia Bay*

Placentia Bay is a large embayment on the southeast coast of Newfoundland (NL) in the northwest Atlantic covering roughly 1750 km of marine coastline (Fig. 1.1). The northwest Atlantic has been identified as a region of accelerated warming and SST on the Newfoundland and Labrador shelf has been increasing by ~0.37 °C per decade (Chen et al. 2020). From 1975–2006, 31 tropical storms passed within 278 km of Placentia Bay (LGL 2007), and more recently the bay experienced two hurricanes (i.e., Igor in 2010 and Leslie in 2012) with Igor being the most intense hurricane in NL to date (Ma et al. 2017). Like many coastal environments, Placentia Bay is also exposed to multiple anthropogenic stressors. As an important marine transport route, crude oil tanker traffic has led to oil spills, creating inputs of polycyclic aromatic hydrocarbons (Kiceniuk 1992). The bay remains ice-free throughout the winter and is high-traffic region important for the Come by Chance refinery, as well as Vale Long Harbour (i.e., metal processing facility) (DFO, 2017). Placentia Bay has been identified as an Ecologically and Biologically Significant Area (EBSA) and is home to cetaceans, seals, and sea birds, as well as endangered species such as the leatherback turtle and harlequin duck (DFO 2019; LGL 2018). The bay also supports essential commercial fisheries (e.g., blue mussel, flounder, cod, and snow crabs) and Atlantic salmon aquaculture operations throughout the central, western and eastern channels (Wells et al. 2019; LGL 2018; DFO 2007).

My thesis research provides the most comprehensive investigation characterizing temporal and spatial patterns in the little studied benthic macrofaunal communities in Placentia Bay, NL to date. Macrofaunal communities in the bay have received little to no attention since the first study conducted 21 years ago. The overarching aim of my thesis research was to assess the influence of various environmental factors on the community structure and functional traits of macrofauna in Placentia Bay using contemporary and historical biological and environmental data. Inherent in this, is the central question of whether macrofaunal communities in Placentia Bay have changed over the last ~20 years. And if so, what environmental drivers may be influencing community structure and functional diversity? Information provided by studies such as this one, aimed at improving our understanding of how communities and habitats have changed over time, within the context of functional traits and ecosystem resilience, can be used in monitoring and management programs, as well as conservation efforts to preserve ecologically and biologically significant ecosystems such as Placentia Bay. It is important to note the inherent limitations imposed by data only taken at specific time points (i.e., historical 1998 vs. contemporary 2019–2020) and this has been taken into consideration when discussing the study's major findings and conclusions. Unfortunately, the lack of continuous long-term time series data for benthic communities in the coastal ocean is all too common making studies such as this even more important and timely.

## Thesis Objectives

The overarching aim of my thesis research was to assess the influence of various environmental factors on the community structure and functional traits of macrofauna temporally and spatially in Placentia Bay, NL. In the first chapter, I used historical and contemporary benthic macrofaunal composition and abundance data (1998 vs. 2019/2020 respectively) taken

21 years apart, to investigate potential changes in community and functional structure in the bay and examine the influence of environmental drivers on observed patterns. I hypothesized that historical and contemporary communities would be different and community patterns would be related to the sedimentary habitat, as it can be influenced by larger-scale oceanographic features. I expected that functional traits of macrofauna would be related to community structure such that they exhibit similar spatio-temporal patterns of variability. I also hypothesized that there would not be a loss of functional traits between historical and contemporary communities. Therefore, I predicted that while species/taxon composition may change, functional roles would remain fulfilled (i.e., no loss of modalities) allowing for any loss of species/taxa to be buffered against (species redundancy hypothesis: Walker 1992).

The objective of my second chapter was to delineate macrofauna communities both temporally and spatially in relation to natural and anthropogenic environmental factors (i.e., total organic matter, chlorophyll-a, phaeopigments, sediment grain size, heavy metals, and PAHs). I hypothesized that the composition and abundance of macrofauna in the bay would not differ between years (i.e., 2019 vs. 2020) as sedimentary habitat features (e.g., grain size, contaminants) important in influencing communities are expected to remain relatively stable temporally (Guerin et al. 2023; Herder et al. 2021; Taghon et al. 2017). However, communities in the bay were expected to differ depending on location and the associated sedimentary habitat, specifically grain size and the level of TOM. I predicted that communities at the head of the bay would be distinct from other areas given it is relatively shallow and occurs in close proximity to river input (i.e., through Swift Current). Thus, the head of the bay was expected to have relatively high levels of total organic matter (e.g., through allochthonous input) and relatively low macrofaunal abundance compared to other areas (Ramey & Snelgrove 2003). Information provided by studies such as this one, aimed at improving our understanding of macrofaunal communities and associated of environmental conditions, can be used in monitoring and management programs (i.e., as a reference for long-term studies), as well as conservation efforts to preserve ecologically and biologically significant ecosystems such as Placentia Bay.

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# Chapter One. What has changed in 20 years? Structure and function of soft-sediment macrofauna in a subarctic embayment, Newfoundland (Canada)

## Abstract

Studies examining biological communities in relation to environmental factors over time are essential for understanding natural sources of community variation (i.e., structure and function), as well as their response to stressors. Placentia Bay, Newfoundland in the Northwest Atlantic has been identified as an ecologically significant area supporting many commercial fisheries and diverse species. This subarctic ecosystem is exposed to multiple stressors including a rise in sea surface temperature, storm events, tanker traffic, and aquaculture. Comparison of macrofauna at eight stations throughout the bay in 1998 (historical data; *n*=41 box cores) and more recently in 2019–2020 (*n*=36 Van Veen grabs) showed significant changes in community composition and abundance (i.e., species/taxon abundance and relative contribution of functional traits [e.g., 8 traits and 36 modalities]) have occurred between historical versus contemporary times. However, despite significant changes to species/taxa composition (i.e., presence/absence), communities did not show a loss of function (i.e., modalities). Contemporary community and functional structure had significantly lower densities, but higher evenness and diversity compared to the historical time point. Historical communities were dominated by highly tolerant (82.3%) subsurface deposit feeders (62.1%) having small body sizes whereas contemporary communities showed an increase in proportion of medium tolerant species/taxa (7.2 vs. 36.7%) and nearly equal proportions of subsurface and surface deposit feeders (26.7 and 29.1%) with small-medium body sizes. These changes are likely a reflection of the large reduction in the relative proportion of polychaetes (91 vs. 58%) with densities of 680 ±80 ind. $m^{-2}$ and 100 ±17 ind. $m^{-2}$ respectively. In contrast, the relative proportion of bivalves (4 vs. 25%), amphipods and

gastropods increased. While it is not unexpected that these changes were linked to changes in the sedimentary habit, they were somewhat counter-intuitive. Interestingly, sediments in the bay have become coarser (6 to 27%; possibly due to increased storms) with a corresponding increase in the percentage of sedimentary organic matter (10.4 to 13.5%) and a decrease in the proportion of highly tolerant polychaete species. Information provided by studies such as this one, aimed at improving our understanding of how communities and habitats have changed over time, can be used in monitoring and management programs. It is also recommended future work examine seasonal variability in the bay to quantify short term temporal patterns.

## Introduction

With global climate change altering ecosystem conditions, information pertaining to both community structure and ecosystem function are necessary to understand and determine important drivers of environmental change and predict the resilience of communities (Vause et al. 2019; Biggs et al. 2015). In the marine environment, soft-sediment benthic macrofauna (here defined as organisms retained on a 500 µm screen) are common biological indicators used in ecological assessments of the coastal oceans worldwide (Drejou et al. 2021; Snelgrove & Butman 1994). These organisms can exhibit high diversity in seafloor sediments and contribute to a variety of essential ecosystem functions including acceleration of organic matter decomposition, bioturbation, and filtration (Chen 2021; Covich et al. 1999). They are an important food source for many species and play a vital role in nutrient cycling and energy flow (Drejou et al. 2021; Covich et al. 2004). Biodiversity (e.g., measured as species richness) has been recognized as an important factor in maintaining ecosystem functioning and the many services associated with the coastal environment (Muntadas et al. 2016; Balvanera et al. 2006). A meta-analysis conducted by Cardinale et al. (2006) found that species loss affects ecosystem functioning (i.e., reduced productivity/biomass), however, the magnitude of the effect depends on which species are lost.

The distribution and community structure of macrofauna are influenced by several abiotic and biotic processes. Given the intimate association soft-sediment macrofauna have with their sedimentary habitat, differences in sediment grain size, organic content, stability, and porosity all contribute to the well-known, characteristically patchy distributions of benthic communities (Snelgrove & Butman 1994; Rhoads & Young 1970). Hydrodynamic conditions or water velocity near the seafloor can influence sediment type. Fine sediments (silty-muddy; 0.06–31 µm grain size) generally occur in areas of weak flow and allow for greater deposition of organic material, whereas coarser sediments (sand; 63–500 µm grain size) are present in fast flowing waters with frequent sediment resuspension (Ramey and Bodnar 2008; Dyer 1986). Fine sediments consequently tend to be richer in organic content resulting from both decreased water velocity, as well as their enhanced ability to bind organic carbon (Snelgrove & Butman 1994). Feeding mode of macrofauna is also influenced by hydrodynamics. Generally, deposit feeders have greater abundances in silty-muddy sediments whereas suspension feeders are associated with coarse sands (Snelgrove & Butman 1994).

Large scale, oceanographic conditions (kms) such as temperature, salinity, surface productivity, and circulation are also important factors influencing coastal benthic communities (e.g., Alexander et al. 2018; Lima & Whether 2012; Olafasson et al. 1994; Morrisey et al. 1992; Barry & Dayton 1991). A recent study in the Northwest Atlantic examining warming based on sea surface temperature (SST) anomalies, projected an increase of 0.4–2.2 °C over the next 50 years (Han et al. 2015). Spatio-temporal SST gradients are important in influencing wind patterns, storm development, and wave activity through ocean-atmosphere interactions (Reguero et al. 2019; Bengtsson et al. 2006). Attributed to rising SST (C2ES 2020), the North Atlantic Basin has seen an increase in the intensity of tropical storms including hurricanes (Méndez-Tejeda & Hernández-Ayla 2023). From 1975–2006, 31 tropical storms passed within 278 km of our study site in Placentia Bay, NL (LGL 2007), and more recently the bay experienced two hurricanes (i.e., Igor in 2010 and Leslie in 2012) with Igor being the most intense hurricane in NL to date (Ma et al. 2017). Moreover, wave activity in terms of wave height has also increased at high latitudes in recent decades (Reguero et al. 2019). Physical disturbance to the benthic habitat by storms and wave action have the potential to influence seafloor substates (Herkul et al.

2016) through erosion and deposition of sediments. Disturbance from storms can also affect circulation patterns (Ma et al. 2017), salinity, and bottom flow.

Using historical and contemporary benthic macrofaunal composition and abundance data (1998 vs. 2019/2020 respectively) taken 21 years apart, the main objective of the present study was to investigate potential changes in community and functional structure in Placentia Bay NL and examine the influence of environmental drivers on observed patterns. It was hypothesized that historical and contemporary communities in Placentia Bay would be different and community patterns would be related to the sedimentary habitat, as it can be influenced by larger-scale oceanographic features. It was expected that functional traits of macrofauna would be related to community structure such that they exhibit similar spatio-temporal patterns of variability. It was also hypothesized that there would not be a loss of functional traits between historical and contemporary communities. Therefore, it was predicted that while species/taxon composition may change, functional roles should remain fulfilled (i.e., no loss of modalities) allowing for any loss of species/taxa to be buffered against (species redundancy hypothesis: Walker 1992).

## Methods

### *Study area and sampling design*

Placentia Bay is a large embayment on the southeast coast of Newfoundland (47.1030 °N, 54.1859 °W) (Fig. 1.1). The bay is ~130 km long and 100 km wide at its southerly directed mouth that is open to the Newfoundland shelf. The inner part of the bay contains three channels divided longitudinally by several islands (Ramey & Snelgrove 2003). Bottom salinity in the bay ranges from 32.2–33.0 (Ramey & Snelgrove 2003; Ramey 2001). Currents in are generally influenced by local factors such as wind, water density, and seafloor topography (Ma et al. 2012; Bradbury et al. 2000). In the spring and fall, prevailing winds are from southwest and west, whereas in the winter they are northwest to west; this shift in prevailing wind direction can influence vertical mixing which in turn affects the distribution of larvae (Ma et al. 2012). In 1998, Ramey and Snelgrove (2003) sampled ten sites distributed throughout different regions of the bay and on the shelf to examine water column and sedimentary variables influencing soft-sediment macrofaunal communities. In 2019 and 2020 eight sites in the bay were sampled as part of the present study (Fig. 1.1).

### *Sample Collection*

Historical samples examined in the present study were collected in 1998 (June and July), at eight sites using a random, nested design at each site (Ramey and Snelgrove 2003; Ramey 2001). Samples were obtained with a rectangular single-spade box corer containing six subcores. A total of six box cores were collected at each site in the bay (total *n*=41 for biological data, *n*=22 environmental data), with fewer samples in the outer bay and shelf (Table 1.1). For each box core, the four subcores (10 cm × 10 cm wide to a depth of 10 cm; 100 cm$^2$) were used to examine the macrofaunal community and one was used for sediment analyses (e.g., carbon, hydrogen, and nitrogen [CHN] and grain size; top 1 cm of sediment) (Ramey and Snelgrove 2003). The four macrofaunal subcores were pooled for community composition analysis since they were not considered independent given that they were not completely separated from each other (Ramey and Snelgrove 2003).

Contemporary samples (i.e., 13–17th September, 2019 and 13–17th October, 2020) were collected using a 0.1 m$^2$ Van Veen grab (Dr. Neves, Ecological Sciences Section, Department of

Fisheries and Oceans, Canada, NL). from eight sites sampled in 1998. Three to six independent grab samples were haphazardly collected at each site, depending on year (total *n*=36), (Table 1.1). Grab samples were subsampled (50 ml of sediment removed) for determination of sedimentary variables including grain size and total organic matter (top 5 and 1 cm respectively).

### *Sample processing*

All macrofaunal samples were fixed in 4% buffered formalin and then transferred to 70% denatured ethanol with Rose Bengal and sieved over a 500 µm mesh screen prior to sorting macrofauna from the sediments using a stereomicroscope (all years). Macrofauna were counted and identified to the lowest practical taxonomic level, usually species. Taxonomic identifications were completed by P. Ramey-Balci (confirmed by P. Pocklington; Ramey and Snelgrove 2003) for the historical samples and L. Treau De Coeli (Université Laval, Quebec) for contemporary samples.

For grain size analysis, sediments were treated with water/peroxide solution to remove organic material. They were then dried and weighed followed by resuspended and disaggregated prior to sieving to separate the sediment grains into coarse (sand: >63µm), fine (silt: <63–3.9 µm), and clay (<3.9 µm) sized fractions (Wentworth scale), (see Danovaro et al. 2010 and adapted by Bureau Veritas: contemporary; Ramey and Snelgrove 2003: historical). The percent sand, silt, and clay in each sample was calculated using dry weights. This analysis was also conducted for additional sediment samples from 2021(unpublished).

Sediments for CHN analysis in the historical samples were freeze dried at -60°C and analyzed in a Perkin Elmer Model 2400 to determine total organic carbon (TOC) (Ramey & Snelgrove 2001; Ramey & Snelgrove 2003). Only total organic matter (TOM) was determined for the contemporary samples where 50–100 mg of dried sediment was weighed and placed in a muffle furnace for 4 hours at 450°C. The TOM was the difference between dry and calcinated sediments which was normalized and expressed as a percentage (Danovaro et al. 2010). To determine total organic matter based on TOC content, historical values of TOC were multiplied by 1.724 as this is a commonly used conversion factor based on the assumption that most soils consist of ~58% organic carbon (Pribyl 2010; Nelson & Sommers 1996).

### *Data Analysis*

#### *Community Structure*

Only infaunal, soft-sediment macrofauna (i.e., organisms >500–1000 µm) were included for community analyses following Gallagher and Grassle (1997) and Taghon et al. (2017a). Hard substrate epifaunal encrusting species (e.g., bryozoans, mussels, and hydrozoans) and meiofauna (i.e., organisms <500–40 µm; e.g., ostracods, copepods, and nematodes) were excluded from the analyses. Additionally, highly mobile species (e.g., mysids and decapods) not reliably sampled using a Van Veen grab or box corer (Taghon et al. 2017a) were omitted. Species/taxa with <10 individuals across all years and samples (rare species) were also removed (Taghon et al. 2017a). Results were examined with rare species included in the dataset and the same trends were observed (i.e., multivariate analysis and community metrics), thus a conservative approach was taken with the removal of rare species. The taxonomic status of species was checked against the continuously updated list in the World Register of Marine Species (http://www.marinespecies.org/index.php). To ensure consistent species/taxon identifications between the historical and contemporary datasets, reference specimens available for the historical data (i.e., 48 commonly abundant taxa: Dr. Ramey-Balci personal collection) were compared with contemporary reference specimens as well as identification notes and images contained in Ramey et al. (2001). This ensured that species/taxon differences between the two data sets were not a result of discrepancies in identification. For example, examination of specimens previously identified as *Cossura longocirrata* (see Ramey and Snelgrove 2003; Ramey 2001) were determined to belong to a morphologically similar species *Cossura pygodactylata herein*.  On occasion, when identifications could not be confirmed and level of taxonomic identification varied between the two datasets, a conservative approach was taken (i.e., that favored grouping rather than splitting taxa) where taxon/species identifications were grouped to a common higher level of classification. For example, the contemporary data included three species in the Family Lumbrineridae (i.e., *Lumbrineris latreilli, Lumbrineris* sp., and *Scoletoma laurentiana*), whereas for the historical data identification was only to Family with more than one species recognized (i.e., Lumbrineridae spp.), therefore in both data sets these were retained/lumped as Lumbrineridae spp. for data analyses. Similarly, *Thyasira gouldii* (in contemporary) and *Thyasir*a sp. (in historical) were as retained as *Thyasir*a sp. since a reference specimen was not available to identify it to species. The sample size/amount of sediment collected by the different gear types varied for the historical versus the contemporary samples (i.e., historical: box corer, 0.04 $m^2$; contemporary: Van Veen grab, 0.1 $m^2$) and therefore species densities for the historical data were re-scaled from 0.04 $m^2$ to 0.1 $m^2$ for data analyses.

### *Functional Traits*

Eight traits were used to describe the functional roles of macrofauna in the bay. The functional traits used represent both morphological (i.e., body size, reproduction), and behavioral roles (i.e., feeding mode, adult movement, bioturbation, living habitat, tolerance, and larval development) to describe their characteristics and functional implications within the community. The eight traits were further broken down into 36 modalities (Table. 1.2). Species/taxa for a given modality were scored using the "Fuzzy coding" procedure (Oug et al. 2012; Bremner et al. 2006; Chevenet et al. 1994). For this the modalities were scored using the following scale, where 0=no affinity (modality not employed), 1=low importance, 2=high importance, and 3=dominant (Oug et al. 2012). For example, when looking at a trait such as feeding mode, species can have more than one modality (e.g., deposit and surface feeding), in this case "1" would be assigned to the less commonly employed modality and "2" to the more commonly employed modality (Oug et al. 2012). If the species/taxon only used one feeding mode (e.g., deposit feeding) a value of 3 was assigned. Modality scores for each trait were assigned based on available databases including the Arctic Trait Base (https://arctictraits.univie.ac.at/), which contained the large majority of the species/taxa in Placentia Bay consistent with its geographic location, as well as The World Register of Marine Species (WoRMS), and Polytrait database (http://polytraits.lifewatchgreece.eu/). This produced a species by trait/modality matrix whereby the modality scores for the species/taxa were multiplied by their abundances in each sample (Oug et al. 2012; Bremner et al. 2006). Following this, the modality scores were summed across all species/taxa present in each sample producing a station by trait/modality matrix for analysis (Oug et al. 2012; Bremner et al. 2006).

### *Univariate statistics*

Species accumulation plots were generated based on richness observed for all historical and contemporary samples to assess if sampling effort was adequate in both studies; this was examined for each major taxonomic group (i.e., amphipods, bivalves, gastropods, and polychaetes) using PRIMER (v7 +PERMANOVA). Pie charts were used to display the proportion of major taxonomic groups including Amphipoda, Bivalvia, Gastropoda, Polychaeta and “other” (proportion of remaining taxa [e.g., Nemerteans, Chaetognatha, Cumaceans]) making up the communities in the historical, compared to contemporary years (i.e., percent of total abundance). The percentage of species/taxa (out of the total species richness for the bay) only sampled in the historical, contemporary, or shared between them were plotted using stacked bar plots. Percent of species/taxa that are tolerant (i.e., defined as tolerant to organic enrichment, pollution, temperature, or salinity changes) or intolerant (i.e., sensitive to organic enrichment, pollution, temperature, or salinity changes) were also compared between years based on values available in the Arctic Trait Base. Community metrics including density (no. of ind. 0.1 $m^{-2}$), richness (e.g., number of species/taxa), evenness (Pielou’s Evenness Index, J'), and diversity (Shannon-Wiener, H´ loge), were calculated in PRIMER v7 and compared between years (historical vs. contemporary) using boxplots. To test for differences in community metrics (and also for sediment variables) between years a student t-test was used when the data were normal and had equal variance or could be normalized through transformation (i.e., log or square root). Normality of the distribution of the datasets was tested using Shapiro–Wilk test and equal variance was examined with Levene’s test of homogeneity. All univariate statistical analyses were performed in JASP (V. 0.16, JASP Team 2022).  For variables where transformations did not normalize distribution and/or variance (e.g., richness, diversity, and evenness [see Appendix Table A1.1—3]), t-tests were used as they are robust against non-normality and the non-parametric (Mann-Whitney U test) showed the same result.

*Multivariate statistics*

Variation in macrofaunal community structure and environmental variables between the historical and contemporary years were examined by conducting separate Principal Coordinates Ordination (PCO) analysis in PRIMER (v7 +PERMANOVA) (Anderson 2017; Clarke & Gorley 2015; Anderson, 2001). For the biological data the PCO was conducted on a Bray-Curtis dissimilarity matrix generated from species/taxon composition and abundance. Prior to this analysis, abundances were fourth root transformed to downweigh the relative influence of the more abundant species/taxa, which would otherwise tend to dominate the dissimilarity matrix (Clarke & Gorley 2015). Bray-Curtis dissimilarity shows the proportion of differences in abundance between the samples where 0 indicates samples are similar and 1 indicates samples are completely dissimilar (Legendre & Legendre 2012). Species/taxa with a correlation coefficient of ≥0.7 (Pearson correlation) with the ordination pattern were overlaid as vectors on the PCO plot, where arrows point in the direction of maximal variation in species abundances, and their length is proportional to their maximal rate of change (Ramette 2007). A Permutational Multivariate Analysis of Variance (PERMANOVA; 9999 permutations) tested the null hypothesis that there is no difference in the centroids (equivalent to mean in univariate analysis) in communities between the historical vs. contemporary years.  Similarity percentage (SIMPER, Clarke 1993) was used to identify the species/taxa contributing to 50% of the dissimilarity between years. These analyses were also conducted on a Sorensen resemblance matrix to compare the presence/absence of species/taxa between years.

To examine differences among samples based on environmental variables (i.e., grain size, total organic matter, and depth) all variables were normalized to Z-scores. This was done to weigh variables equally (i.e., places them on the same scale) which reduces the degree to which any one variable with a larger mean value sways the result. A PCO analysis was run on this matrix based on Euclidean Distance. A PERMANOVA (9999 permutations) tested the null hypothesis that there is no difference in the centroids for environmental conditions between the historical vs. contemporary years.

To examine the relationship between the environmental variables and macrofaunal data, the “Relate” function was used (PRIMER v7) based on ranks, specifically Spearman rank correlation (ρ) (999 permutations) (Clarke et al. 2014; Clarke and Gorley 2015). This analysis tested the null hypothesis that there is no correlation between the two matrices (i.e., thus comparing observed patterns in the environmental vs. biological data). To determine how much of the variation in the biological data can be explained by the environmental data a distance-based linear model (DistLM) was performed, using the environmental data as a predictor and biological data/ resemblance measures as the response. Predictor variables were added to the model via stepwise procedure and the AICc selection criterion was used to evaluate models. An associated distance-based redundancy analysis (dbRDA) plot was then generated based on the DistLM (Legendre & Anderson, 1999) to illustrate the relationships between the environmental predictors best explaining the observed variation.

To compare functional traits between the historical and contemporary years, circular bar plots were used to show the proportion of modalities for each trait such that all modalities for a particular trait add to 100% (“corrplot” package in R Studio; Wei & Simko, 2017). The same univariate and multivariate analyses conducted on species/taxa abundance data were repeated for functional traits with the exception that the PCO was conducted on the Canberra metric. This measure was used instead of Bray-Curtis to overcome issues of arch effects which were seen in exploratory analysis via PCO and metric multidimensional scaling (MDS) (Podani & Miklos, 2002).

## Results

### *Community structure*

In all years, a total of 37,096 individuals belonging to 136 species/taxa were collected. Once rare species/taxa (<10 individuals across all samples) were removed, the dataset contained 36,909 individuals and 77 species/taxa for analysis. The historical samples included 30,663 individuals (64 species/taxa), whereas the contemporary data contained 6,246 individuals (61 species/taxa). Although more samples were collected in the historical (*n*=41) compared to the contemporary years (*n*=36) the total area of the seafloor sampled was greater for contemporary (1.64 $m^2$ vs. 3.6 $m^2$ respectively) (Table 1.1). Overall, the major taxonomic groups including the Amphipoda, Bivalvia, Gastropoda, and Polychaeta made up 90% of the total abundance and >80% of the species/taxa sampled in the bay (historical and contemporary data combined). Species/taxa accumulation curves for the historical and contemporary samples leveled off at ~36 and ~29 samples respectively (Fig. 1.2), and the curves for the four major taxonomic groups (with the exception of amphipods) also reached an asymptote (Fig. 1.3A–D). Species/taxon richness for bivalves and gastropods per sample was greater in the contemporary compared to the historical communities whereas, species/taxon richness from Amphipods and Polychaetes was greater in the historical (Fig. 1.3A–D).

Principal Coordinates Ordination (PCO) analysis of species/taxon composition and abundance explained a total of 44.2% of the variability in the data. Historical and contemporary samples formed two general groups (Fig. 1.4A). A total of 18 species/taxa including 12 polychaetes and 3 bivalves, contributed ~50% of the dissimilarity between these two time points, with the highest contribution from the polychaete *C. pygodactylata* (6.74%) (Table 1.3; also see Fig. 1.4A). Note that *C. pygodactylata* was previously identified as *Cossura longocirrata* (see

Ramey and Snelgrove 2003; Ramey 2001). Variation in macrofaunal community structure between the historical vs. contemporary samples indicated they were significantly different (Permutational multivariate ANOVA, P= 0.0001, pseudo-$F_{1,75}$= 15.4) (Fig. 1.4A). Results were consistent when the dominant species, *C. pygodactylata* was removed from this analysis (Permutational multivariate ANOVA, P= 0.0001, pseudo-$F_{1,75}$=13.4). Similarly, PCO analysis of species/taxon composition (i.e., presence/absence; 45.6% total variability explained) formed two groups and significantly differed between the historical vs. contemporary samples (p, Permanova=0.0001, pseudo-$F_{1,75}$=13.7) (Fig. 1.4B).

Comparison of the major taxonomic groups between the historical and contemporary samples showed the polychaetes made up the greatest proportion of macrofauna collected in both years, however their proportion was much higher in the historical samples (91 vs. 58% respectively) (Fig. 1.5A–B). In contrast, the proportion of amphipods, bivalves, and gastropods were relatively higher in contemporary samples (Fig. 1.5A–B). Moreover, examination of the species making up these major groups found 41 species/taxa (63%) shared between the historical and contemporary years, whereas 14 species/taxa (22%) were only present in the historical samples and 10 species/taxa (15%) were unique to the contemporary samples (Fig. 1.6, Table 1.4). Mean density of individuals making up these major groups were not significantly different ($\alpha$>0.05) for the historical vs. contemporary samples with the exception of polychaetes which were significantly higher ($t_{75}$=-6.694, P=<0.001) in historical samples ($\bar{X}$=680 ±80 ind. $m^{-2}$, range=40–2103 ind. $m^{-2}$) compared to contemporary ($\bar{X}$=100 ±17 ind. $m^{-2}$, *n*=36, range=5–329 ind. $m^{-2}$) (Fig. 1.7). All community metrics examined, with the exception of species richness, differed significantly between years (Fig. 1.8A–D). Species/taxon evenness (J’) and Shannon diversity (H’) were both significantly lower ($t_{75}$=7.072, p=<0.001 and $t_{75}$=6.38, p=<0.001

respectively) in the historical (evenness: 0.56 ±0.03, *n*=41, range=0.25–0.91; diversity: $\bar{X}$=1.7 ±0.091, *n*= 41, range=0.69–2.63) than in the contemporary samples ($\bar{X}$=0.80 ±0.011, *n*=36, range= 0.62–0.91; $\bar{X}$=2.40 ±0.049, *n*=36, range=1.45–2.95). Macrofaunal density was significantly higher ($t_{75}$=-7.691, p<0.001) in the historical ($\bar{X}$=748 ±85 no. ind. $m^{-2}$, *n*=41, range=58–2145 ind. $m^{-2}$) compared to the contemporary samples ($\bar{X}$=174 ±20 no. ind. $m^{-2}$, *n*=36, range=16–512 ind. $m^{-2}$). With the most abundant species removed (*Cossura pygodactylata*) diversity and evenness did not significantly differ between communities.

### *Environmental data*

Total variability explained by the PCO of the environmental variables was 78.7% (Fig. 1.9). Historical and contemporary samples formed two general groups primarily influenced by grain size (Fig. 1.9). Examination of sedimentary environmental variables indicated that the proportion of silt was significantly higher ($t_{55}$=-8.6, P=<0.001) in the historical ($\bar{X}$=58% ±2.34, *n*=22, range= 29–75%) compared to the contemporary samples ($\bar{X}$=37 ±1.25%, *n*=35, range= 24–54%), whereas the proportion of sand was lower significantly ($t_{55}$=9.0, P=<0.001) in the historical ($\bar{X}$=6 ±1.44%, *n*=22, range= 0–22%) vs. the contemporary samples ($\bar{X}$=27 ± 1.53%, *n*=35, range= 11–43%) (Fig. 1.10A). Additionally, sediments have become coarser between all years (Fig. 1.10B). Total organic matter was also significantly lower ($t_{72}$=-2.8, P=0.006) in the historical samples ($\bar{X}$=10.4 ± 0.76%, *n*=38, range= 2.4–15.0%) compared to contemporary ($\bar{X}$=13.5 ± 0.98%, *n*=36, range=3.6–32.7%) (Fig. 1.11).

### *Community structure and environmental drivers*

Patterns observed in the biological resemblance matrix significantly matched those found in the environmental resemblance matrix based on spearman rank correlation (based on RELATE analysis: $\rho$=0.381, P=0.001). Furthermore, using the environmental variables as the predictor of the biological data the combination of silt, sand, and total organic matter were significant in predicting patterns in the biological data (Table 1.5; DistLM). The first two axes of the dbRDA (based on the DistLM) accounted for 88.8% of the fitted variability and corresponded to 22.0% of the total variability of the biological resemblance matrix (Table 1.5). Likewise, sand, silt, and organic matter were significant predictors for presence/absence data and corresponded to 21% of the total variability in the biological resemblance matrix (Table 1.5). A linear regression model indicated a statistically significant relationship between polychaete density and TOM for both historical and contemporary communities ($R^2$=0.26, $F_{1,36}$=12.9, P=<0.001 and $R^2$=0.14, $F_{1,33}$=5.4, P=0.027 respectively) (Fig. 1.12A). The same relationship was found for the density of major taxonomic groups (i.e., amphipods, gastropods, and bivalves]) and TOM (historical= $R^2$=0.15, $F_{1,36}$=6.3, P=0.017 and contemporary= $R^2$=0.34, $F_{1,33}$=17.1, P=<0.001) (Fig. 1.12B).

### *Functional Traits*

Functional composition and relative abundance of the historical vs. contemporary samples (based on the Canberra metric) (Fig. 1.13A) were significantly different (PERMANOVA, P=0.0001, pseudo-$F_{1,75}$=19.5). In comparison, differences in the composition of functional traits (i.e., presence/absence) between the two years was similar (PERMANOVA, P= 0.250, pseudo-$F_{1,75}$=1.6) (Fig. 1.13B). Ten modalities contributed to ~50% of dissimilarity between contemporary and historical samples where high tolerance, small body size, and the subsurface deposit feeding mode were responsible for the greatest contribution to dissimilarity. Furthermore, patterns observed in the functional trait resemblance matrix (Canberra) significantly matched those observed in the community (i.e., taxonomic) resemblance matrix (Bray-Curtis) based on spearman rank correlation (RELATE analysis, ρ= 0.554, P=0.001).

Overall, of the 8 traits considered, half changed in terms of their relative proportions between the historical and contemporary years for modalities relating to feeding, body size, tolerance, and larval development. For example, subsurface deposit feeders represented the dominant feeding mode in the historical samples (62.1%), whereas subsurface and surface deposit feeders were nearly evenly represented in the contemporary samples (26.7% and 29.1% respectively) (Fig. 1.14). Historical samples were also composed of smaller-sized species/taxa compared to (60.6%) the contemporary samples where small-medium body sizes made up a high proportion (39.7%) followed by small sized species/taxa (29.6%) (Fig. 1.14). Tolerance has also changed from being predominantly high (82.3%) in the historical samples to both medium and high in contemporary samples (36.7% and 46.6% respectively) (Fig. 1.14). However, in terms of composition, the percent of tolerant species/taxa was relatively low in the historical versus contemporary years (17.5 vs. 24.6% respectively) (Fig. 1.15).

Examining metrics for functional traits showed that density, evenness, and diversity were significantly different between contemporary and historical samples (Fig. 1.15). Functional richness did not significantly differ. Density was significantly higher (t=-7.8, P=<0.001) in historical samples compared to contemporary. Functional diversity (H´loge) and evenness (J’) were both significantly lower (t=6.1, P=<0.001 and t=6.4, P=<0.001 respectively) in the historical (H´: X̄=2.9 ±0.03, *n*=41, range=2.52–3.20; (J: X̄=0.82 ±0.009, *n*=41, range=0.71–0.91) than in the contemporary samples (H': $\bar{X}$=3.15 ±0.02, *n*=36, range= 2.78–3.29; J': $\bar{X}$=0.89 ±0.004, *n*=36, range=0.82–0.92).

*Functional traits and environmental drivers*

Patterns observed in the functional trait resemblance matrix (Canberra metric) were weakly correlated to those found in the environment resemblance matrix (RELATE analysis, ρ= 0.246, P=0.001). The DistLM indicated that sand and silt were significant in predicting patterns in the functional resemblance data (Table 1.5). Patterns observed in the functional trait resemblance matrix (Sorensen) did not significantly match those observed in the environmental resemblance matrix (Euclidean) based on spearman rank correlation from the RELATE analysis (ρ= -0.01, P=0.54). The environmental variables examined here were not found to be significant predictors of functional trait presence/absence.

## Discussion

Although temporal samples taken at specific time points such as decades apart have inherent limitations that make short-term vs. longer-term community variability difficult to distinguish (Herder et al. 2022; Renaud et al. 2007) they are necessary in understanding and predicting current and future community changes (e.g., Harris et al. 2023; Ramon et al. 2023; Taghon et al. 2017a; Van Geest et al. 2015; May 1984). Therefore, it is pertinent to consider differences in sampling methods and protocols when making comparisons and interpreting results (Taghon et al. 2017a). In our case, yearly sampling effort and sieve size were consistent among years. Furthermore, taxonomic discrepancies were clarified between the two datasets taken 21 years apart using existing reference collections. While the grab used to collect the contemporary samples was larger than the box core used for the historical sampling, density of macrofauna was lower in the contemporary samples (even before the scaling of historical samples to facilitate comparisons); which is the opposite trend than one might expect based on sample size alone.

Species accumulation curves showed that major taxonomic groups were adequately sampled (apart from amphipods which are commonly not well sampled due to their mobility). Thus, an increase in species richness would not be expected with additional sampling. We did however have a discrepancy with respect to sampling season which can result in marked changes in species abundances, especially in temperate regions (Taghon et al. 2017a; Van Geest et al. 2015). Other studies, however, have not found changes in macrofaunal abundances with season (e.g., North Atlantic: Weissberger et al. 2008; Valderhaug & Gray 1984). In the present study, season likely contributes to some of the observed community variation between years, as relatively lower densities may be expected in the fall (Reiss & Kröncke 2005) (sampled in contemporary) prior to the summer reproductive period (sampled in historical). However, lower density in the fall 2019/2020 was taxon specific being only observed for the polychaetes and considerable changes in community composition (i.e., presence/absence of species/taxa) were also observed (even after the removal of rare species); which would be less likely to vary substantially with season. Moreover, significant differences in the community and functional structure in the bay were related to observed differences in the sedimentary habitat, known to be important in structuring benthic communities. Most notably significant differences in grain size and organic matter content occurred between the two time points such that in contemporary years, grain size is coarser and organic matter content is higher at sampling sites throughout the bay.

Overall, benthic macrofaunal communities in Placentia Bay have undergone significant changes in the presence/absence of species/taxa and their relative abundance, with 14 species/taxa found only in historical samples and 11 unique to contemporary times. Furthermore, 63% of species/taxa making up major taxonomic groups were shared, in contrast ~86% of species/taxa were shared between 2019 and 2020 (see Chapter 2). Multivariate analyses indicated that community structure significantly differed between the contemporary and historical time points. The three species contributing most to this dissimilarity were the polychaetes, *Cossura pygodactylata*, Dorvilleidae spp., and *Gyptis bruneli* which showed large reductions (>50%) in their mean densities in the contemporary community relative to the historical one. *Cossura pygodactylata* (Family Cossuridae) was the most abundant species present at both time points, and this family has been observed to vary seasonally. Weissberger et al. (2008), examining macrofaunal communities in the Gulf of Maine found the density of cossurid polychaetes were lowest in the fall but remained a dominant taxon despite this. Similarly in the present study, even with the lower density of *C. pygodactylata* in the contemporary community, it remained the most dominant species in the bay. Although polychaetes were the dominant group for both time points, the relative proportion and density of bivalves, amphipods, and gastropods were generally higher in contemporary relative to the historical community, although higher densities of these taxa were not found to be significantly different between years. Bivalves were responsible for some of the observed dissimilarity between the two time points with *Thyasira* sp., *N. pernula,* and *M. calcarea* having greater mean densities in the contemporary communities. Moreover, there was a significant decrease in macrofaunal density and an increase in evenness and diversity in contemporary relative to historical communities.

Placentia Bay, NL has also undergone significant changes in the sedimentary habitat in terms of grain size. This is in contrast to studies examining changes in the composition of sediment grain size overtime that have indicated that it tends to remain relatively stable (Herder et al. 2021; Taghon et al. 2017a). For example, Taghon et al. (2017a) found that sediment properties in Barnegat Bay, New Jersey, showed few changes in 45 years such that silt/clay content was similar in 1965–1969, 2000–2006, and 2012–2014. Likewise, a previous long-term study in Frobisher Bay, Nunavut, Canada, found that sediment grain size composition did not significantly change over a 50-year period (i.e., between 1967–1976 and 2016) (Herder et al. 2021). In Placentia Bay, contemporary sediments were ~4.5x coarser as compared to the historical time point, which may be a result of possible disturbance events in the bay such as storms. Furthermore, sediments have become coarser in each of the contemporary years (i.e., 2019–2021), and among nearly all sites. September and October are peak months for tropical storm activity in Newfoundland and Labrador (Wood 2021). Newfoundland is subject to numerous extratropical cyclones (Xu et al. 2019) which are a main cause of storm surge events (Xu et al. 2019; Orton et al. 2016). Two hurricanes (i.e., hurricane Dorian in 2019 and hurricane Teddy in 2020) occurred days prior to sampling in contemporary years and affected Atlantic Canada through storm surges, strong winds, waves, and heavy precipitation (Blake 2021; Jones 2019). Disturbance by waves and currents during these events can cause sediment transport (i.e., deposition), as well as removal (i.e., erosion) in coastal regions. While Taghon et al. (2017b) found no change in sediment median particle size after hurricane Sandy, it is possible that sediments in Barnegat Bay were not affected because the bay is protected by two barrier islands that shelter it from the Atlantic Ocean waters. In contrast, Placentia Bay has a large mouth (100 km wide), exposing it to the adjacent shelf environment. Additional sampling in the bay is needed to determine whether the bay has undergone sustained changes in sediment grain size. Placement of current meters at some of the established study sites would also help to confirm whether bottom flows during storm events is a plausible mechanism for the observed changes in sediment type.

In addition to the grain size changes, there has been an increase in sedimentary TOM from historical to contemporary times (i.e., 10.4% ±0.52 vs. 13.5% ±0.98, respectively) which may be a reflection of increased SST (Han et al. 2015) as it can influence the amount and timing of food supply (e.g., primary production) to the benthos. Freshwater input during storm events, and/or from aquaculture in the bay, may be an additional source of organic enrichment (Fuch et al. 2020; Bêche et al. 2006; Haya et al. 2001). Supply of organic matter can vary year-to-year, influenced by both freshwater inputs and climate/weather (Diaz & Rosenberg 1995). Placentia Bay experiences heavy rainfall during storm surges (e.g., ~200mm hurricane Teddy, September 22, 2020), and has freshwater inputs including Swift Current located at the Head of the bay, where TOM was the highest in all years (see Chapter 2 and Ramey et al. 2003).

Functional traits followed trends observed in community structure. Historical traits were influenced by modalities generally associated with polychaetes, while contemporary traits showed changes to their proportions. The three modalities contributing most to dissimilarities based on SIMPER analysis were high tolerance, subsurface deposit feeding mode, and small-medium body size. Historical macrofaunal communities in the bay were dominated by highly tolerant (82.3%) subsurface deposit feeders (62.1%) having small body sizes, whereas contemporary communities had a higher proportion of medium tolerant species/taxa (7.2 vs. 36.7%) and nearly equal proportions of subsurface and surface deposit feeders (26.7 and 29.1% respectively) with small-medium body sizes. Although the proportion of highly tolerant modalities was lower in the contemporary communities where TOM was greater, the proportion of tolerant species/taxa present have increased in contemporary communities (i.e., 17.5 vs. 24.6%), which were mainly polychaetes. The changes in functional trait/modalities contributing to observed dissimilarity, were also influenced by the increased relative proportion of bivalves, and other groups, compared to the historical time. Gusmao et al. (2022) examining functional traits along a sediment gradient found that coarser sediments were dominated by surface modifiers, suspension feeding organisms, with larger bodies, whereas fine sediments associated with higher levels of OM, were dominated by deposit feeders with small bodies. Interestingly in the present study, levels of TOM were found to be elevated in coarser sediments. As most commonly TOM is associated with finer sediment grain sizes (Snelgrove & Butman 1994), this decoupling may also be suggestive of possible effects of disturbance (e.g., storms and organic enrichment) in the bay as described above.

All three environmental variables (sand, silt, TOM) were important predictors of community composition (i.e., presence/absence) and abundance. However, environmental variables were weakly correlated to functional traits and only sediment grain size (silt and sand) was found to be an important predictor. Macrofaunal density had a negative correlation with increasing TOM in both time points. Though organic matter is generally positively related to density (Nestlerode et al. 2020; Davies & Payne 1984), the findings of this study were consistent with previous work conducted in Placentia Bay by Ramey & Snelgrove (2003) which found a negative relationship between organic carbon and macrofaunal density. The relationship was suggested to be driven primarily by low densities at three sites (i.e., Head, Central, and West-1) which had the highest percent of TOC. This trend was observed in contemporary communities with respect to percent of TOM. Offshore sites for both time points had low TOM and greatest mean densities. Ramey & Snelgrove (2003) attributed the low densities to sulphide production in the sediments as well as poor food quality. Similarly, darkened sediments (i.e., hydrogen sulphide accumulation) were seen during contemporary sample collection in both 2019 and 2020. Organic loading can decrease macrofaunal abundance (Snelgrove & Butman 1994; Pearson & Rosenberg 1978), and reductions in the density of tolerant species/taxa observed in contemporary communities in Placentia Bay might suggest an OM threshold has been reached. Studies examining sediment organic carbon concentration on benthic communities in the Northern hemisphere noted a range from 10–35 mg $g^{-1}$ (which is equal to, TOM: 1.7–6.0%; TOC: 1.0–3.5%) the general threshold where benthic communities would be reduced (Walker et al. 2022; Hyland et al. 2005).

## Conclusion

Despite the confound of season, communities and habitats in Placentia Bay have undergone significant changes since 1998. Overall, the initial hypotheses were consistent with the results. Community and functional structure in Placentia Bay did significantly differ between historical and contemporary times and were influenced by the sedimentary habitat. Moreover, trait modalities were maintained between the time points (i.e., no loss of modalities). These findings suggest that communities in Placentia Bay may exhibit greater temporal stability or resilience with respect to functional traits despite the current levels of TOM. A limitation of the present study is the lack of continuous sampling and discrepancy of season between the two time points. Examining seasonal variability should be prioritized to better quantify the extent of year-to-year variation (Holland et al. 1987; Holland 1985) especially for summer months (i.e., June–July). This would allow for a better understanding of shorter-term temporal patterns of macrofauna in relation to environmental variability. It is recommended TOC, nitrogen, and C/N ratios should be examined in future monitoring to compare with the historical values in Ramey and Snelgrove (2003). Such information can provide insight into how levels of OM may have changed over time, as well as food quality and the possible source(s) of OM. Changes in salinity and dissolved oxygen should also be examined as they play an important role in structuring benthic communities and can be affected by large inputs of rainfall (i.e., generally a result of storm surges and hurricanes) (Taghon et al. 2017b).

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## Tables and Figures

**Table 1.1.** Summary of sampling sites indicating latitude and longitude (decimal degrees) for historical and contemporary sampling sites (site/code), mean depth ±SE, and number of samples (no.). Total number of samples: historical=41, contemporary=36). Total sea floor sampled: 1998=1.64 m$^2$, 2019=1.80 m$^2$, 2020=1.80 m$^2$).

| **Site/code** | **Latitude (°N)** | **Longitude (°W)** | **Depth (m)** | **Historical (no.)** | **Contemporary no. (2019/2020)** |
|---|---|---|---|---|---|
| Head/H | 47.7543 | -54.2345 | 63±4.86 | 6 | 6 (3/3) |
| Central/C | 47.5803 | -54.1247 | 202±3.82 | 6 | 6 (3/3) |
| East1/E1 | 47.7475 | -54.0635 | 233±3.36 | 6 | 6 (3/3) |
| East2/E2 | 47.5633 | -54.0428 | 219.5±0.22 | 6 | 3 (0/3) |
| West1/W1 | 47.6448 | -54.2660 | 217±1.49 | 6 | 6 (3/3) |
| West2/W2 | 47.4482 | -54.3298 | 269±17.85 | 6 | 3 (0/3) |
| Outer1/O1 | 47.1800 | -54.3733 | 232±0.67 | 3 | 3 (3/0) |
| Shelf1/S1 | 47.7250 | -54.7967 | 231±1.40 | 2 | 3 (3/0) |

**Table 1.2.** Functional traits and modalities with corresponding abbreviations (abbrev.) and associated ecosystem function. Definitions based on those provided by the Arctic Trait Base (Degen & Faulwetter 2019).

| **Trait** | **Modality** | **Definition** | **Abbrev.** | **Function** |
|---|---|---|---|---|
| Adult Movement | Sessile | No movement | Ses | Foraging ability, predatory avoidance, dispersal abilities |
| | Burrower | Burrows in sediments | Bur | |
| | Crawler | Move along sediment via legs or other appendages | Cra | |
| | Swimmer | Moves above the sediment | Swi | |
| Body Size | Small | <10 mm | Sma | Energetic demand, ability to resistance predation |
| | Small-medium | 10—50 mm | Smamed | |
| | Medium | 50—100 mm | Med | |
| | Medium-large | 100—300 mm | Medlar | |
| | Large | >300 mm | Lar | |
| Larval Development | Pelagic/Planktrophic | Larvae grow in the water column | Pelpla | Fecundity, development, and dispersion insights |
| | Pelagic/lecithotrophic | Larvae with yolk sac, pelagic period is short | Pellec | |
| | Benthic/direct | Larvae have benthic development | Bendir | |
| Reproduction | Asexual | Budding & fission | Ase | Dispersal, continuous reproduction could allow for resilience, production |
| | Sexual: external | External fertilization (e.g., eggs & sperm released into water) | Sexext | |
| | Sexual: internal | Internal fertilization | Sexint | |
| | Sexual: brooding | Internal or external fertilization but eggs are brooded | Sexbro | |
| Bioturbation | Diffusive mixing | Random mixing of particles | Difmix | Impacts on biogeochemical cycles, food acquisition, resistance from disturbance |
| | Surface deposit | Deposition of particles at the surface of sediments (e.g., from defecation) | Surfdep | |
| | Conveyer belt (upward) | Movement of sediment/particles from within sediment to the surface | Conup | |

| | | | | |
|---|---|---|---|---|
| | Conveyer belt (reverse) | Movement of sediment/particles from the surface to deeper within the sediments | Conrev | |
| | None | No bioturbation | Non | |
| Feeding Mode | Subsurface deposit | Feeding from within the sediment | Subdep | Method of resource acquisition, living position, growth requirements |
| | Suspension | Feeding on particles suspended in the water | Sus | |
| | Surface deposit | Feeding on material from sediment surface | Surdep | |
| | Scavenger | Feeds on a variety of particles | Sca | |
| | Predator | Feeds on other organisms | Pre | |
| | Parasite | Uses a host to obtain food | Par | |
| Living Habitat | Free living | Can freely move within/on sediments | Freliv | Position in sediment, food acquisition, preferred environmental conditions |
| | Crevice dwelling | Tend to live in spaces between rocks | Credwe | |
| | Tube dwelling | Create tubes (e.g., sand and mucus) | Tubdwe | |
| | Burrowing | Burrow within sediments | Burr | |
| | Epi/endo/phytic | Live on/in other organisms | Epiendphy | |
| | Attached | Live attached to substrate | Att | |
| Tolerance | Low | Species are sensitive to changes in environment (e.g., organic enrichment, pollution, temperature, and salinity changes) | Low | Species/taxa tolerance to disturbance (e.g., temperature, organic enrichment). Low indicating very sensitive and high indicated tolerant to conditions |
| | Medium | Species are indifferent to changes in the environment | Medi | |
| | High | Species tolerant changes in the environment | Hig | |

**Table 1.3.** Summary results of SIMPER analysis for species/taxa contributing to ~50% of the dissimilarity between historical and contemporary communities including mean density ±SE (no ind. $m^{-2}$) and contribution to dissimilarity (%) for Placentia Bay, Newfoundland. Letters in parenthesis denote P=polychaete, B=Bivalve, O=Other.

| **Species/taxa** | **Historical Density (no. ind. $m^{-2}$)** | **Contemporary Density (no. ind.$m^{-2}$)** | **Dissimilarity (%)** |
|---|---|---|---|
| *Cossura pygodactylata* (P) | 466±64.9 | 29±6.9 | 6.74 |
| *Dorvilleidae* spp. (P) | 30±6.1 | 2±1.5 | 4.47 |
| *Gyptis bruneli* (P) | 16±1.7 | 2±0.7 | 3.51 |
| *Thyasira* sp. (B) | 11±2.2 | 13±2.7 | 2.76 |
| *Prionospio steenstrupi* (P) | 42±8.5 | 18±3.1 | 2.73 |
| *Chaetozone* sp. (P) | 13±2.0 | 5±1.3 | 2.68 |
| *Lumbrineridae* spp. (P) | 20±4.9 | 17±2.5 | 2.63 |
| *Capitellidae* spp. (P) | 13±3.0 | 2±0.5 | 2.63 |
| *Macoma calcarea* (B) | 12±2.4 | 14±2.8 | 2.59 |
| *Aricidea* sp. (P) | 8±1.5 | 1±0.4 | 2.57 |
| *Antalis entalis* (O) | 3±0.9 | 6±1.5 | 2.41 |
| *Cumacean* spp. (O) | 5±1.5 | 3±0.8 | 2.15 |

| | | | |
|---|---|---|---|
| *Nemertea* spp. (O) | 11±1.8 | 5±1.3 | 2.14 |
| *Cistenides hyperborea* (P) | 16±9.2 | 0.3±0.1 | 2.13 |
| *Microphnepthys neotana* (P) | 18±1.9 | 6±0.8 | 2.11 |
| *Nuculana pernula* (B) | 5±0.8 | 10±1.8 | 2.06 |
| *Terebellides stroemii* (P) | 3±0.7 | 0.6±0.2 | 2.04 |
| *Eteone longa* (P) | 3±0.6 | 0.7±0.2 | 1.95 |

**Table 1.4.** Species/taxa unique to historical and contemporary samples with major groups (Amphipoda, Bivalvia, Gastropoda, Polychaeta, other), and their total abundance (total no. ind.).

| **Community** | **Species/Taxa** | **Major Group** | **Total abundance** |
|---|---|---|---|
| Historical | Ampharetidae sp. | Polychaeta | 38 |
| | *Amharete finmarchica* | Polychaeta | 10 |
| | *Dysponetus pygmaeus* | Polychaeta | 30 |
| | *Maldane glebifex* | Polychaeta | 63 |
| | *Dipolydora caulleryi* | Polychaeta | 20 |
| | *Syllides* sp. | Polychaeta | 90 |
| | *Terebellida* sp. B | Polychaeta | 35 |
| | *Artacama proboscidea* | Polychaeta | 73 |
| | *Yoldia* sp. | Bivalvia | 100 |
| | *Turridae* sp. | Gastropoda | 45 |
| | *Protomedeia* sp. | Amphipoda | 30 |
| | *Melita* sp. | Amphipoda | 18 |
| | *Quasimelita formosa* | Amphipoda | 13 |
| | *Bathymedon* sp. | Amphipoda | 58 |

| | | | |
|---|---|---|---|
| Contemporary | *Axinopsida orbiculate* | Bivalvia | 71 |
| | *Curtitoma incisula* | Gastropoda | 39 |
| | *Dipolydora socialis* | Polychaeta | 12 |
| | *Eteone flava* | Polychaeta | 37 |
| | *Megamoera dentata* | Amphipoda | 10 |
| | *Megayoldia thraciaeformis* | Bivalvia | 59 |
| | *Orchomenella minute* | Amphipoda | 27 |
| | *Paratryphosites abyssi* | Amphipoda | 201 |
| | *Priapulus caudatus* | Other | 11 |
| | *Propebela rugulata* | Gastropoda | 23 |
| | *Trachyrhynchus erosus* | Gastropoda | 44 |

**Table 1.5.** Results of DistLM stepwise analysis for species/taxa and functional traits with environmental variables (sand, silt, TOM) as predictors. Results from best models shown below.

| **Data/Resemblance** | **Variable(s)** | **SS (trace)** | **AICc** | **$R^2$** | **Res.DF** | **Pseudo-F** | **P-value** |
|---|---|---|---|---|---|---|---|
| Species/taxa: Bray-Curtis | TOM + sand + silt | 3409.1 | 414.7 | 0.25 | 53 | 2.6 | 0.023 |
| Species/taxa: Sorensen | TOM + sand + silt | 2724.2 | 407.8 | 0.23 | 53 | 2.3 | 0.043 |
| Functional traits: Canberra | Sand + silt | 64.7 | 182.6 | 0.27 | 54 | 2.8 | 0.047 |

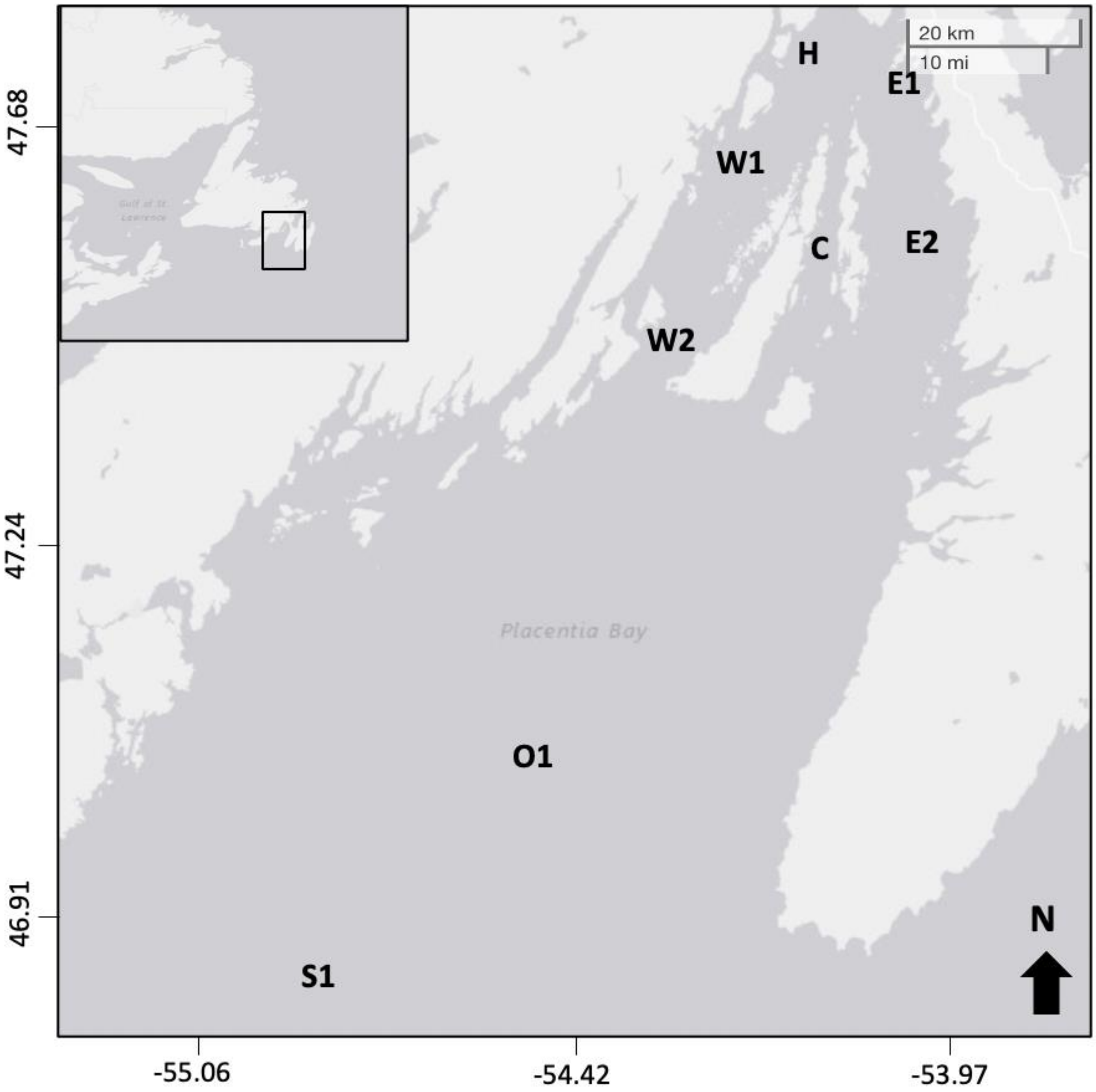

**Figure 1.1.** Sampling sites in Placentia Bay, Newfoundland including H1 at the head of the bay, E1 and E2 in the eastern channel, W1 and W2 (western channel), C1(central channel), O1 (outer bay), and S1 on the continental shelf (also see Table 1.1).

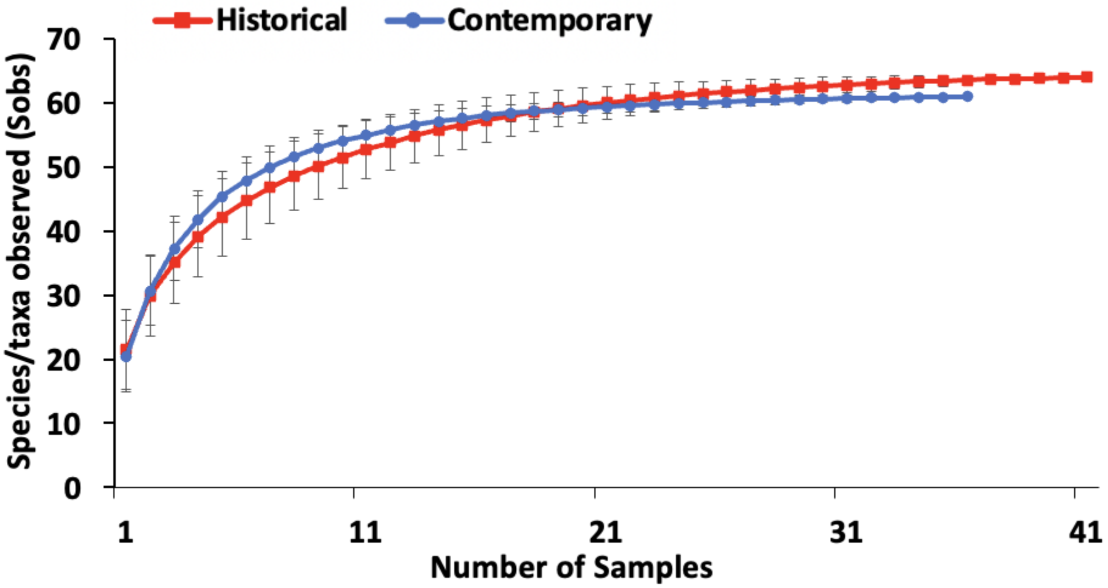

**Figure 1.2**. Species accumulation curve based on species/taxa observations ±SD for historical (1998) and contemporary (2019–2020) samples taken in Placentia Bay, Newfoundland.

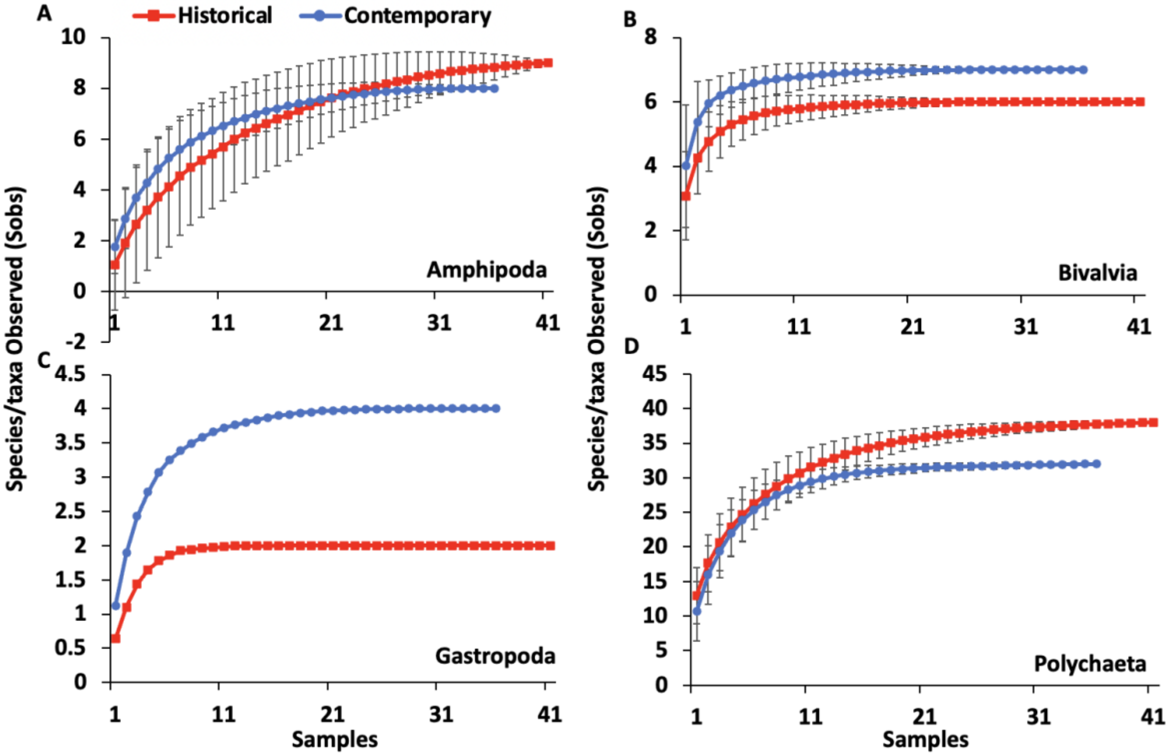

**Figure 1.3.** Species accumulation curve based on species/taxa observations ±SD of major groups (Amphipoda, Bivalvia, Gastropoda, Polychaeta) for historical and contemporary samples.

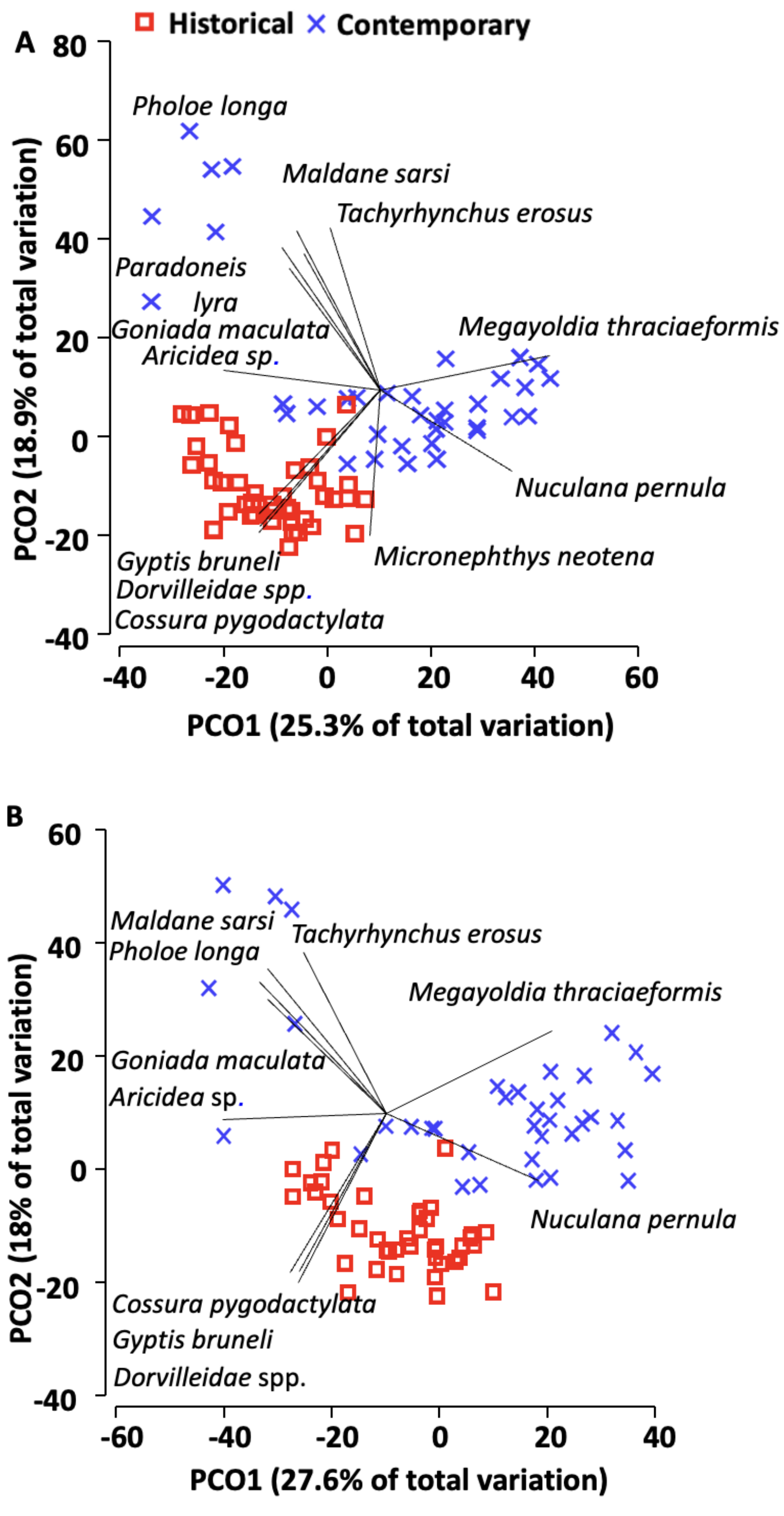

**Figure 1.4.** Principal Coordinates Ordination (PCO) of biological samples of species/taxa A) composition and abundance matrix based on Bray-Curtis similarity data) and B)

presence/absence matrix based on Sorensen resemblance for Placentia Bay, Newfoundland. Differences between the historical and contemporary communities for A and B were statistically significant (pseudo-$F_{1,75}$=15.4 and p, Permanova=0.0001, pseudo-$F_{1,75}$=13.7 and p, Permanova=0.0001 respectively). Species vectors based on Pearson correlation of ≥ 0.7.

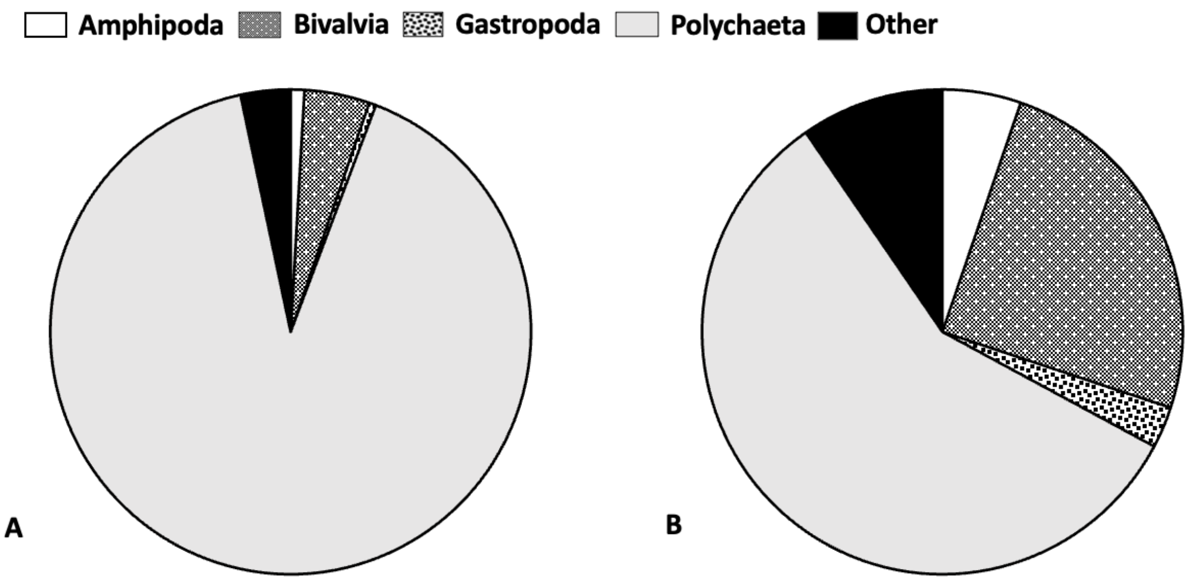

**Figure 1.5.** Pie charts showing the relative abundance (%) of major taxonomic groups (Amphipoda, Bivalvia, Gastropoda, Polychaeta) in Placentia Bay, Newfoundland in A) historical and B) contemporary communities. Other=remaining taxonomic groups present.

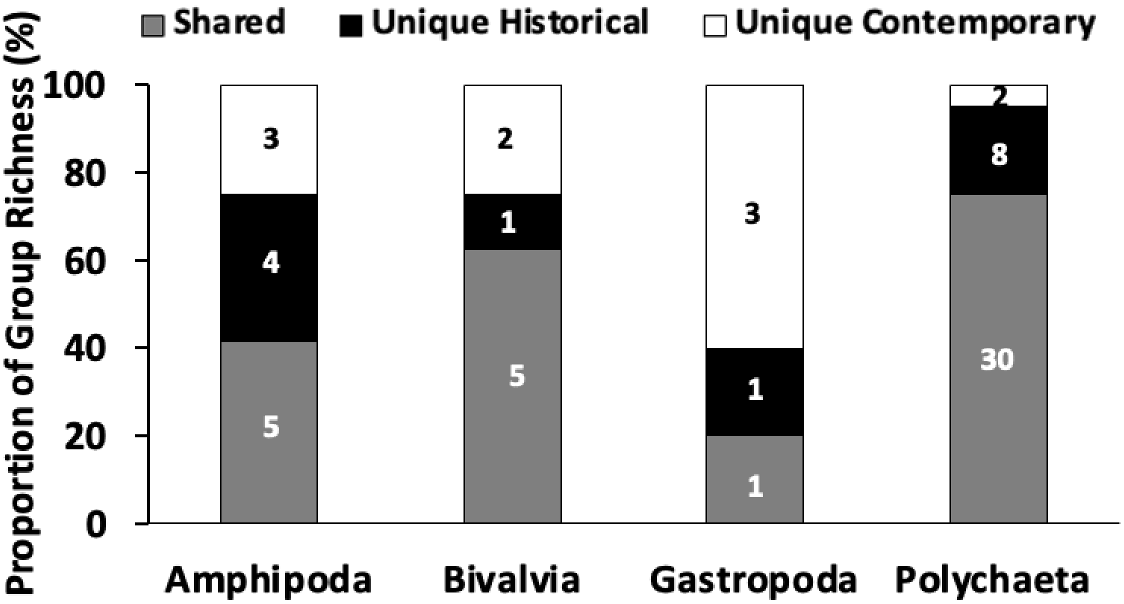

**Figure 1.6.** Proportion (%) of the total number of species/taxa in samples that were unique to either the historical (black) or contemporary (white) samples or shared (gray) between them for each major taxonomic group (Amphipoda, Bivalvia, Gastropoda, Polychaeta) in the Bay. Numbers on bars indicate the number of species/taxa shared or unique to each time point.

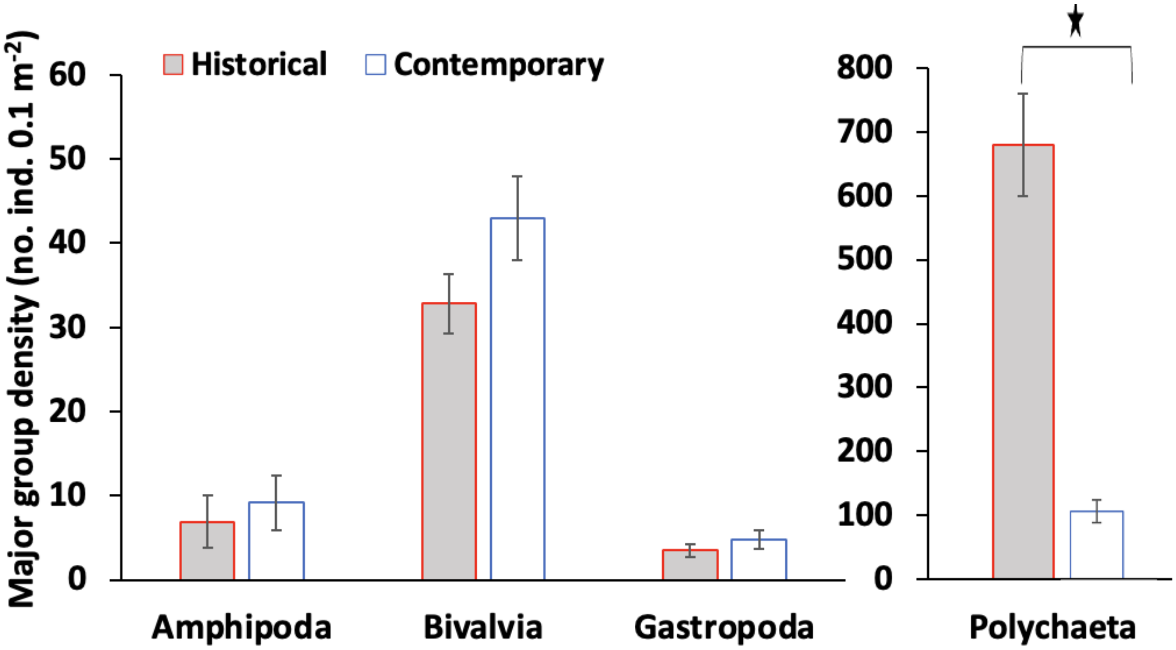

**Figure 1.7.** Density (no. of ind. 0.1 $m^{-2}$ ±SE) of major groups in historical (gray) (*n*=41) and contemporary (white) (*n*=36) communities in Placentia Bay, Newfoundland. Stars indicate that the two groups significantly differed in their means ($\alpha<0.05$).

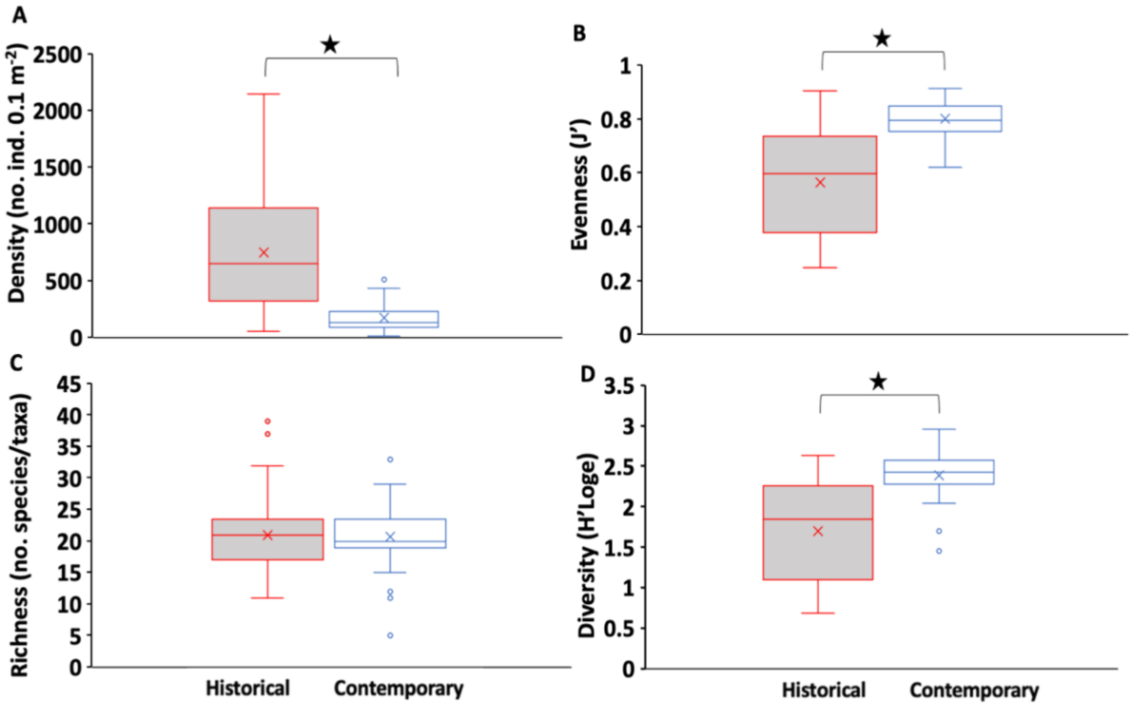

**Figure 1.8.** Box plots comparing A) density (total number of individuals 0.1m$^{-2}$), B) evenness (J'), C) species richness (number of species/taxa 0.1m$^{-2}$), and D) Shannon diversity (H´loge) for the contemporary (*n*=36) and historical (*n*=41) communities in Placentia Bay, Newfoundland. The line dividing the box in half represents the median, and the X represents the mean. The vertical spread of the box depicts the interquartile range (IQR), encompassing the middle 50% of data. Dots denote outliers. Stars indicate that the two groups significantly differed in their means ($\alpha<0.05$). Note that with the most abundant species removed (*Cossura pygodactylata*) diversity and evenness did not significantly differ between communities.

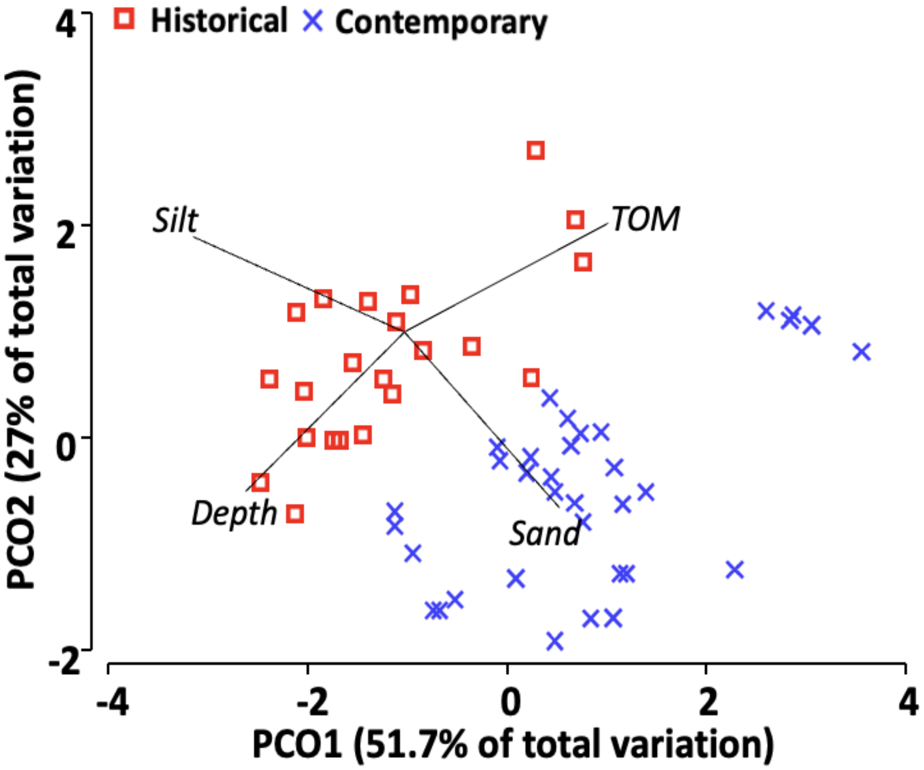

**Figure 1.9.** Principal Coordinates Ordination (PCO) of environmental samples (silt, sand, total organic matter, and depth) based on Euclidean distance for historical and contemporary samples. Abiotic vectors based on Pearson correlation of ≥ 0.7.

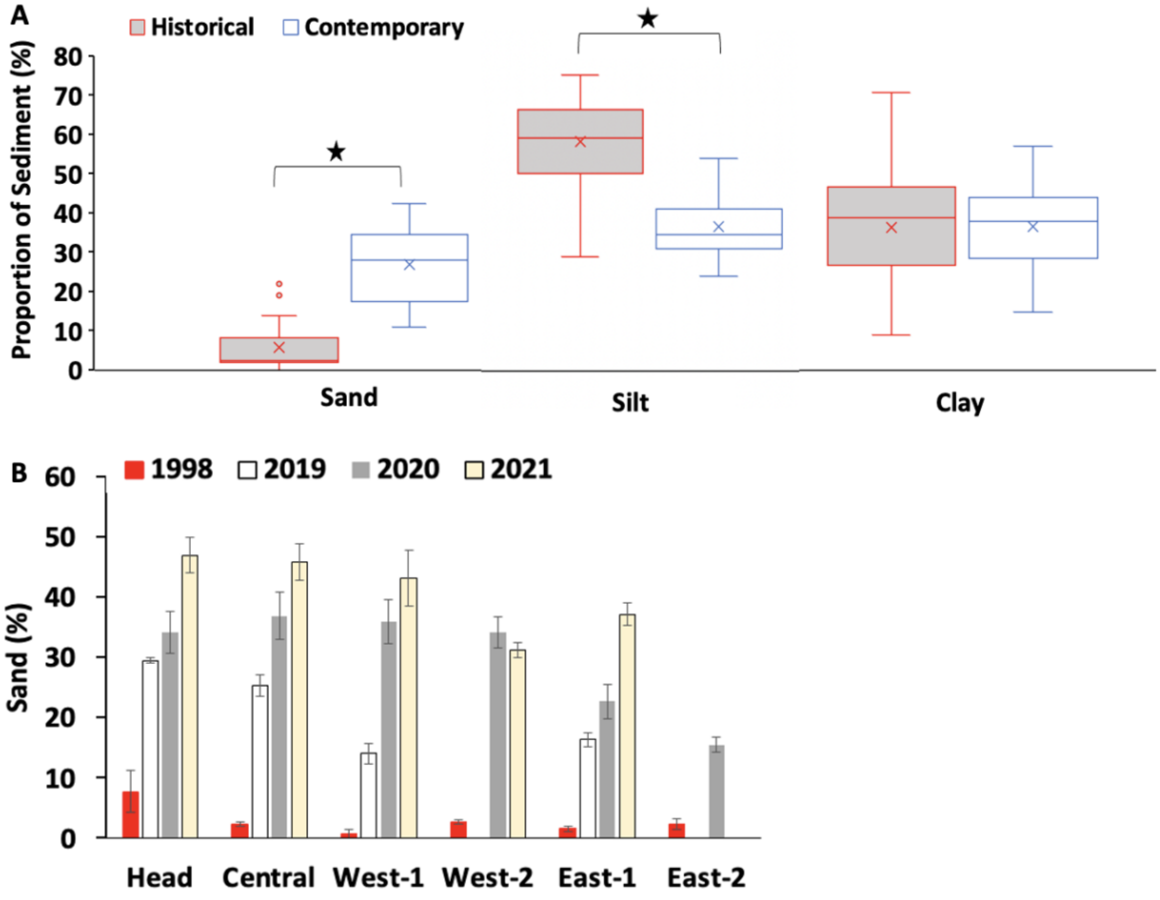

**Figure 1.10.** A) Box plots comparing sand, silt, and clay (%) in historical (*n*=22) and contemporary (*n*=35) samples in Placentia Bay, Newfoundland. Stars indicate that the two groups significantly differed in their means (α<0.05). B) Bar plot showing mean ±SE proportion of sand (%) among inshore sample sites in Placentia Bay for four time points (i.e., red=1998, white=2019, gray=2020, and yellow=2021 unpublished data) (total *n*=60). Sites without bars=no data.

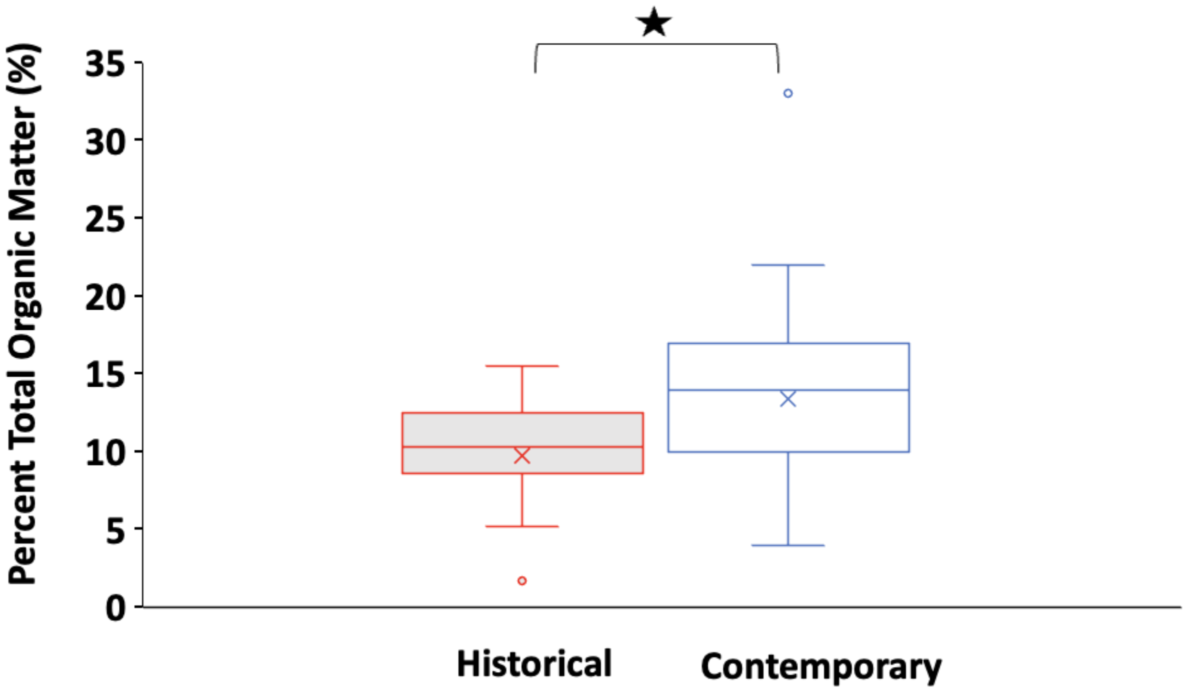

**Figure 1.11.** Box plots comparing total organic matter (%) in historical (*n*=38) and contemporary (*n*=36) samples. Stars indicate that the two groups significantly differed in their means (α<0.05).

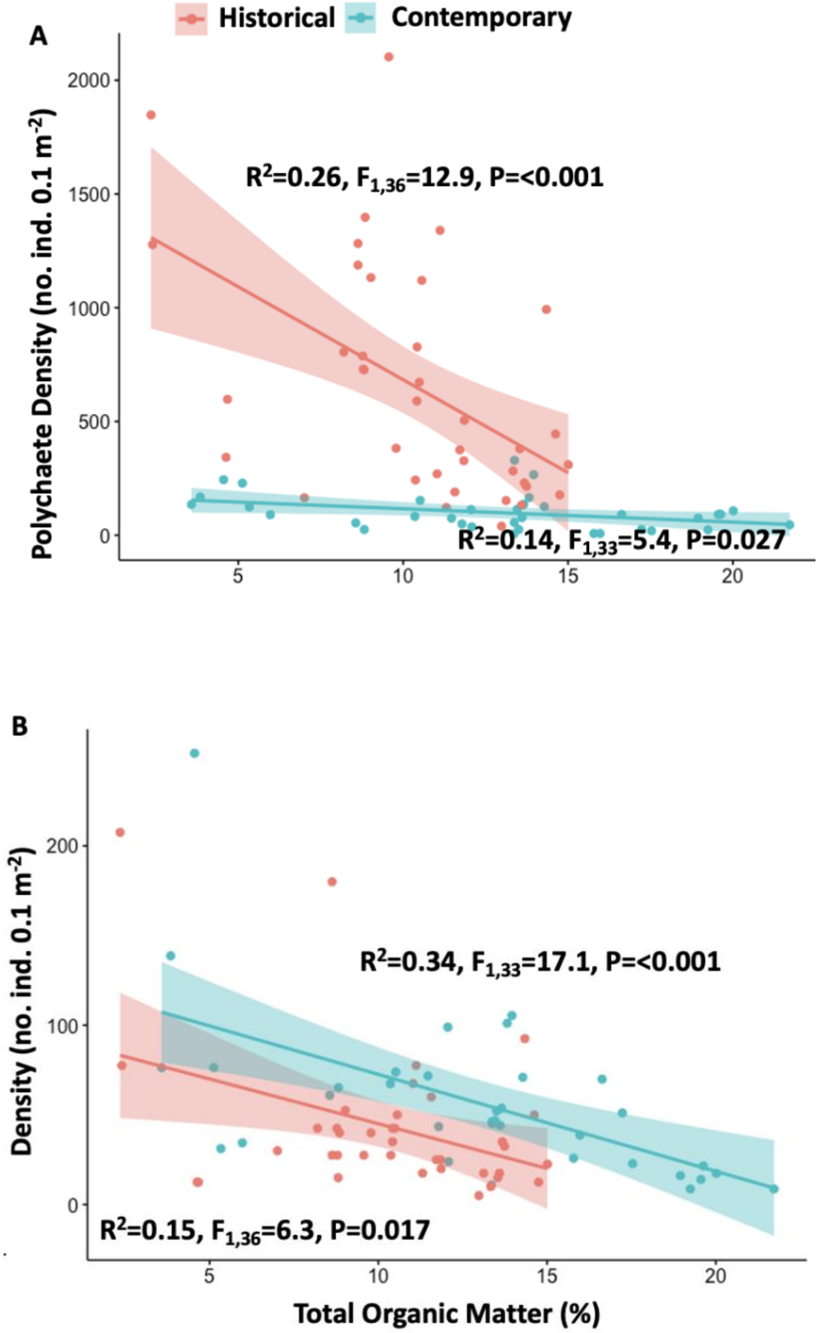

**Figure 1.12**. Relationship between density and total organic matter (%) for A) polychaetes (no. individuals 0.1 m$^{-2}$) and B) major taxonomic groups excluding polychaetes (total number of Amphipoda, Gastropoda and Bivalvia combined). Historical (pink dots), and contemporary (blue dots).

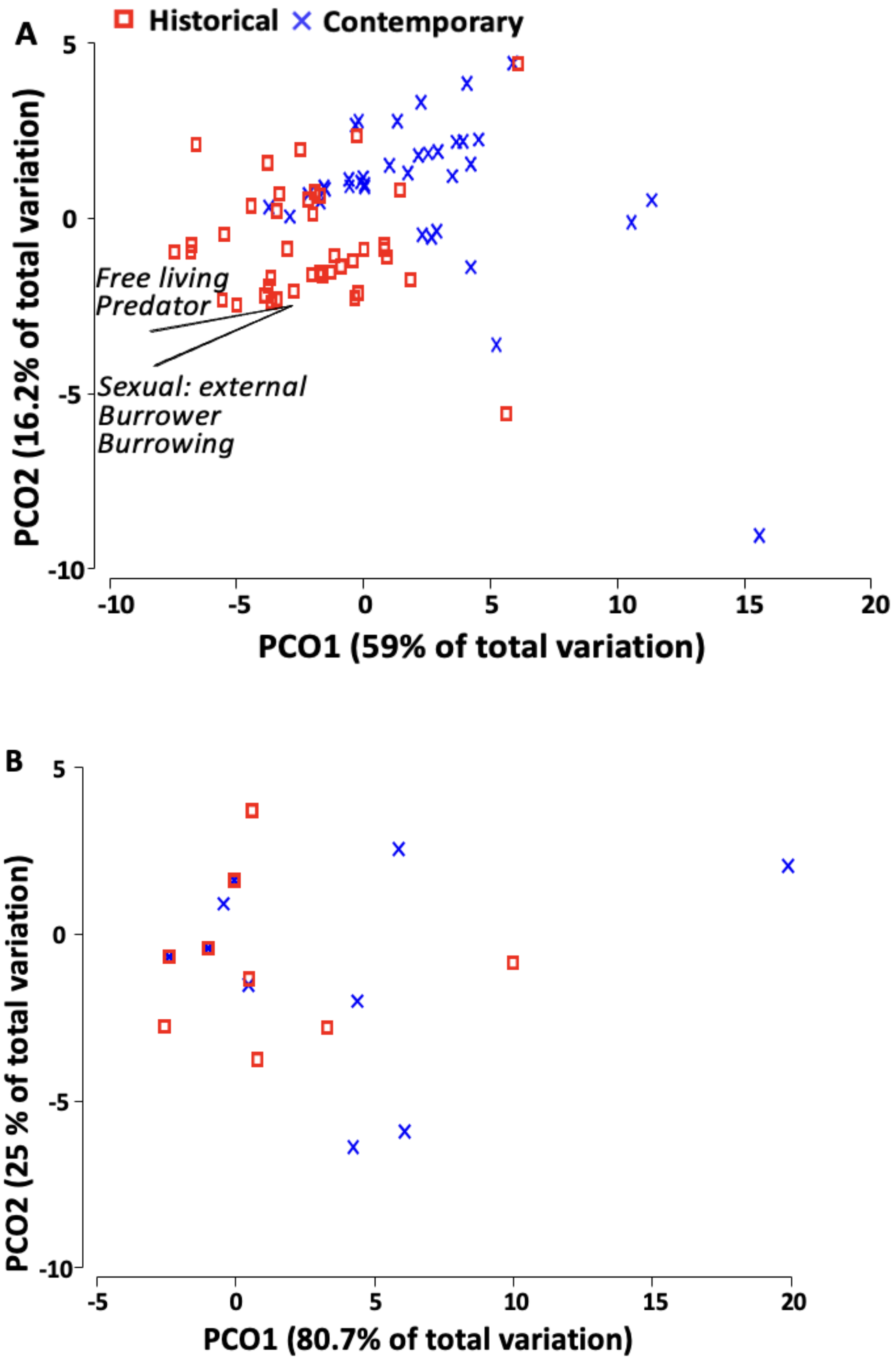

**Figure 1.13.** Principal Coordinates Ordination (PCO) of functional traits A) trait/modality matrix (see methods) based on Canberra similarity and B) trait/modality presence/absence matrix

based on Sorensen resemblance for Placentia Bay, Newfoundland. Community differences between the historical and contemporary communities for A were statistically significant (pseudo-$F_{1, 75}$= 19.5 and p, Permanova = 0.0001). Modality vectors based on Pearson correlation of $\geq 0.7$.

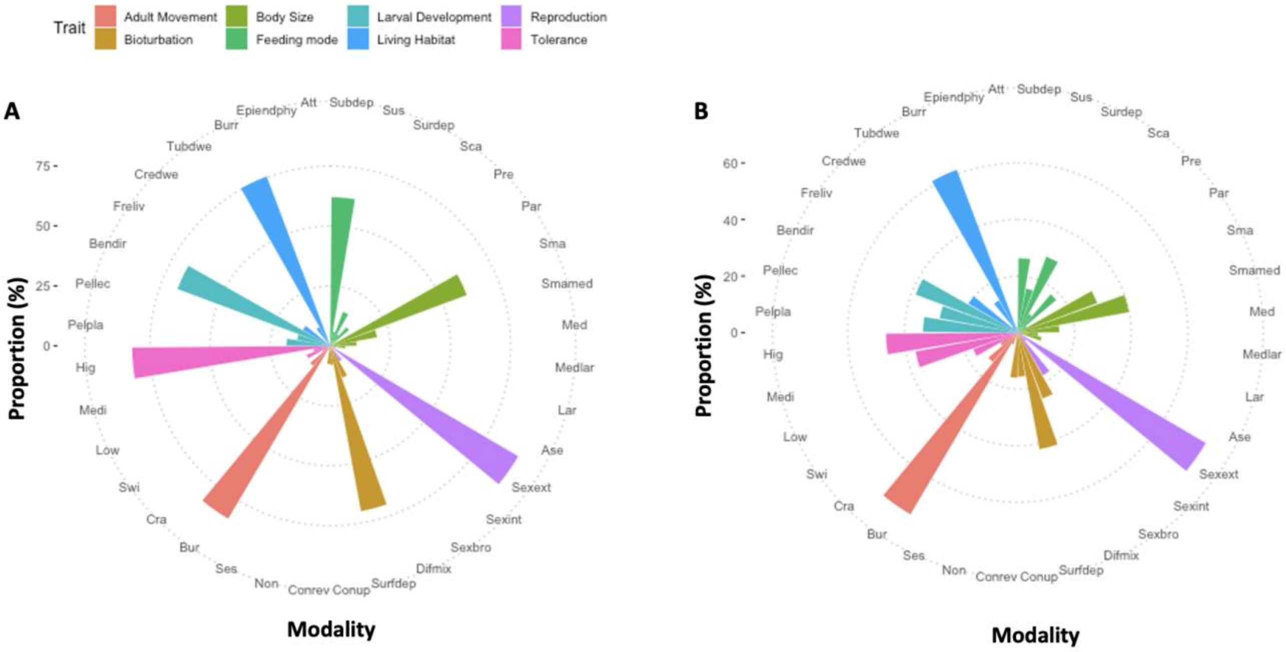

**Figure 1.14.** Circular bar plot showing proportional (%) modality for eight traits (i.e., modalities for each trait add to 100%) for the A) historical and B) contemporary years. Trait modality abbreviations are defined in Table 3.

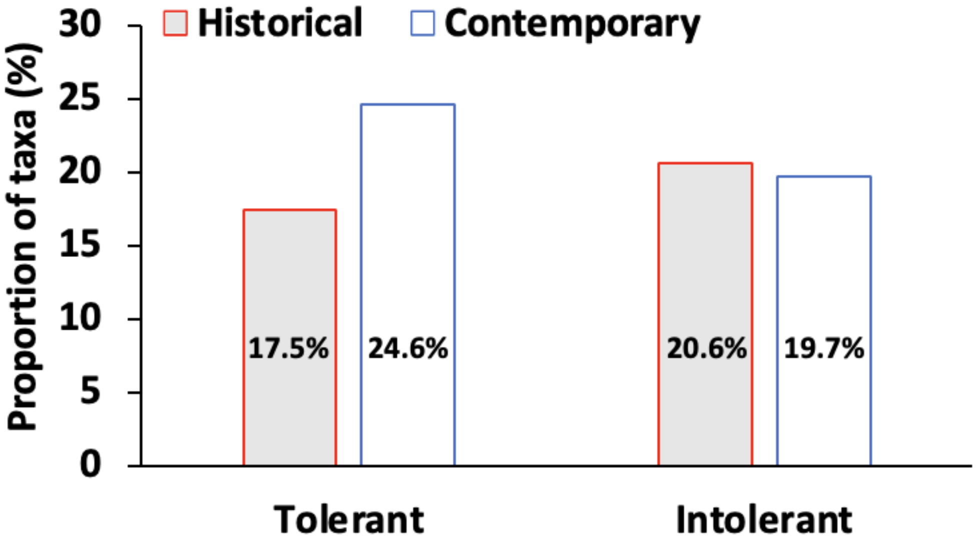

**Figure 1.15.** Percentage of tolerant and intolerant species/taxa in historical and contemporary communities in Placentia Bay, Newfoundland.

## Appendix I

**Table A1.1.** Test results (Shapiro-Wilk, Levene's, Mann-Whitney U, and t-test) conducted on community metric (richness, density, evenness, and Shannon diversity) for contemporary and historical communities. Transformation used to normalize data indicated.

| | **Contemporary** ***n*****=36** | **Historical** ***n*****=41** | | | |
|---|---|---|---|---|---|
| **Response** | **Shapiro-Wilk** | **Shapiro-Wilk** | **Levene's** | **Mann-Whitney U** | **t-test** |
| **Richness** | W=0.957<br>P=0.169 | W=0.932<br>P=0.017 | F=0.104<br>P=0.748 | U=741<br>P=0.980 | t=-0.246<br>P=0.806 |
| **Density** (log) | W=0.973<br>P=0.516 | W=0.973<br>P=0.452 | F=0.511<br>P=0.477 | U=144<br>P=<0.001 | t=-7.691<br>P=<0.001 |
| **Evenness (J')** | W=0.974<br>P=0.530 | W=0.938<br>P=0.027 | F=49.070<br>P=<0.001 | U=1287.0<br>P=<0.001 | t=7.072<br>P=<0.001 |
| **Diversity (H')** | W=0.894<br>P=0.002 | W=0.914<br>P=0.004 | F=38.912<br>P=<0.001 | U=1282.0<br>P=<0.001 | t=6.380<br>P=<0.001 |

**Table A1.2.** Test results (Shapiro-Wilk, Levene's, Mann-Whitney U, and t-test) conducted on functional trait metrics (richness, density, evenness, and Shannon diversity) for contemporary and historical communities. Transformation used to normalize data indicated.

| | **Contemporary *n*=36** | **Historical *n*=41** | | | |
|---|---|---|---|---|---|
| **Response** | **Shapiro-Wilk** | **Shapiro-Wilk** | **Levene's** | **Mann-Whitney U** | **t-test** |
| **Richness** | W=0.588<br>P= <0.001 | W=0.686<br>P=<0.001 | F=4.775<br>df=75<br>P=0.032 | U=943.0<br>P=0.027 | t=-0.118<br>P=0.906 |
| **Density** | W=0.976<br>P=0.598 | W=0.974<br>P=0.456 | F=0.456<br>df=75<br>P=0.502 | U=146.0<br>P=<0.001 | t=-7.773<br>P=<0.001 |
| **Evenness** | W=0.889<br>P=0.002 | W=0.929<br>P=0.014 | F=42.767<br>df=75<br>P=<0.001 | U=1255.5<br>P=<0.001 | t=6.414<br>P=<0.001 |
| **Diversity** | W=0.821<br>P=<0.001 | W=0.918<br>P=0.006 | F=24.868<br>df=75<br>P=<0.001 | U=1266.5<br>P=<0.001 | t=6.090<br>P=<0.001 |

**Table A1.3.** Test results (Shapiro-Wilk, Levene’s, Mann-Whitney U, and t-test) conducted on environmental (TOM, sand, silt) data for contemporary and historical samples. Transformation used to normalize data indicated.

| | **Contemporary** *n*=35 | **Historical** *n*=22 | | | |
|---|---|---|---|---|---|
| **Response** | **Shapiro-Wilk** | **Shapiro-Wilk** | **Levene’s** | **Mann-Whitney U** | **T-test** |
| **Sand** | W=0.957<br>P=0.186 | W=0.729<br>P=<0.001 | $F_{1,55}=3.6$<br>P=0.064 | U=733.5<br>P=<0.001 | $t_{55}=9.2$<br>P=<0.001 |
| **Silt** | W=0.913<br>P=0.009 | W=0.949<br>P=0.305 | $F_{1,55}=3.2$<br>P=0.081 | U=59.5<br>P=<0.001 | $t_{55}=-8.6$<br>P=<0.001 |
| **OM** | W=0.928<br>P=0.018 | W=0.939<br>P=0.046 | F=5.514<br>P=0.022 | U=425.0<br>P=0.005 | t=-2.8<br>P=0.006 |

**Table A1.4.** Summary of major taxonomic groups (Amphipoda, Bivalvia, Gastropoda, Polychaeta, other), species/taxa,, and density (mean ±SE) of macrofauna in Placentia Bay, Newfoundland for historical and contemporary time points. *Indicates species/taxa were grouped together when considering "unique" species/taxa when comparing the historical and contemporary communities (see Fig 5). This method was taken as a conservative approach.

| **Major Group** | **Species/Taxa** | **Historical Density (no. ind.m$^{-2}$) ±SE** | **Contemporary Density (no. ind.m$^{-2}$) ±SE** |
|---|---|---|---|
| Amphipoda | *Aceroides (Aceroides) latipes* | 0.12±0.09 | 0.30±0.14 |
| | *Bathymedon* sp. | 1.40±0.46 | 0.00±0.00 |
| | *Byblis gaimardii* | 0.67±0.40 | 0.09±0.07 |
| | *Hippomedon* sp. | 1.95±1.04 | 0.09±0.07 |
| | *Megamoera dentata* | 0.00±0.00 | 0.27±0.19 |
| | *Melita* sp. | 0.43±0.30 | 0.00±0.00 |
| | *Monocludes* sp. | 0.12±0.085 | 0.51±0.31 |
| | *Orchomenella minuta* | 0.00±0.00 | 0.75±0.23 |
| | *Paratryphosites abyssi* | 0.00±0.00 | 5.59±3.19 |
| | *Pontoporeia femorata* | 1.16±0.50 | 1.56±0.38 |
| | *Protomedeia* sp. | 0.73±0.73 | 0.00±0.00 |
| | *Quasimelita formosa* | 0.31±0.22 | 0.00±0.00 |

|  |  |  |  |
|---|---|---|---|
| Bivalvia | *Axinopsida orbiculata* | 0.00±0.00 | 1.97±1.18 |
|  | *Ennucula* sp. | 1.59±0.44 | 0.99±0.24 |
|  | *Macoma calcarea* | 12.13±2.37 | 14.11±2.78 |
|  | *Megayoldia thraciaeformis* | 0.00±0.00 | 1.63±0.34 |
|  | *Nuculana pernula* | 5.18±0.75 | 9.54±1.81 |
|  | *Thyasira* sp. | 11.10±2.15 | 13.25 ± 2.71 |
|  | *Yoldia hyperborea* | 0.37±0.16 | 1.50±0.31 |
|  | *Yoldia* sp. | 2.44±0.60 | 0.00±0.00 |
| Gastropoda | *Curtitoma incisula* | 0.00±0.00 | 1.08±0.30 |
|  | *Propebla rugulata* | 0.00±0.00 | 1.56±0.38 |
|  | *Retusa obtusa* | 2.38±0.60 | 0.63±0.40 |
|  | *Tachyrhynchus erosus* | 0.00±0.00 | 1.23±0.46 |
|  | *Turridae* sp. | 1.10±0.34 | 0.00±0.00 |
| Polychaeta | Ampharetidae sp. A | 0.92±0.63 | 0.00±0.00 |
|  | *Ampharete finmarchia* | 0.24±0.15 | 0.00±0.00 |
|  | *Apistocranchus typicus* | 0.79±0.32 | 0.63±0.37 |
|  | *Arcteobia anticostiensis* | 0.06±0.06 | 0.54±0.18 |
|  | *Aricidae* sp. | 7.80±1.53 | 1.32±0.39 |

| | | | |
|---|---|---|---|
| **Maldane sarsi* and *Maldane* sp. A grouped together as *Maldane* sp.<br>**Paradoneis lyra* and Paraonidae sp. A grouped together as Paraonidae sp. | *Artacama proboscidae* | 1.77±0.84 | 0.00±0.00 |
| | *Bradabyssa villosa* | 1.28±0.75 | 0.60±0.27 |
| | *Capitellidae* spp. | 12.87±2.95 | 2.09±0.50 |
| | *Chaetozone* sp. | 13.29±1.99 | 5.39±1.27 |
| | *Cistenides hyperborea* | 15.92±9.15 | 0.27±0.12 |
| | *Cossura pygodactylata* | 465.73±64.87 | 28.56±6.88 |
| | *Dipolydora caulleryi* | 0.49±0.49 | 0.00±0.00 |
| | *Dorvilleidae* spp. | 30.12±6.07 | 1.82±1.46 |
| | *Dipolydora socialis* | 0.00±0.00 | 0.33±0.14 |
| | *Dysponetus pygmaeus* | 0.73±0.22 | 0.00±0.00 |
| | *Enipo canadensis* | 0.18±0.13 | 0.42±0.20 |
| | *Eteone flava* | 0.00±0.00 | 1.02±0.34 |
| | *Eteone longa* | 2.99±0.58 | 0.66±0.20 |
| | *Euchone incolor* | 3.29±1.71 | 0.72±0.40 |
| | *Goniada maculata* | 0.24±0.15 | 0.33±0.10 |
| | *Gyptis bruneli* | 16.40±1.66 | 2.24±0.73 |
| | *Lumbrineridae* spp. | 19.82±4.93 | 16.86±2.46 |
| | *Lysilla loveni* | 1.65±0.43 | 0.12±0.07 |

| | | | |
|---|---|---|---|
| | *Maldane glebifex* | 1.52±0.94 | 0.00±0.00 |
| | *Maldane sarsi* | 0.00±0.00 | 1.89±0.81 |
| | *Maldane* sp. A | 0.30±0.20 | 0.00±0.00 |
| | *Micronephtys neotana* | 18.41±1.93 | 6.04±0.81 |
| | *Nephtys ciliata* | 0.31±0.13 | 1.50±0.29 |
| | *Nereimyra aphroditoides* | 8.96±7.04 | 0.36±0.16 |
| | *Paradoneis lyra* | 0.00±0.00 | 0.99±0.45 |
| | *Paraonidae* sp. A | 0.55±0.38 | 0.00±0.00 |
| | *Pherusa plumosa* | 0.49±0.24 | 0.15±0.08 |
| | *Pholoe longa* | 0.55±0.31 | 3.07±1.52 |
| | *Pholoe minuta* | 0.96±0.10 | 0.96±0.41 |
| | *Prionospio steenstrupi* | 41.46±8.52 | 17.50±3.11 |
| | *Scalibregma inflatum* | 0.37±0.19 | 0.03±0.03 |
| | *Scoloplos armiger* | 1.77±0.99 | 2.22±0.71 |
| | *Sphaerodoridium minutum* | 1.65±0.63 | 0.18±0.08 |
| | *Spiochaetopterus typicus* | 0.12±0.09 | 0.42±0.15 |
| | *Syllides* sp. | 2.20±0.85 | 0.00±0.00 |
| | *Terebellides stroemii* | 3.35±0.68 | 0.57±0.16 |

| | | | |
|---|---|---|---|
| | *Terebellidae* sp. B | 0.85±0.40 | 0.00±0.00 |
| Other | *Antalis entalis* | 2.99±0.87 | 6.15±1.53 |
| | Chaetognatha | 0.12±0.09 | 0.18±0.07 |
| | *Ctenodiscus crispatus* | 0.18±0.10 | 0.45±0.13 |
| | Cumacean spp. | 5.24±1.45 | 3.01±0.80 |
| | Echinoidea | 0.55±0.21 | 0.06±0.04 |
| | *Nemertea* spp. | 10.49±1.76 | 4.80±1.25 |
| | *Ophiuridae* | 2.74±0.60 | 1.27±0.40 |
| | *Priapulus caudatus* | 0.00±0.00 | 0.30±0.17 |
| | *Sipunculidea* sp. | 2.44±0.56 | 0.48±0.15 |
| | *Tanaidacea* | 0.31±0.20 | 0.03±0.03 |

**Table A1.5.** SIMPER analyses for trait modalities contributing to ~50% of the dissimilarity between historical and contemporary communities including mean ±SE density (no. mod. 0.1 $m^{-2}$) and contribution to dissimilarity (%) for Placentia Bay, Newfoundland. Dissimilarity is based on fourth root transformed data.

| **Modality** | **Historical Mean Functional Density ±SE** | **Contemporary Mean Functional Density ±SE** | **Dissimilarity (%)** |
|---|---|---|---|
| High (tolerance) | 1575±205 | 182±29 | 5.9 |
| Small (body size) | 1674±216 | 194±28 | 5.7 |
| Subsurface deposit (feeding mode) | 1643±212 | 182±26 | 5.6 |
| Benthic/direct (larval development) | 1663±216 | 236±36 | 5.4 |
| Surface deposit (feeding mode) | 401±48 | 196±24 | 4.9 |
| Sexual external (reproduction) | 2096±240 | 433±48 | 4.7 |
| Burrower (adult movement) | 2033±235 | 441±52 | 4.7 |
| Burrowing (living habitat) | 1839±219 | 378±45 | 2.6 |
| Crawler (adult movement) | 266±31 | 80±9 | 2.6 |
| Medium (body size) | 303±43 | 91±11 | 2.6 |

| | | | |
|---|---|---|---|
| Medium-large (body-size) | 175±24 | 45±6 | 2.5 |

# Chapter Two: Natural and anthropogenic sedimentary characteristics influencing temporal and spatial variation of macrofauna in an Ecologically and Biologically Significant Bay (Newfoundland, Canada)

## Abstract

Characterizing temporal variation in macrofaunal community composition and abundance is essential to understand longer term community changes and environmental drivers creating observed patterns. Benthic macrofauna are important biological indicators used to assess environmental quality in marine ecosystems. Placentia Bay is a large subarctic embayment on the southeast coast of Newfoundland (NL) that has been identified as an ecologically and biologically significant area (EBSA) that is exposed to anthropogenic stressors (e.g., fisheries and hazardous substances). The present study delineates macrofaunal communities in the bay temporally (i.e., September/October 2019 and 2020) and spatially in relation to natural and anthropogenic environmental factors (e.g., total organic matter, sediment grain size, chl-*a*, phaeopigment, heavy metals, and polycyclic aromatic hydrocarbons) at six inshore sites (total *n*=30). The relative proportion of major taxonomic groups, species/taxon abundances (and biomass), and community metrics (i.e., density, richness, evenness, diversity) did not significantly differ between years. Spatially, there were consistent discernible biological and environmental differences among sites. Most notably, the head of the bay had distinct communities characterized by low density, species/taxon richness and diversity along with high levels of total organic matter and heavy metals relative to other sites. At all sites three heavy metals were above the levels where occasional adverse biological effects may be expected, with the highest concentrations observed in the upper western channel where lead also exceeded

quality guidelines. Polycyclic aromatic hydrocarbon concentrations were unrelated to community structure in the bay. This work represents the most comprehensive study conducted in the bay to date and information on annual community variability and inshore biological and environmental spatial patterns can be used as a reference for future monitoring and management of Placentia Bay.

## Introduction

Canada is home to the longest coastline in the world spanning more than 240,000 km (Ganter et al. 2021; Warren & Lulham 2021; Taylor et al. 2014). Coastal ecosystems are among the most productive areas of the ocean. With human populations localized around them they are subject to multiple anthropogenic disturbances (Dobson & Frid 2009; Diaz et al. 2004), including eutrophication, fisheries, hazardous substances, physical disturbance, shipping traffic, and climate change (Nestlerode et al. 2020; Byers & Grabowski 2014). Presently, marine ecosystem management efforts rely on monitoring and assessments to evaluate the condition of the environment (e.g., biological indicators, pollutant/contaminant levels, sedimentary organic matter content) which can serve as an early-warning signal of potential negative impacts (i.e., species loss or ecosystem function) (Drejou et al. 2021; Niemi & McDonald 2004).

The use of benthic macrofauna (herein defined as organisms retained on a 500 µm screen) as bioindicators is an essential component of national and international monitoring and assessment programs (e.g., Ramey et al. 2011; Van Hoey et al. 2010; Directive MSFD: 2008/56/EC, Borja & Dauer 2008; CABIN, CAFF, Directive WFD: 2000/60/EC). Several characteristics of these organisms have contributed to their establishment as bioindicators (Lenihan & Micheli 2001). For instance, benthic macrofauna are primarily sedentary exposing them to prolonged disturbance (Borjaet al. 2000; Dauer 1993). They are trophically diverse and exhibit species-specific tolerances or sensitivity to organic enrichment (Borja & Dauer 2008; Pearson & Rosenberg 1978). As such they can exhibit marked changes related to environmental conditions as they can rapidly respond to stressors (Fuchs et al. 2020; Taghon et al. 2017; Villnas & Norkko, 2011; Borja & Dauer, 2008).

Abiotic and biotic processes influencing the distribution and community structure of macrofauna include small-scale (cm to m's) sedimentary habitat features (e.g., sediment grain size, organic carbon, oxygen, contaminants) and relatively larger-scale (kms) oceanographic conditions (e.g., depth, temperature, salinity, surface production, circulation, disturbance) (Olafasson et al. 1994; Morrisey et al. 1992; Barry & Dayton 1991). Species interactions also play a role in shaping community patterns at smaller scales (mm to m's) (Snelgrove 1999; Olafasson et al. 1994). Studies characterizing community structure (e.g., species composition, abundance, richness, and diversity) of macrofauna in relation to natural and anthropogenic environmental factors form the foundation for conducting mechanistic research aimed at determining specific processes responsible for creating observed community patterns, detecting anthropogenic impacts, and predicting future longer-term community changes (Dreujou et al. 2021; Grassle et al. 2009; Lenihan & Mecheli 2001).

There still exists a general lack of continuous long-term and baseline biodiversity data for many areas including Arctic and subarctic marine benthic environments (Azovsky 2023; Roy et al. 2015). Placentia Bay is a large subarctic embayment on the southeast coast of Newfoundland (NL) in the northwest Atlantic that has been identified as an ecologically and biologically significant area (EBSA) (Fig. 1.1). It is home to cetaceans, seals, and sea birds, as well as endangered species such as the leatherback turtle and harlequin duck (DFO 2019; LGL 2018). A recent study found that the west coast of the bay contains species-rich epifaunal assemblages (Mackin-McLaughlin et al. 2022). The bay also supports essential commercial fisheries (e.g., blue mussel, flounder, cod, and snow crabs) and Atlantic salmon aquaculture operations throughout the central, western, and eastern channel (Wells et al. 2019; LGL 2018; DFO 2007). Like many coastal environments it is exposed to multiple anthropogenic stressors. The bay is an important marine transport route that has been experiencing growth in vessel traffic as well as coastline activities (Mackin-McLaughlin et al. 2022; DFO 2007). Crude oil tanker traffic has led to oil spills, creating inputs of polycyclic aromatic hydrocarbons (PAHs) (Kiceniuk 1992). On- and nearshore mining and industrial processing plants also occur at Come by Chance and Vale Long Harbour (DFO 2007). Soft-sediment benthic infaunal communities in Placentia Bay have received little to no attention since the first study conducted more than 20 years ago (Ramey & Snelgrove 2003; Ramey 2001).

The aim of the present study was to delineate macrofauna communities both temporally and spatially in relation to natural and anthropogenic environmental factors (i.e., total organic matter, chlorophyll-a, phaeopigments, sediment grain size, heavy metals, and PAHs). It was hypothesized that the composition and abundance of macrofauna in the bay would not differ between years (i.e., 2019 vs. 2020) as sedimentary habitat features (e.g., grain size) important in influencing communities are expected to remain relatively stable temporally (Guerin et al. 2023; Herder et al. 2021; Taghon et al. 2017). However, communities in the bay would differ depending on location and the associated sedimentary habitat, specifically grain size and the level of TOM. It was predicted that communities at the head of the bay will be distinct from other areas given it is relatively shallow and occurs in close proximity to river input (i.e., through Swift Current). Thus, the head of the bay was expected to have relatively high levels of total organic matter (e.g., through allochthonous input) and relatively low macrofaunal abundance compared to other areas (Ramey & Snelgrove 2003). Information provided by studies such as this one, aimed at improving our understanding of macrofaunal communities and associated of environmental conditions, can be used in monitoring and management programs (i.e., as a reference for long-term studies), as well as conservation efforts to preserve ecologically and biologically significant ecosystems such as Placentia Bay.

## Methods

### *Study area and sample collection*

Placentia Bay is a large subarctic embayment located on the southeast coast of Newfoundland (Chapter 1, Fig. 1) that has been identified as an EBSA (DFO 2016). As such, it is a unique area with respect to marine biodiversity including endangered species (e.g., blue whale and leatherback turtle) and supporting critical life stages (e.g., capelin spawning grounds) (Mackin-McLaughlin et al. 2022; DFO 2004). The Bay is ~130 km long and 100 km wide at its mouth. The innermost part of the bay contains three deep channels (100's of m deep) divided longitudinally by several islands. Circulation is generally cyclonic, flowing into the bay on the eastern side and out on the western side (Ma et al. 2012). For a more complete description of the study area see Chapter 1.

Soft-sediment benthic macrofauna were sampled using a 0.1 $m^2$ Van Veen grab. Three independent samples were collected from four sites in Placentia Bay in 2019 (13–17th September) and six sites in 2020 (13–17th October) by the Department of Fisheries and Oceans, Canada (Dr. Neves, Ecological Sciences Section, NL) (total $n$=30). Sites sampled in both years included the head of the bay, Central, East-1, and West-1 (total $n$=24) (Chapter 1, Fig. 1.1). In 2020, the East-2 and West-2 were also sampled (2020 total $n$=18) (Chapter 1, Table. 1.1). Each grab sample was subsampled (50 ml of sediment removed) for determination of sedimentary variables including grain size (top 5 cm), total organic matter (TOM) (top 1 cm), chl-*a*, phaeopigments, and contaminants (note: heavy metals and polycyclic aromatic hydrocarbons

[PAHs] were only examined in 2020). The remaining grab sample was used to examine macrofauna and was fixed in formalin and then transferred to 70% ethanol.

### *Sample and data processing*

#### *Macrofauna*

Samples were sieved over a 500 µm mesh screen prior to sorting macrofauna from the sediments using a stereomicroscope. Macrofauna were counted and identified to species where possible, and biomass was determined from wet weight (g 0.1 m$^{-2}$). Taxonomic identifications were completed by L. Treau De Coeli (Université Laval, Quebec). Only infaunal, soft-sediment macrofauna (i.e., herein defined as organisms retained on a >500 µm sieve) were used for community analyses (see Gallagher and Grassle 1997; Taghon et al. 2017). Epifaunal encrusting species such as bryozoans, mussels, and hydrozoans as well as meiofauna (i.e., organisms <500–40 µm; e.g., ostracods, copepods, and nematodes) were excluded. Highly mobile species such as mysids and decapods were also omitted since they are not reliably sampled using a Van Veen grab (Taghon et al. 2017). Species/taxa with <10 individuals, across all years and samples (rare species) were also removed (Taghon et al. 2017; also see Chapter 1). The taxonomic status of species was checked against the World Register of Marine Species (http://www.marinespecies.org/index.php). Densities and biomass were re-scaled to 0.1 m$^{-2}$ to account for the removal of sediment (50 ml) for sedimentary analyses.

#### *Environmental variables*

Determination of grain size and contaminants were conducted by Bureau Veritas services following modified procedures generalized below and adapted from Danovaro et al. (2010), Total organic matter, chl-*a*, and phaeopigment determination was performed by the Department of Fisheries and Oceans, Canada, NL following methods adapted by Danovaro et al. (2010). For sediment grain size analysis, sediments were first treated with 10% $H_2O_2$ for 24–48 hours to remove organic matter. They were then dried in an oven at 105°C for 24 hours, weighed, and sieved on a series of standard sieves (i.e., sand:>63μm, silt:<63–3.9 μm, and clay <3.9 μm). The percent sand, silt, and clay in each sample was calculated using dry weight. Sediment samples for TOM were homogenized, placed in individual aluminum cups, and dried at 60°C for 24 hours. After this, they were weighed and placed in a muffle furnace for 4 hours at 450°C. Total organic matter was calculated as the difference between the initial dry weight and that of calcinated sediments (normalized and expressed as a percentage). For chl-*a*, 5–10 ml of 90% acetone was added and vortexed in the dark prior to sonication at 30 sec intervals for 3 mins. The samples were then centrifuged, and 3 mL of the supernatant was obtained for spectrophotometric determination. For phaeopigment determination, the acetone extract was acidified with 200 μL of 0.1 N HCL. Chl-*a* and phaeopigment concentrations were then calculated (see Danovaro et al. 2010). Sediments for heavy metals and PAHs were freeze dried and homogenized via acid-washed mortal and postal prior to sending them for processing. A total of 19 heavy metals ( (i.e., aluminum, arsenic, barium, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, selenium, strontium, thallium, tin, uranium, vanadium, and zinc) and 21 PAHs (i.e., 1-methylnapthalene, 2-methylnapthalene, acenaphthene, ancenaphythylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(b/j)fluoranthene, benzo(g,h,j)perylene, benzo(j)fluoranthene, benzo(k)fluoranthene, chrysene, dibenzo(a, h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, perylene, phenanthrene, and pyrene) were analyzed. For nine PAHs, all values fell below detection limits and therefore were removed prior to statistical analysis (i.e., 1-methylnapthalene, 2-methylnapthalene, acenaphthene, ancenaphythylene, anthracene, benzo(j)fluoranthene, benzo(k)fluoranthene, dibenzo(a, h)anthracene, fluorene, naphthalene).

### *Data Analysis*

#### *Univariate*

Species/taxon accumulation plots were generated for 2019 and 2020 (shared sample sites). The proportion of major taxonomic groups making up the communities in 2019 vs. 2020 was examined with stacked bar plots (shared sites only: Head, Central, East-1, West-1). Bar plots (mean ±SE) and t-tests were used to compare the density of major taxonomic groups between years ($n$=12 each group year$^{-1}$). Mean ±SE for density (no. ind. 0.1 m$^{-2}$), richness, Pielou's evenness (J'), and Shannon-Wiener diversity (H´loge) were compared between years and among sites (2020) (calculated using PRIMER v7) and plotted. Environmental variables were also only compared between years ($n$=11–12 each group year$^{-1}$) and among sites in 2020 by plotting mean ±SE ($n$=2–3). T-tests were used to examine differences in community metrics (i.e., density, richness, evenness, and diversity) and environmental variables (i.e., sand, silt, TOM, chl-*a*, and phaeopigments) between years but not sites in 2020 since sample size was small ($n$=3). Assumptions of normality and homogeneity of variance were checked using Shapiro-Wilk and Levene's tests respectively (JASP V. 0.16, JASP Team 2022). If data were not normal with equal variance transformations were applied (i.e., log or square root) (Appendix Table A2.4–5). For variables where transformations did not normalize distribution and/or variance (i.e., TOM, Shannon diversity), a t-test was used as they are robust against non-normality and the non-parametric Mann-Whitney U test showed the same result.

*Multivariate*

Characterization of spatial patterns of macrofaunal species/taxon composition and abundance in the bay were conducted using all sites sampled in both years (*n*= 30), whereas yearly comparisons only utilized shared sampling sites (i.e., Head, Central, East-1, and West-1 sampled in both years). Variation in macrofaunal community structure was examined using Principal Coordinates Ordination (PCO) (PRIMER v7 +PERMANOVA) (Anderson 2017; Clarke & Gorley 2015; Anderson, 2001). For macrofaunal data the PCO was conducted on Bray-Curtis dissimilarity matrix generated separately for species/taxon abundances and biomass, as well as presence/absence data (based on Sorensen resemblance). Species/taxa were overlaid as vectors on the PCO plot (i.e., ≥0.7 Pearson correlation with ordination pattern) where arrows point in the direction of maximal variation in species abundances and their length is proportional to their maximal rate of change (Ramette 2007). To determine whether spatial patterns observed in the presence/absence and biomass matrix matched the species/taxon abundance matrix (all sites 2019 and 2020), the "RELATE" procedure was used. A Permutational Multivariate Analysis of Variance (PERMANOVA; 9999 permutations) tested the null hypothesis that there is no difference in communities and the sedimentary environment between 2019 vs. 2020 (shared sites only).

Examination of community structure in relation to the sedimentary environment focused on samples collected in 2020 since contaminants were only available for this year and biological communities were found to be similar between years (also see results). For this, environmental variables (i.e., grain size, depth, TOM, chl-*a*, phaeopigment, heavy metals and PAHs) were normalized to Z-scores. Where metal and PAH concentrations (mg·kg$^{-1}$) were non-detectable, a value of 0 was assigned, although this does not necessarily mean the true value was zero, it was not high enough to be detected. A PCO analysis was run on this matrix based on Euclidean Distance. To examine the relationship between environmental variables and macrofaunal data, the "Relate" function was used (PRIMER v7) based on Spearman rank correlation (ρ) (999 permutations) (Clarke and Gorley 2015; Clarke et al. 2014). This analysis tested the null hypothesis that there is no correlation between the two matrices (i.e., thus comparing observed patterns in the environmental vs. biological data).

## Results

### *Temporal and spatial patterns in community structure and sedimentary environment (2019 and 2020)*

Overall, in both years, a total of 4643 individuals belonging to 98 species/taxa were collected. Once rare species/taxa (<10 individuals across all samples) were removed, the dataset contained 4482 individuals and 45 species/taxa for analysis. Species accumulation curves showed that species/taxa leveled off at about nine samples for both years (Fig. 2.1). The major taxonomic groups (i.e., Amphipoda, Bivalvia, Gastropoda, Polychaeta) made 94% of the total species/taxa sampled and polychaetes were the most dominant group (2019=61% vs. 2020=55% respectively) followed by bivalves (24% vs. 26% respectively) (Fig. 2). Moreover, bivalves were absent from the head of the bay in both years, with the exception of one individual in a single sample (Fig. 2.2). Of the species/taxa making up the major groups between the years, 31 species/taxa (86.1%) were shared. Mean density (i.e., no. ind. $m^{-2}$) for the major taxonomic groups examined did not differ between years, with the exception of amphipods which were significantly lower (U=26.0, P=0.008) in 2019 ($\bar{X}$=1 ind. $m^{-2}$ ±0.36) compared to 2020 ($\bar{X}$=5 ind. $m^{-2}$ ±1.6) (Fig. 2.3). Community metrics (i.e., species/taxon richness, density, evenness, and diversity) and sedimentary environmental variables (with the exception of sand, silt, and chl-*a*/phaeopigment ratio, see Fig. A2.4 and Appendix Table A2.6) did not significantly differ between 2019 and 2020 (p>0.5).

Principal Coordinates Ordination analysis of species/taxon composition and abundance explained a total of 60.7% of the variability in the data. Axis 1 explained 46.2% of the total variation and separated the head of the bay samples from all other sites (Fig. 2.5A). Seven species/taxa were correlated with the observed grouping of the head of the bay. (Fig. 2.5A). Axis 2 explained 14.5% of the total variation and represented variability among sites and samples, with West-1 generally separating out from the remaining sites (Fig. 2.5A). Three species of bivalves were correlated to the grouping of the West-1 samples (i.e., *Nuculana pernula, Megayoldia thraciaeformis,* and *Thyasira gouldii*). The polychaetes C*ossura pygodactylata*, *P. steenstrupi,* and *Scoletoma laurentiana* were generally correlated with the grouping of the Eastern Channel and West-2 site with C. *pygodactylata* having the greatest abundance at East-1 and East-2 (Fig. 2.5A). This axis also indicated some variability between years at the head of the bay and in the central channel which was more similar to the eastern channel and West-2 sites in 2019. Multivariate analysis of species/taxon composition and abundance for 2019 vs. 2020 samples indicated that they were not significantly different (P>0.5). Community patterns observed in composition and abundance were also seen for the presence/absence and biomass resemblance matrices (based on RELATE analysis: ρ= 0.981, P=0.001; ρ= 0.953, P=0.001 respectively) (see Appendix Fig. A2.1A). There were no new species correlated to PCO groupings using presence/absence or biomass but fewer species emerged as important, emphasizing those which contributed to differences between the head of the bay and the rest of the sites (see Appendix Fig. A2.1A–B). Additionally, biomass analysis generally prioritized larger bodied species such as *N. pernula* and *T. gouldii* (see Appendix Fig. A2.1B). Multivariate analysis of environmental variables for 2019 vs. 2020 samples indicated that they were significantly different (Pseudo-$F_{1,21}$=4.73, P=0.0001).

### *Community Structure (2020)*

Overall, a total of 2590 individuals belonging to 77 species/taxa were collected in 2020 (*n*=18). With the removal of rare species, the dataset contained 2447 individuals and 36 species/taxa for analysis. PCO analysis of species/taxon composition and abundance explained 67.1% of the total variation. Patterns observed in the PCO were consistent with the previous analysis containing both sampling years (i.e., 2019 and 2020), however fewer species were correlated to the observed groupings in 2020 (Fig. 2.5B). In addition to *C. pygodactylata*, *the* polychaete *Eteone flava* and the cumacean *Leucon (Leucon) nasica* also contributed to grouping the Central channel, West-2, and Eastern channel sites along axis 2 (Fig. 2.5B). Bivalve species (i.e., *N. pernula*, *M. thraciaeformis*, and *T. gouldii*) were important at West-1 (Fig. 2.5B). Community metrics showed that the sites at the Head, Central, and West-1 sites had low densities compared to the other sites ($\bar{X}$=74 ind. $m^{-2}$ ±9.87, *n*=9 vs. $\bar{X}$=198 ind. $m^{-2}$ ±36.78, *n*=9 respectively) (Fig. 2.6A). Species/taxon richness and diversity was lowest at the head of the bay (Fig. 2.6C–D). Similar values of evenness were observed for all sites ($\bar{X}$= 0.83 J' ±0.014, *n*=18) (Fig. 2.6B).

### *Environmental Data (2020)*

Comparison of sedimentary environmental variables between sites (Fig. 2.7A–G) showed that grain size at the eastern channel sites (i.e., East-1 and East-2) had the lowest proportion of sand (Eastern channel: X̄=19% ±2.14, *n*=6; all other sites: X̄=35% ±1.51, *n*=12) and the highest proportion of clay (X̄=44% ±1.54, *n*=6; X̄=34% ±1.21, *n*=12) compared to all other sites (Fig. 2.7A–B). Total organic matter was highest at the head of the Bay compared to all other sites (X̄=20% ±0.73, *n*=3; X̄=13% ±1.50, *n*=14 respectively) (Fig. 2.7A). Concentrations of chl-*a* did not appear to vary greatly amongst sites (Fig. 2.7C). Phaeopigment concentrations were highest at East-2 (Fig. 2.7D). The concentration of several heavy metals were generally higher at the Head and West-1 sites, relative to all other sites (Fig. 2.8A–K). Total variability explained by the PCO of the environmental variables was 40.1% (Fig. 2.9). The samples from head of the bay and West-1 separated out from the remaining sites along axis-1 (37% of variation explained) where water depth and contaminants were most correlated to the observed groups along this axis. Axis-2 mainly represented within and between site variability (27% of variability explained). Overall, the samples from the head of the bay were most distinct from all other sites, followed by samples from West-1. (Fig. 2.9). Four heavy metals were most important in separating the head of the bay from all other sites including molybdenum, uranium, cadmium, and thallium were correlated to this grouping (Fig. 2.9). Several other heavy metals were important at West-1 such as nickel, lead, copper, arsenic, vanadium, selenium, and chromium.

### *Community Structure and Environmental Drivers (2020)*

Patterns observed in the community composition and abundance resemblance matrix (Bray-Curtis similarity) significantly matched those found in the environmental data based on the RELATE analysis (ρ= 0.401, P=0.003). However, since the PAHs did not appear important based on PCO analyses (see Fig. 2.7), this analysis was also conducted with the removal of PAHS which resulted in a stronger correlation with the community composition and abundance matrix (based on RELATE: ρ= 0.61, P=0.001). The PAHs were also analyzed separately to ensure that they were not significantly correlated to community data in the absence of the other variables, and patterns observed in the community composition resemblance matrix did not match those found based on the PAHs (based on RELATE ρ= -0.081, P=0.67).

## Discussion

The major taxonomic groups making up the macrofaunal community in Placentia Bay were similar in September 2019 and October 2020 and ~86% of species/taxa were present in both years. This is despite the finding that environmental variables varied between these two years and sediment grain size has become slightly coarser at all sites from 2019 (21% ±2.04) to 2020 (34%± 2.28). Multivariate analyses showed no temporal differences in community structure and species accumulation curves showed that species/taxa were adequately sampled in both years, thus an increase in species richness would not be expected with additional sampling. Consistent with this, density, richness, evenness, and diversity were also similar, but results should be interpreted with caution given that comparisons are based on a limited number of sites sampled in both years (i.e., Head, Central, West-1, and East-1). Polychaetes were the dominant taxon present, followed by bivalves. The most abundant species included the polychaetes *C. pygodactylata* (15% of total macrofauna), *P. steenstrupi* (10%), and *S. laurentiana* (8%), and the bivalves, *T. gouldii* (9%), *M. calcarea* (8%) and *N. pernula* (6.8%) which made up ~60% of the total macrofaunal abundance. Of these species, *C. pygodactylata, N. pernula* and *P. steenstrupi* are generally considered to be indifferent or tolerant to environmental change (e.g., organic enrichment, pollution, temperature and salinity changes) (Degen & Faulwetter 2019; Berger & Naumov 2001*;* Borja et al. 2000; Pearson & Rosenberg 1978).

The sedimentary environment was primarily composed of fine sediments (i.e., silt: range=24–46% and clay: range=27–57%) and TOM was higher (8.6–21.7%) than generally found in other coastal bays and harbours  (e.g., ~1.7–3.5%) (Guerin et al. 2023: New Brunswick, Canada; Taghon et al. 2017: New Jersey, USA; Valderhaug & Gray 1984: Oslofjord, Norway), suggesting an input from freshwater sources such as Swift Current and/or aquaculture operations. High levels of sedimentary TOM can result in low oxygen conditions (Diaz & Rosenberg 2008; Pearson & Rosenberg 1978) and qualitative observations of the sediments in Placentia Bay, below the first few mm to cm, were black and smelled of sulfur (i.e., Head, Central, West-1 and East-1). A previous study in Placentia Bay conducted by Ramey and Snelgrove (2003), at the same sites as examined herein, also indicated possible sulfide accumulation in the sediments at relatively lower levels of TOM (1998: ~7–15.7% [based on 4.1–9.1% TOC values]) than observed in the present study (8.6–21.7%). Moreover, one of the dominant species of bivalves in Placentia Bay, *T. gouldii*, has been observed to contain bacteria chemosymbionts (based on samples collected from Bonne Bay, NL; Batstone et al. 2014), which allow it to thrive in sediments containing hydrogen sulfide as symbionts utilize reduced compounds (Dufour & Felbeck 2006).

Spatial patterns of macrofaunal composition and abundance, however, did reveal variability among sampling sites and observed patterns were primarily related to water depth and heavy metals in the sediments. Communities were distinct at the shallowest site at head of the bay, as well as in the upper western channel (West-1). The head of the bay contained species/taxa not found at any other site (i.e, polychaetes: *M. sarsi* and *P. lyra*) or only occurred rarely elsewhere (1 or 2 individuals) (i.e., polychaetes: *P. longa, G. maculata, A. suecica;* gastropods: *T. erosus, R. obtusa*). These two sites also exhibited the lowest density of macrofauna (followed by the central channel), and species/taxon richness and diversity were lowest at the head of the bay. Compared to other areas the sedimentary environment these two sites contained relatively high levels of chromium, arsenic, copper, and lead. In a study by Callier et al. (2009), concentrations of heavy metals in two marinas on the south coast of England and west coast of France found copper (English marina: $\bar{X}$=~45–170 mg·kg$^{-1}$ vs. Placentia Bay $\bar{X}$=26–38 mg·kg$^{-1}$), cadmium ($\bar{X}$=~0.12–0.35 mg·kg$^{-1}$ vs. $\bar{X}$=0–0.50 mg·kg$^{-1}$), zinc ($\bar{X}$=~145–250 mg·kg$^{-1}$ vs. $\bar{X}$=65–71 mg·kg$^{-1}$), and lead ($\bar{X}$=~40–70 mg·kg$^{-1}$ vs. $\bar{X}$=22–41 mg·kg$^{-1}$) concentrations were linked to changes in macrofaunal community composition and decreased abundance. In the same study, *C. pygodactylata* was the dominant species at sites with relatively lower concentrations of heavy metals but was nearly absent from areas with higher concentrations (Callier et al. 2009: see Table 4, Figure 4). In Placentia Bay, this same polychaete was not observed in samples from the upper western channel (West-1) (2020) which contained some of the highest concentrations of these heavy metals, despite the fact that it was the most dominant species in the bay. Several other heavy metals also reached their highest concentration either at the head of the bay or in the upper western channel (Fig. 2.9). Among these, the concentration molybdenum peeked at the head of the bay. Accumulation of this metal in marine sediments has been used as an indicator of hypoxia (Boothman et al. 2022), given that it can adsorb into organic matter (Boothman et al. 2022; King et al. 2018). Despite potential concerns of oil exposure in Placentia Bay from shipping traffic, PAHs were unrelated to community structure.

Newfoundland is naturally rich in metals including nickel, copper, cobalt, and iron which are processed by local mining industries (e.g., Vale Long Harbour and Voisey Bay). The source these of metals (i.e., natural versus anthropogenic) in the environment can be difficult to distinguish as they can occur naturally at high concentrations (e.g., aluminum and iron; Dauvin 2008) or as a result of human activity. Based on the interim sediment quality guidelines (ISQGs) established by the Canadian Council on Ministers of the Environment (CCME) that were available for six metals, concentrations of chromium, copper, and arsenic in the bay were above the ISQGs but below the probable effect levels (PELs). Concentrations in this range are occasionally associated with adverse biological effects (CCME 1999). Although at higher levels (i.e., >300 $mg \cdot kg^{-1}$) than observed in the present study, copper has been found to negatively affect diversity and recruitment of benthic fauna (Josefson et al. 2008; Trannum et al. 2004; Burd 2002; Olsgard et al. 1999). Concentrations of lead and nickel in Placentia Bay also exceeded the ISQG (CCME 1999) and ERL (Long et al. 1995) respectively in the upper western channel which is upstream of several potential anthropogenic sources of heavy metals (i.e, Vale Long Harbour metal processing plant and aquaculture operations) located in the eastern channel where water flows into the bay. Waste resulting from aquaculture (e.g., fish faeces and feed) may contain metals such as zinc and copper as well as others to a lesser extent including arsenic, cadmium, chromium, and lead (Kingsbury et al. 2023; Sutherland et al. 2001; Naylor et al. 1999). While concentrations of heavy metals in the bay did not exceed levels where adverse biological effects are frequently observed, it is noteworthy that they were related to inshore community patterns. A study by Guerin et al. (2023) in St. John harbour, New Brunswick, Canada found that heavy metals, even below PELs, played a role in influencing macrofaunal community structure in addition to other factors such as depth and TOC. Of the five PAHs with available ISQGs, none of them exceeded the CCME guidelines (CCME 1999). However, the East-1 site had the highest average of phenanthrene ($\bar{X}$= 0.067 mg·kg-1) with one of the samples surpassing the ISQG of 0.0867 $mg \cdot kg^{-1}$.

This study represents the most comprehensive examination of benthic macrofaunal communities in Placentia Bay after more than two decades and is the first assessment of contaminants in the sediments. While macrofaunal community composition and diversity in the bay has changed substantially since it was last studied in 1998 (see Chapter 1), spatial patterns among different regions of the bay have remained remarkably consistent. Ramey (2001) also found that the macrofaunal community at the head of the bay was unique compared to the other areas, however, the species important in distinguishing this community have changed. For example, while the dominant bivalve (*M. calcarea*) was present in all samples taken at the head of the bay in 1998, it was not collected (nor any other bivalve species) from this site in the present study; even though it was generally more abundant in the present study than in 1998 (see Chapter 1). Moreover, mean species/taxon richness and diversity were highest at the head of the bay in 1998, whereas contemporary communities at this site had the lowest observed values for both metrics (species/taxon richness: 30 vs. 11; diversity: 2.5 vs. 1.9, respectively). A potential explanation for these findings could be that storm activity (e.g., wave action and sediment movement) may be more pronounced at the head of the bay due to its shallower depth relative to other sites (Posey et al. 1996). In both years prior to sampling, two hurricanes (i.e., hurricane Dorian in 2019 and hurricane Teddy in 2020) affected Newfoundland through storm surges, strong winds, and waves (Blake 2021; Jones 2019). Posey et al. (1996), found that surface dwelling organisms (i.e., surface deposit and suspension feeders) were the only group to experience significant reductions in abundance following a storm event off of the Gulf of Mexico and coast of Florida, USA.

## Conclusion

Consistent with the initial hypotheses, there were no significant differences in macrofaunal community composition and abundance between years (2019 vs. 2020), and communities did differ spatially among locations in Placentia Bay, Newfoundland. Specifically, the head of the bay was most distinct, characterized by low macrofaunal density, richness, and diversity, as well as a high levels of TOM. Community patterns were related to depth and concentration of heavy metals in the sediments (2020). The head of the bay and West-1 generally had the highest concentrations of heavy metals, however, arsenic, chromium, and copper were above ISQGs at all sites. At West-1, lead and nickel also exceeded guidelines such that they were at levels that are occasionally associated with adverse biological effects. This research indicates the need for consistent monitoring and carefully designed studies to better quantify temporal changes in TOC and heavy metals in relation to potential anthropogenic sources and effects on benthic communities in the bay. Overall, there is a general lack of information pertaining to heavy metal concentrations in marine sediments and species-specific effects on benthic macrofauna despite their use as biological indicators (Ramey et al. 2011; Borja and Dauer 2008). More information is needed with respect to acceptable contaminant levels and possible biological outcomes as development continues in coastal regions such as Placentia Bay.

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## Tables and Figures

**Table 2.1.** Range of heavy metal concentrations (mg·kg$^{-1}$) for arsenic, cadmium, chromium, copper, lead, nickel, and zinc in Placentia Bay, Newfoundland with corresponding interim sediment quality guidelines (ISQG) and probable effect levels (PEL) based on the Canadian Council of Ministers of the Environment (CCME 1999), and sediment quality guidelines for effect range-low (ERL) and effect range-median (ERM) (Long et al. 1995). ISQG/ERL's and PEL/ERM's pertain to occasional and frequently observed adverse biological effects respectively. ******=ISQGs exceeded at all sites, *****=ISQG and ERL exceeded at West-1, – = no data.

| | | **CCME (1999)** | | **Long et al. (1995)** | |
|---|---|---|---|---|---|
| **Metals** | Range in Placentia Bay | ISQG | PEL | ERL | ERM |
| ******Arsenic | 13–35 | 7.24 | 41.6 | 8.2 | 70 |
| Cadmium | 0–0.52 | 0.7 | 4.2 | 1.2 | 9.6 |
| ******Chromium | 41–59 | 52.3 | 160 | 81 | 370 |
| ******Copper | 24–38 | 18.7 | 108 | 34 | 270 |
| *****Lead | 16–42 | 30.2 | 112 | 46.7 | 218 |
| *****Nickel | 28–33 | – | – | 20.9 | 51.6 |
| Zinc | 64–75 | 124 | 271 | 150 | 410 |

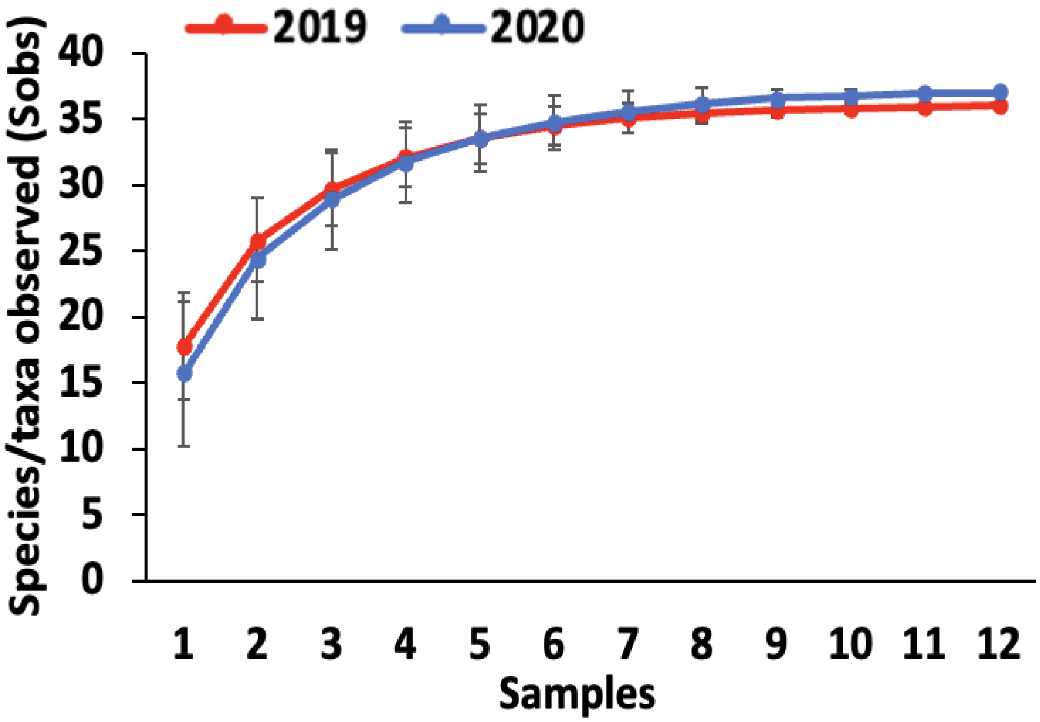


**Figure 2.1.** Species accumulation curve based on species/taxa ± SD for 2019 (red) and 2020 (blue) for the same sites sampled in both years (i.e., Head, Central, East-1, West-1) in Placentia Bay, Newfoundland.

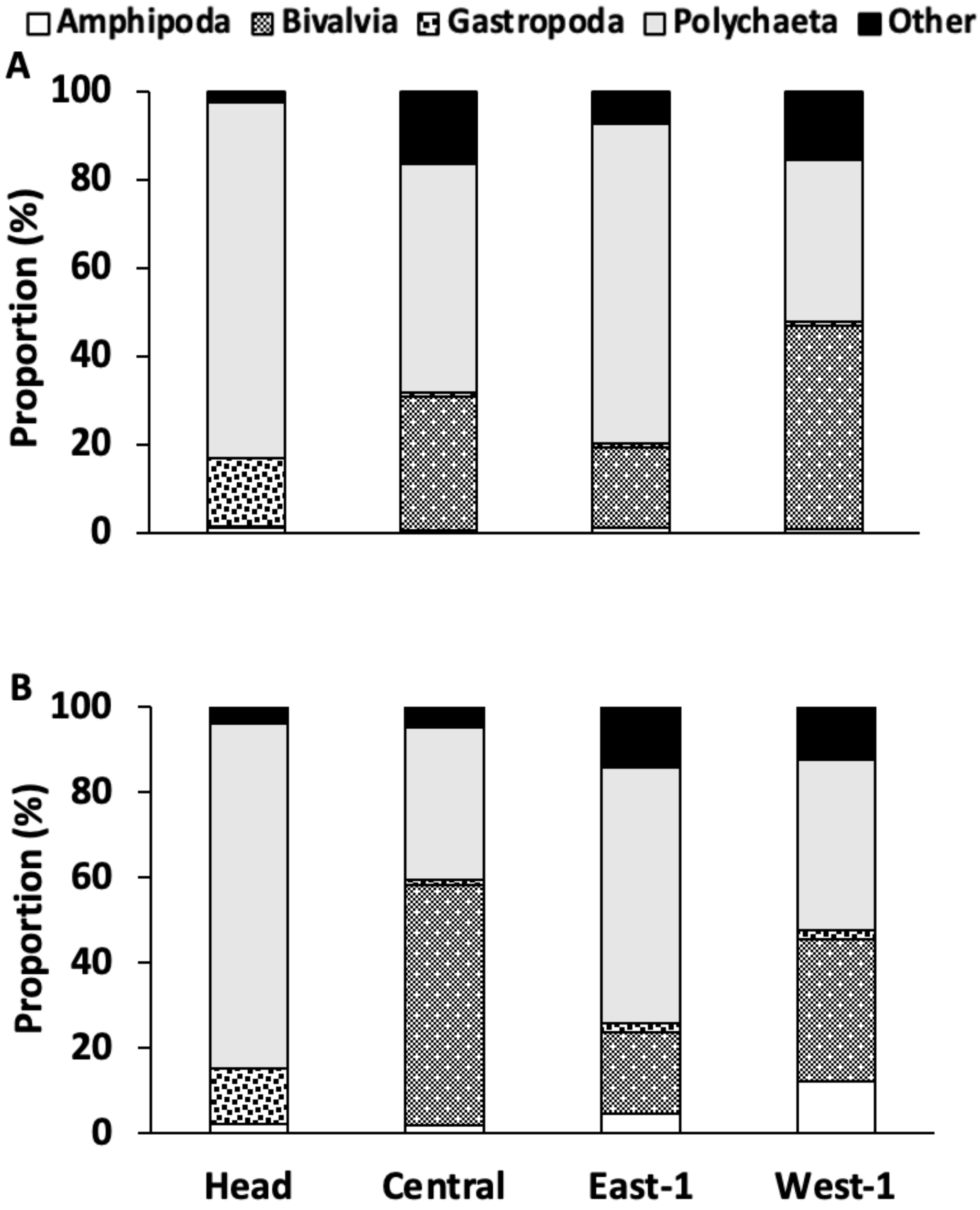


**Figure 2.2.** Stacked bar graph depicting relative abundance (%) of major taxonomic groups (Amphipoda, Bivalvia, Gastropoda, Polychaeta) at each site (*n*=3) in Placentia Bay, Newfoundland in A) 2019 and B) 2020 based on total macrofaunal abundance (no. ind. site$^{-1}$). Other= taxa not included in the major groups indicated (e.g., Nemerteans, Chaetognatha, Cumaceans).

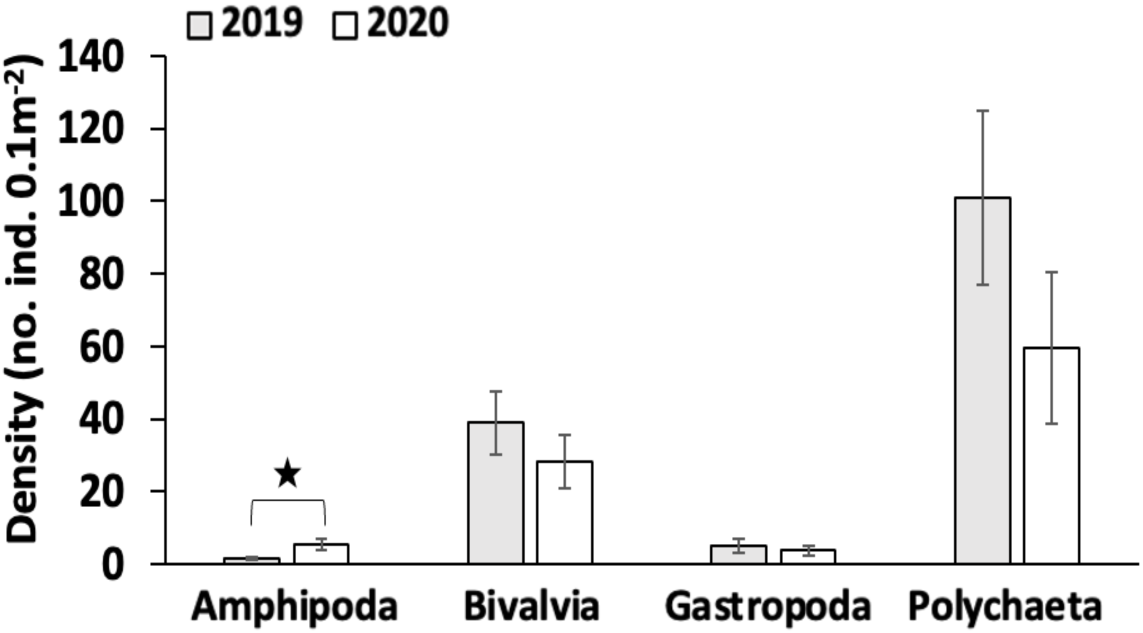


**Figure 2.3.** Mean ±SE of major taxonomic groups in 2019 (*n*=12) and 2020 (*n*=12) making up the communities in Placentia Bay, Newfoundland for the same sites sampled in both years (i.e., Head, Central, East-1, West-1). Stars indicate that the two groups significantly differed in their means (α<0.05).

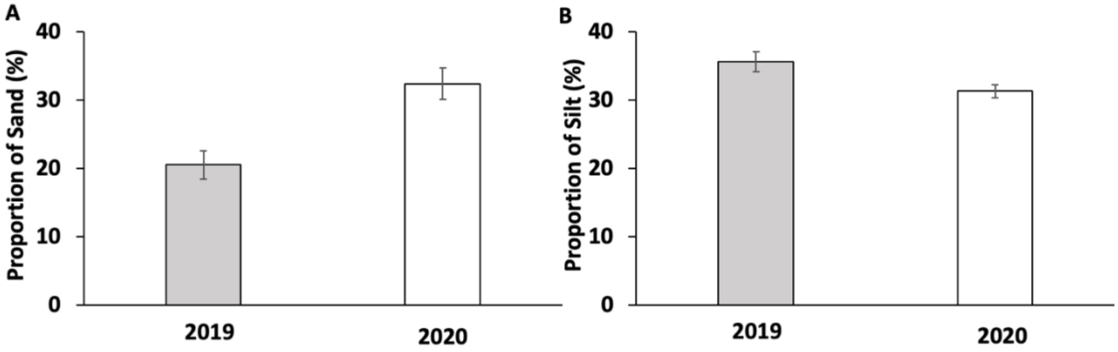


**Figure 2.4.** Plots showing mean ±SE A) proportion of sand (%) B) proportion of silt (%) at shared sampling sites in 2019 (*n*=11) and 2020 (*n*=12) in Placentia Bay, Newfoundland.

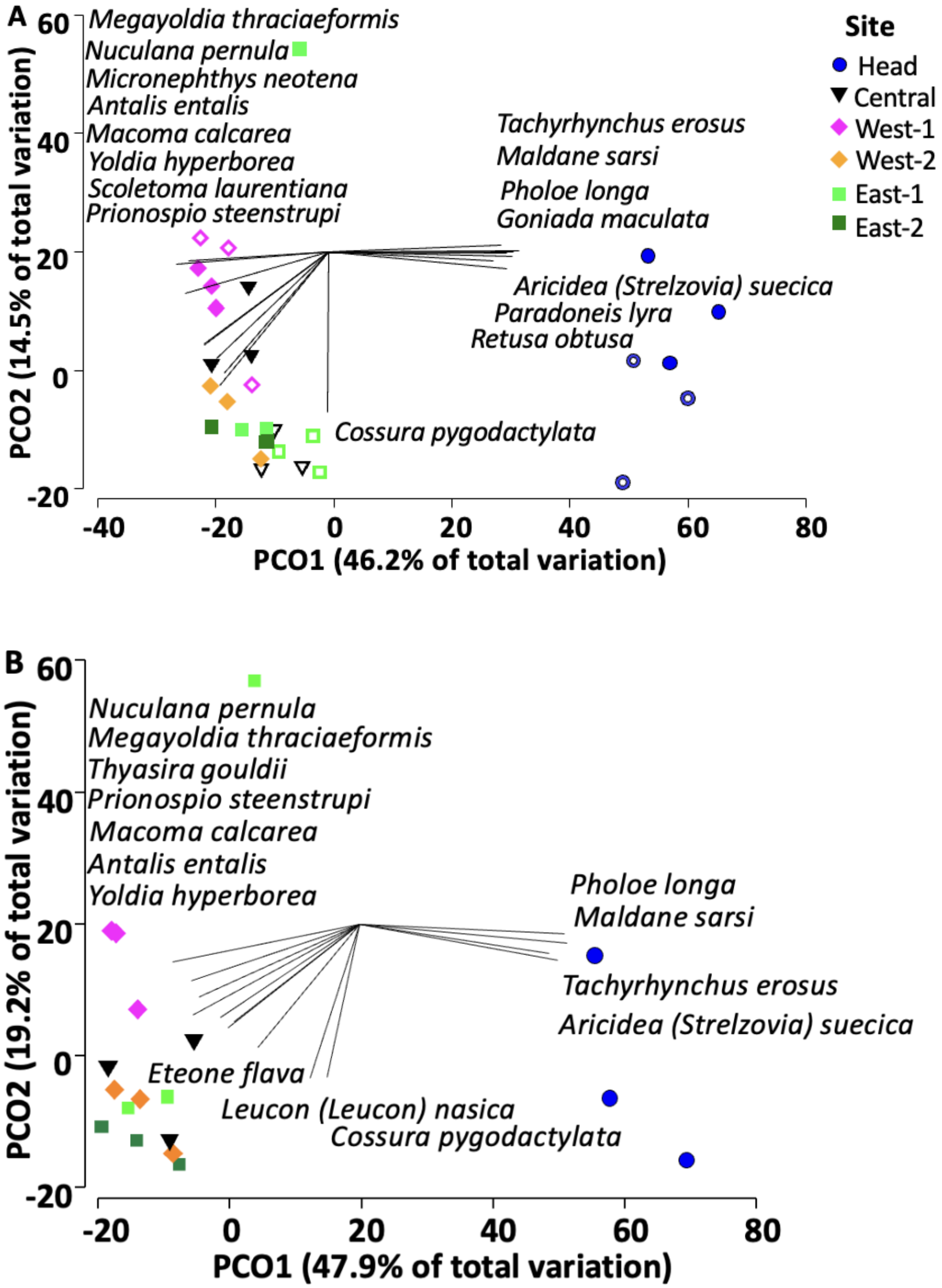


**Figure 2.5.** Principal Coordinates Ordination (PCO) of biological samples of species/taxon composition and abundance matrix based on Bray-Curtis similarity for sites in Placentia Bay, Newfoundland for A) 2019 and 2020 (total *n*=30) and B) 2020 (total *n*=18). Colored symbols

indicate different sites (no fill=2019; filled=2020). Species vectors based on Pearson correlation of $\geq 0.7$.

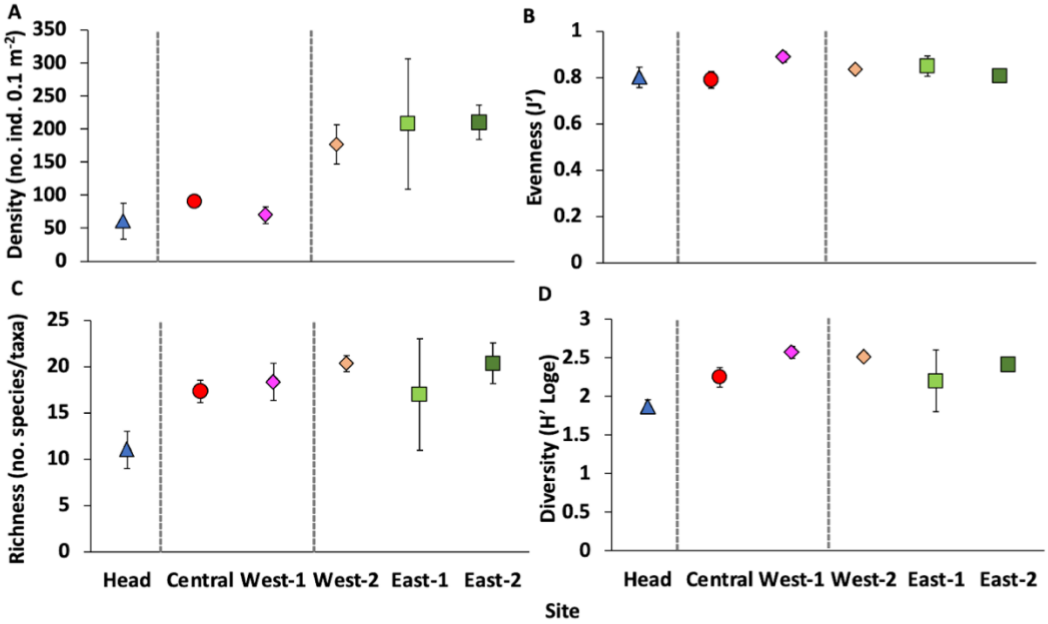


**Figure 2.6.** Plots showing mean ±SE A) density (total number of individuals 0.1m$^{-2}$), B) evenness (J'), C) richness (no. species/taxa), D) Shannon diversity (H' loge) at sampling sites for 2020 in Placentia Bay, Newfoundland, total *n*=18 (2020).

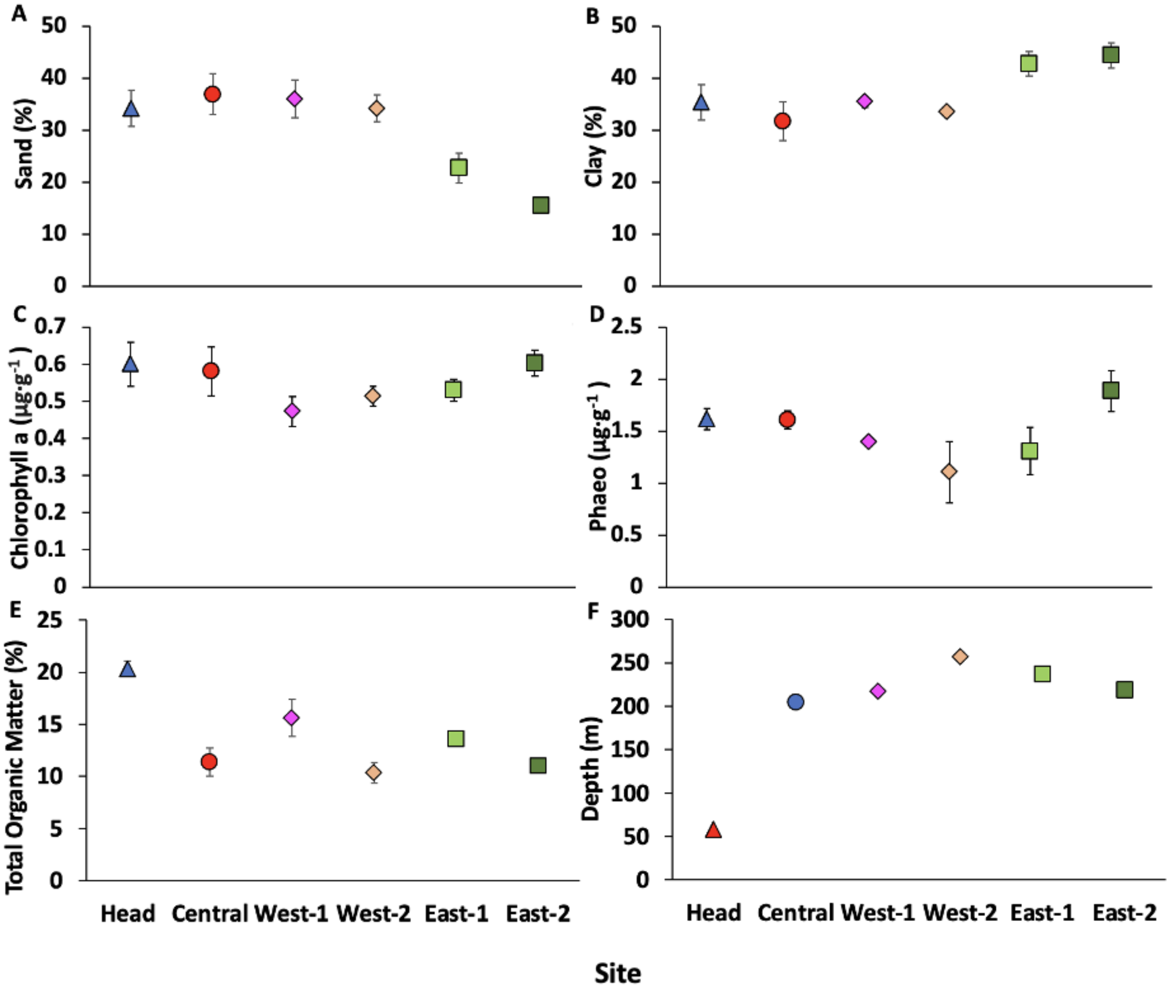
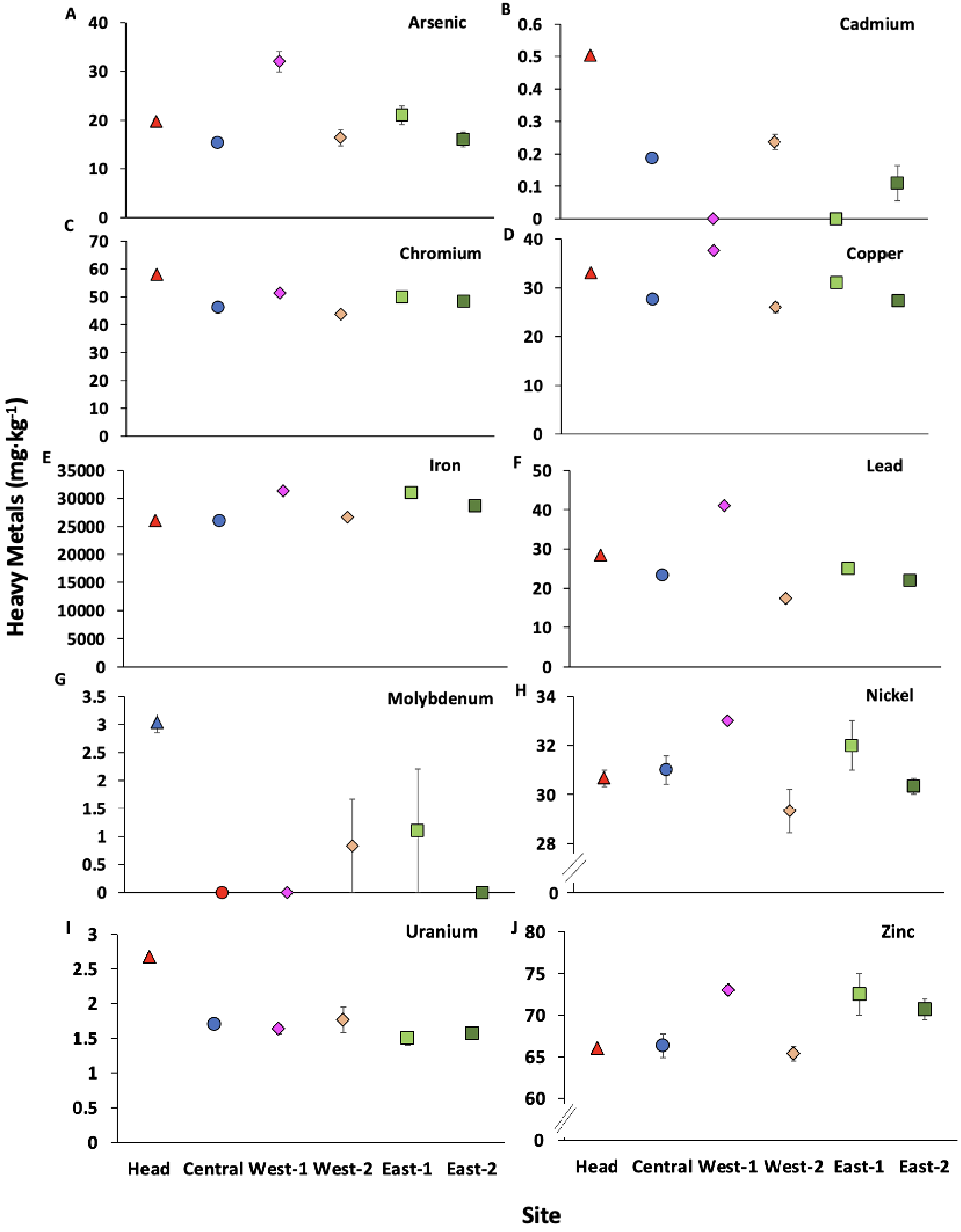


**Figure 2.7.** Plots comparing mean ±SE (*n*=18) for environmental variables including A) sand (%), B) clay (%), C) chl-*a* (µg·g$^{-1}$), D) phaeopigment (µg·g$^{-1}$), E) total organic matter (%), and F) depth (m) for sampling sites in 2020 in Placentia Bay, Newfoundland.



A
Arsenic
40
30
20
10
0
B
Cadmium
0.6
0.5
0.4
0.3
0.2
0.1
0
C
Chromium
70
60
50
40
30
20
10
0
D
Copper
40
30
20
10
0
E
Iron
35000
30000
25000
20000
15000
10000
5000
0
F
Lead
50
40
30
20
10
0
G
Molybdenum
3.5
3
2.5
2
1.5
1
0.5
0
H
Nickel
34
32
30
28
0
I
Uranium
3
2.5
2
1.5
1
0.5
0
J
Zinc
80
75
70
65
60
0
Heavy Metals (mg·kg-1)
Head
Central
West-1
West-2
East-1
East-2
Head
Central
West-1
West-2
East-1
East-2
Site

**Figure 2.8**. Plots comparing mean ±SE (*n*=17) for heavy metals including A) arsenic, B) cadmium, C) chromium, D) copper, E) lead, F) molybdenum, G) nickel, H) thallium, I) uranium and J) zinc (mg·kg$^{-1}$) for sampling sites in 2020 in Placentia Bay, Newfoundland. Note scale on y-axis differs among plots. Arsenic, chromium, and copper exceeded ISQGs at all sites and West-1 exceeded ISQGs and ERL for lead and nickel respectively (see Table 2.1).

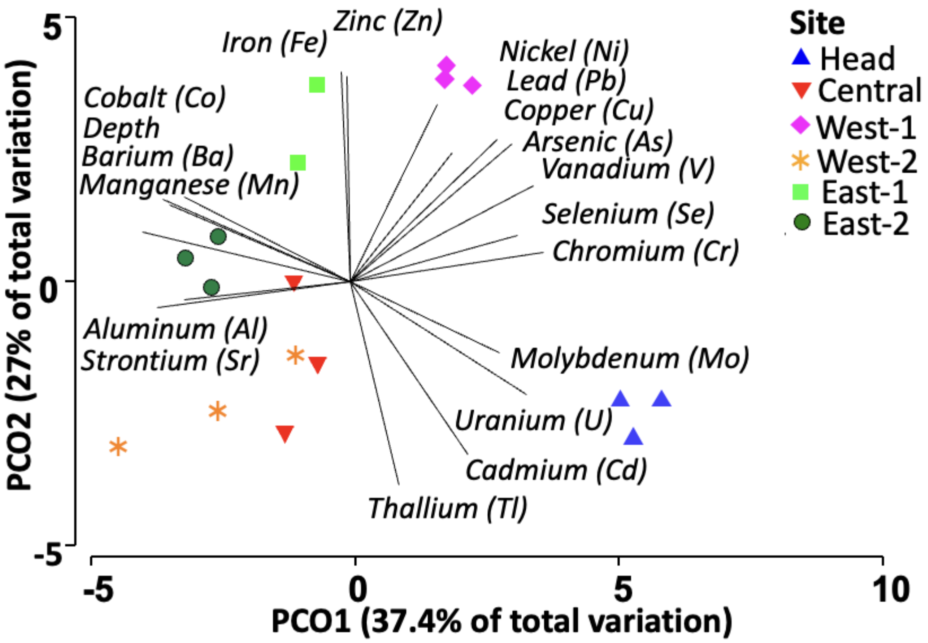


**Figure 2.9.** Principal Coordinates Ordination (PCO) of environmental samples based on Euclidean distance for samples collected in 2020 (total *n*=17). Colored symbols indicate different sites. Environmental vectors based on Pearson correlation of ≥ 0.7.

## Appendix II.

**Table A2.1**. Mean ±SE metal (mg·kg$^{-1}$) concentrations with correlations ≥ 0.7 for environmental groupings (i.e., based on Fig. 2.8) for sampling sites in 2020 (i.e., Head, Central, West-1, West-2, East-1, and East-2).

| Metal | Head | Central | West-1 | West-2 | East-1 | East-2 |
|---|---|---|---|---|---|---|
| Aluminum | 48666.7±666.7 | 51666.7±333.3 | 48666.7±333.3 | 52333.3±333.3 | 52500±500 | 53333.3±333.3 |
| Arsenic | 19.7±0.7 | 15.3±0.9 | 32.0±2.1 | 16.3±1.7 | 21.0±1.9 | 16.0±1.5 |
| Barium | 346.7±3.3 | 393.3±6.7 | 383.3±8.8 | 393.3±8.8 | 415.0±5.0 | 406.7±3.3 |
| Cadmium | 0.5±0.01 | 0.19±0.009 | 0.0±0.0 | 0.2±0.02 | 0.0±0.0 | 0.1±0.06 |
| Chromium | 58.0±0.6 | 46.3±1.5 | 51.3±0.9 | 43.7±1.5 | 50.0±1.0 | 48.3±1.5 |
| Cobalt | 7.3±0.03 | 7.6±0.0 | 7.9±0.06 | 8.2±0.2 | 8.3±0.3 | 8.2±0.1 |
| Copper | 33.0±0.0 | 27.7±0.9 | 37.7±0.3 | 26.0±1.0 | 31.0±1.0 | 27.3±0.3 |
| Iron | 26000±577.4 | 26000±577.4 | 31333.3±666.7 | 26666.7±333.3 | 31000±1000 | 28666.7±333.3 |
| Lead | 28.3±0.3 | 23.3±0.3 | 41.0±0.6 | 17.3±0.7 | 25.0±1.0 | 22.0±0.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Manganese | 400.0±0.0 | 473.3±3.3 | 453.3±6.7 | 490.0±5.8 | 490.0±10.0 | 513.3±3.3 |
| Molybdenum | 3.03±0.19 | 0±0 | 0±0 | 0.83±0.83 | 1.1±1.1 | 0±0 |
| Nickel | 30.7±0.3 | 31.0±0.6 | 33.0±0.0 | 29.3±0.9 | 32.0±1.0 | 30.3±0.3 |
| Selenium | 3.0±0.06 | 2.3±0.06 | 2.9±0.03 | 1.4±0.7 | 2.3±0.0 | 0.7±0.7 |
| Strontium | 163.3±6.7 | 253.3±6.7 | 210.0±5.8 | 243.3±6.7 | 195.0±15.0 | 226.7±3.3 |
| Thallium | 0.5±0.01 | 0.4±0.02 | 0.3±0.02 | 0.4±0.02 | 0.3±0.02 | 0.4±0.003 |
| Uranium | 2.7±0.03 | 1.7±0.06 | 1.6±0.07 | 1.8±0.19 | 1.5±0.1 | 1.6±0.03 |
| Vanadium | 100.0±0.0 | 79.3±0.9 | 100.0±0.0 | 78.3±0.9 | 91.5±0.8 | 83.3±0.7 |
| Zinc | 66.0±0.0 | 66.3±1.5 | 73.0±0.6 | 65.3±0.9 | 72.5±2.5 | 70.7±1.2 |

**Table A2.2**. Mean ±SE polyaromatic hydrocarbon carbons (mg·kg$^{-1}$) concentrations in sediments at sampling sites in Placentia Bay, Newfoundland.

| PAHs | Head | Central | West-1 | West-2 | East-1 | East-2 |
|---|---|---|---|---|---|---|
| Benzo(a)anthracene | 0.016±0.0078 | 0±0 | 0.022±0 | 0±0 | 0.022±0.0005 | 0.021±0.0017 |
| Benzo(a)pyrene | 0.016±0.0078 | 0.021±0.00058 | 0.022±0 | 0±0 | 0.022±0.0005 | 0.021±0.0017 |
| Benzo(b)fluoranthene | 0.033±0.0012 | 0.024±0.00088 | 0.034±0.00033 | 0.013±0.0068 | 0.030±0.0005 | 0.028±0.0018 |
| Benzo(b/j)fluoranthene | 0.033±0.0012 | 0.024±0.00088 | 0.034±0.00033 | 0.013±0.0068 | 0.030±0.0005 | 0.034±0.0071 |
| Benzo(g,h,i)perylene | 0.030±0.00058 | 0.025±0.00088 | 0.034±0.00033 | 0.014±0.0069 | 0.026±0 | 0.026±0.0015 |
| Chrysene | 0.030±0.001 | 0.023±0.0012 | 0.032±0.00033 | 0.011±0.0058 | 0.030±0.0020 | 0.027±0.0022 |
| Fluoranthene | 0.061±0.0022 | 0.043±0.0020 | 0.064±0.0045 | 0.028±0.0032 | 0.070±0.010 | 0.057±0.0029 |
| Indeno(1,2,3-cd)pyrene | 0.024±0.00058 | 0.019±0.00067 | 0.027±0.00033 | 0.011±0.0056 | 0.021±0 | 0.021±0.0012 |
| Perylene | 0.12±0.0058 | 0.051±0 | 0.11±0.010 | 0.043±0.0075 | 0.050±0.0010 | 0.058±0.0044 |
| Phenanthrene | 0.036±0.0015 | 0.029±0.0031 | 0.048±0.012 | 0.020±0.0020 | 0.067±0.030 | 0.044±0.0023 |
| Pyrene | 0.050±0.0015 | 0.036±0.0015 | 0.052±0.0048 | 0.023±0.0024 | 0.055±0.013 | 0.044±0.0020 |

**Table A2.3**. Mean ±SE for depth, sediment grain size (sand, silt, clay), total organic matter (TOM), chl-*a*, chl-*a*/phaeopigment ratio, bottom and surface chl-*a* in Placentia Bay, Newfoundland for 2019 (*n*=3, except for sediments at the head of the Bay where *n*=2).

| **Site** | **Sample size (*n*)** | **Depth (m)** | **Sand (%)** | **Silt (%)** | **Clay (%)** | **TOM (%)** | **Chl- *a*** | **Phaeo** | **Chl-*a*/ Phaeo** | **Bottom chl-*a*** | **Surface chl-*a*** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Head | 3 | 63 | 29.5 ±0.5 | 33.5 ±1.5 | 36.5 ±1.5 | 19.4 ±0.2 | 0.98 ±0.3 | 3.1 ±1.2 | 0.3 ±0.01 | 0.27 ±0.02 | 2.6 ±0.04 |
| Central | 3 | 188 | 25.3 ±1.8 | 39.7 ±3.5 | 35.0 ±4.0 | 13.9 ±0.2 | 0.69 ±0.06 | 2.4 ±0.3 | 0.3 ±0.01 | 0.2 ±0.007 | 2.3 ±0.07 |
| Shelf | 3 | 232 | 26.1 ±3.8 | 45.0 ±1.2 | 24.1 ±1.8 | 5.5 ±0.3 | 1.5 ±0.2 | 5.4 ±0.5 | 0.3 ±0.01 | 0.3 ±0.002 | 2.3 ±0.05 |
| East-1 | 3 | 237 | 16.3 ±1.2 | 33.0 ±3.1 | 50.7 ±3.8 | 13.5 ±0.09 | 0.5 ±0.19 | 1.7 ±0.6 | 0.3 ±0.009 | 0.2 ±0.002 | 2.5 ±0.04 |
| West-1 | 3 | 223 | 14 ±1.7 | 35.7 ±1.5 | 50.3 ±0.3 | 16.1 ±0.3 | 0.6 ±0.06 | 1.4 ±0.1 | 0.4 ±0.01 | 0.2 ±0.003 | 2.7 ±0.02 |
| Outer | 3 | 233 | 32.3 ±2.9 | 48.6 ±1.1 | 19.1 ±2.6 | 4.0 ±0.3 | 2.3 ±0.5 | 7.9 ±1.5 | 0.3 ±0.01 | 0.2 ± 0.0007 | 2.0 ±0.08 |

**Table A2.4**. Mean ±SE for depth, sediment grain size (sand, silt, clay), total organic matter (TOM), chl-*a*, chl-*a*/phaeopigment ratio, bottom and surface chl-*a*, and total metals and PAHs sites in Placentia Bay Newfoundland in 2020 (*n*=3 for all variables except total metals and PAHs where *n*=2).

| **Site** | **Sample size (*n*)** | **Depth (m)** | **Sand (%)** | **Silt (%)** | **Clay (%)** | **TOM (%)** | **Chl-*a*** | **Phaeo** | **Chl-*a*/Phaeo** | **Total metals (mg/kg)** | **Total PAHs (mg/kg)** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Head | 3 | 58 | 34.2 ±3.5 | 30.3 ±0.2 | 35.3 ±3.4 | 20.3 ±0.7 | 0.6 ± 0.06 | 1.6 ±0.1 | 0.4 ±0.01 | 142.9 ±1.2 | 285.9 ±2.3 |
| Central | 3 | 204 | 36.9 ±4.0 | 31.4 ±1.3 | 31.7 ±3.7 | 11.4 ±1.4 | 0.6 ± 0.07 | 1.6 ±0.09 | 0.4 ±0.04 | 283.3 ±1.7 | 566.6 ±3.5 |
| East-1 | 3 | 237 | 22.7 ±2.9 | 35.0 ±1.3 | 42.7 ±2.4 | 13.6 ±0.19 | 0.5 ± 0.03 | 1.3 ±0.2 | 0.5 ±0.07 | 300.2 ±1.0 | 600.6 ±2.0 |
| East-2 | 3 | 219 | 15.4 ±1.2 | 40.6 ±1.1 | 44.3 ±4.4 | 18.2 ±7.2 | 0.6 ± 0.04 | 1.9 ±0.2 | 0.3 ±0.02 | 296.0 ±10.2 | 592.0 ±20.5 |
| West-1 | 3 | 217 | 35.9 ±3.6 | 28.7 ±2.6 | 35.4 ±1.0 | 15.6 ±1.8 | 0.5 ± 0.04 | 1.4 ±0.03 | 0.3 ±0.02 | 295.5 ±2.1 | 591.0 ±4.3 |
| West-2 | 3 | 257 | 34.1 ±2.6 | 32.7 ±2.2 | 33.5 ±0.8 | 10.3 ±1.0 | 0.5 ± 0.03 | 1.9 ±0.3 | 0.3 ±0.04 | 334.0 ±1.2 | 668.1 ±2.4 |

**Table A2.5.** Test results (Shapiro-Wilk, Levene's, Mann-Whitney U, and t-test) conducted on community metric (richness, density, evenness, and diversity) for 2019 vs. 2020 for shared sample sites. Transformation used to normalize data indicated.

| | 2019 *n*=12 | 2020 *n*= 12 | | | |
|---|---|---|---|---|---|
| **Response** | **Shapiro-Wilk** | **Shapiro-Wilk** | **Levene's** | **Mann-Whitney U** | **t-test** |
| Richness | W=0.963<br>P=0.823 | W=0.965<br>P=0.853 | $F_{1,22}$=0.520<br>P=0.478 | U=86.5<br>P=0.418 | $T_{22}$=0.989<br>P=0.333 |
| Density | W=0.696<br>P=<0.001 | W=0.914<br>P=0.239 | $F_{1,22}$=0.345<br>P=0.563 | U=104.0<br>P=0.068 | $T_{22}$=1.27<br>P=0.218 |
| Evenness | W=0.930<br>P=0.379 | W=0.891<br>P=0.121 | $F_{1,22}$=0.8<br>P=0.380 | U=46.0<br>P=0.143 | $T_{22}$=-1.63<br>P=0.118 |
| Diversity | W=0.839<br>P=0.027 | W=0.976<br>P=0.962 | $F_{1,22}$=1.044<br>P=0.318 | U=72.0<br>P=1.000 | $T_{22}$=0.020<br>P=0.984 |

**Table A2.6.** Test results (Shapiro-Wilk, Levene’s, Mann-Whitney U, and t-test) conducted on environmental variables (sand, silt, TOM, chl-*a*, phaeopigment, and chl-*a*/phaeo ratio) data between 2019 vs. 2020 for shared sample sites. Transformation used to normalize data indicated.

| | 2019 *n*=11 | 2020 *n*= 12 | | | |
|---|---|---|---|---|---|
| **Response** | **normality** | **normality** | **Levene’s** | **Mann-whitney U** | **t-test** |
| Sand | W=0.913<br>P=0.262 | W=0.935<br>P=0.431 | $F_{1,21}$=0.126<br>P=0.726 | U=16.0<br>P=0.002 | $T_{21}$=-3.85<br>P=<0.001 |
| Silt | W=0.951<br>P=0.652 | W=0.962<br>P=0.818 | $F_{1,21}$=1.08<br>P=0.311 | U=103.0<br>P=0.024 | $T_{21}$=2.51<br>P=0.020 |
| TOM<br>No transformation | W=0.832<br>P=0.022 | W=0.950<br>P=0.643 | $F_{1,22}$=3.77<br>P=0.065 | U=83.0<br>P=0.551 | $T_{22}$=0.372<br>P=0.551 |
| Chl-*a*<br>No transformation | W=0.869<br>P=0.064 | W=0.946<br>P=0.579 | $F_{1,22}$=5.73<br>P=0.026 | U=91.0<br>P=0.291 | $T_{22}$=1.095<br>P=0.285 |
| Phaeopigment<br>No transformation | W=0.854<br>P=0.041 | W=0.887<br>P=0.107 | $F_{1,22}$=7.88<br>P=0.010 | U=92.0<br>P=0.266 | $T_{22}$=1.57<br>P=0.130 |
| Chl-*a/*phaeo ratio | W=0.882<br>P=0.093 | W=0.900<br>P=0.158 | $F_{1,22}$=2.50<br>P=0.128 | U=32.0<br>P=0.020 | $T_{22}$=-2.49<br>P=0.021 |

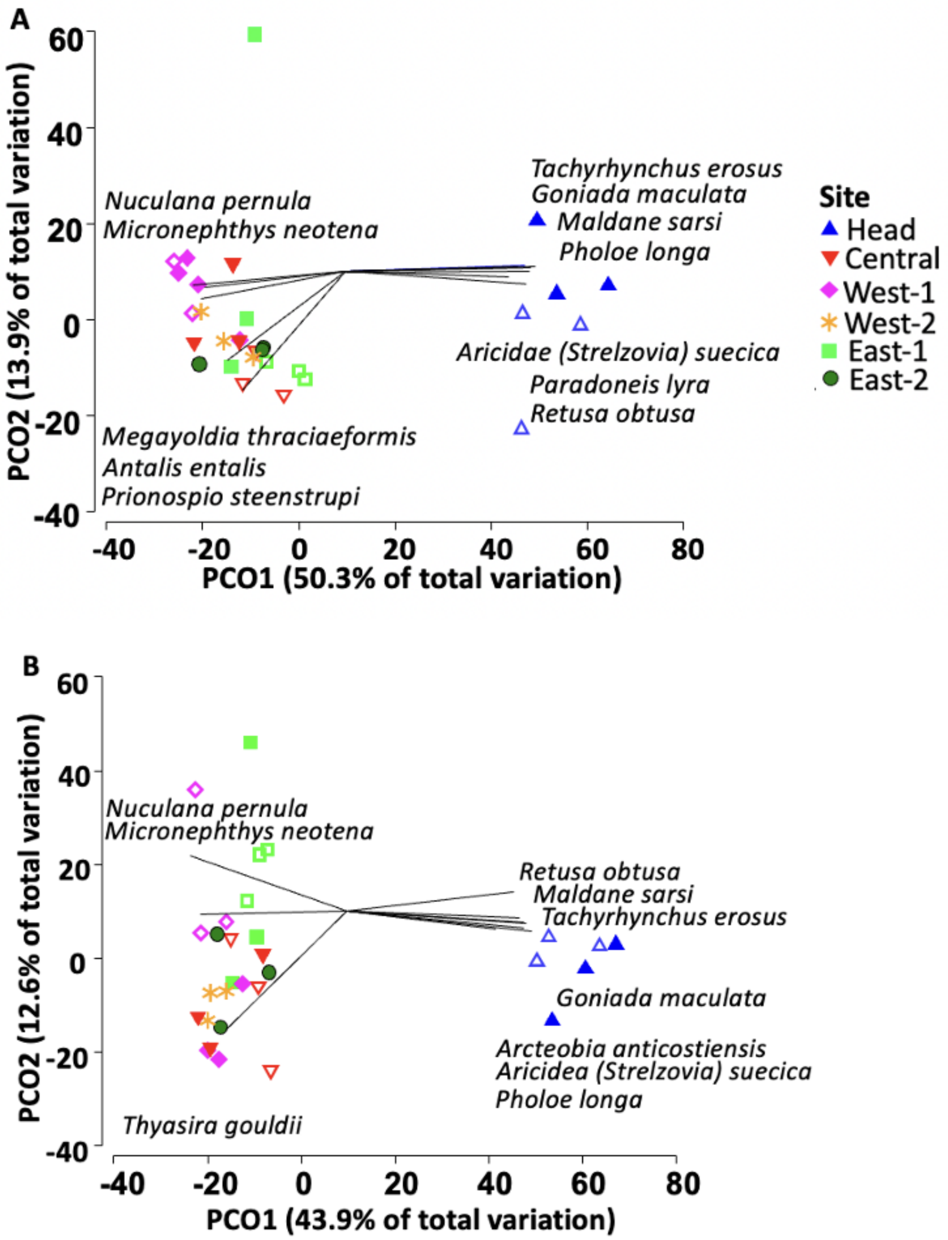


**Figure A2.1.** Principal Coordinates Ordination (PCO) of biological samples of species/taxa A) presence/absence matrix based on Sorensen resemblance, and B) biomass (g) for sites in

Placentia Bay, Newfoundland for A) 2019 and 2020 (total *n*=30). Colored symbols indicate different sites (no fill=2019; filled=2020). Species vectors based on Pearson correlation of ≥0.7.

## Thesis Conclusion

Studies such as this one, examining short and long-term spatio-temporal patterns in community structure, provide a means to understand biological and environmental changes (Macdonald & Cote, 2014). Quantifying these changes is key in identifying future change in communities and habitat due to anthropogenic or other stressors in Placentia Bay. The aim of my thesis was to assess the influence of environmental factors on community structure and functional traits of macrofauna using historical (1998) and contemporary (2019–2020) data for the EBSA Placentia Bay, Newfoundland. Overall, the initial hypotheses were consistent with the results. Community and functional structure in Placentia Bay did significantly differ between historical and contemporary times and were influenced by the sedimentary habitat. Moreover, trait modalities were maintained between these two time points (i.e., no loss of modalities). Examining contemporary communities (2019–2020) indicated that there was no significant difference in macrofaunal community composition and abundance between years, but communities did differ spatially among locations in the bay.

Interestingly, there has been a large reduction in polychaete densities between historical to contemporary time points and an increase in the relative proportion of other major taxonomic groups, most notably the bivalves. The historical macrofaunal community and associated functional modalities had significantly higher density, but lower evenness and diversity compared to contemporary years. Specifically, historical macrofaunal communities in the bay were dominated by highly tolerant (82.3%) subsurface deposit feeders (62.1%) having small body sizes whereas contemporary communities showed an increase in proportion of medium tolerant species/taxa (7.2 vs. 36.7%) and nearly equal proportions subsurface and surface deposit feeders (26.7 and 29.1%) with small-medium body sizes. While macrofaunal community composition and diversity in the bay has changed substantially, spatial patterns among inshore sites have remained remarkably consistent. Spatially, sites within the bay showed clear biological and environmental differences. The head of the bay was most distinct and had the lowest density, richness, and diversity and high TOM relative to other sites.

Placentia Bay, NL has shown significant changes in its sedimentary habitat from historical to contemporary times with respect to grain size and TOM. Sediments in the bay have become coarser and TOM has increased. Sediment grain size is generally expected to be stable over time (Herder et al. 2021; Taghon et al. 2017) suggesting this change might be a result of disturbance in the bay such as wave action from storms. Changes in sediments could also be due to potential changes in flow or current. In both 2019 and 2020, days prior to sampling, hurricanes affected Newfoundland through storms, wave action, and heavy precipitation. Disturbances from such events in coastal regions may alter the sedimentary habitat through erosion and deposition as well as input of freshwater sources (i.e., precipitation). Additional sediment grain size data (2019–2021) showed that sediments have also become coarser at all sites within the bay. With respect to inshore macrofaunal communities examined in 2020, depth and heavy metals were correlated to observed patterns. The head of the bay and West-1 generally had the highest concentrations of heavy metals, and arsenic, chromium, and copper were above ISQGs at all sites. At West-1, lead and nickel also exceeded guidelines such that they were at levels that are occasionally associated with adverse biological effects. Despite potential concerns of oil exposure in Placentia Bay from shipping traffic, PAHs were unrelated to community structure.

A limitation of the present study is the lack of continuous sampling and discrepancy of season between historical and contemporary time points. Examining seasonal variability should be prioritized to better quantify the extent of year-to-year variation (Holland 1985; Holland et al. 1987) especially for summer months (i.e., June–July). Such information can provide insight into how levels of organic matter may have changed over time, as well as food quality and the possible source(s) of organic matter. Placement of current meters at established study sites is also recommended to confirm whether bottom flows during storm events is a plausible mechanism for the observed changes in sediment type. Changes in salinity and dissolved oxygen should also be examined as they play an important role in structuring benthic communities and can be affected by large inputs of rainfall (i.e., generally a result of storm surges and hurricanes) (Taghon et al. 2017). Despite the influence heavy metals may have on benthic communities, there is a general lack of information pertaining to their concentrations in marine sediments and species-specific effects (Ramey et al. 2011; Borja and Dauer 2008). More information is needed with respect to acceptable contaminant levels and possible biological outcomes as development continues in coastal regions such as Placentia Bay.

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