

TEMPORAL AND SPATIAL ANALYSIS OF THE RELATIONSHIP BETWEEN HYPOXIA,
TEMPERATURE, AND BENTHIC BIODIVERSITY IN THE ST LAWRENCE ESTUARY AND GULF

by

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ABSTRACT

This thesis explores temporal changes, and spatial patterns of marine benthic communities in Canada's Lower St. Lawrence Estuary (LSLE) and Gulf of St. Lawrence (GSL) and finds benthic biodiversity consistent with responses to an altered environment. Dissolved oxygen in the LSLE has slowly and continuously declined for decades with no seasonal or episodic return to normal oxygen levels. Temporal analysis of sedimentary fauna from the region confirmed significant reductions in benthic species richness, evenness, and Shannon diversity between 1980 and 2005/6, but higher abundance in 2005/6 samples, a pattern seen in studies of succession following disturbance. These analyses identified multiple contributors to this shift beyond opportunistic species, including species displaying a range of tolerances for organic disturbance and minimal capacity to cope with hypoxia. Surprisingly, mean richness and Shannon diversity in the LSLE exceeded that in the GSL in locations of lowest oxygen concentration, noting no documentation of environmental shifts by 2005 in the GSL. Comparison of stations grouped by oxygen concentrations indicated a clear biological threshold in the LSLE by 2005 that excluded most omnivores and subsurface deposit feeders. Analysis of benthic communities in 2015 indicated significantly higher diversity and differences in assemblages between those near the southwest coast of Newfoundland and those at the intersection of the Laurentian and Esquiman Channels. Port au Port Bay stations supported the highest number of families tolerant of eutrophication and hypoxia. A shift to a higher proportion of surface deposit feeders and to organisms more tolerant of disturbance characterized stations in the Laurentian/Esquiman Channel in 2015 compared to nearby stations in 2006. These changes in benthos, combined with significant reductions in oxygen concentrations and rising temperatures in the eastern GSL suggest increasing risk of benthic community alteration in this region. Changes in LSLE and GSL benthos reported here indicate a need for ongoing efforts to reduce sources of environmental alteration in these ecologically important regions.

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Table of Contents

ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
LIST OF FIGURES	vi
LIST OF TABLES	viii
LIST OF ABBREVIATIONS	x
CO-AUTHORSHIP STATEMENT	xi
CHAPTER 1: GENERAL INTRODUCTION	1
1.1 The Importance of Biodiversity for Benthic Ecosystem Functioning	1
1.2 Hypoxia as a Major Threat to Biodiversity	2
1.3 Challenges in Documenting Hypoxia Effects on Biodiversity in Contrasting Habitats ..	4
1.4 Hypoxia and Eutrophication Effects in Estuaries	6
1.5 Hypoxia and Biodiversity in the St. Lawrence Estuary and Gulf of St Lawrence	8
1.6 Purpose of This Research: Benthic Biodiversity Responses to Hypoxia	11
Hypotheses:	11
1.7 References	12
CHAPTER 2: TEMPORAL ANALYSIS OF HYPOXIA IMPACTS ON THE ST. LAWRENCE ESTUARY AND GULF (1980, 2005, AND 2006)	20
2.1 INTRODUCTION	20
2.2 METHODS	25
2.3 RESULTS	28
2.4 DISCUSSION	31
2.5 CONCLUSION	39
2.6 FIGURES AND TABLES	41
2.7 REFERENCES	50
CHAPTER 3: SPATIAL ANALYSIS OF HYPOXIA IMPACTS ON THE ST. LAWRENCE ESTUARY AND GULF (2005 AND 2006)	56
3.1 INTRODUCTION	56
3.2 METHODS	62
3.3 RESULTS	67
3.4 DISCUSSION	71
3.5 CONCLUSIONS	79
3.6 FIGURES AND TABLES	80
3.7 REFERENCES	98
CHAPTER 4: REVISITING HYPOXIA IMPACTS ON THE ST. LAWRENCE ESTUARY AND GULF: A DECADE LATER	103
4.1 INTRODUCTION	103
4.2 METHODS	105
4.3 RESULTS	108
4.4 DISCUSSION	111
4.5 CONCLUSION	115
4.6 TABLES AND FIGURES	117

4.7 REFERENCES.....	126
CHAPTER 5: SUMMARY OF THIS THESIS	130
5.1 Benthic responses in a permanently hypoxic region	130
5.2 Biodiversity and ecosystem function changes in the LSLE and GSL.....	133
5.3 Taxonomic Sufficiency to Detect Hypoxic and Eutrophic Effects on Benthic Biota	134
5.4 Limitations of this thesis.....	136
5.5 Areas for future research	137
5.6 References.....	138
APPENDICES	140

LIST OF FIGURES

Figure 2.1. Stations sampled in the Lower St Lawrence estuary in 1980 (Ouellet 1982) and resampled on 2005 and 2006 cruises.....	41
Figure 2.2a. Comparison of mean station density in 1980 and 2005/6 measured by Individual/0.125 m ² . Error bars represent standard error of the mean.....	42
Figure 2.2b. Comparison of mean station density in 1980 and 2005/6 of the four most abundant phyla measured by Individual/0.125 m ² . Error bars represent standard error of the mean.....	42
Figure 2.2c,d. Mean univariate measures of richness, evenness, and Shannon diversity of c)species and d)families from 1980, 2005 and 2006 cruises. Vertical bars indicate the standard error of each mean.....	43
Figure 2.3a. Mean abundance of of functional groups measured by Individuals/0.125 m ² . SDF=surface deposit feeders, SBDF=subsurface deposit feeders, FF=filter feeders, C=carnivores, O=omnivores, ND=unidentified specimens.....	44
Figure 2.3b Ratio of SDF (surface deposit feeders) to O (Omnivores) based on mean abundance shown in Figure 2.3a.....	44
Figure 3.1 Upper map of North America shows the location of the study area of the 2005/6 cruises that occurred in Quebec, Canada. Lower map shows stations sampled during the 2005 and 2006 research cruises. LSLE refers to the Lower St Lawrence Estuary.....	80
Figure 3.2. Most westerly stations of the 2005 cruise in the Lower St Lawrence estuary. Station 23 was sampled during both the 2005 and 2006 cruises.....	81
Figure 3.3a Dendrogram of station groupings based on family similarity by the CLUSTER procedure. Stations differentiated into Groups at different levels of similarity. IC_B was not placed in a group and not considered further.....	82
Figure 3.3b Mean oxygen % saturation of groups formed through CLUSTER. The location of each group within the LSLE and GSL is indicated in brackets.....	83
Figure 3.4a Distance based redundancy analysis of the relationship between environmental factors and species assemblages. The vector direction and	

length is proportional to the amount of variation each environmental factor contributes to the variation in biota accounted for by each axis. Station 23.1 refers to samples obtained at station 23 in 2005, and 23.2 samples were obtained in 2006.....84

Figure 3.4b. Distance based redundancy analysis of the relationship between environmental factors and Family assemblages at the 2005 and 2006 stations. The vector direction and length are proportional to the amount of variation each environmental factor contributes to the variation in biota accounted for by each axis. Station 23.1 refers to samples obtained from station 23 in in 2005, and station 23.2 refers to samples from station 23 obtained in 2006.....85

Figure 3.5. Functional Group composition at each station showing percentage abundance of each group. Station 23_1 refers to station 23 samples obtained in 2005, and 23_2 refers to station 23 samples obtained in 2006. Stations from top to bottom of figure are presented approximately in a west to east sequence, from head of the LSLE to the Esquiman channel.....86

Fig 3.6 PERMANOVA+ tests of difference of species a) richness, b) evenness and c) Shannon diversity between pairs of oxygen groups. Bars indicate standard error of each mean. Only oxygen groups with different letters are significantly different.....88

Fig 3.7 PERMANOVA+ tests of difference of family a) richness, b) evenness and c) Shannon diversity between pairs of oxygen groups. Bars indicate standard error of each mean. Only oxygen groups with different letters are significantly different.....88

Figure 3.8. Mean abundance of surface deposit feeders (SDF) and omnivores (O) in the 4 oxygen groups and the SDF to omnivore ratio. Error bars represent errors of the mean abundance for each functional group.....90

Figure 4.1. Upper map of eastern North America shows the location of the 2015 cruise in Quebec, Canada. Lower map shows stations sampled from June 15-17, 2015 aboard the RV Coriolis II in the Gulf of the St. Lawrence River. Red symbols indicate 2015 stations. Blue symbols indicate 2006 stations used for comparison.....117

Figure 4.2 Results of PERMANOVA + testing of differences in mean family a) richness, b) evenness and c) Shannon diversity between pairs of 2015 cruise stations. Bars represent standard errors of each mean. Only stations with different letters are significantly different.....118

Figure 4.3. Temporal qualitative comparison of richness, evenness, and Shannon diversity between a) station IC sampled in 2006 and B4 sampled in 2015 and b) station 16 sampled in 2006 and station T3 sampled in 2015. Latitude and longitude of each station is shown.....119

Figure 4.4. CLUSTER analysis dendrogram of stations based on similarity of family composition. Letters A, B and C refer to groups generated through the CLUSTER procedure.....120

Figure 4.5 CLUSTER analysis dendrogram of 2015 cruise stations grouped together based on similarity of functional group composition. Letters A, B, C, and D refer to the groups generated through CLUSTER.....121

Figure 4.6 Mean a)SDF, b)omnivores and c)SBDF of the stations sampled on the 2015 cruise. Error bars represent standard error of each mean.....122

Figure 4.7 Temporal qualitative comparison of Functional Group Density between a) station IC sampled in 2006 and station B4 sampled in 2015, and b) station 16 sampled in 2006 and station T3 sampled in 2015.....123

LIST OF TABLES

Table 2.1 Latitude and longitude of stations of 1980, 2005, and 2006 cruises.....45

Table 2.2. Results of the PERMANOVA procedure comparing benthic composition of a) species and b) families in 1980, 2005 and 2006.....45

Table 2.3 PERMANOVA+ Pair-wise testing of differences in a) species and b) family composition between 1980, 2005 and 2006, showing t values and probability (P) for each year compared. Significant results shown in bold.....46

Table 2.4 SIMPER determination of the a) species and b) families with the greatest dissimilarity in abundance between 1980 and 2005. Mean dissimilarities between time periods were 79.51 for species and 56.54 for families.....47

Table 2.5 Bioturbation ratings and contribution to dissimilarity of species that either decreased or Increased the most in abundance between 1980 and 2005. Reworking is scored from 1-5 for degree of alteration of sediment and Mobility is scored from 1-4 with higher scores indicating more activity. Ratings obtained

from or based on Queiros et al. (2013). Species no longer present in 2005/6 were identified as eliminated, while species only present in 2005/6 are marked as new.
.....49

Table 2.6. ANOSIM test of pair-wise difference in functional group compositions between years. Significant results are bolded. For meaning of abbreviations see section 2.2.....50

Table 3.1. Latitude and longitude of stations of 2005 and 2006 cruises used in the spatial analysis.....91

Table 3.2 Grouping of stations of the Lower St Lawrence Estuary and Gulf of St Lawrence according to their % saturation of oxygen (based on Belley et al., 2010).....91

Table 3.3 Results of CLUSTER analysis by similarity of family composition. The 3 most abundant families of each group, the mean abundance of each family and the family’s percentage contribution to the total abundance are shown.....92

Table 3.4a Results of Step-wise distance-based linear modeling (DISTLM) of differences in benthic species assemblages due to environmental factors from 2005/2006 data. Selection criteria was AICc. P is significance level. Proportion of correlation for each variable and cumulative total correlation with species assemblages are shown.....94

Table 3.4b. Results of Step-wise distance-based linear modeling (DISTLM) of differences in benthic family assemblages due to environmental factors from 2005/2006 data. Selection criteria was AICc. P is significance level. Proportion of correlation for each variable and cumulative total correlation with family assemblages are shown.....94

Table 3.5 Results of PERMANOVA+ tests of differences in composition of a) species and b) families among oxygen groups.....95

Table 3.6. SIMPER results showing the a) species and b) families that most contributed to the benthic dissimilarity between the Hypoxic and High oxygen groups. Mean dissimilarity between species was 82.87% and between families was 73.12%.....96

Table 3.7 Results of PERMANOVA+ comparisons of functional group composition between pairs of oxygen groups. Significantly different pairs

are shown in bold.....97

Table 4.1. Coordinates of stations sampled June 15-17, 2015, aboard the RV Coriolis II in the Gulf of St. Lawrence.....124

Table 4.2. Family-level SIMPER results for 2015 cluster data indicating the three largest contributors to each group. See Figure 4.4 for the list of samples in each group. Samples were collected June 15-17 aboard the RV Coriolis II in the Gulf of St. Lawrence.....124

Table 4.3 Functional group SIMPER results for 2015 cluster data indicating the largest contributors to each group. See Figure 4.5 for the list of samples in each group. Samples were collected June 15-17 2015 aboard the RV Coriolis II in the Gulf of St. Lawrence.....125

LIST OF ABBREVIATIONS

BEF - biodiversity and ecosystem functioning

C - carnivores

GSL- Gulf of St. Lawrence

LSLE- Lower St. Lawrence Estuary

ND- non-defined benthos

O - omnivores

OMZ - oxygen minimum zone, oxygen < 5mg/ml

SBDF - sub surface deposit feeders

SDF- surface deposit feeders

WoRMS - World Register of Marine Species

CO-AUTHORSHIP STATEMENT

The research presented in this thesis was designed by R. Steinhart, based on unpublished historical data and some new data, with assistance from committee members P.V.R. Snelgrove and P. Archambault. P.V.R. Snelgrove helped with thesis organization, writing, and presentation of data. All statistical analyses were conducted by R. Steinhart, with assistance from P.V.R. Snelgrove and P. Archambault.

CHAPTER 1: GENERAL INTRODUCTION

1.1 The Importance of Biodiversity for Benthic Ecosystem Functioning

Multiple studies suggest a causal link between benthic sedimentary biodiversity and ecosystem functioning. Laboratory experiments document a positive relationship between diversity of infaunal invertebrates and rates of nutrient regeneration (Ieno et al., 2006). Meta-analyses of in situ studies conclude that exponential increases in ecosystem functioning relate to benthic biodiversity (Danovaro, 2008; Loreau, 2008; Mora, et al., 2014) through increased rates of flow of energy and materials between abiotic and biotic components of ecosystems. Snelgrove (1997, 1999) reviewed and emphasized the extent of critical services provided by benthic biodiversity. However, meta-analysis of freshwater ecosystems (Covich et al., 2004) and recent field studies in marine systems suggest that the effects of changes in benthic biodiversity on ecosystem functioning depend more on the functional roles or specific biological traits of individual species rather than on species richness alone (Belley & Snelgrove, 2016; Biles et al., 2003).

Functional richness, but not taxonomic richness, improved community stability following experimental disturbance by benthic transplantation (Wahl et al., 2011). Similarly, the particularly negative effects of bottom trawling on biogeochemical cycles (Pusceddu et al., 2014) can cause ecosystem collapse associated with the elimination of ecosystem functions rather than species changes per se. Because rare functions tend to occur in high diversity species assemblages (Guillemot et al., 2011), even small decreases in biodiversity can lead to significant declines in secondary production (Micheli et al., 2014). These strong relationships between biodiversity and ecosystem functioning (BEF) also suggest an important role for benthic functional redundancy in vulnerable marine communities. Multiple studies conclude that functional redundancy increases functional stability in an ecosystem, because a species that fills a similar functional role to one that

disappears can help compensate for the loss of that species (Duarte, 2000; Levin et al., 2001; Montoya et al., 2012).

Recent syntheses recognize the importance of interactions between species and functional groups within a community or ecosystem in biodiversity-function relationships (Snelgrove et al., 2014). Even a single function such as carbon sequestration requires the interaction of multiple processes, and this interdependence of functions adds an additional dimension to the importance of benthic and functional diversity. Furthermore, the strongly context dependent nature of the biodiversity-ecosystem function relationship can vary across different abiotic gradients and through degrees of disturbances such as hypoxia (Norrko et al., 2015); this variation complicates understanding of this crucial relationship and emphasizes the need for further context and gradient centered BEF research.

In summary, marine ecosystems depend on benthic biota and benthic biodiversity for ecosystem functioning and essential services. Depending on conditions, spatial scales, and time frames, biodiversity may provide: (1) functional redundancy, (2) functional richness, (3) essential and unique species contributions, (4) multi-functionality, (5) complex species and functional group interactions, and (6) varying contributions to functions depending on context and degree of disturbance.

1.2 Hypoxia as a Major Threat to Biodiversity

Hypoxia in various marine environments threatens benthic and pelagic ecosystems. Diaz and Rosenberg (2011) reported at least 500 hypoxic or dead zones globally in 2011, and concluded that oxygen levels in the ocean have changed more than any biotic factor over the past 50 years. Hypoxia deleteriously affects the physiology of a wide variety of marine fauna. Major effects involve increasing or decreasing respiration, blood alkalosis (Fritsche, 1990; Hagerman & Uglow, 1985), and hypercapnia, which can reduce immune defenses and lead to increased vulnerability to infections (Diaz & Rosenberg, 2011; Tanner et al., 2006). Additional responses include alteration of movement and

behaviour patterns to avoid hypoxic conditions (Wannamaker & Rice, 2000), and decreasing rates of settlement, growth, and survival of some species (Baker & Mann, 1992; Secor & Gunderson, 1998). Furthermore, by reducing the maximum size of organisms, low oxygen can reduce functional efficiency and productivity (Norkko et al., 2013).

Although Levin and Gage (1998) argue that above an oxygen minimum threshold of 0.45 ml/l, oxygen concentration cannot predict macrobenthic biodiversity, numerous studies report serious consequences of hypoxia for sessile benthic organisms at concentrations of ≤ 2 ml of O₂/liter (Vaquer-Sunyer & Duarte, 2008). Below that concentration, benthic organisms behave aberrantly, such as by abandoning burrows (Diaz et al., 2008). Even 2mg/l (1.50 ml/l) of oxygen, a concentration often considered the lowest oxygen level that does not produce hypoxic effects, may limit ecosystem productivity; Timmerman (2012) modelled increased ecosystem biomass growth at an oxygen concentration of 4 mg/l, compared to 2 mg/l. Hypoxia and anoxia also reduce benthic biodiversity, increase the frequency of mass mortality events, and cause significant declines in secondary production (Diaz & Rosenberg, 2008; Melzner et al., 2013). In the Baltic Sea, for example, hypoxia has led to extensive benthic habitat loss and faunal loss with critical disturbance of benthic food webs (Conley et al., 2009). Because ocean warming increases organism metabolism, increased temperature could increase the oxygen concentration threshold affecting benthic biodiversity.

Hypoxia can also indirectly affect benthic marine organisms by altering their habitat. Hypoxia can affect sediment-water oxygen exchanges, decreasing essential re-oxidation processes and thereby increasing sulphide concentrations to levels toxic to many benthic macrofaunal species (Middelburg & Levin, 2009). Hypoxia also alters nitrogen cycles and fluxes; ammonium concentrations can increase in sediment and water as nitrification (re-oxidation) efficiency decreases (Middelburg & Levin, 2009). Similarly, hypoxia impairs iron and manganese re-oxidation and dissolved ions escape from the

sediment, depleting affected elements relative to normoxic sediments (Middelburg & Levin, 2009).

These hypoxia-driven sediment changes impair functioning of macrofauna that support bioturbation, bio-irrigation, and related biogeochemical processes (Middelburg & Levin 2009). In the Gulf of Mexico “dead zone”, community structure shifted and affected bioturbation; lower oxygen areas favoured dilators over compacters, reducing sediment shear strength and increasing its erodibility (Briggs et al., 2015). Increased hypoxic sedimentary habitats in the Baltic Sea released large amounts of dissolved inorganic phosphorus and reduced dissolved inorganic nitrogen, contributing to major diversity losses (Conley et al., 2009). As a result, Baltic Sea hypoxia not only results from, but also causes, exacerbates, and accelerates eutrophication impacts, increasing the susceptibility of this ecosystem to more frequent future episodes of hypoxia and eutrophication (Conley et al., 2009). In summary, research increasingly demonstrates the self-sustaining nature of hypoxia, where hypoxia extended in time and space alters benthic habitats towards conditions that favour continued or increased hypoxia, in a positive feedback loop that increasingly threatens biodiversity. This feedback loop illustrates the complexity of hypoxia effects on benthic functioning, which limits understanding of the relative importance of reduction in vulnerable species, direct abiotic changes, and the interaction between these two major factors on ecosystem functioning (Hillebrand and Matthiessen, 2009).

1.3 Challenges in Documenting Hypoxia Effects on Biodiversity in Contrasting Habitats

Distinct combinations of factors can cause hypoxia in different habitats, and hypoxia affects diversity differently in contrasting habitats, complicating understanding of the relationship between biodiversity and hypoxia. Hypoxia in the open ocean, distinct from mechanisms in coastal and estuarine environments, most often links to natural oxygen reduction, sometimes exacerbated by long-term climate changes (Ekau et al., 2010). Some consequences of hypoxia in pelagic habitats differ from effects in benthic habitats

because of the mobility of pelagic species and the vertical and horizontal expansions of oxygen minimum zones (OMZs) in the open ocean (Prince et al., 2006; Stramma et al., 2012). These distinctions limit generalizations about hypoxic benthic habitats from the many studies of large OMZs, though studies where OMZs intersect seafloor habitats offer considerable insights on persistent effects on benthos.

Anthropogenic and natural causes can both contribute to hypoxia, complicating understanding of current marine oxygenation patterns and in predicting future patterns of abiotic changes. Eutrophication associated with excess nutrient runoff often causes hypoxia in semi-enclosed seas such as the Gulf of Mexico. Continental shelves and upper slopes may experience oxygen minima as a result of recurrent strong upwelling of nutrients that increase phytoplankton production, which leads to rapid mass decomposition events and hypoxia when phytoplankton sink to the bottom (Helly & Levin, 2004). La Niña events can exacerbate hypoxia-inducing upwelling, particularly when these events coincide with seasonal oxygen minima (Nam et al., 2011) complicating prediction of hypoxia effects on diversity. The naturally generated hypoxic episodes that add to long-term abiotic fluctuations of marine habitats will select for tolerant organisms, such as oxygen regulating or conforming euphausiid species (Tremblay et al., 2020), in contrast to the increased frequency of anthropogenic episodes that are too recent for organisms to have evolved adaptations (Diaz & Rosenberg, 2008); distinguishing these two forms of hypoxia can help to inform conservation efforts.

Shorter time periods of studies to understand the effects of hypoxia on biodiversity (Baird et al., 2004) are important contributors to marine ecology, but few studies re-visit the same habitat over longer spans of time that could offer more information. Although some studies utilize a global or large-scale survey approach of hypoxic areas (Helly & Levin, 2004) or rely upon data and projections from published studies (Diaz & Rosenberg., 2008), these approaches cannot predict or fully account for specific changes

in particular ecosystems or communities because of differences in temporal patterns of hypoxic effects on different habitats, such as rate of oxygen depletion, or continuous versus punctuated episodes.

In summary, wide variation in the causes, development, and consequences of hypoxia in various contrasting marine environments limit extrapolation of understanding and research from one ecosystem to another, and therefore hamper understanding of the relationship between marine hypoxia and biodiversity. Therefore, the section below on hypoxia and its relationship to biodiversity solely considers estuarine and related gulf or coastal areas that form the focus of this thesis.

1.4 Hypoxia and Eutrophication Effects in Estuaries

As transitional ecosystems between freshwater rivers and oceans, estuaries provide essential ecosystem services, such as habitat provisioning for fish migrating between rivers and oceans, and nurseries for larval and juvenile development (Barbier et al., 2011). Geochemical functions include transformation of nutrients and industrial runoff that benefit downstream habitats, through filtering and detoxification of water by filter feeders and vegetation before it enters coastal and open ocean environments (Paerl et al., 1998). Estuaries can reduce flooding in coastal areas and reduce the impact of severe climate events (Barbier, 2015).

Unfortunately, several estuarine characteristics that contribute to critical ecosystem functions and services increase their vulnerability to disturbances. Estuarine ecosystems typically vary more in environmental characteristics than truly marine and freshwater shallow ecosystems, with rapid and significant fluctuations in abiotic factors (Laprise & Dodson, 1994; Levin et al., 2001). The industrial and agricultural run-off concentrated in estuarine and coastal areas increases vulnerability to eutrophication, particularly where circulation limits dilution and dispersion of anthropogenically elevated nutrients. (Altieri & Gedan, 2015). As far back as 2008, 415 coastal locales showed changes in

eutrophication, and researchers classified 169 areas as hypoxic as a result of the demands eutrophication placed on oxygen concentrations (Selman et al., 2008). Persistent effects of human activity, especially leaching of chemicals from land, exacerbated by extreme weather events that increase runoff, add to the increasing number of hypoxic coastal environments (Breitburg et al., 2018).

Hypoxia in estuarine benthic environments can arise from external drivers such as decomposition of organic compounds in runoff from adjacent watersheds runoff, or primary production driven events in the surface waters within the estuary such as phytoplankton blooms (Paerl et al., 1998). Given the lower salinity that characterizes estuarine environments compared to open coastal habitats, estuaries typically release less phosphorus bound to clay, and phosphorus limitation can exacerbate nitrogen pollution and, consequently, eutrophication (Howarth et al., 2011). Eutrophic estuaries can favour algal plankton blooms that contribute to hypoxia, but simultaneously reduce abundances of benthic macroalgae and rooted aquatic plants because of reduced water clarity, with cascading effects through to apex predators. Hypoxia can reduce estuarine ecosystem resilience and the transfer of energy in estuarine ecosystems to higher trophic levels, suggesting the potential for bottom-up trophic collapse; hypoxia diverts energy flow away from consumers and towards microbial decomposition pathways (Baird et al., 2004). The positive correlation between seafloor biodiversity and dissolved oxygen (Seitz et al., 2009) underscores the need to understand biodiversity changes linked to decline in dissolved oxygen saturation in order to minimize bottom-up effects of benthic decline on higher trophic levels.

Some studies predict elevated vulnerability of estuaries and coastal areas to climate change impacts given well documented increases in the extent and duration of eutrophication and anoxic “dead zone” formations in these habitats (Altieri & Gedan, 2015). Unfortunately, the confounding effects of non-hypoxia factors on biodiversity and functioning complicate the detection, quantification, and further understanding of

hypoxic effects on BEF, even within a single group of ecosystems such as estuaries. Distinct habitats significantly affect biodiversity patterns and ecosystem functioning through many factors. Benthic systems vary in sediment type, temperature, currents, depth, and the amount and type of organic carbon and nutrients delivered from adjacent habitats; all can affect biodiversity. Benthic biodiversity studies must also consider relationships to land, eutrophication, industrialization, fishing and other disturbances, and latitude/evolutionary history, which can roughly predict biodiversity trends (Roy et al., 2000). Because estuaries receive water flow from watersheds that vary in degrees of industrialization, estuaries can serve as “barometers” of anthropogenically-induced change. Their sensitivity to anthropogenic changes offers potential early evidence of biodiversity change that can inform conservation plans.

Temporal studies of diversity, which compare community composition and taxonomic and functional diversity in the same location over extended periods of time, simplify the problems of multiple confounding influences in contrasting habitats. In 1980, Ouellet (1982) sampled the benthic biodiversity in the St. Lawrence Estuary, a region with increasing hypoxia over the last 90 years. Further sampling in 2005 and 2006 offers a unique opportunity to evaluate how increasing hypoxia has impacted benthic biodiversity in the same habitats over a 25-year time span, potentially shedding light on strategies to address biodiversity conservation in areas of increasing hypoxia. These sampling efforts also allow comparisons between the benthic assemblages in this estuary and the less industrialized Gulf of St Lawrence, and a meaningful comparison of related but contrasting contiguous areas.

1.5 Hypoxia and Biodiversity in the St. Lawrence Estuary and Gulf of St Lawrence

The St. Lawrence Estuary, the largest estuary in the world, drains the Great Lakes and flows to the Atlantic Ocean through the province of Quebec, Canada. The St. Lawrence River transitions into an estuary near Ile d'Orleans, Quebec, and continues almost 250 km northeast where it widens into the saline Gulf of the St. Lawrence (GSL) (Fisheries

and Oceans Canada, 2017). Thousands of jobs throughout Quebec depend on commercial fishing and aquaculture, located at many ports of essential economic and social importance along the estuary (Fisheries and Oceans Canada, 2014). The habitat disturbances from these intense anthropogenic activities in the lower St Lawrence estuary (LSLE) threaten the diverse range of highly productive habitats that support a diverse range of species.

From the 1930s through 1984-2003, the St. Lawrence estuary became increasingly hypoxic; the drop from an average 125 $\mu\text{mol/L}$ to 65 $\mu\text{mol/L}$ over 50-70 years corresponded to almost a 50% decrease in oxygen saturation (Gilbert et al., 2005). Concurrent warming of deep estuarine waters by 1.65 $^{\circ}\text{C}$ during this time suggests that changes in the proportions of water mixing could explain about half to two thirds of the loss of oxygenated waters. Specifically, Gilbert et al. (2005) found that the proportion of warm, saline, hypoxic waters of the North Atlantic Central water mass, flowing into the estuary increased from 28% to 48%, and the influx of colder, less saline, oxygen-rich water from the Labrador Current decreased proportionally. Major changes in ocean circulation linked to climate change, such as a northward shift in the Gulf Stream could change the relative proportions of cold normoxic and warm hypoxic water entering the estuary. A pycnocline that extends from 100 to 250 meters depth separates the landward flowing deep hypoxic layer from the seaward flowing shallow layer, preventing the deep hypoxic layer from mixing or receiving oxygen inputs from the shallow normoxic layer.

Considerable evidence indicates that eutrophication has also contributed significantly to the development of hypoxia in the LSLE. Pelagic and benthic marine organisms increased significantly in abundance in the estuary over the past 40 years. Two LSLE sediment box core samples included the eutrophication tolerant foraminifera *Brizalina subaenariensis* and *Bulimina exilis*, and increases in $\delta^{13}\text{C}_{\text{ORG}}$ (from -24.8 to -24.0‰ and from -24.5 to -23.5‰). Both findings provide evidence of eutrophication beginning in

the late 1960's (Thibodeau et al., 2006). The shift in isotope proportions to favour C¹³ point to higher marine primary production (Burnett and Schaeffer, 1980), given that primary production utilizes C¹² preferentially. Some of the reduction in oxygen levels in the LSLE were likely driven by effluent-based eutrophication from the heavily industrialized areas of the LSLE and eroded coastal areas (Martineau et al., 2002); higher productivity would drive increased rates of oxidative decomposition of organic matter. Without replenishment of oxygen from the upper layers, increased organic import and re-mineralization at the sediment would lead to progressive hypoxia.

Gilbert et al. (2005) estimated the estuary average oxygen saturation from 1984-2003 to be only 20.7%. This level of oxygen can place organisms under great physiological stress, leading to changes in metabolic rates that can impact movement, growth, and reproduction (Laprise et al., 1994). Furthermore, the 1.65 °C average increase in estuary water temperature (Thibodeau et al., 2010a) reduces oxygen solubility and could indirectly increase the impact of hypoxia by raising the rate of benthic respiration and aerobic organic re-mineralization (Gilbert et al., 2005), as well as pelagic respiration (Bourgault et al., 2012). Shifts in LSLE macrofaunal assemblages to more hypoxia-tolerant species such as *Brizalina subaenariensis* and *Bulimina exilis*, (Thibodeau et al., 2006) and *Ophiura* sp. (Belley et al, 2010) add to the evidence of hypoxic stress.

Continuing risks of oil spills from shipping (CBC News, 2015; ABC News, 2022) in the LSLE/Gulf area increases the importance of knowledge regarding the trajectory of changes in macrofaunal communities, including potential impacts on benthic biodiversity over time and space and the health of the ecosystem (Puente & Diaz, 2008). The importance of this region for biodiversity and ecosystem functioning, and the significance of threats to the estuary underscore the decision to designate the St. Lawrence estuary as an Area of Interest under consideration for designation as a Marine Protected Area (Fisheries and Oceans Canada, 2015)

1.6 Purpose of This Research: Benthic Biodiversity Responses to Hypoxia

This research focuses on the Gulf of St. Lawrence and adjacent estuary with the aim of contributing new knowledge on estuary responses to threats, taking advantage of data from 1980 (Ouellet, 1982) and more recent sampling in 2005-2006 that details changes in spatial patterns in LSLE benthic community composition during those sampling periods. More recent data that includes detailed abiotic measurements link physical factors to biodiversity patterns in the benthos of the St. Lawrence Estuary and Gulf. Examination of temporal changes helps to identify diversity changes across the same region of the LSLE in relation to ongoing abiotic natural and anthropogenic habitat changes, rather than those associated with spatial variability across distinct habitats. These abiotic variables can, in turn, help to interpret temporal changes in benthic community composition. A fourth research cruise conducted in 2015 extends the examination of temporal patterns of diversity changes to span a 35-year period.

This research examines how hypoxic changes affect biodiversity and macrofaunal assemblages along the estuary and Gulf, and how these changes vary in both space and time for family, species, and functional group diversity, noting a particular gap in knowledge of functional group responses.

Hypotheses:

In order to address these questions, we test five alternate hypotheses

H_{a1}: The St. Lawrence Estuary benthic taxonomic and functional richness and diversity has significantly decreased over the past 25 years, based on comparisons of benthic community composition and diversity in 1980 and 2005/6 (Chapter 2). H_{a2}: Family, species, and functional group diversity respond differently over time to hypoxic and normoxic areas based on comparisons of species, family, and functional group diversity and composition between 1980 and 2005/6 (Chapter 2). H_{a3}: Hypoxia and higher temperature correlate negatively with biodiversity, as determined through spatial analysis of the relationships between the benthos and environmental factors of the St

Lawrence Estuary and Gulf of St Lawrence (Chapter 3).H_a4: Hypoxia significantly affects macrofaunal assemblages when controlling for sediment grain size and water temperature (Chapter 3).

Ha5: Benthic diversity and composition has not changed in the past 10 years in the Gulf of St Lawrence, based on comparisons of diversity measures and benthic composition in 2005/6 and 2015 (Chapter 4).

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CHAPTER 2: TEMPORAL ANALYSIS OF HYPOXIA IMPACTS ON THE ST. LAWRENCE ESTUARY AND GULF (1980, 2005, AND 2006)

2.1 INTRODUCTION

Variation in benthic macrofaunal community composition often correlates with factors unrelated to differences in bottom water oxygen concentration (Snelgrove & Butman, 1995), and interactions between oxygen concentrations and other environmental factors complicate comparisons of benthic communities to determine the biotic effects of hypoxia (Ahmerkamp et al., 2017; Ahmerkamp et al., 2020). Studies have documented variation in sediment composition, such as the degree of cohesion or water permeability among particles, as an important factor that can modify oxygen concentrations within the seabed (Hicks et al., 2017) and potentially alter the effect of hypoxia on benthic communities.

Whereas diffusion provides the central mechanism of water-sediment exchange of oxygen and solutes in cohesive (muddy) sediments, advection of pore water often plays a major role in regulating exchanges between bottom-water and permeable (sandy) sediments, increasing the exchange rates significantly compared to diffusive mechanisms in cohesive sediments (Ahmerkamp et al., 2020). The interaction of bottom currents and sediment ripples can lead to pressure gradients across the water-sediment interface that increases pore water flow rates and advection into the permeable sediment. This advection enhances oxygen penetration, and therefore the depth of the redox potential discontinuity (RPD), and simultaneously influences the relative amount of aerobic and anaerobic respiration occurring in the sediment (Ahmerkamp et al., 2017). Oxygen penetration was much greater and oxygen uptake much lower in a sandy site of the Celtic Sea compared to cohesive muddy sites (Hicks et al., 2017). This documented variation in sediment composition as an important factor that can modify oxygen concentrations within the seabed can potentially alter the effect of hypoxia on benthic communities.

Snelgrove and Butman (1995) reviewed evidence that sediment type can alter the availability of organic input that subsequently influences the distribution of deposit and suspension feeders. The proportion of cohesive muddy sediments correlates positively with higher content of organic matter, which may favour deposit feeders (Lee et al., 2019). Differences in the average organic matter content of particular sediment types could alter the rate of aerobic respiration and intensify or lessen the effects of low oxygen concentration and impact the proportion of hypoxia resistant organisms. Sediment surface area per sediment volume, but not grain size correlates positively with microbial abundance that, in turn, can also favour deposit feeders (Ahmerkamp et al., 2020). Boundary layer flow rather than grain size alone can alter pore water characteristics, which can influence sediment oxygen levels and potentially benthic assemblages, independently of boundary oxygen concentration (Ahmerkamp et al., 2017). In summary, sediment type, microbial activity, boundary flow, organic content, and the interactions between these factors all contribute to boundary layer and sediment oxygen concentrations, and therefore complicate efforts to measure and understand the biotic effects of hypoxic conditions, and generalize those findings to other geographic regions, including those with similar bottom oxygen concentrations.

Temperature can also significantly alter the tolerance of organisms to hypoxic conditions through two major mechanisms: the rate of oxygen consumption of the organism, and the solubility of oxygen in water. Benthic communities in regions with similar oxygen levels, but differing in overall or seasonal temperature, may experience different impacts (Vaquer-Sunyer & Duarte, 2011).

Temporal studies on changes in benthic diversity and composition following the development of hypoxia in a single region reduce the difficulties of separating hypoxia effects from the influence of other factors, such as temperature and sediment type, on

benthic communities. For example, a 14-month study found specific changes in normoxic communities during hypoxic periods, with loss of intolerant and persistence of unexpectedly tolerant species during hypoxic periods (Chu et al., 2018); The degree of tolerance to hypoxia of sessile species limited the rate of community recovery because sessile species could not migrate to normoxic areas. By studying the same eutrophic estuary that experienced significantly different degrees of hypoxia over two summers, Baird et al. (2004) documented greater benthic invertebrate losses, and a shift in energy flow from consumer to microbial trophic levels that correlated with more depleted oxygen levels.

Hale et al. (2016) demonstrated the benefits of sequential measurement of benthic changes over extended time in the northeast Atlantic estuary of Narragansett Bay. They found large losses (50%) of richness and declines in rare species, biomass, secondary productivity rates, and bioturbation depth through comparison of benthos over 20 years. Subsequent analysis of data gathered over more than 55 years in this same estuary correlated changes in nitrogen concentration, sediment pollutants, oxygen decline, and significant benthic diversity loss and composition changes (Hale et al., 2018).

Some long-term studies help in understanding how hypoxia develops. The many short-term studies of Chesapeake Bay's seasonal hypoxic zone did not identify the primary factors contributing to oxygen decline and the temporal patterns of hypoxia development. Only after using 25 years of data, Testa and Kemp (2014) found evidence that a combination of algal production, possibly from eutrophication, and changes in physical forcing affecting stratification and replenishment of oxygen strongly and solely determine the extent of summer hypoxia.

By incorporating 27 years of measurements from 1985 to 2012 within Chesapeake Bay, Du et al. (2018) supported Testa and Kemp's (2014) findings by modelling variation in

the vertical exchange time (VET), which is inversely proportional to the rate of water column mixing. Using data collected over an extended period enabled the researchers to propose that climate change reduced wind forcing, and increased temperature, precipitation, and subsequently, river input. The increase in climate-change related factors can explain the inefficacy of previous efforts to reduce nutrient input to reduce hypoxia and benthic changes (Boesch et al., 2001); only careful temporal studies could highlight the need to further reduce eutrophication in Chesapeake Bay to balance climate related changes.

Unfortunately, most studies of hypoxia-associated biotic changes focus on seasonal hypoxia that cannot provide information on the increasing number of regions experiencing multi-season and decades-long, or permanent, hypoxia; the shift to longer durations of hypoxia likely results from greater eutrophication, as found in the Amvrakikos Gulf of Greece (Kountoura & Zacharias, 2011), and many interacting aspects of climate change (rising temperature, increased precipitation, and increased river runoff affecting stratification). When hypoxia extends across many seasons and years without intervening normoxic episodes, hypoxia-intolerant organisms presumably have no opportunity to recover or return to these areas as they could with seasonal or episodic forms of hypoxia. Organisms tolerant to continuous and often increasingly severe forms of hypoxia or anoxia thus may become dominant in permanently hypoxic regions, but such inferences require studies of this process. Furthermore, studies of the benthos of permanently hypoxic regions can lead to the discovery of unique organisms that can help understand adaptation to hypoxic conditions (Sergeeva & Zaika, 2013).

The St. Lawrence estuary Canada provides an opportunity to explore changes in benthic diversity and composition that have developed within a hypoxic environment, considering hypoxia as oxygen concentrations less than 2 mg/L or 62 $\mu\text{Mol/L O}_2$. The LSLE oxygen concentration reported in the 1990s (55-85 $\mu\text{mol/l}$ oxygen saturation), have persisted for decades since, with mean oxygen levels of 56.4 $\mu\text{mol/l}$ in 1993 and 58.6

umol/l in 2003 (Gilbert et al., 2005; Gilbert et al., 2007). The decline in oxygen concentration has partly resulted from a significant increase in the landward flow of warmer hypoxic bottom water, potentially attributable to climate change induced changes in ocean circulation (Gilbert et al., 2005; Gilbert et al., 2007). Eutrophication, evidenced by a large increase in species tolerant of both organic content and hypoxia (Thibodeau et al., 2006), combined with stratification of the estuarine flow that prevents mixing of more oxygenated surface water with bottom water, likely represents the other main driver of declining oxygen levels. Biogeochemical modelling predicted that increased NO₃ concentrations in runoff from human activities along the St. Lawrence River would lead to oxygen level reductions below 30% and even 20% oxygen saturation in the Lower St Lawrence Estuary (LSLE) and northwest region of the Gulf of St Lawrence (GSL) (Lavoie et al., 2015).

A detailed survey of the benthic communities along the St Lawrence estuary conducted by Ouellet (1982) in 1980 provides an opportunity to evaluate long-term benthic community changes. The relatively normoxic levels in the LSLE in 1980 aligns with an average range in the 1970's of 95-120 umol/l, followed by an average decline in oxygen concentration of approximately 1 umol/l per year (Gilbert et al, 2005). Cruises in 2005 and 2006 re-sampled the same sites investigated by Ouellet in 1980, providing an opportunity to compare benthic communities after a period of continuous and gradual oxygen depletion. Even if the benthic community along the LSLE in 1980 differed from the time before oxygen depletion and eutrophication began, this benthic community was very likely far less altered than the benthos sampled during cruises in 2005 and 2006, noting continuous and increasingly pervasive hypoxia since at least the early 1990's.

From previous studies of environmental (Gilbert et al., 2005; Thibodeau et al., 2006) and benthic (Belley et al, 2010) changes in the LSLE we hypothesize that the decrease in oxygen between 1980 and 2005/6, together with eutrophication, has caused a shift in

both species and family composition to favour more disturbance tolerant opportunistic benthos, and reduced diversity indices. To test this idea, we quantify temporal changes in the macrobenthic communities using historical data from the region and investigate the existence and degree of adaptation to a hypoxic and eutrophic environment.

2.2 METHODS

Research Cruise 1980

Complete details of the 1980 cruise methods were published previously (Ouellet, 1982), however we provide a brief summary here. The 1980 research cruise took place from June 2 to June 11 aboard the MV St. Barnabe, and sampled seven stations (Figure 2.1) targeting three main sites, 25, 24, and 23 (Figure 2.1), each containing 5 closely situated (<1 km apart) sub-stations (except 3 stations at 25). Samples were obtained at two stations, 24.75 and 23.6, between the main stations, and at 22 and 21, stations nearer to the mouth of the estuary. Three replicate grabs were sampled at each station (see Oullet, 1982 Appendix 1, p. 149-52 for latitude and longitude of the stations and substations, and Fig. 1, p. 9 for a full map of the sites).

Sampling Protocol 1980

A Charcot-Picard dredge sampled sediment and infauna at 3 sub-stations of station 25. Because of the large weight of the filled dredge, which can remove 120 L of sediment, all other samples were obtained with a van Veen grab (0.125 m²). The lighter van Veen penetrates different sediment types to approximately similar depths (Gallardo, 1965). The grab penetrated the seabed 12-14 cm, sampling a surface area of 0.125 m². Three replicates were assumed to provide sufficient numbers of organisms to estimate species abundances and other diversity measures accurately. In order to extract and retain the maximum number of organisms, sediment grabs were processed on board through nested sieves of mesh size 7 mm and 0.8 mm, prior to preserving samples in seawater and 4% formaldehyde. Organisms were identified to the lowest possible taxonomic level.

Research Cruises 2005/06

The 2005 and 2006 cruises utilized the research/survey vessel Coriolis II. Sampling took place from August 20-26, 2005, and from August 14- 22, 2006. The 7 sites sampled originally in 1980 by Ouellet, stations 25, 24.75, 24, 23.6, 23, 22 and 21 were resampled in 2005 and station 23 was sampled again in 2006 (Figure 2.1). Table 2.1 lists the coordinates of each station utilized for the temporal study.

Sampling Protocol 2005/6

During the 2005/6 cruises, no substations were sampled; three replicate van Veen grabs of 0.125 m² were obtained at each of the seven stations visited in 2005, and three replicates were obtained from station 23 during 2006 sampling. Both cruises followed sampling protocols utilized by Ouellet (1982).

Following collection, sediments were sieved onboard the ship through a 1 mm mesh sieve and preserved in 10% buffered formalin solution. In the lab, organisms were examined under a binocular dissecting microscope and identified to the lowest possible taxonomic level to provide density and diversity estimates for each site. We updated species names using the World Register of Marine Species (WoRMS Editorial Board, 2020), the most accurate and current taxonomic information source for marine species names.

Statistical Methods

We directly compared the 1980, 2005, and 2006 macrobenthic faunal data because, aside from the 3 substations in 1980, all 3 cruises used the same collection method, minimizing potential differences resulting from methodologies, as opposed to any biological change. We used PRIMER-E version 6 with the PERMANOVA+ add-on (Anderson et al., 2008; Clarke and Gorley, 2006) for data analysis, including data transformation, matrix calculation, and multivariate testing (detailed below). We performed all tests at both the family and species level. Analysis at two levels of taxonomic classification clarified whether temporal changes in benthic composition and

diversity were significantly different at species level compared to family level, and therefore whether family level analyses provided taxonomic sufficiency to detect environmentally induced benthic changes.

Univariate Analysis

Calculation of family and species level richness, Shannon diversity, and Pielou's evenness for stations sampled in 1980 and resampled in 2005/2006 were performed and provided standard measures of diversity. The diversity measures were compared between years through transformation into Euclidian distance matrices and then analyzed through PERMANOVA+ tests (Anderson et al., 2008).

Multivariate Analysis

We used multivariate statistical methods to compare the 7 stations sampled originally in 1980 and resampled in 2005 and 2006. All species and family data were 4th root transformed prior to calculation of a Bray-Curtis resemblance matrix. PERMANOVA+ tests with an alpha level of 0.05 were conducted with 999 permutations for the 7 stations and 3 years. Further pairwise tests using a one-way PERMANOVA+ with year as the factor were performed to clarify which stations differed between which years of comparison. SIMPER analysis determined those organisms most responsible for differences in composition of assemblages among sampling times.

Based chiefly on information from the World Register of Marine Species, (WoRMS Editorial Board, 2020) (with supplemental information if needed for individual organisms) we divided the biota into six functional groups: surface deposit feeders (SDF), subsurface deposit feeders (SBDF), filter feeders (FF), carnivores (C), omnivores (O), and unclassifiable (ND). A 4th root transformation in each case preceded calculation of a Bray-Curtis resemblance matrix based on the functional groups. One-way ANOSIM tests with year as factor evaluated changes in functional group composition between 1980, 2005, and 2006.

2.3 RESULTS

Univariate Analysis

The overall mean abundance of benthic macrofauna at stations in 2005/6 was approximately three times greater than in 1980 (Figure 2.2a). An almost four-fold increase in average abundance of Annelida largely underscored this large increase. In parallel, Arthropoda and Mollusca increased, but by a much smaller percentage over time (Figure 2.2b). Although Echinodermata increased over four times in abundance, their absolute abundance, and that of Arthropoda and Mollusca were considerably less than Annelida, contributing much less to the temporal increase in macrofaunal abundances.

Some of the PERMANOVA+ tests of univariate diversity measures detected differences between years at different stations, noting significant interaction between years and stations. Separate pair-wise testing of stations between years clarified temporal changes. In contrast to the large increase in benthic abundance between 1980 and 2005/6, species-level richness among years (Figure 2.2c) declined significantly ($P=0.001$) between 1980 and 2005 at stations 25 and 23, with a borderline non-significant decline at station 24. A significant reduction in species richness also occurred between 1980 and 2006 at station 23, the only 2006 station included in the temporal study. Evenness differed significantly among years ($p=0.01$). Shannon diversity also decreased significantly ($p=0.001$) between 1980 and 2005 at stations 25 and 23. We also observed a significant interaction for Shannon diversity between year and stations ($p=0.02$), because of a significant difference between stations 25 and 23 in 1980. Pair-wise testing indicated no other significant differences between stations.

Mean values of both family richness and family Shannon diversity were also greatest in 1980 (Figure 2.2d), although the 2006 data included only a single 1980 station with 3 replicates. PERMANOVA+ testing of family-level diversity indices among years confirmed

significant reductions in richness between 1980 and 2005, at station 25 ($P=0.021$), and between 1980 and 2006, family richness also decreased significantly at station 23 ($P=0.018$). A significant interaction was driven by different family level richness between stations 25 and 24, and 25 and 23 in 1980. Pielou's evenness increased significantly between 1980 and 2006 ($p=0.002$), whereas Shannon diversity decreased significantly between 1980 and 2005 ($P=0.002$).

Multivariate Analysis

PERMANOVA+ comparison of both species and family assemblages showed significant differences among years, stations, and their interaction (Table 2.2a,b). Further pairwise tests to analyze benthic changes between years separately showed no significant difference between 2005 and 2006. Species and family benthos differed significantly between 1980 and 2005 for stations 25, 24.75, 24, and 23, and changed significantly between 1980 and 2006 at station 23, the only 2006 site in common (Table 2.3a,b). SIMPER also identified the 14 species most responsible for dissimilarity between stations (Table 2.4a) including five species (*Scoletoma impatiens*, *Melinna cristata*, *Trochochaeta watsoni*, *Thyasira gouldi*, and *Ampharete acutifrons*) no longer detected in 2005, and three additional species (*Ampharete lindstroemi*, *Trochochaeta multisetosa*, and *Amphiura sundevalli*) that appeared only in 2005. Four species were more abundant in 2005 than 1980. Polychaetes comprised 12 of the 14 species that contributed most to temporal dissimilarity, demonstrating both increasing and decreasing abundance effects of environmental changes between 1980 and 2005/6, depending on the species.

The greater mean similarity of 2005 biota to 1980 (43.5%) compared with the 2006 assemblages (34.2%) should not be interpreted as a change in community composition between 2005 and 2006, given the non-significant PERMANOVA+ comparison of assemblages between those years at station 23

SIMPER identified the 14 families (Table 2.4b) most responsible for the family level dissimilarities that developed in benthic composition between 1980 and 2005. All families decreased in abundance during this time span, except for two polychaete families, Spionidae and Oweniidae. Many families decreased by 50% or more in abundance, including the mollusc families Thyasiridae, Nuculanidae and Nuculidae. Only one family (Photidae) sampled in 1980 was not detected in 2005.

The species replaced or reduced in abundance tended to be more active sediment reworkers scoring 3-4/5 on a comparative scale and generally 2-3/4 on motility ratings. In contrast, the species that increased in abundance over time were less motile, and did less sediment reworking, scoring 1-2/4 and living mostly at the surface. Table 2.5 summarizes differences in abundances of taxa that rework sediment or differ in motility from 1980 to 2005/6 (categories/ratings from Queiros et al., 2013).

Functional Group Analysis

Mean functional group abundances increased for all functional group categories, with the exception of the unclassified group, reflecting an overall increase in benthic abundance over time. The composition of the functional groups changed greatly between sampling periods (Figure 2.3a), whose significance was confirmed by ANOSIM (Table 2.6). Surface deposit feeders (SDFs) were six times more abundant in 2005 compared to 1980, ($R=0.266$, $p<0.001$) a far greater difference than in any other functional group. Moreover, the ratio of SDFs to omnivores, the second most abundant functional group, increased from 1.35 in 1980 to 4.07 in 2005, (Figure 2.3b) indicating a major replacement of omnivores with SDFs. Carnivore abundances also increased significantly between 1980 and 2005 ($R=0.261$, $p=0.002$) and between 1980 and 2006 ($R=0.201$, $P=0.004$). Omnivores increased significantly between 1980 and 2005 ($R=0.099$, $p=0.019$) and between 1980 and 2006 ($R=0.169$, $p=0.003$), In 2006, SDF abundance remained greater than in 1980, but much lower than the 2005 station average ($R=0.134$, $p<0.001$); in contrast 2006 samples had the highest abundances of filter feeders, and the lowest SDF/O ratio, with omnivores as the dominant functional group. However, all

2006 results must be interpreted cautiously, noting that only station 23 was sampled in 2006.

2.4 DISCUSSION

Overall, this temporal study confirmed our prediction that in this region of increasing hypoxia we would find significantly altered macrobenthic communities at the head of the LSLE. Our analysis confirms our expectation of an increased proportion of hypoxia-adapted and eutrophication-tolerant benthos, in particular SDF annelids potentially outcompeted less tolerant components of the 1980 assemblage; these changes are consistent with biotic changes in other hypoxic and or eutrophic environments (Baker & Mann, 1992; Pearson & Rosenberg, 1978; Secor & Gunderson, 1998).

Somewhat unexpectedly, the average abundance of organisms in the LSLE increased from 1980 to 2005/6; after major environmental disturbances, temporary increases in abundance of opportunistic fauna commonly occur, followed by a reduction to pre-disturbance densities (Pearson & Rosenberg, 1978). This increase potentially reflects increased primary production (Thibodeau et al., 2006) associated with increased nutrient loading, and long-term responses of hypoxia-tolerant organisms to eutrophication without episodes of defaunation. In this temporal study, the large increase in benthic abundance along with reductions in all three diversity indices at both the species and family level from 1980 to 2005/6 conforms to the Pearson and Rosenberg (1978) model of benthic succession after disturbance and confirms that a large change in benthic community structure occurred. While the lack of buffering of the initial fixative used in the 1980 study could have reduced the viability and identification of arthropods and especially molluscs, (the acidic nature of formaldehyde can dissolve CaCO_3 in their shells), their relative increase over time mirrors increases in all other phyla and suggests that the unbuffered fixative did not alter the main findings of the temporal study.

The spatial extent of the reduction in richness, evenness, and Shannon diversity spanned from station 23 to station 25, a distance of ~79 kilometers, and provides a functional biotic measurement of the geographic scope of environmental change sufficiently great to evoke significant benthic change. The largest changes in univariate indices occurred at stations 24 and 25; these stations had the lowest oxygen levels of any station measured in both sampling periods. Although station 23 also had notably lowered species richness in 2006, it is situated closer to the middle of the estuary, with a low but not strongly hypoxic oxygen saturation of 24.39% that suggests less eutrophication (Helly & Levin, 2004; Thibodeau et al., 2006). Given this station's location in the deepest part of the estuary, it may experience reduced food supply at the seafloor compared with other stations, and the combination of low oxygen and reduced food sources could reduce species richness; low oxygen has been shown to sometimes have a greater impact when combined with other habitat factors (Vaquer-Sunyer & Duarte, 2011).

Overall, two main changes contributed most to reductions in family and species richness, evenness, and Shannon diversity, and the unexpected increase in LSLE abundance. First, an increase in abundance of polychaetes in contrast to all other taxa such as molluscs explained much of the faunal shift. Second, within the polychaetes, surface dwelling species such as *Ampharete lindstroemi* replaced mobile, deep-sediment adapted active bioturbators, such as the lumbrinerid *Scoletoma impatiens*, a change also reflected in the shift to SDF over time.

Given the well-documented intolerance of crustaceans and some molluscs to hypoxia, the disappearance of the crustacean family *Photidae* was not surprising. However, we did not expect increases solely in polychaete families and reduction in all other families, including the molluscs, given the lower tolerances of many polychaetes to hypoxia compared to echinoderms and especially molluscs, the phylum generally most tolerant

to hypoxia (Gray et al., 2002; Vaquer-Sunyer & Duarte, 2008). However, these researchers emphasized important differences between laboratory studies of sensitivity to oxygen and actual hypoxic events, where additional factors such as higher temperature and sulphide concentration can affect survival (Vaquer-Sunyer & Duarte, 2011); in our case, eutrophication likely added a complicating factor for organisms coping with hypoxia. As filter feeders, some Thyasiridae are highly sensitive to organic enrichment (Borja et al., 2000), perhaps explaining their 85% decline in abundance. However some Thyasiridae tolerate enrichment, and some tolerate hypoxia (including some *Thyasira gouldi*) through mechanisms such as chemosymbiosis from bacteria living in their gills (Batstone et al., 2014), limiting our ability to interpret the decline in abundance. Moreover, their taxonomy is in flux (S. Dufour, pers. comm.), limiting the reliability of identification to species level in this study. Nuculanidae survived severe hypoxic levels of 0.05-0.11 ml/L in Baja (Hendrickx et al., 2016), far below the minimum LSLE level; the smaller reduction in their abundance (50%) compared to Thyasiridae aligns with a hypoxia tolerant family. In contrast, some polychaetes tolerate excess organic matter, so eutrophication apparently played an important role in determining family level gain and loss over the 25 years between studies (see also Dean, 2008; Gray, 1979).

Species level changes were even greater than family composition shifts over time. SIMPER showed that, of the 14 species most responsible for temporal changes, 5 were no longer detected in our sampling, and 3 species newly appeared in the 2005/6 samples, with most of these changes occurring within the same family or even genus of polychaetes. Understanding these temporal changes requires recognizing key differences between the altered environment of the LSLE and environments where most published studies of hypoxic effects on benthos took place. Most such studies document the effects of relatively sudden, short-term discrete hypoxic 'events' that defaunate or greatly reduce biota in swaths of seabed, such as seasonal hypoxia in which severe drops in oxygen can reduce levels to 10% saturation or less (Baban et al., 2016; Diaz &

Rosenberg, 1995; Seitz et al., 2009). After short-term hypoxic events, initially opportunistic polychaetes with modest hypoxia tolerance re-colonize sites through flexible development that includes larval forms and rapid genetic selection related to r-selection strategies (Gray, 1979; Vaquer-Sunyer & Duarte, 2008). After both experimental defaunation (Lu & Wu, 2000) and natural defaunation following hypoxia and re-oxygenation (Diaz and Rosenberg, 1995), polychaetes dominated recoveries, and species richness temporarily exceeded pre-disturbance levels followed by a decrease in species and in overall abundance, essentially following the Pearson-Rosenberg (1978) successional model. Eventually, community composition in each case closely resembled the pre-hypoxic pattern.

However, in our study, the St. Lawrence estuary reached a 21% saturation minimum in 2005, an average saturation of 20.7% from 1984-2003, and the lowest recorded mean oxygen level over that time period of 18.6 % in 1993 (Gilbert et al, 2005). Such conditions should not cause major mortality or defaunation episodes. Furthermore, the temporal pattern of hypoxia and eutrophication differed from many previous studies. Oxygen in the estuary has declined at a slow average rate of ~1µmol/L per year, declining from 125 µmol/L to 65 µmol/L over 50-70 years (Gilbert et al., 2005), a much more gradual process than reported in many studies. The persistence of hypoxia over decades as a result of the combined effect of a pycnocline above the deep hypoxic waters that limits mixing and eutrophication (see Chapter 1) precludes the eventual return or new arrival of hypoxia-intolerant organisms, in contrast to episodic hypoxia events in which conditions eventually improve through seasonal mixing. Instead, gradually increasing selection pressure favours phenotypic characteristics that improve tolerance and better adapt species to compete within hypoxic and eutrophic conditions, above and beyond opportunistic reproduction.

Consistent with the slow reduction in oxygen to low but non-lethal levels, the temporal changes in LSLE species suggests that rapid reproduction is not the only or, in some

cases, not the main adaptation in this estuary. The appearance of *Ampharete lindstroemi*, and the disappearance of the two other Ampharetidae members, *Melinna cristata* and *Ampharete acutifrons*, suggests the presence of successful alternative adaptations. This process of replacement within one family and even within the same genus would not likely occur if reproductive strategy alone determined persistence, because their similar reproductive strategies presumably lead to similar responses to disturbances. Instead, evidence suggests that replacement within Ampharetidae likely reflects differences in hypoxia tolerance; the ciliated branchiae of *A. lindstroemi* increase surface area and water flow that increases efficiency of oxygen extraction compared to the branchiae of *A. acutifrons*, which lack cilia (Parapar et al., 2012).

Although past studies considered *A. lindstroemi* sensitive to disturbances such as eutrophication (Borja et al., 2000), as an SDF, *A. lindstroemi* lives at the sediment surface, where higher oxygen saturation occurs compared to the deeper sediments in which *Thyasira gouldi* lives; this latter species was absent by 2005;. This adaption to living at the sediment surface may also account for the ability of *A. lindstroemi* to colonize hypoxic environments, despite high sensitivity to organic disturbance; *M. cristata*, which tolerates excess organic matter, and *A. acutifrons*, a species considered indifferent to eutrophication (Borja et al, 2000), both disappeared. *A. lindstroemi*, a weak bioturbator with low mobility, replaced *A. acutifrons* and *M. cristata*; both are stronger bioturbators (Queiros et al., 2013) Superficial bioturbators with low mobility may also gain a competitive advantage through reduced oxygen needs linked to their reduced movements (Childress and Seibel, 1998). A significant reduction in bioturbation in the LSLE suggested by the temporal shift favouring weak bioturbators is supported by Belley et al. (2010), who found significantly deeper bioturbation traces in normoxic than in hypoxic regions of the LSLE.

The increase in average abundance of both polychaetes *Heteromastus filiformis* and *Myriochele heeri* potentially reflects their ability to survive and cope with low oxygen

rather than simply a capacity to re-colonize an environment. Diaz and Rosenberg (2008) noted that *H. filiformis* and multiple *Myriochele* species survived oxygen concentrations as low as 0.9 ml/L in the Kattegat Strait in 1988, far below the minimum in the LSLE. Unlike the surface deposit feeders, *A. lindstromi*, *Spiophanes kroyeri* and *Myriochele heeri*, which increased most in abundance, *H. filiformis* is a subsurface deposit feeder whose high tolerance of hypoxia apparently enables it to cope with the more severe hypoxia that occurs below the sediment water interface.

The arrival of *Trochochaeta multisetosa* and increases in abundance of *S. kroyeri* and *H. filiformis* may reflect their tolerance to hypoxia, but also eutrophication, the other major environmental disturbance that occurred in the LSLE during the study period. *S. kroyeri* tolerates disturbance, and some spionid polychaetes increase in abundance following increased inputs of sewage or other organic material (Dafforn et al., 2013; Gray, 1979; Lenihan et al., 2003), because they can reproduce quickly enough to cope with the smothering effects of organic rich sediments (Gray et al., 2002). Spionids might form a symbiotic relationship or depend on capitellid polychaetes in adapting to heavy organic contamination (Gray, 1979) and co-occur in organically enriched estuaries in Australia (Dafforn et al., 2013) as well as in the LSLE. The capitellid *H. filiformis*, a more aggressive secondary opportunist, exemplifies a highly adaptive species that tolerates both hypoxia and organic disturbance. Similarly, *T. multisetosa* demonstrated tolerance to both oxygen and organic disturbance and completely replaced *Trochochaeta watsoni*. *Amphiura sundevalli* was the only echinoderm that increased in abundance according to the SIMPER analysis. Previous experimental work showed slightly better tolerance of hypoxia by echinoderms than annelids, through various adaptations (Vaquer-Sunyer and Duarte, 2008), including raising of their discs above the sediment surface to access more oxygen, and shifting to anaerobic metabolism. Although *A. sundevalli* is considered indifferent to organic pollution disturbance, *Odontaster validus*, a surface living asteroid, significantly increased in abundance in an experimentally enriched environment (Lenihan et al., 2003). *Ophiura* sp. in the same class as *A. sundevalli* occurred in

significantly greater abundance in the hypoxic region of the LSLE than in the normoxic areas of the GSL in 2006/2007 field sampling, supporting the shift towards hypoxia tolerant brittle stars (Belley et al., 2010).

Several of the observed temporal changes in biota were inconsistent with published groupings of species mostly based on their degree of tolerance to organic excess input and chemical pollutants (Borja et al., 2000). This outcome suggests that eutrophication may not have played as large a role in determining biotic change as hypoxia. Our temporal comparison shows that a more profound difference in biotic composition occurred than would be apparent from a biotic index alone used as a 'snapshot' of the degree of disturbance in 2005, demonstrating the importance of measuring changes in this benthic community over time.

Queiros et al. (2013) developed a classification scheme based on bioturbation characteristics for each family or species, rating degree of mobility on a scale of 1 to 4, and amount of sediment reworking on a scale of 1-5; higher numbers identified increasing movement or bioturbation activity. Available bioturbation and mobility ratings for any of the 14 abundant species in our study indicated that the species and families that disappeared or decreased in abundance were generally more mobile and more active bioturbators than the species and families that increased over time. The largest contributor to dissimilarity among species that disappeared, *Scoletoma impatiens* rated 4 and 3 for reworking and mobility, respectively. By comparison, *A. lindstroemi*, a new species in the LSLE that contributed most to dissimilarity over time, received only a reworking score of 2 and mobility score of 1.

In interpreting the temporal shifts in dominance of benthos it is important to consider differences in sample processed protocols used in 1980 and 2005/6. The finer 0.8 mm mesh used to sieve specimens in 1980 compared to the 1 mm mesh used in 2005/6 could have biased comparisons by increasing abundances of smaller specimens in 1980

and larger benthos in 2005. However, comparing the sizes of dominant specimens from each sampling period, offers no clear evidence of such a bias. *S. kroyeri* (a polychaete much less than 1 mm in diameter) increased in abundance in the 2005/6 study, as did the small polychaete *M. heeri*, whereas *T. gouldi* (diameter 1- 2.55 mm) was not detected in 2005. (WoRMS Editorial Board, 2020)

The increase in abundance of each functional group (except for ND) in the St. Lawrence estuary between 1980 and 2005 was unexpected, given the significant decline in oxygen saturation over the same time period. The continued input of excess organic material to the estuary, which contributed to the continued decline in oxygenation in the LSLE (Thibodeau, et al., 2006), likely explains this significant increase in overall abundance in an environment without defaunation as occurs in severely hypoxic regions (Dafforn et al., 2013; Gray 1979; Gray et al., 2002; Pearson & Rosenberg, 1978). However, the percentage increase in abundance over time varied among feeding guilds. The tripling of the SDF/O ratio between 1980 and 2005 suggests a change in dominance reported previously in the LSLE (Belley et al., 2008) and other hypoxic environments (Diaz & Rosenberg, 1995; Levin et al., 2009; Middelburg & Levin, 2009; Wu, 2002).

Therefore, the increase in functional group abundance skewed so much towards SDFs that it reinforces our prediction that the LSLE hypoxia exerts significant influence over biotic composition. Previous studies reported that hypoxic environments favour SDFs over other trophic groups because of the competitive advantage their adaptations confer (Levin et. al, 2009); unlike deeper-living infauna, SDFs can avoid oxygen-depleted sediments. Some SDFs, such as *Prionospio pinnata*, have evolved more efficient respiratory structures through increased surface area, more enzymes subtypes to facilitate anaerobic metabolism, and show induced changes such as hemoglobin increase following hypoxic exposure (Sturdivant et al. 2012). Many SDFs, such as *S. kroyeri* and *T. multisetosa*, which proved more adaptive in the LSLE, tolerate excess

organic input (Dean, 2008), possibly as a result of their rapid reproduction that helps them cope with the smothering effect of high organic input (Gray 1982; Gray et al 2002).

The loss of several mobile and strong sediment-reworking bioturbators, *Scoletoma impatiens*, *M. cristata*, and *A. acutifrons*, likely reduced the degree of bioturbation activity in the estuary markedly, further decreasing sediment oxygen saturation, given the important role of bioturbation in increasing sediment porosity and oxygenation (Queiros et al., 2013; Zhang et al., 2017). This reduction in sediment oxygen saturation presumably further reduced infaunal survival, and further reduced bioturbation, in a synergistic self-amplifying process. The loss of bioturbators could therefore represent both a result and further cause of sedimentary hypoxia. The further reduction in sedimentary oxygen saturation could account for the smaller percentage increase in sessile SBDFs compared to omnivores, and likely contributed to the strong dominance of SDFs at the head of the LSLE.

2.5 CONCLUSION

Chapter 2 explored the temporal and spatial patterns of benthos in a somewhat unusual hypoxic region, namely an estuary that has become hypoxic slowly but continuously over decades, without seasonal or episodic returns to normoxic levels. Eutrophication contributes to the development of hypoxia and increases the complexity environmental change. While the declines in richness, evenness, and Shannon diversity that occurred between 1980 and 2005/6 aligns with many studies of opportunistic responses to hypoxia and eutrophication, some temporal biotic changes differed from those usually reported in altered environments.

The large number of species replacements that occurred between 1980 and 2005/6 within polychaete families and even within one genus suggested strong competition between closely related organisms; fewer studies report competition in response to

disturbances as opposed to the sudden appearance and short-term dominance of opportunistic polychaetes. This difference in response to disturbance likely reflects difference in selection between short severe disturbances (such as sewage or oil leaks, seasonal episodes of < 10% oxygen saturation) and prolonged, moderate (18-21% oxygen saturation) disturbance that occurred in the LSLE without either defaunation or returning to normoxic conditions. This distinction was only evident in comparing species in 1980 and 2005/6 and emphasizes the importance of temporal studies within the same habitat that can yield information not available through spatial studies alone. Determining whether distinct mechanisms of assemblage formation has occurred during other long-term disturbances will require further research but would help future assessments of the health of benthic communities.

Eutrophication and hypoxia each apparently exerted somewhat different selective pressure, because persisting and newly detected species in the LSLE possessed a range of tolerance to eutrophication but in previous studies showed somewhat more consistent tolerance or competitive advantages within hypoxic environments. Species in the estuary in 2005 seemed at least minimally adapted to cope with one of these factors and a greater capacity to cope with the other. The LSLE environment would appear relatively healthy using standard scoring such as AMBI than the significant temporal changes reported here would suggest, further emphasizing the importance of temporal studies. We presented evidence that suggests risks to functional richness and ecosystem functions in the estuary, pointing to the importance of exploring current functional diversity measures and ecosystem functions within the estuary and Gulf.

2.6 FIGURES AND TABLES

Figures

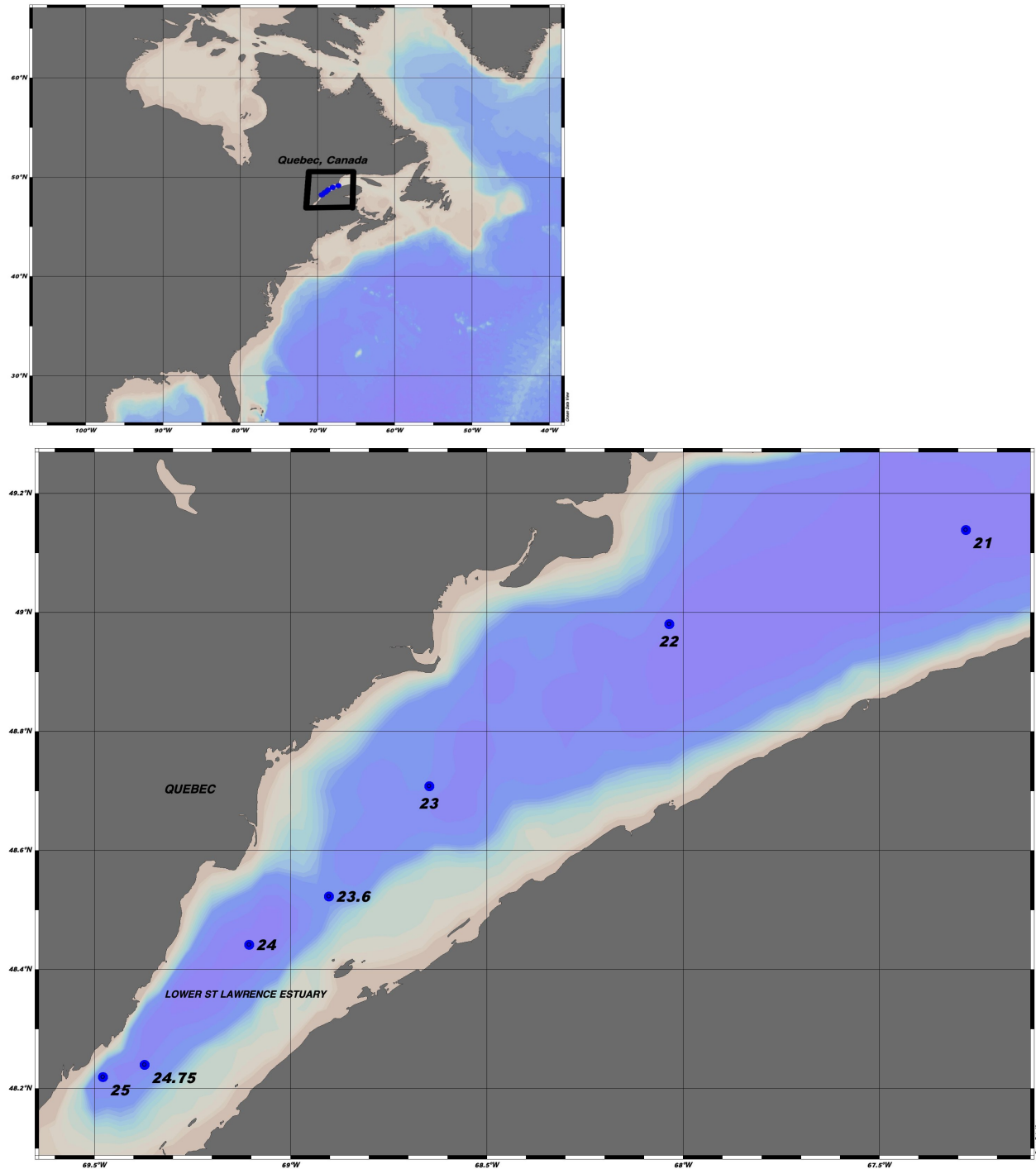


Figure 2.1. Upper map of eastern North America shows the 1980 and 2005/6 study area located in Quebec, Canada. Lower map shows stations sampled in the Lower St. Lawrence estuary in 1980 (Ouellet 1982) and resampled during the 2005 and 2006 research cruises.

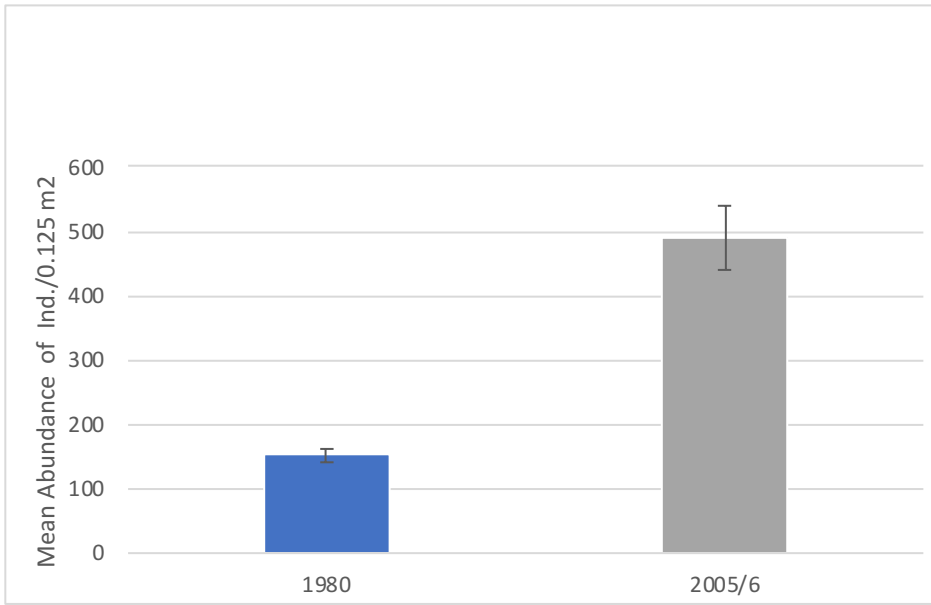


Figure 2.2a. Comparison of mean station density in 1980 and 2005/6 measured by Individual/0.125 m². Error bars represent standard error of the mean.

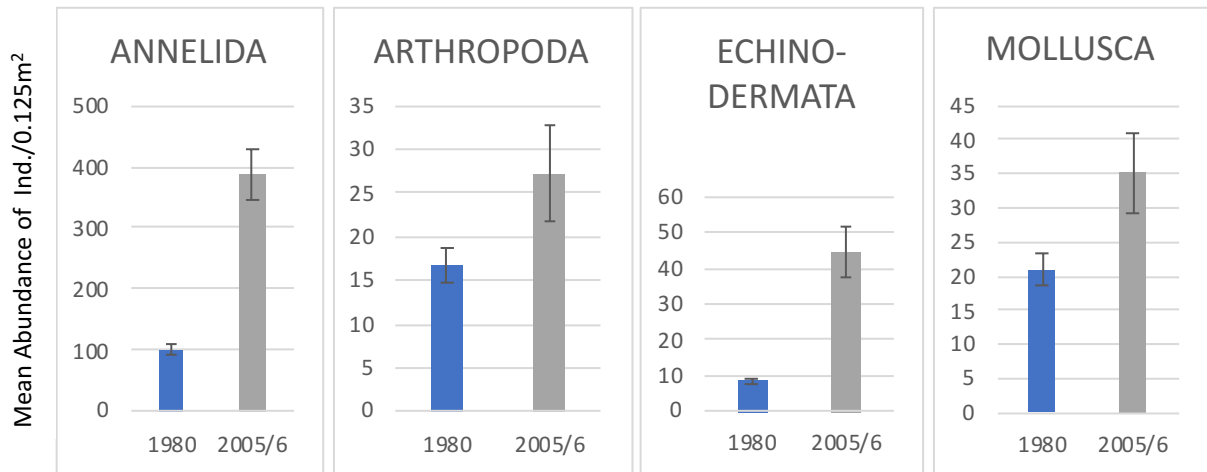


Figure 2.2b. Comparison of mean station density in 1980 and 2005/6 of the four most abundant phyla measured by Individual/0.125 m². Error bars represent standard error of the mean.

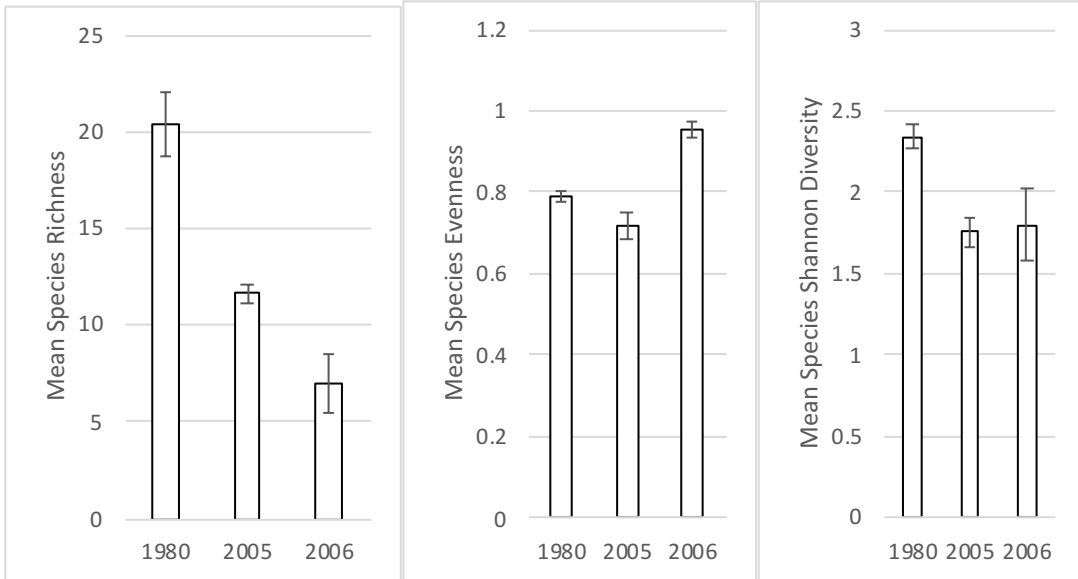


Figure 2.2c Mean univariate measures of richness, evenness and Shannon diversity of species from 1980, 2005 and 2006 cruises. Error bars represent standard error of the mean.

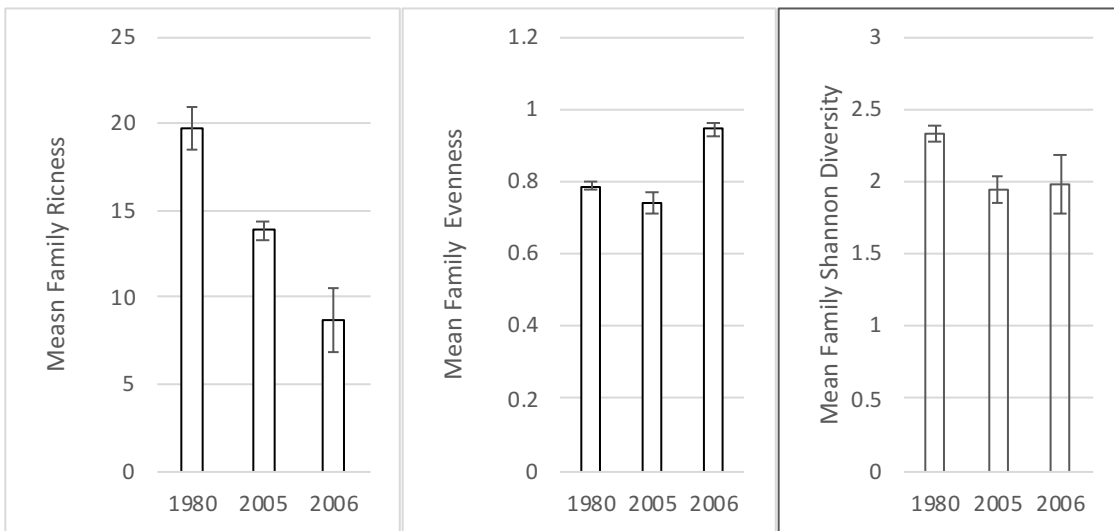


Figure 2.2d Mean univariate measures of richness, evenness and Shannon diversity of families from 1980, 2005 and 2006 cruises. Error bars represent standard error of the mean.

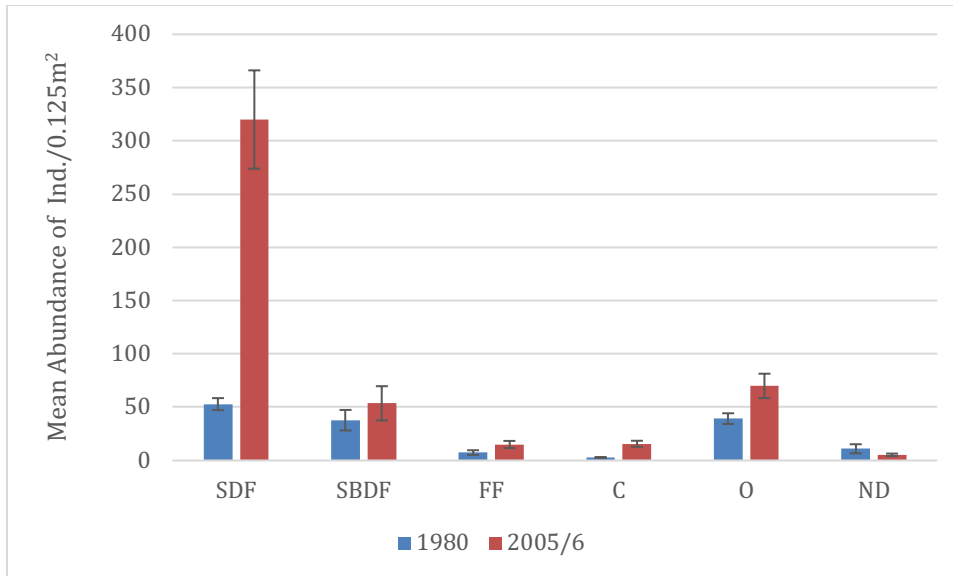


Figure 2.3a. Mean abundance of of functional groups measured by Individuals/0.125 m². Error bars represent standard error of the mean. SDF=surface deposit feeders, SBDF=subsurface deposit feeders, FF=filter feeders, C=carnivores, O=omnivores, ND=unidentified specimens.

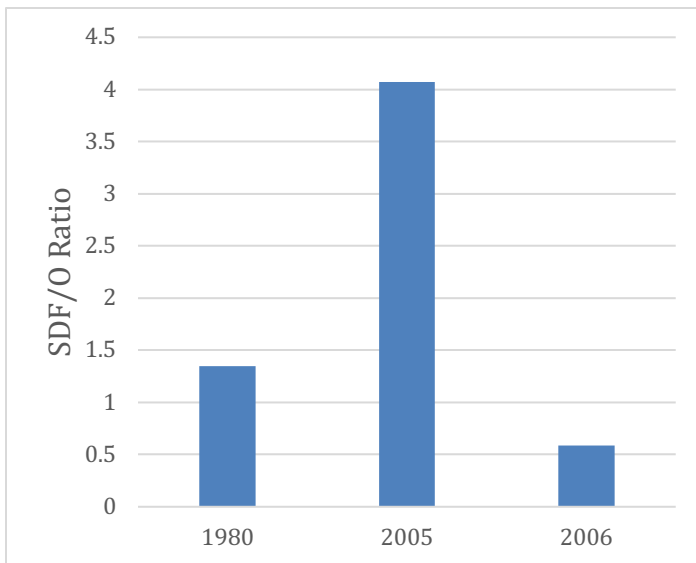


Figure 2.3b Ratio of SDF (surface deposit feeders) to O (Omnivores) based on mean abundance shown in Figure 2.3a

Tables

Table 2.1. Latitude and longitude of stations of 1980, 2005, and 2006 cruises.

1980 Cruise			2005 Cruise		2006 Cruise	
Station No.	Latitude	Longitude	Latitude	Longitude	Latitude	Longitude
25	48.2194	-69.4778	48.2328	-69.4505		
24.75	48.2403	-69.3722	48.2426	-69.3521		
24	48.4417	-69.1056	48.4313	-69.1008		
23.6	48.5228	-68.9028	48.5446	-68.8933		
23	48.7084	-68.6472	48.6983	-68.6512	48.7006	-68.6479
22	48.9806	-68.0361	48.9308	-68.0914		
21	49.1389	-67.2805	49.1202	-67.2822		

Table 2.2. Results of the PERMANOVA procedure comparing benthic composition of a) species and b) families in 1980, 2005 and 2006.

a) Species

Source	df	SS	MS	Pseudo-F	P (perm)
Year	2	29690	14845	22.69	0.001
Station	6	28253	4708.9	7.20	0.001
Year x Station	6	10395	1732.6	2.65	0.001
Residuals	28	18317	654.16		
Total	42	1.0318E5			

b) Families

b) Family

Source	df	SS	MS	Pseudo-F	P (perm)
Year	2	9952.5	4976.3	8.55	0.001
Station	6	23106	3850.9	6.62	0.001
Year x Station	6	8880.5	1480.1	2.54	0.001
Residuals	28	16292	581.87		
Total	42	66522			

Table 2.3. PERMANOVA+ Pair-wise testing of differences in a) species and b) family composition between 1980, 2005 and 2006, showing t values and probability (P) for each year compared. Significant results shown in bold.

a) Species

Station	25		24.75		24		23.6		23		22		21	
Pairs	t	P	t	P	t	P	t	P	t	P	t	P	t	P
2005, 1980	4.4	0.02	3.11	0.027	3.44	0.02	1.9	0.23	3.69	0.02	2.7	0.25	2	0.24
2005, 2006	-	-	-	-	-	-	-	-	0.98	0.62	-	-	-	-
1980, 2006	-	-	-	-	-	-	-	-	3.3	0.02	-	-	-	-

b) Families

Stations	25		24.75		24		23.6		23		22		21	
Pairs	t	P	t	P	t	P	t	P	t	P	t	P	t	P
2005, 1980	3.1	0.01	2.47	0.025	2.38	0.01	2	0.25	2.09	0.01	1.8	0.22	1.28	0.26
2005, 2006	-	-	-	-	-	-	-	-	1.21	0.32	-	-	-	-
1980, 2006	-	-	-	-	-	-	-	-	2.41	0.02	-	-	-	-

Table 2.4 Average abundance per 0.125 m² and SIMPER determination of the a) species and b) families with the greatest dissimilarity in abundance between 1980 and 2005. Mean dissimilarities between time periods were 79.51 for species and 56.54 for families.

a)

Class	Species	Abundance (1980)	Abundance (2005)	Dissimilarity	Contribution	Cumulative %
					%	
Polychaeta	<i>Ampharete lindstroemi</i>	0	2.46	4.82	6.06	6.06
Polychaeta	<i>Scoletoma impatiens</i>	1.86	0	3.55	4.46	10.53
Polychaeta	<i>Spiophanes kroyeri</i>	1	1.8	3.21	4.03	14.56
Polychaeta	<i>Myriochele heeri</i>	0.82	2.21	3.17	3.99	18.55
Polychaeta	<i>Melinna cristata</i>	1.6	0	3.04	3.82	22.37
Polychaeta	<i>Trochochaeta multisetosa</i>	0	1.6	2.99	3.75	26.13
Polychaeta	<i>Trochochaeta watsoni</i>	1.58	0	2.97	3.74	29.86
Ophiuroidea	<i>Amphiura sundevalli</i>	0	1.41	2.68	3.37	33.24
Polychaeta	<i>Ceratocephale loveni</i>	1.21	1.56	2.53	3.18	36.41
Bivalvia	<i>Thyasira gouldi</i>	1.32	0	2.46	3.1	39.51
Polychaeta	<i>Maldane sarsi</i>	1.36	0.31	2.36	2.97	42.48
Polychaeta	<i>Ampharete acutifrons</i>	1.12	0	2.24	2.81	45.3
Polychaeta	<i>Onuphis opalina</i>	1.29	0.56	2.2	2.77	48.07

Table 2.4b)

Class	Family	Abundance (2005)	Abundance (1980)	Dissimilarity	Contribution %	Cumulative %
Bivalvia	Thyasiridae	0.2	1.3	2.62	4.63	4.63
Polychaeta	Nereididae	0.93	1.26	2.51	4.44	9.07
Polychaeta	Spionidae	1.07	1	2.44	4.31	13.38
Polychaeta	Onuphidae	0.33	1.29	2.41	4.27	17.64
Polychaeta	Maldanidae	0.78	1.6	2.29	4.06	21.7
Polychaeta	Lumbrineridae	0.83	1.86	2.27	4.02	25.72
Polychaeta	Trochochaeti	0.95	1.58	2.06	3.65	29.37
Polychaeta	Oweniidae	1.44	0.82	2.06	3.65	33.02
Bivalvia	Nuculidae	0.45	0.89	1.82	3.21	36.23
Malcrostica	Photidae	0	0.86	1.79	3.17	39.4
Ophiuroidea	Amphiuridae	0.84	0.98	1.79	3.16	42.56
Bivalvia	Nuculanidae	0.44	0.78	1.76	3.11	45.67
Bivalvia	Yoldiidae	0.27	0.73	1.51	2.67	48.34
Polychaeta	Capitellidae	0.75	0.96	1.5	2.66	51

Table 2.5. Bioturbation ratings and contribution to dissimilarity of species that either decreased or Increased in abundance between 1980 and 2005 for which ratings of bioturbation were available. Reworking is scored from 1-5 for degree of alteration of sediment and Mobility is scored from 1-4 with higher scores indicating more activity. Ratings obtained from or based on Queiros et al. (2013). Species no longer present in 2005/6 were identified as NP while species only found in 2005/6 are marked as new.

Species Reduced in Abundance in 2005/6	Bioturbation Ratings		Contribution to Dissimilarity
	Reworking	Mobility	
<i>Scotolema impatiens</i> NP	4	3	4.46%
<i>Melinna cristata</i> NP	3	1	3.82%
Thyasiridae (family level ratings)	3	2	3.10%
Maldanidae (family level ratings)	3	2	2.97%
<i>Amphorete acutifrons</i> NP	3	2	2.81%
<hr/>			
Species Increased in Abundance in 2005/6	Bioturbation Ratings		Contribution to Dissimilarity
	Reworking	Mobility	
<i>Amphorete lindstroemi</i> (new)	2	1	6.06%
<i>Spiophanes kroyeri</i>	3	1	4.03%
<i>Myriochele heeri</i>	2	1	3.99%
<i>Tochochaeta multisetosa</i> (new)	3	2	3.75%
<i>Ceratocephale loveni</i>	3	3	3.18%
<i>Heteromastus filiformis</i>	3	2	2.50%

Table 2.6 ANOSIM test of pair-wise difference in functional group compositions between years. Significant results are bolded. For meaning of abbreviations see section 2.2

Pairings	Functional Group											
	SDF		SBDF		FF		C		O		ND	
	R	p	R	p	R	p	R	p	R	p	R	p
2005, 2006	0.13	<0.001	-0.01	0.51	-0.01	0.62	0.01	0.28	-0.01	0.61	0.08	0.013
2005, 1980	0.27	<0.001	0.05	0.16	0.02	0.69	0.26	0.002	0.099	0.02	0.06	0.056
2006, 1980	0	0.42	0	0.45	0.02	0.25	0.2	0.004	0.169	0	0.02	0.25

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CHAPTER 3: SPATIAL ANALYSIS OF HYPOXIA IMPACTS ON THE ST. LAWRENCE ESTUARY AND GULF (2005 AND 2006)

3.1 INTRODUCTION

Although the temporal analysis of changes in benthic composition in the Lower St. Lawrence Estuary (LSLE) between 1980 and 2005/6 described in Chapter 2 minimizes the problem of confounding studies of different sites and environments, it does not address the specific relationship between oxygen concentrations and diversity changes or the effects of different degrees of hypoxia on benthic composition at species, family, and functional group levels. In order to evaluate relationships between benthic communities and corresponding environmental factors, we therefore undertook spatial comparisons.

Exploring the spatial relationships between benthic diversity and environmental characteristics can help to clarify oxygen thresholds for hypoxic effects. Many studies identify an oxygen concentration of 2 mg/l or 1.5 mg/l as the threshold for normal metabolic function, below which hypoxic effects often begin. However, this threshold was derived from studies of major fishery death and disturbances, and Vaquer-Sunyer and Duarte (2008) documented a wide range of tolerances for oxygen depletion in marine organisms. Their review reported a median lethal oxygen concentration, LC_{50} , ranging from 8.6 mg/l for larval crustaceans to complete tolerance of anoxia in a species of oyster, tested at 20 °C, where the overall LC_{50} was 1.60 mg/l. This concentration corresponds to a level of just 20% below the level at which physiologists assume hypoxia starts. They also reported a large range in median sublethal oxygen level, SLC_{50} , the threshold at which organisms alter their physiological or behaviour functions. A cod species increased its ventilation rate below 10.2 mg/l oxygen, whereas a shrimp species switched to anaerobic respiration at 0.085 mg/l. Many studies report an overall mean SLC_{50} of 2.61 mg/l, and a median SLC_{50} of 2.24 mg/l, indicating that the majority of organisms show adaptive shifts such as avoidance of hypoxic water, increased

ventilation, reduced activity, shift to anaerobic metabolism, or reduced growth well above the standard threshold of 2 mg/l. These changes in behaviour and physiological functioning can lead to reduced survival or abundance through impairment of the ability of organisms to compete, which in turn can affect ecosystem functions, especially if reduced oxygen alters key species.

Along with the oversimplification of thresholds for hypoxia that ignore both large variation in tolerance across species and the sublethal effects of hypoxia, most laboratory-based hypoxia research ignores the interaction of non-oxygen environmental drivers with hypoxia in measuring biotic effects. Increases in ocean temperature through global warming has, in turn, increased the severity and expanse of hypoxic areas, and their disruptive effects on marine ecosystems through multiple direct and indirect mechanisms (Vaquer-Sunyer & Duarte, 2011). Increases in ocean surface temperatures have increased stratification and reduced vertical mixing in the water column sufficiently to significantly impair the normal replenishment of oxygen depleted deeper water, potentially reducing nutrient supplies to surface waters and associated production (Sarmiento et al., 1998).

In particular, temperature can alter the effect of hypoxia on survival by increasing metabolic rates and oxygen demands of organisms, while simultaneously reducing oxygen solubility. A meta-analysis concluded that increased temperature reduced the threshold for lethal oxygen concentration by 16%, and reduced survival time by 74% (Vaquer-Sunyer & Duarte, 2011). Hypoxia also reduces organism tolerance to thermal stress, increasing the difficulty of predicting biodiversity alterations within hypoxic regions (Pörtner & Knust, 2007). In addition, more rapid heterotrophic nutrient cycling and eutrophication in estuaries through temperature increase can raise productivity beyond the ability of grazers to limit algal blooms and further exacerbate hypoxic events (Altieri & Gedan, 2015). Better understanding of these complex interactions between

temperature, production, consumption, and oxygen levels will require comparative field studies in different regions.

A major reduction in benthic biomass (1200%), diversity, and abundance (500%) occurred at oxygen concentrations of 3 mg/l far above the standard 2mg/l level during seasonal hypoxia in Corpus Christi Bay estuary (Ritter & Montagna, 1999). Omnivores disappeared at this oxygen concentration and were replaced by suspension feeding polychaetes. Even below 5 mg/l oxygen concentrations, they observed reductions in benthic community indicators of biomass, diversity, and abundance, suggesting a 5 mg/l optimum oxygen level in this estuary. The authors hypothesized that the summer high temperatures of 29.8-30 °C increased respiration rates, which amplified the disruptive effects of hypoxia sufficiently to account for major losses of benthic species, emphasizing the need to consider the biotic effects of oxygen depletion within the larger environmental context of a given region.

As noted in Chapter 2, cohesive (muddy) and permeable (sandy) sediments can vary considerably in the mechanisms and average depth of oxygen penetration (Ahmerkamp et al., 2017; Ahmerkamp et al., 2020), because cohesive sediments depend upon diffusion, whereas permeable sediments also allow oxygen penetration by advection. Furthermore, the typically deeper penetration of oxygen from advection into permeable sediments, on average contrasts with shallower and more variable oxygen penetration depth in permeable sediments. Through bioturbation, which strongly increases permeability, living organisms alter oxygen penetration, which adds further difficulty to understanding the complex biotic effects of hypoxia. Differences in these variables can potentially alter the effects of a specific level of hypoxia on benthic species' diversity, survival, and functions. Knowledge of the effect of sediment type on benthic responses to oxygen depletion can improve our understanding of the biological costs of hypoxia.

Acknowledging the challenges associated with spatial analysis of differences in the benthos between sites with variation in multiple environmental factors such as temperature and sediment type, inclusion of environmental variability could contribute significant knowledge on interactions between hypoxia, other environmental factors, and benthic assemblages. By considering spatial differences in benthic communities within the LSLE and Gulf of St. Lawrence (GSL), multivariate analysis can contribute useful information on the relationship between various environmental factors and biodiversity across a gradient of oxygen concentrations that range from moderately hypoxic to normoxic.

Seasonal hypoxia typically develops rapidly, lasting weeks to months (Du et al., 2018; Testa & Kemp, 2014). During such events, varying degrees of loss of hypoxia intolerant organisms occurs, and recovery during normoxic periods can depend more on opportunism (short life cycles), rather than on hypoxia tolerance (Gray et al., 2002). Studies of brief intermittent hypoxia cannot help in generalizing the long-term effects of hypoxia on benthic diversity, composition, and function that emerge from an accumulation of benthic loss and gain over many years (Ritter & Montagna, 1999).

Permanent hypoxic zones do not provide normoxic time periods that allow hypoxia intolerant species to return, and may also lack periods of rapid widespread benthic loss that provide opportunistic species to recolonize sediments. These unique aspects of permanent hypoxic zones provide an opportunity to improve understanding of adaptations to hypoxia, and the resulting effects on benthic communities. The expectation of an increase in the number of long-term and permanent hypoxic areas in the future adds urgency to the need to understand hypoxia effects on benthic ecosystems.

The permanent hypoxic zone in the Black Sea provides an opportunity to consider the effects of chronic hypoxia on biotic benthic composition. In this ecosystem, oxygen

concentration declines rapidly with depth from normoxic levels of 4.39 mg/l at 100-meter depth to 0.97 mg/l at 150 metres and 0.26 mg/l at 200 meters, forming a gradient that favours different species within different depth layers (Sergeeva & Zaika, 2013). Although nematodes dominate community composition down to 120 meters depth in one region, cnidarians and polychaetes become increasingly dominant in sediments at 130-150 meters depth. From 150-250 meters, a unique hypoxia-tolerant meiobenthic polychaete (*Vigtorniella zaikai*) increases significantly in abundance. The shift in dominance of biota at different depths illustrates the functional effects of different permanent levels of hypoxia on benthos. The discovery of hypoxia-tolerant organisms in new regions could potentially provide an indicator of long-term or severe hypoxia.

Helly and Levin (2004) reviewed large, permanently hypoxic seafloor regions known as oxygen minimum zones or OMZs, defined as regions with oxygen concentrations below 0.5 mg/l that typically occur at depths between 200 and 1500 m. The extent of these areas far exceeds known areas of hypoxia in coastal locations and enclosed seas resulting primarily from anthropogenic influences. Benthic communities of the OMZ of the Oman region of the Arabian Sea provide a type of natural experiment that reveals important differences in hypoxia tolerance among taxa that studies of locations with only intermittent oxygen reductions might not elucidate (Levin et al., 2000). The lowest levels of species richness, Shannon diversity, and evenness coincided with the lowest oxygen level.

The LSLE is also a region of permanent hypoxia, with the lowest annual mean oxygen concentration reported in recent decades of 56.4 $\mu\text{mol/l}$ or 1.25 ml/l (Gilbert et al., 2005). Jutras et al. (2020) provided an updated study of LSLE oxygenation, and determined that the decline in oxygen in the LSLE of 50 $\mu\text{mol/kg}$ between 1970 and 1995 was followed by a further reduction of oxygen content by 22 $\mu\text{mol/kg}$ between 2008 and 2018, mostly as a result of an increased volume of lower oxygenated North

Atlantic Central Waters entering the LSLE, and a decrease in the volume of higher oxygenated Labrador Current waters entering the estuary.

Therefore, the LSLE provides an opportunity to study a eutrophication driven, permanently hypoxic estuary, and provides a rare opportunity to study the effects of an uninterrupted moderate level of hypoxia that developed gradually over decades. Spatial comparisons of benthic response to hypoxia in the LSLE and GSL provide novel information on biotic responses to contrasting levels of permanently hypoxic, low oxygen, and normoxic habitats. By comparing benthic composition at species, family, and functional levels, this study also explores differences in adaptive ability that yield competitive advantages at different taxonomic levels. The differing effects of hypoxia at different taxonomic levels have important implications for BEF relationships and clarifies the degree of taxonomic sufficiency, and associated cost and effort, to detect hypoxia induced biotic changes.

Utilizing the standard 2 mg/l threshold for hypoxia could significantly underestimate the number of marine environments affected in a region, the spatial extent of these effects, and the degree of disturbance experienced by benthic communities. Selection of this threshold would, in turn, hamper conservation efforts to implement programs to mitigate the extent of the disturbance before major impacts on diversity occur. Rather than using a single threshold for all species based on mass mortality, some experts recommend developing an understanding of the various oxygen thresholds that predict the non-lethal as well as lethal effects of hypoxia specific to different taxonomic groups (Vaquer-Sunyer and Duarte, 2008).

The spatial analysis in our study contributes to this goal by examining changes in diversity and benthic composition at different oxygen concentrations and determining the degree to which hypoxia influences biotic composition. Specifically, we approach hypoxia not as a single effect, occurring only when oxygen drops below 2 mg/l, but as a

persistent source of disturbance of benthic communities across oxygen gradients. By considering diversity and composition of benthic species, families, and functional groups, our study can help to clarify the different thresholds for hypoxic effects of various taxa and differing responses of these taxa to different degrees of hypoxia. Although the absence of an organism at a specific oxygen level might indicate lethal effects, differences in abundance of the same organism at other oxygen concentrations can identify sublethal effects of hypoxia that contribute to changes in benthic composition by altering an organism's ability to cope and/or compete. This knowledge will widen understanding of oxygen thresholds among taxonomic groups that could be utilized in predicting and potentially mitigating hypoxic threats to diversity.

We hypothesize from published data of benthic changes in hypoxic and eutrophic areas that comparison of hypoxic regions of the LSLE with the GSL will show reduced univariate indices and evidence of large shifts in microbenthic structure towards hypoxic and eutrophic tolerant species, families, and functional groups. We further hypothesize that oxygen saturation will predict diversity indices and community composition in the hypoxic LSLE but will not effectively predict these variables in normoxic study regions.

3.2 METHODS

Research Cruises 2005 and 2006

Cruises during 2005 and 2006 utilized the research/survey vessel Coriolis II. Sampling took place from August 20-26, 2005, and from August 14- 22, 2006. Eleven stations were sampled in 2005, ranging from the innermost station 25 in the LSLE to the outermost station 18 in the Houguedo Strait near the southwest coast of Anticosti Island (Figures 3.1 and 3.2). The depth of these stations varied between 281 and 378 m. Selection of sampling sites during the 2005 cruise focused on the LSLE, and seven stations coincided with the stations sampled by Ouellet (1982) in 1980 (see Section 2.2). The 2006 cruise sampled nine stations, all located within the GSL except for station 23. Station 17, the most westerly site, was south of Anticosti Island in the deep part of the Laurentian

Channel, at 411 metres depth. Two stations, CA and TCA, were located along Anticosti Channel north of the island. Most of the sites sampled on this cruise were located along the shallower Esquiman Channel that parallels the west coast of Newfoundland, up to the most northerly and easterly station, TCE, near Hawke's Bay, Newfoundland. The 2006 sampling spanned a wide range of submarine features, resulting in sampling depths from 257 to 434 metres, encompassing a larger range of sampling depths than the 2005 cruise. Table 3.1 provides a complete list of the latitude and longitude coordinates of each station utilized in the spatial analysis.

Sampling Protocol

Chapter 2 provides a full description of the protocol for obtaining sediment samples and processing of sediments. In summary, sampling at the stations visited in 2005 and 2006 followed sampling protocols utilized by Ouellet (1982), obtaining 3 replicate van Veen grab samples of .125 m² at each station. Sediments were sieved onboard the ship through a 1 mm mesh sieve and preserved in 10% buffered formalin solution. In the lab, organisms were identified to the lowest possible taxonomic level to provide density and diversity estimates for each site, using taxonomic names verified in the World Register of Marine Species (WoRMS Editorial Board, 2020).

Depth, temperature, salinity, and % oxygen saturation profiles were obtained using an SBE 19 Sea-Bird CTD (conductivity, temperature, and depth) probe (Sea-Bird Scientific). Sediment subsamples were collected from grabs at each station to determine sediment composition, mean grain size, and % total organic matter. Bottom water samples were collected at each station with Niskin bottles attached to a rosette sampler. The following laboratory procedures were carried out to quantify the relevant environmental factors used in the spatial analysis.

Winkler assays, as modified by Carpenter (1965) and Carritt and Carpenter (1966), provided dissolved oxygen concentrations in bottom water samples. This assay can

detect the low levels of dissolved oxygen expected in the most hypoxic waters (<20% saturation) of this region (Belley et al., 2010).

Sediment total organic matter (%) was determined for each site using sequential loss on ignition (LOI) (Dean, 1974). Heating of 1 g of surface sediment at 500 °C for 12 hours was assumed to have oxidized organic carbon to carbon dioxide. Measured loss in weight thus provided an estimate of organic carbon in sediment samples. Determination of grain size involved separation of particles to eliminate clumping or flocculation of particles. Subsamples of 1 g of surface sediment were treated with 10 ml of 1g/L sodium hexametaphosphate solution [(NaPO₄)₆ or 'calgon'] to disperse floccules of the charged clay particles. Together with 40 ml of distilled water, sediments were then shaken for three hours to separate particles. Ultrasonic treatment before and during analysis increased particle separation (Blott, 2001). The sample was then processed with a Beckman Coulter LS 13 320 Laser Diffraction Particle Size Analyzer, which differentiates particles ranging from 0.04 µm to 2 mm in size (Blott et al., 2004; Pye & Blott, 2004). Mean grain size and distribution of particle sizes in each size class were determined using GRADISTAT particle size analysis software.

Statistical Methods

We used PRIMER-E version 6 with the PERMANOVA+ add-on (Anderson et al., 2008; Clarke and Gorley, 2006) for data analysis, including data transformation, matrix calculation, and multivariate testing. We performed all tests at both the family and species level to determine if family level analysis provided taxonomic sufficiency to detect environmentally induced benthic changes.

Univariate Analysis

Calculation of species and family richness, Shannon diversity index, and Pielou's Evenness index for all stations provided standard measures of diversity. The diversity indices were transformed into Euclidian distance matrices. We then compared these indices between stations using a one-way PERMANOVA+ test.

Multivariate Analysis

All environmental data were $\log(x+1)$ transformed and normalized prior to the calculation of a Euclidean distance resemblance matrix, the most commonly used distance matrix for multivariate analysis of environmental data. All species and family data were 4th root transformed prior to calculation of a Bray-Curtis resemblance matrix because Bray-Curtis matrices are more appropriate for taxonomic data.

CLUSTER analysis grouped the stations by degrees of similarity between families, and SIMPER calculated the families in each group that contributed most to dissimilarity.

Distance-based linear modeling (DISTLM) using a step-wise selection procedure and AICc selection criteria determined which environmental variables best accounted for variation patterns in the biotic assemblages, and how much of the variation they explained. Distance-based redundancy analysis (dbRDA) using the DISTLM results displayed the best fitted linear combination of environmental factors to account for biotic variation

Functional Group Analysis

Based primarily on information from the World Register of Marine Species, (WoRMS Editorial Board, 2020) (with supplemental information if needed for individual organisms) we divided the biota into six functional groups: surface deposit feeders (SDF), subsurface deposit feeders (SBDF), filter feeders (FF), carnivores (C), omnivores (O), and unclassified (ND). After 4th root transformation of all functional group data, we produced a Bray-Curtis resemblance matrix. The proportion of total abundance of each function group was calculated for each station. Further analysis of functional group composition was based on oxygen group differences.

Analysis of Pooled Data from Oxygen and Temperature Station Groupings

Belley et al. (2010) showed differences in benthic bioturbation activity among regions with differing oxygen saturation levels in the LSLE and GSL, and we hypothesized that we would find univariate and benthic structural differences among regions differing in oxygen concentrations. We divided the oxygen saturation range of 21.04% – 58.13% measured in the study region into 4 levels based on the approach of Belley et al. (2010) to form 4 oxygen groups: hypoxic, low, intermediate, and high.(Table 3.2).

Univariate Analysis of Pooled Oxygen Groups

We tested the differences in mean univariate values among oxygen groups using PERMANOVA+ to compare family and species level richness, Shannon diversity, and Pielou's evenness between oxygen group pairs.

Multivariate Analysis of Pooled Groups

Through pair-wise PERMANOVA+ tests we examined variation in species and family composition between oxygen group pairs. Through SIMPER we then determined the families and species most responsible for differences among assemblages among oxygen groups. We utilized PERMANOVA+ to evaluated functional group frequency differences among oxygen groups based on pairwise tests.

3.3 RESULTS

In reporting and discussing results in this chapter, references to 2005 data consider stations within the LSLE and the western portion of the GSL ending at station 18. The 2006 data considers stations within the GSL except for station 23, which was sampled in both 2005 and 2006. Therefore comparisons between these two years in this chapter focus on spatial variation in benthic assemblages between the LSLE and GSL. This chapter therefore represents a spatial study that complements temporal comparisons presented in Chapter 2 that used some, but not all of the data from the 2005 cruise.

Univariate Analysis of Species and Family Composition

Unexpectedly, the 2005 stations mostly within the LSLE (Appendix 1) possessed higher overall richness than the 2006 stations located in the GSL, and somewhat higher Shannon diversity, whereas higher evenness characterized the GSL stations. PERMANOVA+ on the species-level diversity indices showed highly significant differences in richness, evenness, and Shannon indices between 2005 and 2006 stations ($p=0.001$ for all). PERMANOVA tests confirmed significantly higher family-level richness and Shannon diversity at 2005 stations compared to 2006, and higher Pielou's evenness at 2006 stations. ($p=0.001$ for all family tests except $p=0.005$ for Shannon diversity). Differences in richness and evenness were more pronounced at the species than at the family level.

Multivariate Analysis of Family and Species Composition

CLUSTER analysis at the family level formed 6 distinct groups (Figure 3.3a); Table 3.3 lists the major families of each group. At approximately 47% similarity, stations from the Anticosti and the upper Esquiman channels in Group B and stations at the estuary mouth of Group C differentiated from all the other stations. Family composition at the stations from south of Anticosti Island and at the intersection of the Esquiman and Laurentian Channels, forming Group E were distinct from Group D stations in the lower oxygen level region in the middle of the estuary, and Group F stations from the hypoxic

and coldest area at the head of the estuary, whose most abundant two families are unique to that group. Station IC, a lone sample, grouped with both mid-Estuary and Laurentian-Esquiman stations, but was placed within group E because stations 17 and 16 were most similar to IC. Group G, at mid Esquiman Channel containing BOI formed a unique family assemblage that did not cluster with other stations. The mean oxygen values of the groups spanned a wide range of oxygen % saturation, from 21.27 % saturation in group F to 54.14 % saturation in group E (Figure 3.3b).

Distance-based linear modeling (DISTLM) produced the best-fitted model for species-level data incorporating the environmental variables (Table 3.4a) that combined, explain 49.9% of the variation in the biota. Modeling the same variables using family-level data reduced the amount of explained variation by only 0.7% compared to species-level data (Table 3.4b), explaining 49.2% of the variation in the biota. Overall, the environmental factors in the model showed a similar linear relationship with both species and family composition.

The distance based redundancy analysis (dbRDA) of the relationship between environmental factors and species composition showed the highest correlation between environmental factors of temperature and longitude and benthic composition along axis 1, and the highest correlation of benthos with depth and % clay on axis 2 (Figure 3.4a). The dbRDA showed significant and similar correlation to family level assemblages when fitted to the same environmental factors (Figure 3.4b) as those in the species analysis; temperature and longitude dominated dbRDA1 (35.7% of fitted variation), and depth and % clay dominated dbRDA2, similar to the results for species analysis, but accounting for slightly less biotic spatial variation (26.5%).

Spatial Analysis of Functional Group Composition

Functional group composition divided sharply into two main groups (Figure 3.5) that corresponded to location within either the hypoxic and low oxygen regions of the LSLE

or GSL. The percentage abundance of each functional group indicates a very clear shift spatially (Appendix 2) from SDFs, the most abundant functional groups in LSLE stations 25 through 21, to omnivores at station 20 at the mouth of the estuary (Figures 3.1 and 3.2). The reduction of SDF within the GSL was less consistent than the replacement of omnivores as the dominant group in the LSLE, given the higher abundances of SDFs at stations 17, IC, and CA within the GSL relative to omnivores.

When grouping stations by oxygen levels, the hypoxic group surprisingly showed both the highest mean richness as well as highest mean Shannon diversity at the species level (Figure 3.6). Highest evenness scores occurred in the high oxygen grouping for both species and families. PERMANOVA+ confirmed the unexpected level of richness of 12.5 in the hypoxic group was significantly higher ($p=0.001$) than the High oxygen group richness of 6.6, intermediate richness of 7.16 ($p=0.007$) and low group richness of 8.72 ($p=0.04$). Species-level Shannon diversity did not differ significantly among oxygen groups, despite large differences in richness, perhaps resulting from significantly higher evenness for the high oxygen group than for all other groups.

The unexpectedly high family richness in the hypoxic group (Figure 3.7), differed significantly only from the high oxygen group, with no clear difference between the hypoxic group, and the low and intermediate oxygen stations. Shannon diversity also did not differ among groups, perhaps because significantly higher evenness in the high oxygen group countered the higher richness of the low and hypoxic oxygen groups.

Testing of differences in species composition between oxygen groups through PERMANOVA+ (Table 3.5a) confirmed a pronounced dissimilarity between the Hypoxic group and each of Low, Intermediate, and high oxygen stations. The Low and Intermediate oxygen group faunal composition each differed from the high group. Family composition (Table 3.5b) showed less differences between groups, as the Hypoxic and Low groups were almost, but not significantly different ($p=.075$); the

families still differed between composition of high oxygen stations and each of the hypoxic, low and intermediate stations.

SIMPER identified the species and families most responsible for differences between oxygen groups, with the greatest species level mean dissimilarity between the hypoxic and high groups (82.87%) (Table 3.6a). High percentage abundances of *Spiophanes kroyeri*, *Ampharete lindstoemi* and *Trochochaeta multisetosa*, all in the hypoxic group, were most responsible for the dissimilarity between these groups, in contrast with low abundances or complete absence of *S. kroyeri* and *T. multiseotsa* in the high oxygen group. The presence or absence of hypoxia tolerant species largely determined species-level dissimilarity between groups, as opposed to high abundances of any one species in the high oxygen group.

SIMPER results showed that hypoxic and maximum oxygen saturation groups also differed most at the family level (dissimilarity=73.12%). 'Specialized' hypoxic group members were primarily responsible for these differences given that the three most abundant families Spionidae, Oweniidae, Trochochaetidae, as well as Trichobranchidae were essentially absent from the high oxygen group (Table 3.6b). The hypoxic and maximum groups were almost 10% more dissimilar at the species level than at the family level.

A strong shift at hypoxic stations towards SDFs produced an SDF/O ratio of 12.91 (Figure 3.8), contributing to the unexpectedly highest richness and Shannon diversity at the hypoxic stations. Omnivores dominated all other oxygen groups, although to a lesser degree, with the largest O/SDF ratio of 1.90 in the intermediate group, reflecting much greater evenness in the composition of these groups than at hypoxic stations.

From PERMANOVA+ testing, we found significant differences in functional group composition between the hypoxic group and both intermediate and high oxygen groups.

though not between hypoxic and low oxygen stations ($p=0.0692$) (Table 3.7).

Intermediate and high oxygen groups were significantly different, likely owing to the highest abundance of omnivores occurring at intermediate stations. This pattern is reflected in the intermediate group possessing the lowest SDF/O ratio; therefore, the hypoxic and intermediate stations had the biggest difference in their SDF/O ratio of any pair, 12.91 compared to 0.53 (Figure 3.8).

3.4 DISCUSSION

The highest levels of richness and Shannon diversity at stations 25, 24, 24.75 and E5 at the head of the LSLE represented the most significant and unexpected result of this spatial study, given well-documented eutrophication and hypoxia in this region. At sites near and within the GSL, richness and diversity generally decreased. This surprising result contrasts the temporal decline in richness in the LSLE in comparison with 1980, as discussed in Chapter 2. The comparison of univariate indices among the oxygen saturation groupings illustrates the paradoxical effects of hypoxia on benthos of the LSLE.

Despite clearly greater species richness in the hypoxic group compared to any other group, evenness and oxygen saturation correlated positively as expected, reflecting the limited abundance of some families and species in the hypoxic group. Therefore, despite highest Shannon diversity at the hypoxic stations, the difference was not statistically significant, likely reflecting the lowest evenness at this station. The inverse relationship between univariate indices and oxygen levels at both taxonomic levels contradicts our hypothesis of a positive relationship between oxygen levels, species richness, and Shannon diversity. SIMPER identified the chief drivers of differences in composition of the hypoxic and maximum groups as polychaete surface dwellers and feeders that frequently tolerate organic loading (Dafforn et al., 2013; Dean, 2008), such as *S. kroyeri* and *A. lindstroemi*, the two species that differed most in abundance between the hypoxic and high oxygen groups.

Numerous studies have documented rapid reproduction and resulting increased abundance of opportunistic organisms in locations subject to excess organic input (Pearson and Rosenberg, 1978; Gray, 1979; Gray et al., 2002). While our study does not document the full effect of organic enrichment, anthropogenically stimulated productivity of the LSLE likely played a significant role in sustaining the unusually high diversity in the hypoxic environment decades after oxygen saturation began to decline. Once hypoxic and low oxygen environments became established in the estuary, surface-adapted, hypoxia-tolerant organisms that can survive and outcompete deeper dwelling infauna presumably benefited from a sustained increased source of organic matter they could access from the sediment surface. In this way, eutrophication and hypoxia could act synergistically to establish a long-term, highly altered benthic community with paradoxically higher richness than unaffected regions.

Temperature also potentially contributed to the unexpectedly diverse biota in the hypoxic group, which corresponded to the LSLE region with the coldest water temperature of 4.9-5.2 °C, whereas water temperature in the warmest stations in the GSL exceeded 5.5°C. A meta-analysis by Vaquer-Sunyer and Duarte (2011) documented 74% mean decreased survival times in hypoxic conditions in marine benthic organisms exposed to high water temperatures. In contrast, the lower temperature in the LSLE, by reducing benthic average metabolic rates, could have helped hypoxia tolerant organisms increase in richness and diversity.

The highest benthic richness and diversity observed in the most disturbed environmental area in the LSLE contradicts the Pearson-Rosenberg model (1978) that predicts abundance peaking near the source of organic pollution, but with lowest richness at the site of disturbance, and increasing to background levels away from the pollution source. While temporarily increased levels of richness have been reported during and after environmental changes, these communities typically return to pre-

disturbance levels over time. In contrast our study involves an area of continuous hypoxia over decades. The complex community composition resulting from this unusual form of environmental disturbance may explain the inverse situation at the main hypoxic region disturbance in the LSLE (Chapter 2). The slow decline in oxygen over time, without major mortality or defaunation events, apparently enabled gradual community changes more compatible with long-term competition and establishment of taxa with adaptations to cope with hypoxia, rather than favoring only the strongest opportunists. The varied adaptations exhibited by the dominant species at the head of the estuary could increase diversity beyond the level typical of severely hypoxic or anoxic environments that develop over short time spans. This inversion of the typical model of biotic changes along a hypoxic and organic pollution gradient could therefore provide some new understanding of biotic change in the continuous presence of these two interlinked environmental modifiers over decades.

The Pearson-Rosenberg model also predicts increased richness and diversity towards relatively undisturbed regions of the GSL. However, noticeably lower richness but greater evenness characterized stations 20, 19, and 18 at the mouth of the estuary, at the transition area into the Gulf relative to the stations closer within the estuary. Highly variable diversity indices characterized the stations along the Esquiman Channel as well, which runs parallel to the west coast of Newfoundland.

Based on field studies between 1992 and 1994, Desrosiers et al. (2000) reported considerable variability in the overall availability of organic sources in the GSL, both in terms of timing of primary production deposition at the sediment interface, and the amounts that reached the seafloor at various stations. They inferred that the mouth of the estuary and western end of Anticosti Island near stations 20, 19, and 18 received variable amounts of organic matter from the gently sloping Laurentian Channel at the Anticosti Gyre. Organic inputs decreased towards the mouth of the Esquiman Channel where it intersects the Laurentian Channel, a pattern compatible with the lowest

richness and diversity in the GSL at station 16. Near-bottom flow also influences organic resources at the sediment-water interface (Snelgrove and Butman, 1995), potentially adding to small-scale variability.

Levin and Gage (1998) in their literature review emphasized the primary importance of oxygen concentrations as a determinant of benthic composition within hypoxic regions, but at sites above the oxygen threshold for survival, organic matter was the most significant determinant of benthic diversity. The greater richness and diversity in the estuary in comparison with the Gulf could result from the cumulative effects of adaptations to hypoxic and eutrophic alterations within the estuary, and the added influence of limited and variable organic inputs through parts of the Gulf. Belley et al. (2010) found a greater density of bioturbation traces in the hypoxic region of the LSLE than in the normoxic stations of the GSL; he largely attributed the higher trace density to the higher activity level of *Ophiura* sp., a SDF that likely outcompeted the suspension feeders that occurred only at moderate and highly oxygenated regions of the LSLE and GSL. This thesis adds to those findings by confirming the shift in community composition to favour SDF in the hypoxic region, and by finding greater richness in the hypoxic and eutrophic area.

Although the hypoxic and eutrophic stations of the LSLE are not directly comparable to areas of time-limited organic pollution, multiple studies (Clark et al., 2015; Mckinley et al., 2011) report unexpectedly greater richness in disturbed than in unaffected environments that may help to interpret our findings. Dafforn et al. (2013) reported greater abundance and richness of SDF polychaetes in Australian estuaries contaminated with both metals and organic enrichment than in uncontaminated habitats. They attributed this pattern to two factors, namely the increase in SDFs in response to organic enrichment, and the comparatively oligotrophic uncontaminated estuaries that contained much less organic matter and supported fewer families. Clark et al. (2015) also concluded that interaction of variables can create atypical biotic

patterns and therefore block detection of significant disturbances. Parallels exist in the reversal of expected diversity characteristics between the LSLE and Australian estuaries.

In contrast to the unexpected greater richness and Shannon diversity in the LSLE and hypoxic stations particularly, the high proportion of SDFs at stations 21-25 affirms documented patterns of functional group change that favour surface dwellers within eutrophic and hypoxic regions (Belley et al., 2010; Borja et al., 2000; Dafforn et al., 2013; Lenihan et al., 2003). The vast imbalance in functional group composition of the hypoxic group provides perhaps the clearest indicator of the degree to which the upper LSLE has undergone major alteration of the benthos. The SDF/O ratio of the hypoxic group is 24 times greater than the intermediate, and 20 times that of the high oxygen group ratio. Pronounced difference in abundance of SDFs between the hypoxic and other groups ranged from 3.3-7.8 times greater. Previous studies of hypoxic environments that report a shift to SDFs (Belley et al., 2010; Levin et al., 2009), besides noting advantages of SDF in hypoxic and eutrophic regions, also recognized the poor capacity of omnivores to tolerate increasingly hypoxic and potentially toxic sedimentary environments (e.g. hydrogen sulfide).

Hypoxia leads to shallower vertical distributions of organisms within sediments (Levin et al., 2009) because many infaunal species emerge from their burrows and/or shed metabolically costly camouflage (Riedel et al., 2014; Wu, 2002), decreasing oxygen uptake but leaving them vulnerable to predation, and potentially further decreasing their abundance. This large difference in functional groups between regions serves as evidence of a form of biological 'threshold' of tolerance for hypoxia and eutrophication within the hypoxic group stations that virtually excluded deeper dwelling benthos. The resulting uneven functional group composition in the hypoxic, low, and intermediate oxygen groups (the intermediate group had the highest omnivore level in contrast), may leave insufficient members of the other functional groups at these stations to maintain overall functions and services (Hale et al., 2016).

Significant differences in both species and family composition between assemblages in both low and high oxygen environments corroborates the univariate evidence that even reduced, though not fully hypoxic levels of oxygenation can alter benthic assemblages. The lower proportion of mobile bioturbators in the low oxygen group could amplify differences in composition associated with seafloor oxygen concentrations by further reducing sediment oxygenation. The difference between hypoxic and low oxygen species assemblages disappears at the family level, indicating that even within the same family, environmental differences select for species between these neighbouring regions. We saw the same process of intra-family competition occur temporally in the LSLE when comparing 1980 and 2005/6 (Chapter 2), emphasizing the powerful selection for hypoxic and eutrophic tolerant benthos.

The sudden shift to omnivores in the GSL lacked a clear environmental driver in our study, in contrast to the compatibility of strong dominance of SDF and diminished presence of omnivores in the estuary with known environmental alterations. Surprisingly, stations 19 and 18 supported the greatest abundance of omnivores in the entire study area, despite their proximity to the estuary. However, previous studies may provide some indirect information to help account for dominance of omnivores in the GSL. Desrosiers et al. (2000) reported significant seasonal variation and limited nutrient input into the Anticosti Gyre region near stations 20 and 19 in 1993 and 1994, which favours omnivores as bioturbators in that they can utilize food sources within the sediment. Combined with the increase in the proportion of omnivores from 18% in 1994 to a range of 40-76% in 2005/6 provides evidence of a shift in functional group composition within the Gulf consistent with a general reduction in nutrient input into some regions of the GSL over the 12 years between studies. Alternatively, the normoxic level at station 19 and high oxygenation at station 18 could possibly provide a sufficient level of oxygen in the sediment to allow deeper bioturbating omnivores to dominate these stations.

In contrast to the univariate studies that provided evidence for strong selection for tolerant benthos within the hypoxic and eutrophic area of the LSLE, the multivariate analyses of the overall relationships between environment and biota across the whole area of study (from the head of the LSLE to the Esquiman channel) indicated a considerably diminished role for oxygen in accounting for biotic composition in the lower part of the estuary and especially in the GSL where normoxic conditions prevailed.

Clustering strongly differentiated the family assemblage of stations 25, 24, E5 and 24.75 at the hypoxic head of the estuary, with only 31.5% similarity to the other groups. The dominant families, Spionidae, Oweniidae, and Ampharetidae are all SDFs that previous studies identify as taxa well adapted to hypoxic conditions and or to eutrophication, to varying degrees (Borja et al., 2000). The absence of mobile highly active bioturbators, lumbrinerid and nereidid polychaetes, that dominate almost all other stations, emphasizes that threshold oxygen levels of these stations might exclude species poorly adapted to eutrophication and hypoxic sediments.

Ampharetids dominate the middle of the LSLE (group D), which spans stations 23.6 to 21, where oxygen levels are not hypoxic, but are lower than the rest of the LSLE and GSL. Benthos that can benefit from significantly lower levels of organic enrichment compared to eutrophication towards the head of the LSLE should characterize this region (Thibodeau et al., 2006); this scenario accounts for the dominance of *A. lindstoemi*, a species that is hypoxia tolerant but highly sensitive to eutrophication disturbance. The other groups varied in the degree to which omnivores dominated family composition, indicating taxa indicative of a progressively less altered environment deeper within the sediments favourable to bioturbators.

Whereas temperature played an important role in DistLM distance-based linear modeling at both the species and family level, oxygen concentrations predicted much less of the biotic variation in these models than expected from its apparent influence on biota in the LSLE. This difference likely reflects the limited strong threshold effect of

oxygen upon biotic assemblages only within the lower estuary that does not extend into comparatively normoxic habitats; in contrast, the lowest temperatures in this study at the estuary head parallel the effect of oxygen on benthic composition at the hypoxic head of the estuary, and unlike oxygen levels it also remains strongly correlated with GSL benthos at normoxic stations.

Depth could also influence family composition, in that it can affect hydrodynamics at the sea floor and therefore availability of food (Snelgrove and Butman, 1995). Percentage clay, was the third most important factor influencing family composition and silt was fourth in proportion of predicting species composition; because clay retains more organic material than coarser sediments (Lee et al., 2019), it can influence biotic composition, especially if some regions of the GSL receive variable and limited organic input (Desrosiers et al., 2000). The reduced oxygen permeability of some clay sediments compared to silt sediments (Hicks et al., 2017; Revsbech et al., 1980) may partially explain the importance of % clay for family structures, and % silt in species assemblage composition. The lower silt and higher clay content of the estuary sediments presumably favours SDF; conversely, the slightly more oxygen permeable higher silt and lower clay sediments in the GSL would favour deeper-dwelling species, facilitating a deeper RPD and infaunal bioturbation which, in turn, can increase sediment oxygenation and further deepen RPD. Furthermore, the lower clay content of the GSL sediments compared to those in the LSLE, presumably retains less organic content (Lee et al., 2019), thereby favouring deep bioturbating omnivores.

dbRDA showed that oxygen correlated only weakly with biota when modelling for environmental factors that best predicted assemblages across the largest number of stations. Most stations did not align along the oxygen saturation vector, but instead along vectors for temperature, longitude, depth, and sediment, irrespective of taxonomic levels. This pattern aligns with the limiting effect of oxygen on benthic

survival and competition only in study locations within the hypoxic or near-hypoxic range.

3.5 CONCLUSIONS

The unexpected finding of significantly greater richness and higher (but not statistically significant) Shannon diversity in the altered region of the LSLE than in the unaltered GSL builds on previous work that provided considerable evidence (Chapter 2) of a distinction between the biotic shift in the estuary over time and the rapid colonization of heavily disrupted areas by opportunists. A more complete understanding of the effects of other permanent hypoxic regions on microbenthic structures will require further research to determine whether chronic hypoxia leads to different benthic communities than those documented after short episodes of lowered oxygen.

The surprising spatial pattern challenges the assumption that greater richness or diversity compared to presumably undisturbed environments reliably indicates an undisturbed environment. Questioning this assumption can prevent researchers from missing atypical shifts in diversity and biotic composition in altered environments as has occurred in the LSLE. Because most biotic responses to disturbance involve increases in limited number of species that cope well with disturbance, evenness may offer a sensitive measure of community-altering events, given reduced evenness in the disturbed head of the LSLE. Furthermore, the high proportion of SDFs, generally known to be hypoxia tolerant, combined with the near absence of filter feeders and omnivores in a habitat, could potentially provide a bioindicator to clarify ambiguous and unexpected patterns, such as those in the LSLE.

The analysis of environmental-biotic relationships in the spatial study strongly supported a threshold effect of hypoxia that excluded species intolerant of low oxygen concentrations. Differences in benthic assemblages between low and maximum oxygen stations adds new understanding of the disruptive effects on benthic communities that even reduced, though not truly hypoxic oxygen levels, can exert. This finding points to

the need to look for biotic changes and not rely solely on oxygen concentrations to infer healthy or altered environments.

3.6 FIGURES AND TABLES

Figures

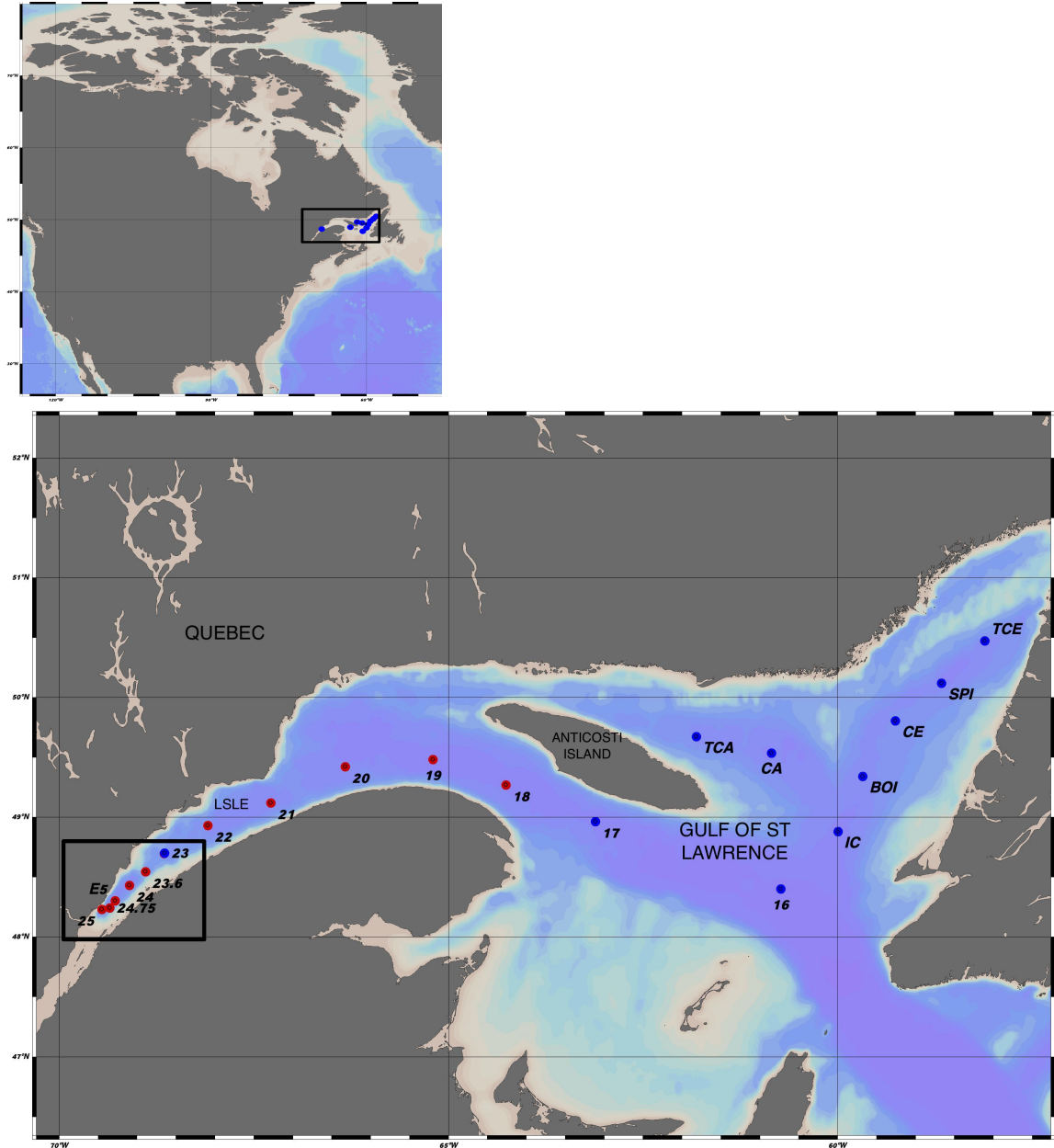


Figure 3.1. Upper map of North America shows the location of the study area of the 2005/6 cruises that occurred in Quebec, Canada. Lower map shows stations sampled during the 2005 and 2006 research cruises. LSLE refers to the Lower St Lawrence Estuary. Red symbols indicate 2005 stations sampled. Blue symbols indicate 2006

stations sampled. Station 23 was sampled on both cruises. The box indicates the area shown in Figure 3.2

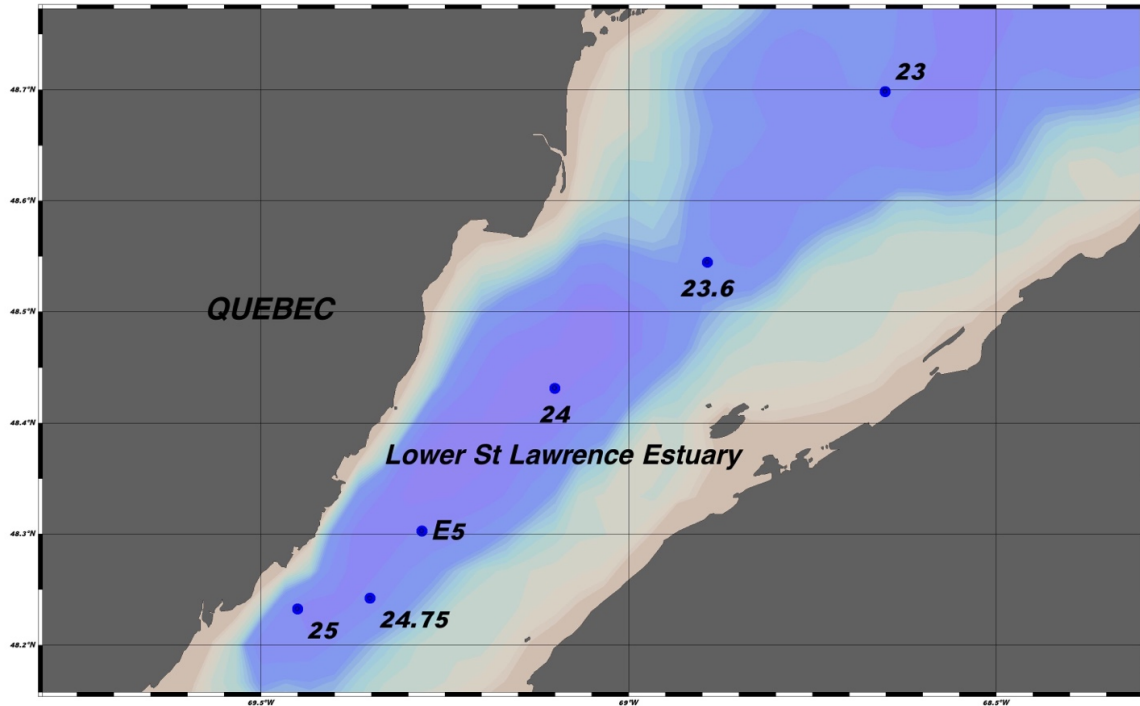


Figure 3.2. Western-most stations of the 2005 research cruise in the Lower St. Lawrence estuary. (See box in Figure 3.1) Station 23 was sampled during both the 2005 and 2006 cruises.

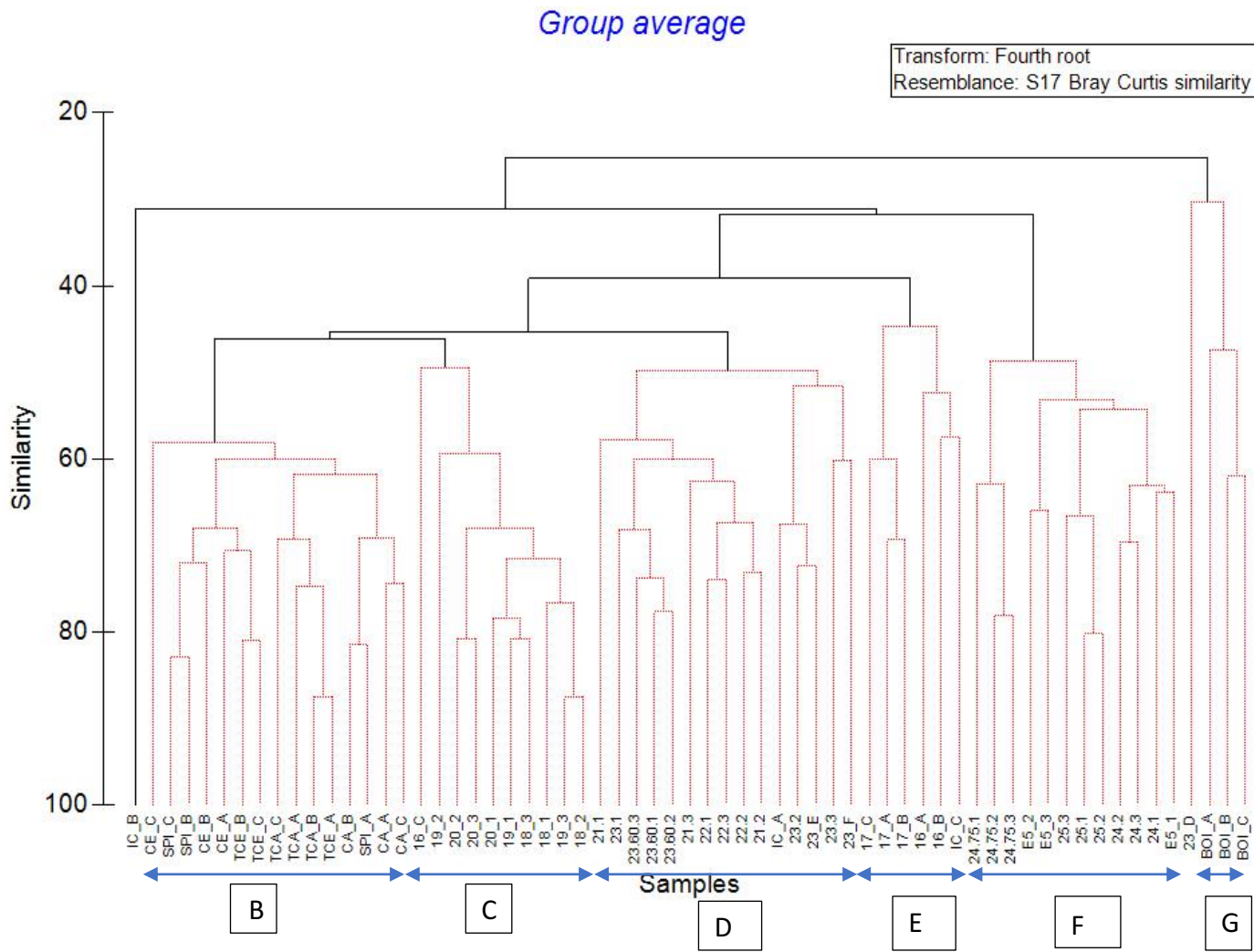


Figure 3.3.a Dendrogram of station groupings based on family similarity by the CLUSTER procedure. Stations differentiated into Groups at different levels of similarity. IC_B was not placed in a group and not considered further.

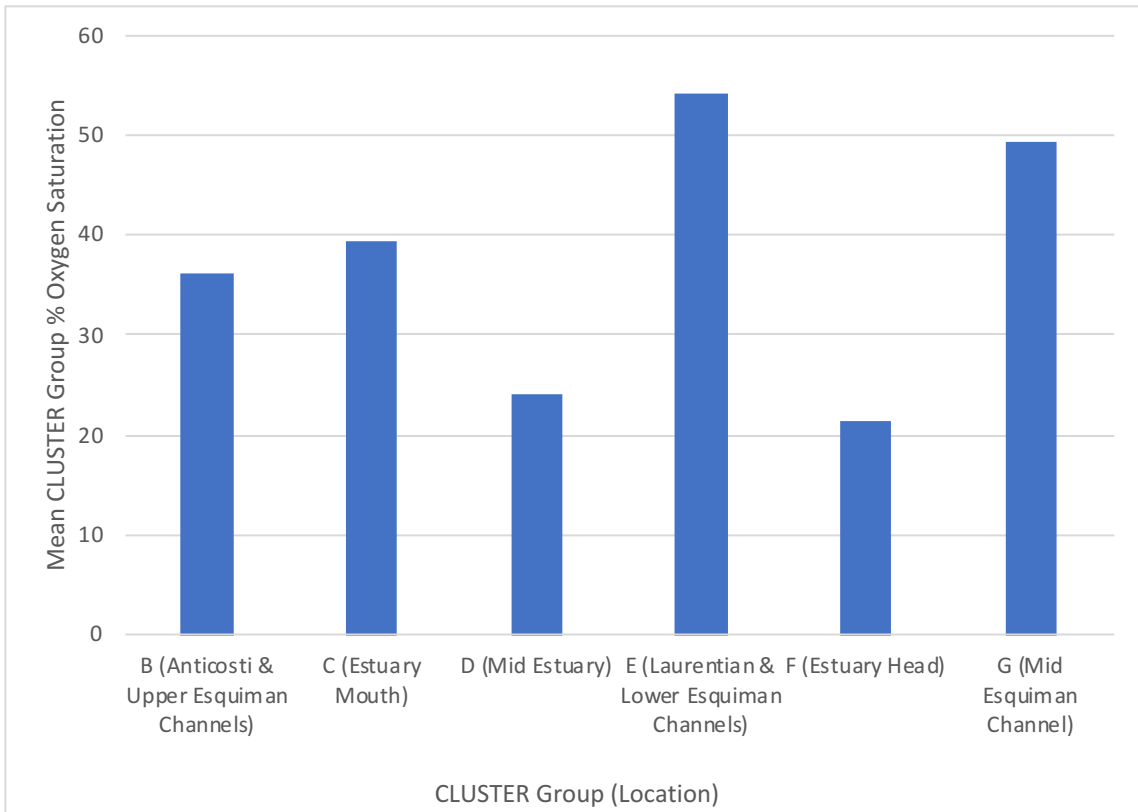


Figure 3.3.b Mean oxygen % saturation of groups formed through CLUSTER. The location of each group within the LSLE and GSL is indicated in brackets.

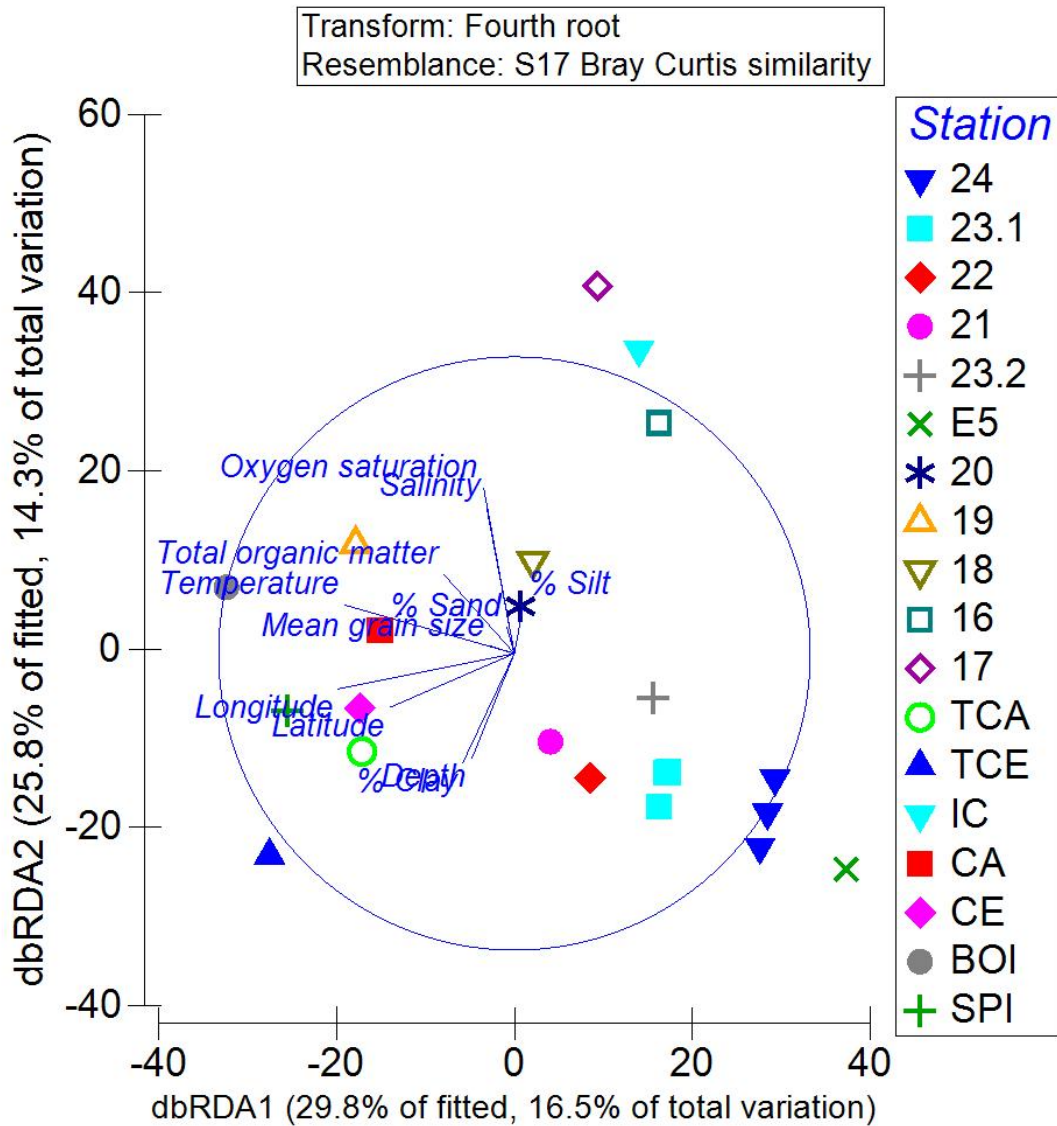


Figure 3.4a Distance based redundancy analysis of the relationship between environmental factors and species assemblages. The vector direction and length are proportional to the amount of variation each environmental factor contributes to the variation in biota accounted for by each axis. Station 23.1 refers to samples obtained at station 23 in 2005, and 23.2 samples were obtained in 2006.

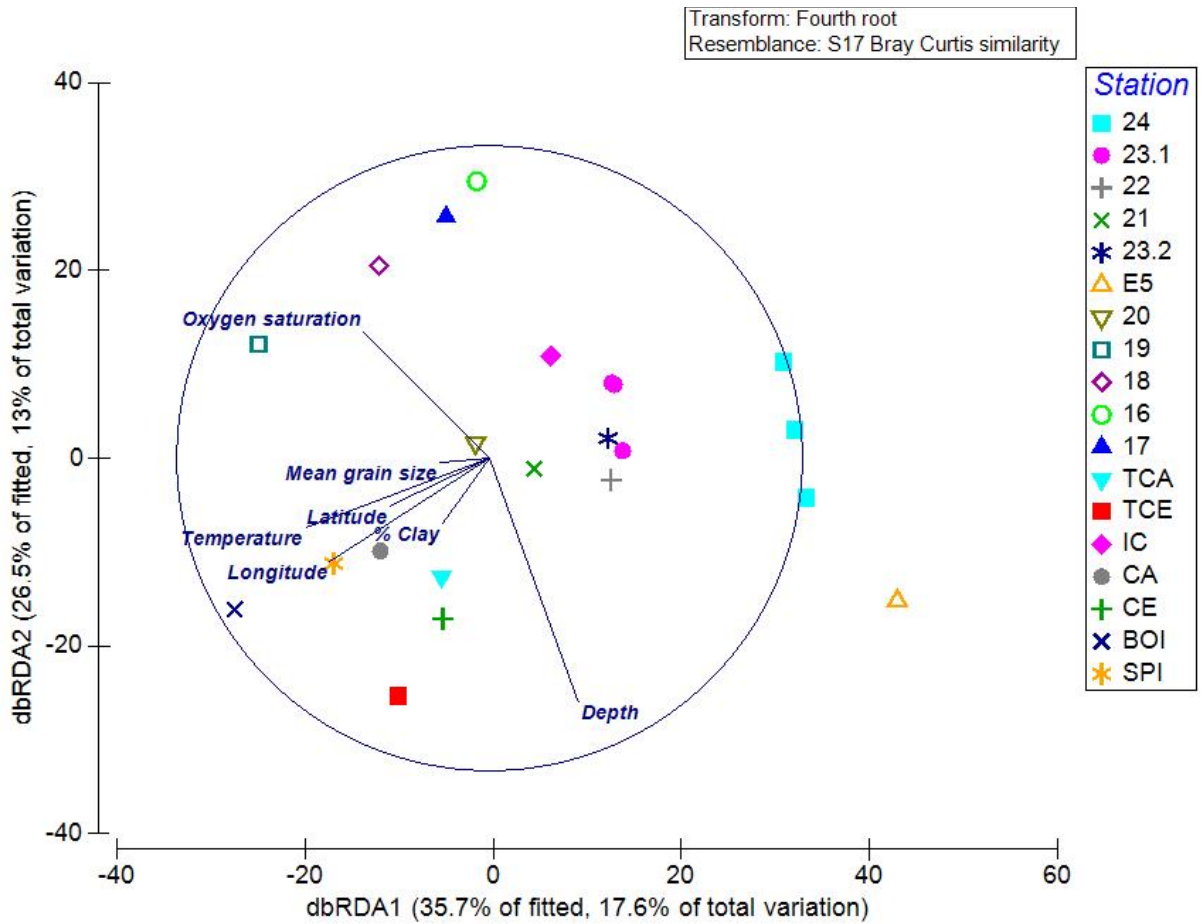


Figure 3.4b. Distance based redundancy analysis of the relationship between environmental factors and Family assemblages at the 2005 and 2006 stations. The vector direction and length is proportional to the amount of variation each environmental factor contributes to the variation in biota accounted for by each axis. Station 23.1 refers to samples obtained from station 23 in in 2005, and station 23.2 refers to samples from station 23 obtained in 2006.

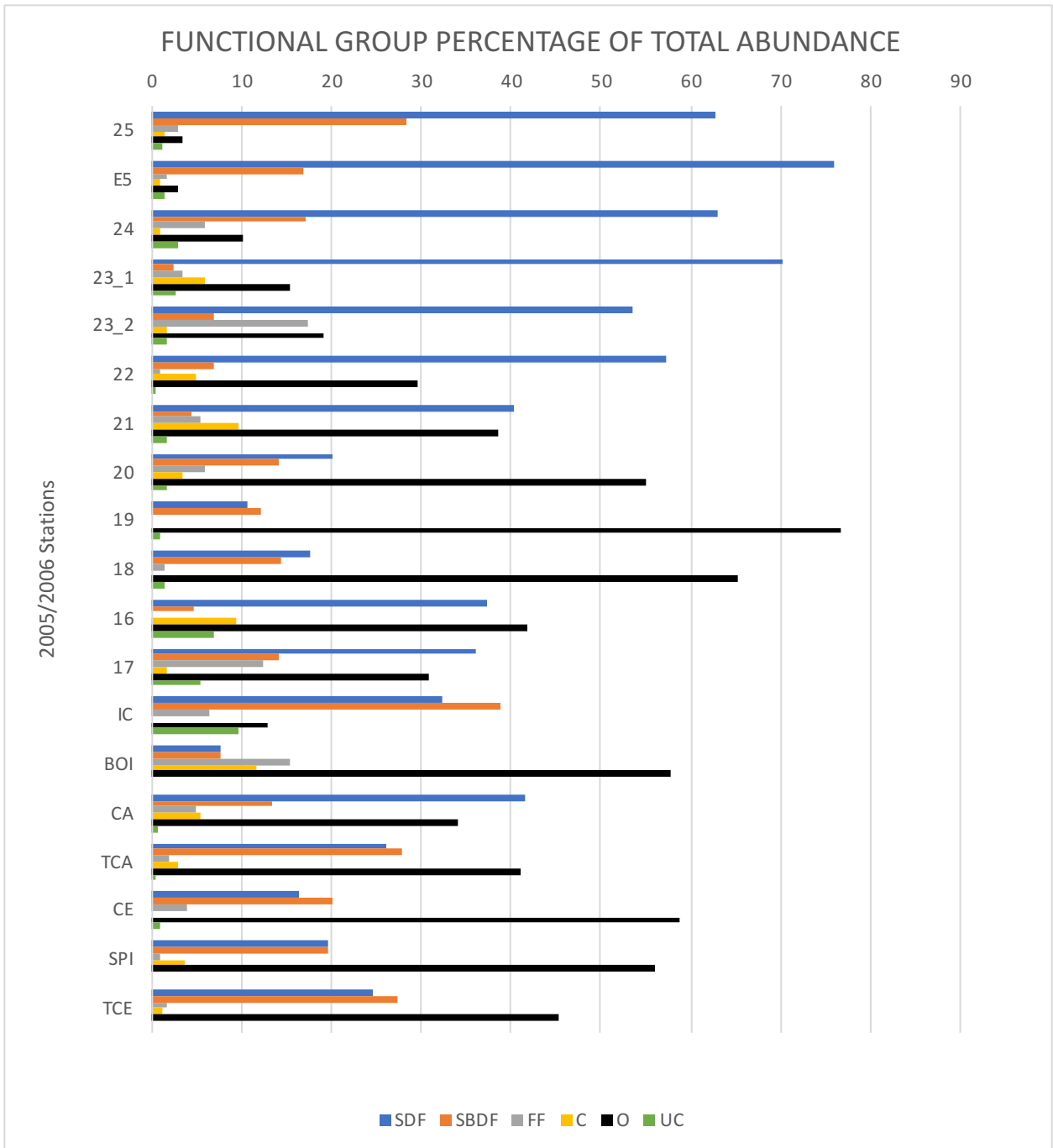
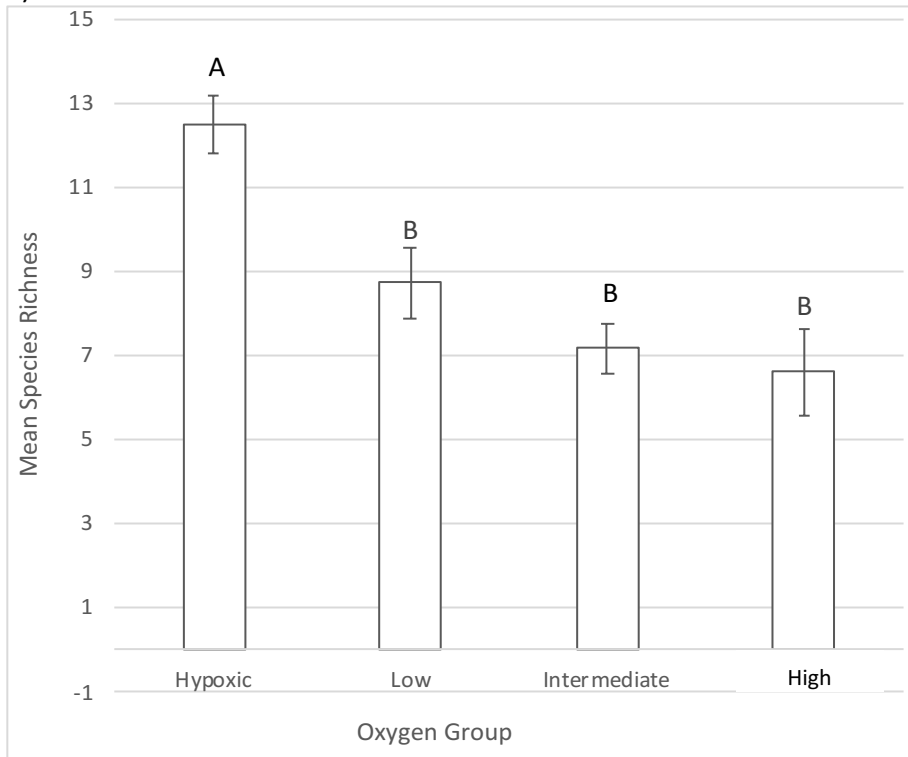
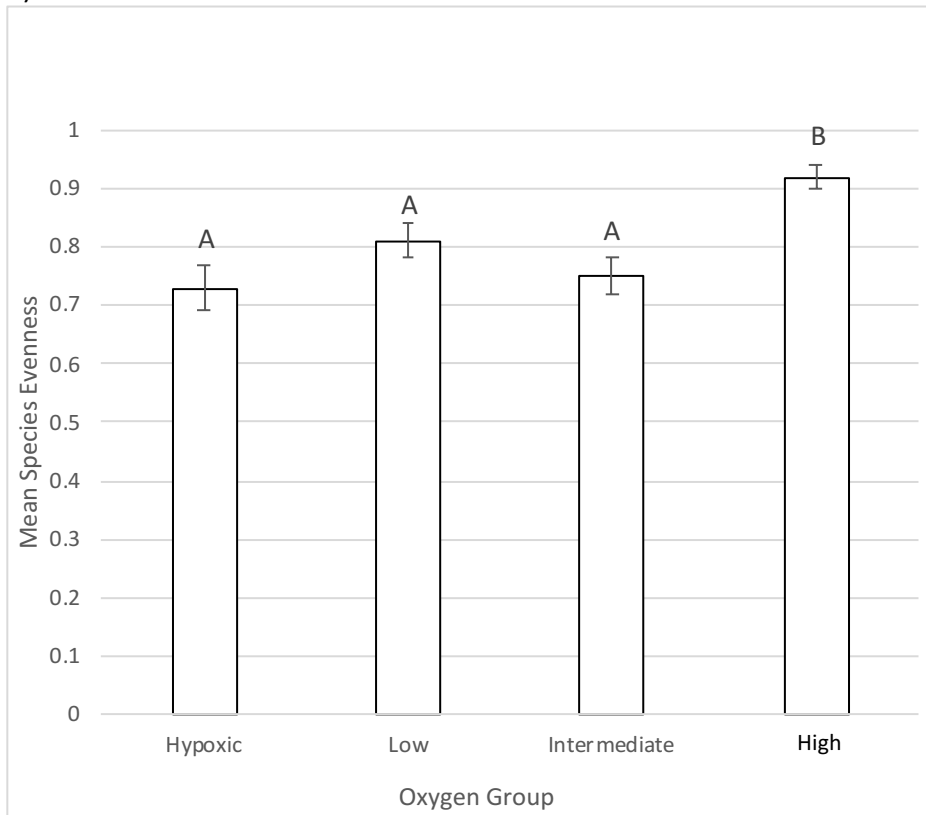


Figure 3.5. Functional Group composition at each station showing percentage abundance of each group. Station 23_1 refers to station 23 samples obtained in 2005, and 23_2 refers to station 23 samples obtained in 2006. Stations from top to bottom of figure are presented approximately in a west to east sequence, from head of the LSLE to the Esquiman channel.

a)



b)



c)

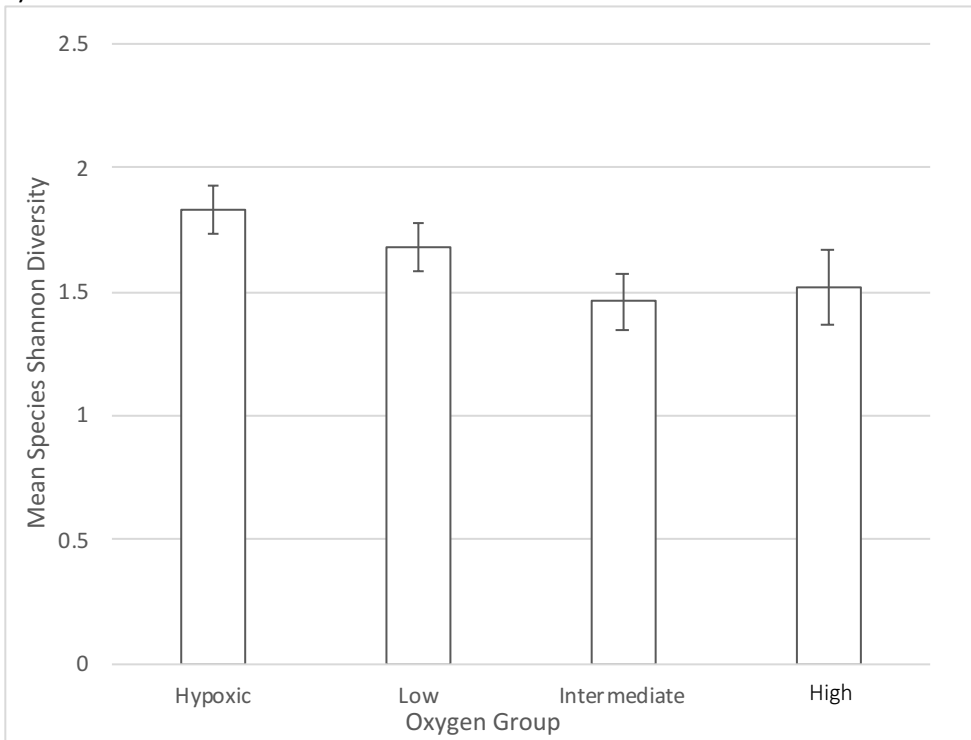
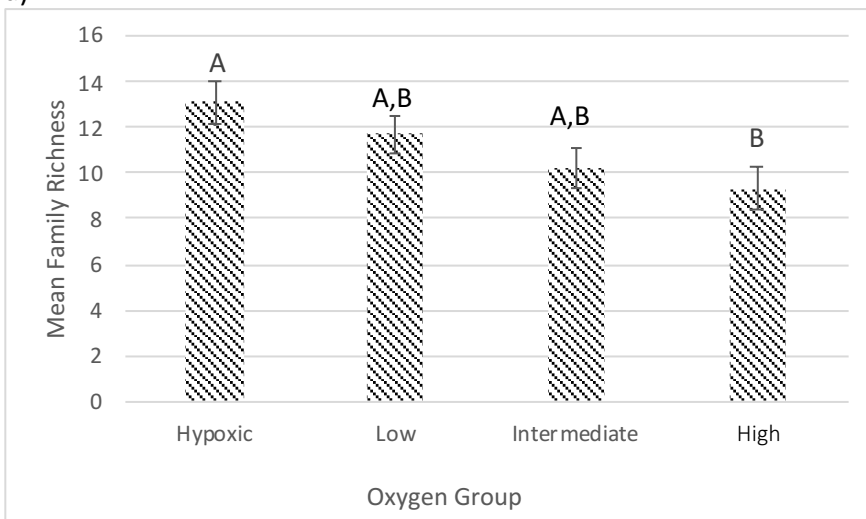
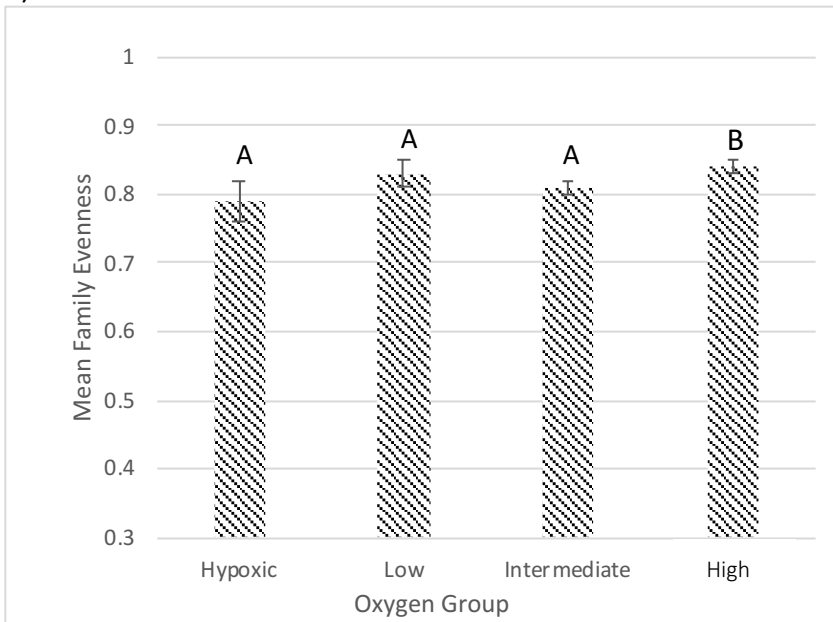


Figure 3.6. PERMANOVA+ tests of difference of species a) richness, b) evenness and c) Shannon diversity between pairs of oxygen groups. Bars indicate standard error of each mean. Only oxygen groups with different letters are significantly different

a)



b)



c)

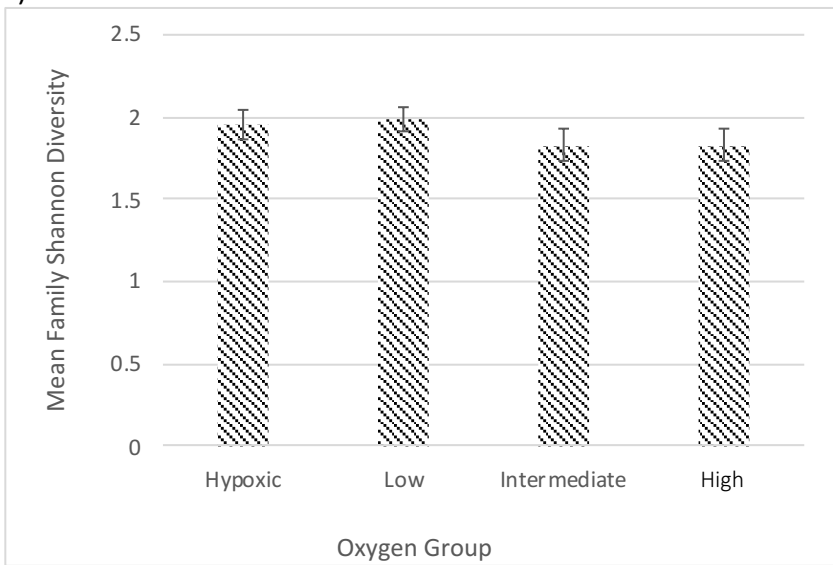


Figure 3.7. PERMANOVA+ tests of difference of family a) richness, b) evenness and c) Shannon diversity between pairs of oxygen groups. Bars indicate standard error of each mean. Only oxygen groups with different letters are significantly different.

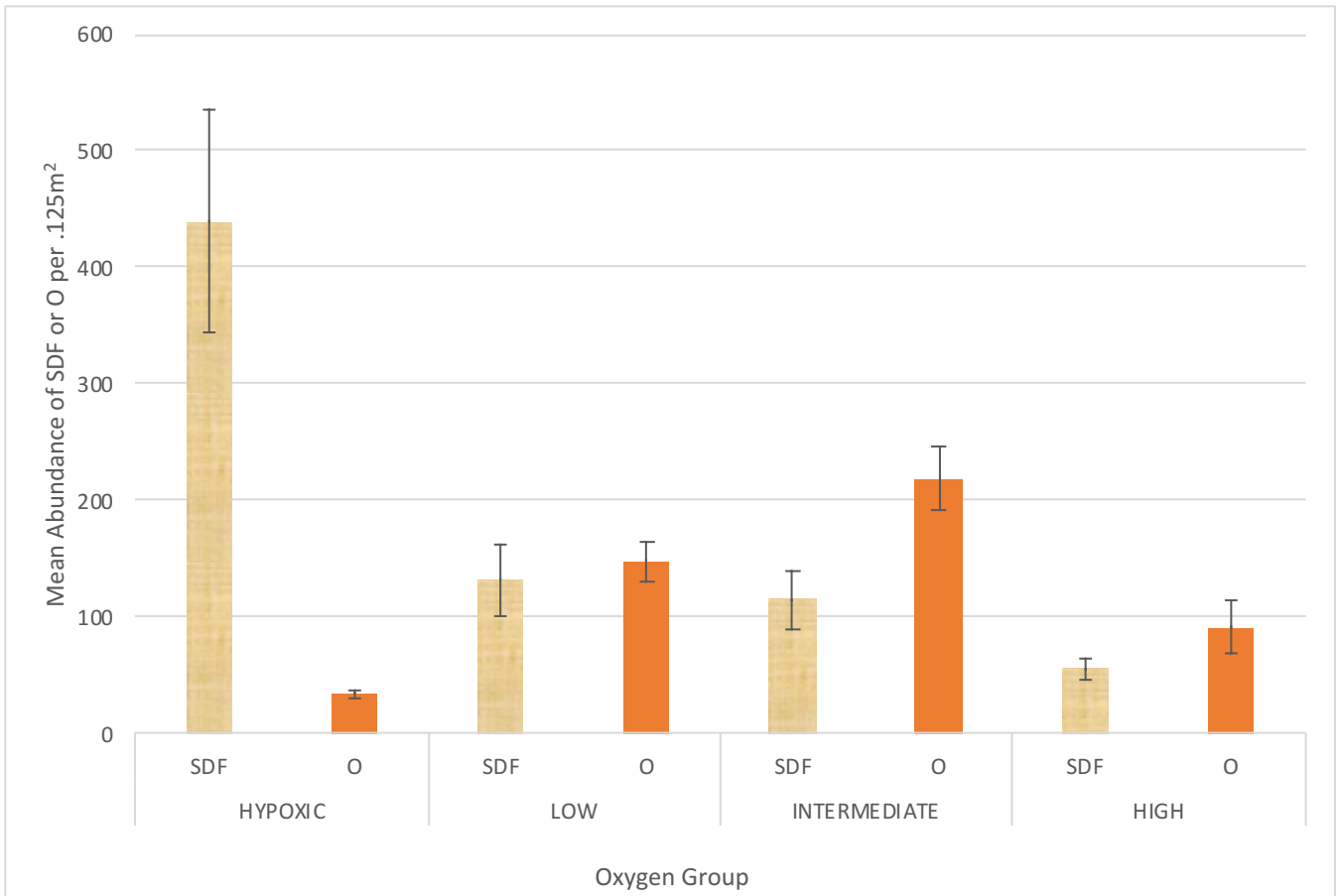


Figure 3.8. Mean abundance of surface deposit feeders (SDF) and omnivores (O) per .125m² in the hypoxic, low, intermediate and high oxygen groups. Error bars represent error of the mean.

Tables

Table 3.1. Latitude and longitude of stations of 2005 and 2006 cruises used in the spatial analysis.

2005			2006		
Station No.	Latitude	Longitude	Station No.	Latitude	Longitude
25	48.23283	-69.45055	23	48.7006	-68.647917
24.75	48.2426167	-69.3521	17	48.9661	-63.114517
E5	48.30285	-69.281483	TCA	49.6741833	-61.81805
24	48.4313333	-69.100833	CA	49.53595	-60.850433
23.6	48.5446667	-68.893333	16	48.4008333	-60.7337
23	48.6983333	-68.651167	IC	48.88	-59.9982
22	48.9308167	-68.091417	BOI	49.3409833	-59.679817
21	49.1201667	-67.282167	CE	49.8052333	-59.26
20	49.4236667	-66.324833	SPI	50.1208667	-58.667217
19	49.4836667	-65.199	TCE	50.4722	-58.113267
18	49.2705	-64.2645			

Table 3.2. Grouping of stations in the Lower St Lawrence Estuary and Gulf of St. Lawrence according to their % oxygen saturation, based on Belley et al., 2010.

Oxygen Level	Stations
Hypoxic <22%	25, E5, 24, 23_1
Low 22-29%	23_2, 22, 21, 20, SPI, TCE
Intermediate 30-42%	19, CA, TCA, CE
High >42%	18, 17, 16, ICE, BOI

Table 3.3. Results of CLUSTER analysis by similarity of family composition. The 3 most abundant families of each group, the mean abundance of each family and the family's percentage contribution to the total abundance are shown.

	3 Most Abundant Families	Mean Abundance	Contribution %
Group Stations	B (Anticosti and Upper Esquiman Channels)		
	TCA, CA, TCE, CE, SPI		
	Lumbrineridae	1.8	19.39
	Capitellidae	1.71	16.99
	Nereididae	1.62	15.16
Group Stations	C (Estuary Mouth)		
	20, 19, 18		
	Lumbrineridae	1.57	19.95
	Nereididae	1.71	18.3
	Amphiuridae	1.54	17.8
Group Stations	D (Mid-Estuary)		
	23.6, 23.2, 22, 21		
	Ampharetidae	1.76	17.85
	Lumbrineridae	1.09	10.45
	Nereididae	1.43	14.18
Group Stations	E (Laurentian and Lower Esquiman Channels)		
	17, 16, IC*		
	Lumbrineridae	1.38	18.99
	Amphiuridae	1.22	18.28
	Cirratulidae	1.25	18.26

Table 3.3 continued

Group	F (Estuary Head)		
Stations	25, 24.75, E5, 24		
	Spionidae	2.24	17.68
	Oweniidae	1.2	10.81
	Ampharetidae	1.37	8.5
Group	G (Mid-Esquiman Channel)		
Stations	BOI		
	Thyasiridae	1.09	44.1
	Onuphidae	0.91	22.75
	Nereididae	0.84	19.15

Table 3.4.a Results of step-wise distance-based linear modeling (DISTLM) of differences in benthic species assemblages due to environmental factors from 2005/2006 data. Selection criteria was AICc. P is significance level. Proportion of correlation for each variable and cumulative total correlation with species assemblages are shown.

Variable	P		Cumulative
Temperature	0.001	0.13006	0.13006
Salinity	0.001	0.10985	0.23991
Longitude	0.001	0.0592	0.29908
Silt	0.001	0.0461	0.34518
Depth	0.001	0.0463	0.39152
Latitude	0.008	0.0318	0.42337
neg Salinity	0.071	0.0209	0.40249
Oxygen saturation	0.005	0.033707	0.4362
Mean grain size	0.005	0.032992	0.46919
% Clay	0.008	0.029723	0.49891

Table 3.4.b Results of step-wise distance-based linear modeling (DISTLM) of differences in benthic family assemblages due to environmental factors from 2005/2006 data. Selection criteria was AICc. P is significance level. Proportion of correlation for each variable and cumulative total correlation with family assemblages are shown.

Variables	P	Proportion	Cumulative
Temperature	0.001	0.126	0.126
Depth	0.001	0.132	0.258
% Clay	0.001	0.069	0.327
Longitude	0.001	0.056	0.382
Mean Grain Size	0.001	0.043	0.425
Oxygen % Saturation	0.003	0.039	0.464
Latitude	0.012	0.028	0.492

Table 3.5. Results of PERMANOVA+ tests of differences in composition of a) species and b) families among oxygen groups.

a)

Species Composition Oxygen Group Comparison	t value	Probability	Permutations
Hypoxic, Low	1.5884	0.008	997
Hypoxic, Intermediate	1.9979	0.002	997
Hypoxic, High	2.3048	0.001	998
Low, Intermediate	1.0967	0.294	998
Low, High	2.1534	0.001	999
Intermediate, High	1.862	0.002	999

b)

Family Composition Oxygen Group Comparison	t Value	Probability	Permutations
Hypoxic, Low	1.3512	0.075	999
Hypoxic intermediate	2.1291	0.002	998
Hypoxic, High	2.6071	0.001	999
Low, Intermediate	0.85066	0.681	999
Low, High	1.8861	0.003	998
Intermediate, High	1.9416	0.002	997

Table 3.6. SIMPER results showing the a) species and b) families that most contributed to the benthic dissimilarity between the Hypoxic and High oxygen groups. Mean dissimilarity between species was 82.87% and between families was 73.12%.

a)

Species	Hypoxic Av.Abund	Maximum Av.Abund	Av.Diss	Diss/SD	Contrib%	Cumulative %
<i>Spiophanes kroyeri</i>	2.87	0	7.04	1.75	7.57	8.50
<i>Ampharete lindstroemi</i>	2.56	0.71	5.79	1.38	5.49	15.48
<i>Trochochaeta multisetosa</i>	2.08	0.11	4.94	1.75	5.32	21.45
<i>Myriochele heeri</i>	1.91	0.26	4.62	1.67	5.57	27.02
<i>Nucula delphinodonta</i>	1.65	0.62	3.71	1.20	4.47	31.49
<i>Terebellides stroemi</i>	1.32	0	3.48	1.26	4.20	35.69
<i>Ophiura sarsi</i>	1.32	0	3.26	1.28	3.93	39.62
<i>Ceratophale loveni</i>	0.70	1.10	3.19	1.02	3.85	43.47
<i>Heteromastus filiformis</i>	1.17	1.01	3.00	1.12	3.62	47.10
<i>Amphiura sundevalli</i>	0.65	1.13	2.97	1.00	3.58	50.68

b)

Family	Hypoxic Av.Abund	Maximum Av.Abund	Av.Diss	Diss/SD	Contrib%	Cumulative%
<i>Spionidae</i>	1.71	0	5.54	1.72	7.57	7.57
<i>Oweniidae</i>	1.27	0.15	4.01	2.19	5.49	13.06
<i>Trochochaetidae</i>	1.24	0.07	3.89	1.85	5.32	18.38
<i>Ampharetidae</i>	1.55	0.67	3.89	1.42	5.31	23.7
<i>Maldanidae</i>	1.26	0.26	3.72	1.62	5.08	28.78
<i>Nuculidae</i>	1.09	0.37	3.1	1.31	4.24	33.02
<i>Lumbrineridae</i>	0.56	1.23	2.86	1.33	3.92	36.94
<i>Amphiuridae</i>	0.38	0.91	2.71	1.2	3.71	40.65
<i>Trichobranchidae</i>	0.79	0	2.68	1.28	3.66	44.31
<i>Nereididae</i>	0.42	0.66	2.52	1.05	3.45	47.76
<i>Cirratulidae</i>	0.82	0.8	2.52	1.19	3.45	51.21

Table 3.7. Results of PERMANOVA+ comparisons of functional group composition between pairs of oxygen groups. Significantly different pairs are shown in bold.

Oxygen Group Pairs	t Value	Probability	Permutations
Hypoxic, Low	1.6438	0.0692	96331
Hypoxic, Intermediate	2.2955	0.0128	96482
Hypoxic, High	2.058	0.0223	96607
Low Intermediate	0.7629	0.6335	96128
Low, High	1.521	0.1015	96524
Intermediate, High	2.435	0.0037	96447

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CHAPTER 4: REVISITING HYPOXIA IMPACTS ON THE ST. LAWRENCE ESTUARY AND GULF: A DECADE LATER

4.1 INTRODUCTION

Despite less evidence of significant disturbances to benthic communities in the central or eastern Gulf of St Lawrence (GSL) comparable to those in the Lower St. Lawrence Estuary (LSLE) to date, accumulating evidence suggests ongoing environmental change. These changes could significantly alter benthic macrofaunal composition within the GSL. The progressive increase in temperature and decrease in oxygenation of the waters entering the GSL could represent one potential cause of such change. Recent studies confirm the hypothesis that increasing flow from much less oxygenated warmer subtropical waters from the Gulf Stream has partially replaced the weakened Labrador Current, which brings cold, well-oxygenated water into the Laurentian Channel and Gulf (Claret et al., 2018). The authors concluded that this change in source water has likely caused significant decreases in oxygenation of waters at the Laurentian Channel mouth and in the Cabot Strait, corresponding to the entry point for much of the water flowing into the GSL. At the Cabot Strait, oxygen concentrations decreased by approximately 28 μM ($\sim 1\text{mg/L}$) between 1960 and 2015 (Claret et al., 2018). Stortini et al. (2016) characterized the Cabot Strait as slightly hypoxic with oxygen concentrations as low as 50 μM , and emphasized that as water flows into the GSL, progressive loss of oxygen occurs through respiration and oxidation of organic matter as the water reaches the head of the channels. This trend suggests even lower oxygen concentration than 50 μM in recent years. Gilbert et al. (2005) proposed that this process of reduction of the Labrador Current contributions to GSL and LSLE waters and replacement by the warmer, less oxygenated North Atlantic Central Waters contributed significantly to the development of hypoxia at the LSLE.

The GSL has recently experienced higher temperatures than during any previous periods of measurement. The GSL average surface temperature reached a new record high in 2015, (Galbraith et al., 2016) and continued to increase in 2016. The surface water entering the GSL through the Cabot Strait increased to a record high in 2016, 2.2 °C above average, and reached a record high by 2.5 °C in the Esquiman Channel (Galbraith et al., 2017). Deep water temperatures throughout the Gulf also reached record highs at 150 to 300 metres depth in 2015. For the first time in a century, the temperature below 250 m rose above 6 °C in Esquiman Channel and the central Gulf region, reducing bottom environments to temperatures of 5-6 °C, with record high temperatures in the deep part of Esquiman Channel (Galbraith et al., 2016)

The increase in temperature and decrease in oxygen concentration presents a dual threat to biotic communities because both environmental alterations impact growth and survival. These variables also create a potentially synergistic disturbance to benthic biota, because higher temperatures contribute to de-oxygenation of seawater through reduced solubility, and creates further oxygen stress on organisms by raising their metabolic rate and an associated temperature driven increase in mean lethal oxygen level (Vaquer-Sunyer & Duarte, 2011). Researchers hypothesize that the enhanced respiration resulting from rising deep-water temperature contributed to the development of hypoxia in the LSLE (Genovesi et al, 2011). The combination of rising temperature and lowered oxygen levels can increase the vulnerability of communities to eutrophication that can occur near to the coast and beyond, leading to shifts in richness, diversity, and functional group memberships (Stortini et al., 2016).

These documented environmental shifts in the eastern GSL raise the question of associated changes in benthic composition of the GSL in response to increased temperature and decreased oxygenation. Resolving this question requires resampling stations sampled in 2006 to evaluate potential changes in benthic community structure. This resampling approach avoids the complication of sampling different spatial locations

and confounding factors such as sediment and depth that might confound interpretation. However, temporal studies alone cannot elucidate the relative influence of factors such as temperature, oxygen concentration, and organic input. Spatial analysis of benthic communities among sites could quantify the relative influence of various environmental factors on benthic species composition, including possible biotic effects of ongoing temperature and oxygenation changes in the eastern GSL. In order to explore possible benthic changes in the eastern GSL in response to the decrease in oxygen concentrations and increase in temperature subsequent to the 2006 study (Chapter 3), we conducted a research cruise in 2015.

Utilizing both temporal and spatial approaches through resampling the eastern GSL in 2015 provides greater understanding of benthic community patterns and any disturbance to benthic communities. The Introductions of Chapters 2 and 3 provide a detailed discussion of the importance of temporal and spatial studies of benthic changes respectively in relation to environmental change. The 2015 cruise sampled stations in the eastern Gulf of St Lawrence at or near some of the same sites that were assessed in 2006 to evaluate whether the benthic communities of the eastern area of the GSL have changed significantly since 2006, and to identify any environmental driver(s) of such change. Clarifying whether changes occurred in the benthic communities of the GSL between 2006 and 2015 will help in understanding the biotic effects of climate change related environmental shifts. We hypothesize that no significant changes occurred in the benthic communities or environments in this region of the Gulf, based on the absence of definitive data published on benthic changes in the eastern GSL.

4.2 METHODS

Research Cruise 2015

Sampling was completed from the RV Coriolis II from June 15-17, 2015 at 13 stations in the Gulf of the St. Lawrence (Table 4.1). Weather and ocean conditions limited sampling stations to a smaller geographic region than originally planned, concentrating near the

southwest coast of Newfoundland (Fig. 4.1). B3 stations were situated 2.75-5 km from the coast, within eastern Port au Port Bay. The northern extension of the triangular mass of the Port au Port Peninsula separates and protects this bay from direct ocean waves. B2 stations were approximately 80 km from the coast, but very near the western tip of the Port au Port Peninsula, at the northwest boundary of St George's Bay. T3 and B4 were approximately 130 km west of the coastline at the start of Esquiman Channel, near its intersection with the Laurentian Channel.

Sampling Protocol

We collected a total of 13 box cores (0.35 m² surface area), subsampling with four 10 cm diameter push cores that we sectioned into 0-2 cm, 2-5 cm, and 5-10 cm vertical fractions. Each core fraction was sieved through a 500-µm mesh, and then fixed in a 10% formalin seawater solution. In the lab, we transferred samples into 80% ethanol stained with rose bengal. Given the much larger surface area of sediment sampled by box cores than by the Van veen grab in the 2006 study, and the coarser sieve used during the 2006 study (1000 µm), these differences add potential complications when comparing data from these two cruises that we explore in the Discussion. Samples were then examined under a dissecting microscope to separate them by phylum. Organisms were later identified to family level under a dissecting microscope, or a light microscope when necessary. Time constraints eliminated any opportunity to undertake planned environmental sampling.

Statistical Methods

Primer-E version 6 with PERMANOVA+ add-on (See Chapter 2 Statistical Methods) was used for transformation of data into matrices, principal components analysis, and multivariate testing.

Univariate Analysis

We calculated family richness, Shannon diversity index, and Pielou's Evenness index for each station, and used PERMANOVA to test for significant differences between the univariate indices among stations, and to determine which pairs of stations differed significantly. We compared the univariate indices for stations B4 with the indices for station IC, the nearest station from the 2006 cruise and compared the indices of station T3 with station 16 from the 2006 cruise (Figure 3.1). No stations from the 2006 cruise were sufficiently close to compare with the diversity indices for stations B2 and B3.

Multivariate Analysis

The biological data was 4th root transformed prior to calculation of a Bray-Curtis resemblance matrix, which we subsequently used in the CLUSTER procedure to group substations with similar family composition. We then utilized SIMPROF to create a similarity profile indicating the major contributors to each cluster analysis.

Functional Group Analysis

Based chiefly on information from the World Register of Marine Species, (WoRMS Editorial Board, 2020) (with supplemental information if needed for individual organisms) we divided the biota into six functional groups based largely on feeding: surface deposit feeders (SDF), subsurface deposit feeders (SBDF), filter feeders (FF), carnivores (C), omnivores (O), and unclassified (ND). All functional group data were 4th root transformed prior to producing a Bray-Curtis resemblance matrix for the functional groups, and then we performed a Cluster analysis with similarity profile analysis (SIMPROF). We also calculated and qualitatively compared the mean overall abundances of the six functional groups for stations T3-16, and B4-IC. CLUSTER analysis determined sites with the greatest similarity in functional group composition, and SIMPROF determined the major contributors to each cluster. All stations from both 2006 and 2015 cruises were sampled during summer, and we standardized all organism densities to individual/0.125 m², acknowledging differences in sampling gear that may have influenced estimates.

4.3 RESULTS

Univariate Analysis

Considerably higher family richness (Figure 4.2) characterized stations B2 and B3 near the Newfoundland coast compared to B4 and T3, (Figure 4.1) the stations located further out in the Gulf of the St. Lawrence. Family richness at the B3 stations, nearest the coast, had the highest values on average, approximately 33% greater than at T3, the station farthest into the channel with the lowest average richness. Similarly, distinctly higher Shannon diversity indices characterized both B2 and B3 compared to B4 and T3; diversity at station B3 was 17% greater than at station T3. The mean evenness of all stations was high, ranging from 0.98-0.99.

PERMANOVA confirmed significant differences in richness and Shannon diversity among the stations (Figure 4.2), driven by significantly higher indices at each station compared to T3, as well as significantly higher richness and diversity at B3 than at B4 indicating that B3 richness and Shannon diversity was indeed the highest overall. Somewhat surprisingly, PERMANOVA detected significant differences in evenness, despite the narrow range of values, a result entirely driven by lower B3 evenness (0.98) relative to the other stations (evenness of 0.99).

The two stations near the convergence of Esquiman and Laurentian Channels, B4 and T3, were geographically proximate to two of the 2006 stations to allow for temporal univariate indices comparison (Figure 4.3). Station T3 (2015 cruise) was close to station 16 (2006 cruise), and station B4 (2015 cruise) was close to station IC (2006 cruise). Comparison of the univariate indices from stations B4 and IC showed a decrease in mean richness over time, with 10 % higher richness at station IC sampled in 2006 than station B4, and 6% higher diversity and 10% higher mean evenness at B4 in 2015. In contrast, mean richness, evenness, and diversity at station T3 in 2015 were 28%, 11%, and 24% higher, respectively, than at station 16. However, average family indices in

2006 for richness (8.67), evenness (0.94) and Shannon diversity (1.98) linked to below average diversity indices for station 16 families, in contrast to average richness and diversity measures at station IC.

Multivariate Analysis

At 40% similarity, CLUSTER analysis of family composition grouped stations into three large clusters confirmed as significant with SIMPROF, with two additional lone stations, T3b.3 and T3d.1 (Figure 4.4). Stations B4 and T3 formed a channel station cluster (Group A), station B3 formed a coastal station cluster (Group B), and station B2 separated from the other stations to form a cluster of samples between the coast and channel (Group C). The groupings indicate more distinct family composition among coastal stations than for Laurentian/Esquiman Channel stations.

SIMPER analysis identified Spionidae, Paraonidae, and Capitellidae (listed in order of descending contribution) as the most abundant families within Group A comprised of the channel stations (Table 4.2). Cossuridae, followed by Cirratulidae, and Nephtyidae comprised the most abundant families in B3 nearest the coast that was unique in family composition, sharing none of the three most common families with either of the other clusters. In contrast, Group C, composed of station B2 samples between coast and channel, was distinguished by its largest contributor Yoldiidae; it shared Spionidae and Capitellidae with Group A. SIMPER calculated the similarities within groups A, B, and C to be 52.63%, 69.34% and 53.96%, respectively.

Functional Group Analysis

CLUSTER analysis of functional group composition identified 4 clusters at approximately 67% similarity (Figure 4.5), a much higher similarity level than the family-level analysis that produced distinct clusters at approximately 47% (Figure 4.4). Coastal stations of B3 in Group A through SIMPER contained a slightly greater numbers of SBDFs compared to SDFs (Table 4.8). SBDFs were slightly greater than SDFs in Group B stations between the

coast and channel formed by two replicates, B2a.3 and B2c.2, which included numerous filter feeders. Group C, which includes B2 samples and the remainder of station B3 coastal replicates was characterized by slightly more SBDFs than SDFs. SDFs and SBDFs heavily dominated (41.19% and 30.47% respectively) Group D, comprised of the B4 and T3 channel samples, and the majority of station B2 replicates grouped between the coast and channel.

Overall, SDFs and SBDFs dominated benthic functional groups identified during the 2015 research cruise (Appendix 3). In particular, we observed a surprisingly large reduction in omnivores that dominated many of the GSL stations in 2006. Furthermore, the mean abundances of both SDFs and SBDFs at stations of B3 were markedly higher than at any other sites (Figure 4.6); SDFs were approximately three times more abundant and SBDFs were observed more than four times as frequently at B3 than at any other group of stations. SBDFs were the dominant functional group at the two stations near the Newfoundland coast (except B2a), whereas SDFs dominated the two stations further from the coast into the GSL. The highest ratios of SDF/O occurred at B2 stations, and particularly B3 located much closer to shore, reflecting the lowest omnivore counts. In contrast the SDF/SBDF ratio was lowest at the coastal stations, and highest at station T3.

In order to examine patterns of functional group composition over time, we examined stations 16 and T3, sampled in 2006 and 2015 respectively, and stations IC and B4, sampled in 2006 and 2015 (Figure 4.7). Within these four stations, SDFs were much more abundant at both 2015 stations than at the 2006 stations, whereas omnivores were approximately 2.7 times more frequent at station 16 than at T3, and similar in abundance at B4 and IC. As a result, the SDF/O ratio at the T3 station, sampled in 2015, was approximately 12 times higher on average than the ratio at station 16, and the ratio at station B4 was 2.4 times higher than at IC. Unexpectedly, the SDF/SBDF ratio at station 16 was approximately 2.3 times larger than at T3, because SBDFs were generally far more numerous at the 2015 stations than at the 2006 stations.

4.4 DISCUSSION

Collectively, the findings presented here do not support our null hypothesis that the composition of the benthic community of the eastern GSL in 2015 were unchanged relative to the composition documented in 2006. The B3 stations nearest the Newfoundland coast showed the most evidence of alteration of any region sampled, with much greater benthic abundances than at any other stations, a finding compatible with documented responses to organic pollution (Pearson & Rosenberg, 1978). To sustain such a large quantity of benthic macrofauna compared to the other sites requires a much larger supply of organic material, suggesting greater input of anthropogenic input than elsewhere in the region. The SDF/O ratio at B3, which ranged from 30 to 162, was also much higher than at any other stations, a pattern of functional group dominance previously documented at hypoxic and eutrophic sites (Diaz & Rosenberg, 1995; Levin et al., 2009; Middelburg & Levin, 2009; Wu, 2002). The high values of family richness and Shannon diversity and lowest value of evenness at B3 further suggest an altered environment; these findings mirror differences in richness, diversity, and functional groups between the hypoxic/eutrophic stations and the normoxic stations at the head of the LSLE. Greater eutrophication at B3 would increase the chance of hypoxia at these stations.

The coastal environment of B3 supported a unique set of families not found at the other sites, including second-order opportunistic organisms that comprised the two most abundant families (Borja et al., 2008; Tweedley et al., 2014). One species of Cossuridae living in the Arabian Sea OMZ has an enlarged respiratory apparatus (Lamont & Gage, 2000), and a species of this family occurs in high abundance within an organically enriched hypoxic area of the South-East Arabian coast (Baban, et al., 2016). *Cossura coasta* also appeared after significant eutrophication within a tropical estuary (Martin et al., 2011). Cirratulidae, the prototypical SBDF, increase in abundance within a brief time

in enriched sediments because they tolerate hypoxic conditions more than many other species. Two intertidal species, *Cirriiformia tentaculata* and *Cirratulus cirratus*, use extracellular haemoglobins to cope physiologically with hypoxia (Dales & Warren, 1980). The co-occurrence of these two opportunistic disturbance-adapted polychaete families at B3 increases the likelihood that benthic composition has shifted in the region of eastern Port au Port Bay to adapt to reduced oxygen and increased temperature previously documented in this environment (Galbraith et al., 2016, 2017; Stortini et al., 2016). While we cannot conclude that these changes in benthic community structure represent adaptations to disturbance, they coincided with a time period when climate-related environmental changes increasingly altered the eastern Gulf of St. Lawrence environment.

A significant increase in dead scallops in fishing catches in Port au Port Bay, starting in 2012, that preceded the collapse of the local scallop fishery by 2013, further suggests environmental disturbance near B3 stations. Residents attributed the widespread scallop mortality to oil and especially polycyclic aromatic hydrocarbons leaking into the bay from abandoned oil wells, as well as climate change (Morrill & Cook, 2015). However, testing of sediments and *Mytilus edulis* (blue mussels) in the bay found no evidence of major contamination of Port au Port Bay sediments or the mussels with petroleum (Cook et al., 2018), and the cause of the scallop decline remains a mystery.

Most of the dominant species at the other stations sampled are well adapted to hypoxia and or organic enrichment. The dominant family of stations B4 and T3, Spionidae, typically tolerates both organically enriched and hypoxic environments (Borja et al., 2008; Tweedley et al., 2014), and may form a symbiotic relationship with the third most abundant family Capitellidae to cope with organic enrichment (Gray, 1979). Capitellidae, like Spionidae, commonly occur in disturbed environments, and their co-occurrence in the channel stations is consistent with eutrophication (Shivarudrappa et al., 2011).

The mollusc family Yoldiidae, the only non-polychaete in the list of major contributors from the SIMPER analysis, dominated B2. These detritus feeders typically cannot tolerate organic enrichment, although some species appear indifferent (Borja et al., 2008). Given the importance of Spionidae and Capitellidae at B2, benthic composition nonetheless indicates biotic change, but Yoldiidae suggests less disturbance at B2 than at B4 and T3.

Temporal comparisons provided some evidence of a shift in biota towards increased tolerance of environmental disturbance. Overall, spionids dominated family composition at 2015 stations T3 and B4, which also included the opportunistic Capitellidae, whereas clustering of the 2006 study (Chapter 3) reported Lumbrineridae and Amphiuroidae as the most common families at stations IC and 16. Lumbrineridae appear generally indifferent to organic disturbance, reflecting limited hypoxia tolerance, whereas some Amphiuroidae are sensitive to disturbance and less likely to occupy hypoxic or eutrophic environments than spionids (Borja et al., 2008; Tweedley et al., 2014). Only cirratulids at 2006 stations near the intersection of the Laurentian and Esquiman channels (Group E) tolerate disturbance well. We therefore suggest that the temporal analysis of family composition supports an environmental shift to eutrophic and/or hypoxic conditions, though we also acknowledge that spatial differences such as sediment, depth, or organic input may have contributed to faunal differences. A definitive answer would require further research to clarify whether biotic differences between the 2006 and 2015 stations link closely to temperature change, lowered oxygen levels, or other alterations of the environment that has occurred during the nine-year interval between samplings.

The functional group composition of the stations of the GSL studied in 2015 represents a significant shift in functional group composition from 2006, when the overall SDF/O ratio was 0.59 (Figure 3.5, Appendix 2), and omnivores were the most abundant feeding group at 9 of the 11 stations sampled in the GSL. In contrast, the SDF/O at all stations of

B3, and B2b, T3b and T3c even exceeded the large 12.91 ratio that characterized the hypoxic region of the LSLE. The temporal comparison between specific sites confirms this shift, noting that the SDF/O ratio of the B4 and T3 sites in 2015 was several times higher than the corresponding 2006 sites of IC and 16. These comparisons between 2006 and 2015 must be viewed cautiously given two potentially important differences in methodology. The use of a 0.5 mm mesh to sieve specimens in 2015 compared to the 1 mm mesh used in 2006 could bias data towards larger benthos in 2006. Whereas the highly abundant Spionidae at T3 and B4 (2015) were similar in size (0.5 mm average diameter) to the Lumbrineridae at stations IC and 16 (2006), the Capitellidae that dominated in 2015 average only 0.45-6 mm in diameter (WoRMS Editorial Board, 2020). The smaller mesh therefore adds potential bias that would result in a relative increase in these opportunistic organisms, but we have no evidence it would change our overall findings. Furthermore, surface area sampled by van Veen grabs in 2006 (0.125 m²) was much smaller than the 2015 box core sample area of 0.35 m² which may also sample deeper in the sediment. However, the higher relative abundance of SDF in 2015 compared to 2006 suggests that differences in gear did not bias sampling towards deeper infaunal benthos.

The increasing temperature of water entering the Cabot Strait (Claret et al., 2018), can lead to increased hypoxic events (Burt et al., 2013) and increasing hydrogen sulphide sediment levels (Sturdivant & Shimizu, 2017). Rising marine temperatures also increase the risk of eutrophication (Lewandowska et al., 2012). In conjunction with upwelling in the middle of the Esquiman Channel that can bring nutrients to the surface (sensu Helly and Levin, 2004), long-term increases in primary production may have further reduced oxygen levels at B3 and T4. The generally superior adaptations of SDFs to hypoxia and eutrophication compared to omnivores aligns with these environmental changes (Dafforn et al., 2013; Lenihan et al., 2003); increased primary production following an Antarctic glacier retreat strongly favoured deposit feeders at the cost of reduced abundances of suspension feeders (Sahade et al., 2015). Although these findings may

simply reflect differences in environmental factors such as sediment type and organic content, the similarity of the 2015 functional group composition of channel sites and the 2005 dominance of SDF in the hypoxic area of the LSLE offers further evidence of increased disturbance at stations sampled in 2015.

4.5 CONCLUSIONS

Both the spatial and temporal analysis of the benthos from the 2015 cruise contradict the hypothesis of unimpacted macrobenthic structure of the eastern GSL, and each approach provided evidence that increasing hypoxia and rising temperatures in the eastern GSL are altering community composition of this region. The unique composition of the near-coast B3 stations, characterized by the highest richness and diversity, the greatest tolerance for disturbance, and an extremely high abundance of SDFs and SBDFs, sets this location as one likely undergoing major environmental changes. This region may be developing atypical patterns of biotic change as was seen in the altered region of the LSLE. The collapse of the scallop fishery in Port au Port Bay where B3 stations are situated further suggests environmental degradation and the urgency to further examine the environmental and benthic health of this bay.

Temporal comparisons with the 2015 cruise, unlike those presented in Chapter 2 that compared the same stations on 1980 and 2005/6 cruises, only utilized nearby stations sampled from 2006. Because these stations differ slightly in location from those sampled in 2015, interpretations therefore require caution. Furthermore, although differences in sampling gear (grabs versus box corers) complicate comparisons, we believe the dominant taxa are less vulnerable to gear differences (e.g. bow wave effects) than smaller organisms, but we must interpret these results with caution. Nevertheless, increases in disturbance tolerant organisms at the 2015 stations relative to 2006 stations indicate possible changes in benthic composition near the mouth of the

Esquiman/Laurentian channel. The large shift between 2006 and 2015 that increased disturbance tolerant functional groups further points to a biotic change.

Numerous studies identify the Gulf of St. Lawrence as an area of scientific interest with respect to evaluating the effects of climate change, noting rapidly shifting temperature and oxygen levels in the eastern GSL potentially associated with shifts in major ocean currents entering the Gulf. These large environmental shifts therefore increase the probability of biotic change; together with findings from the 2015 cruise, this chapter supports the hypothesis that the GSL is transitioning into to a disturbed environment with altered benthic composition and reduced ecological health. The importance of this potential large-scale change justifies further research on the environmental and benthic characteristics of the eastern GSL to test this hypothesis.

4.6 TABLES AND FIGURES

Figures

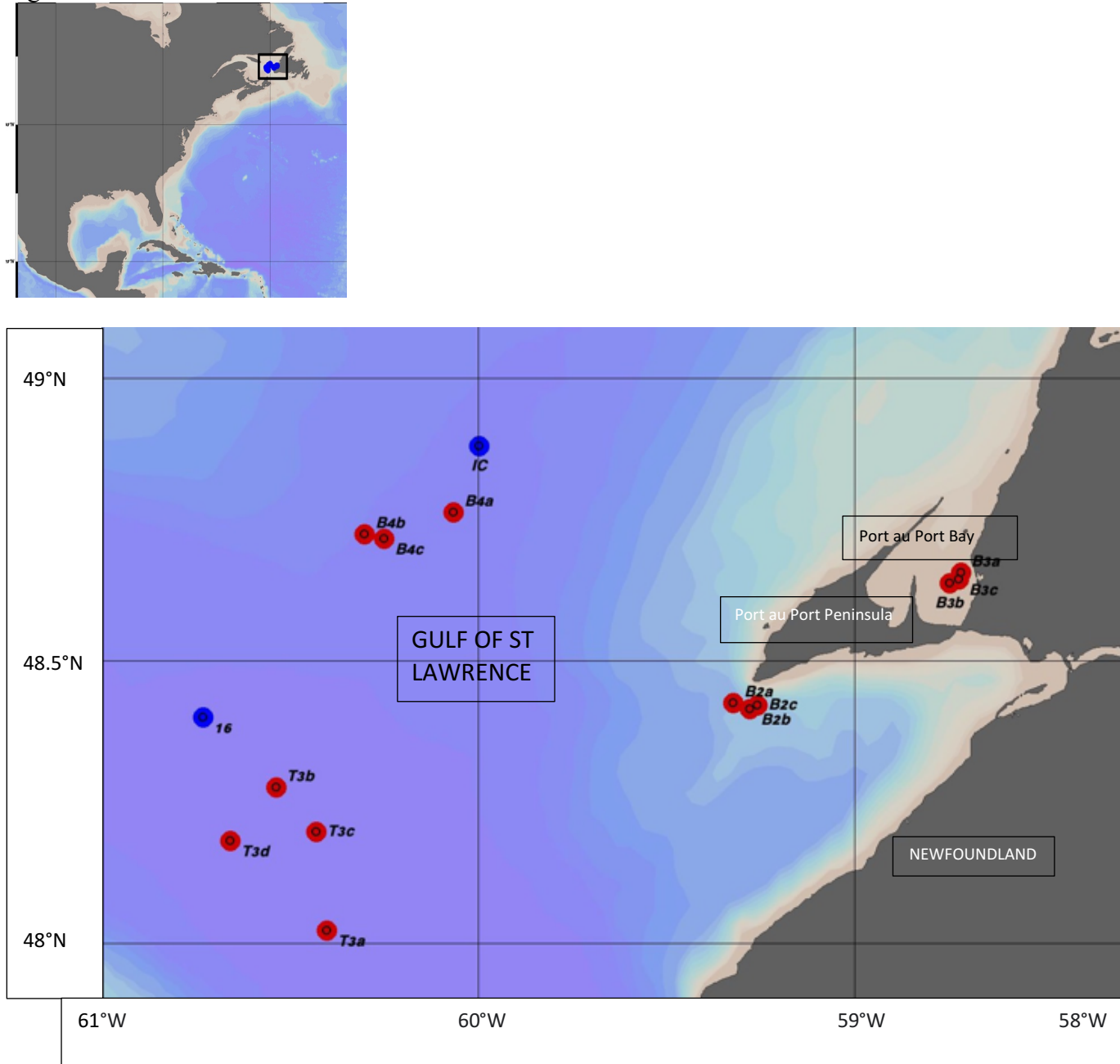


Figure 4.1. Upper map of eastern North America shows the location of the 2015 cruise in Quebec, Canada. Lower map shows stations sampled from June 15-17, 2015 aboard the RV Coriolis II in the Gulf of the St. Lawrence River. Red symbols indicate 2015 stations. Blue symbols indicate 2006 stations used for comparison.

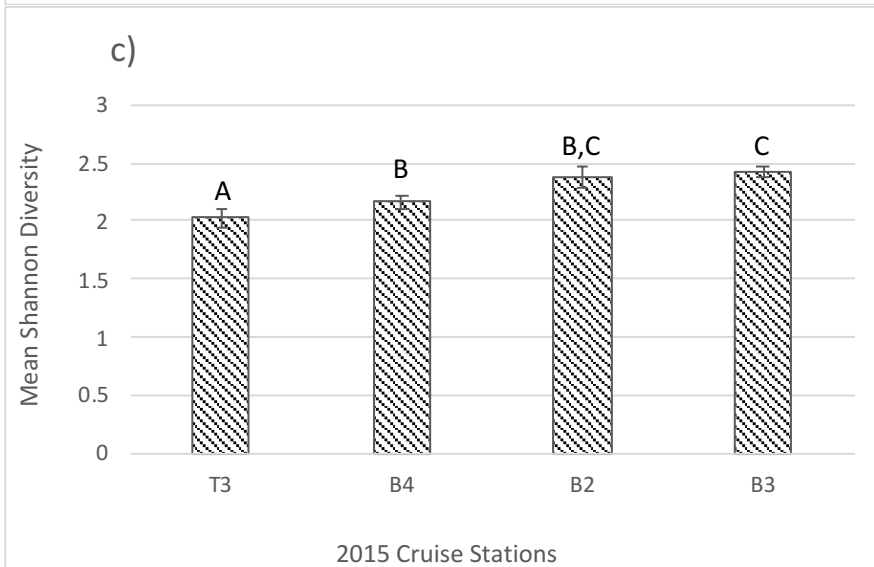
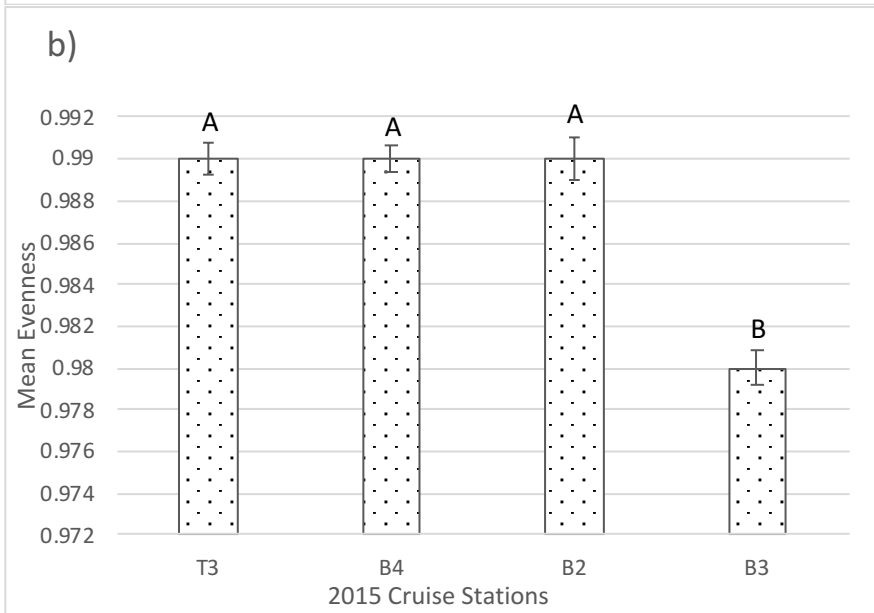
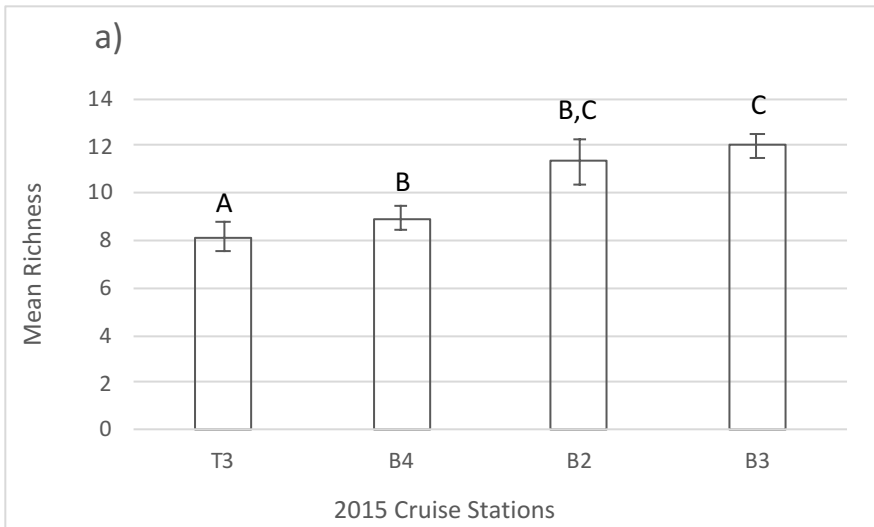


Figure 4.2. Results of PERMANOVA + testing of differences in mean family a) richness, b) evenness and c) Shannon diversity between pairs of 2015 cruise stations. Stations with different letters differ significantly. Error bars represent standard error of the mean.

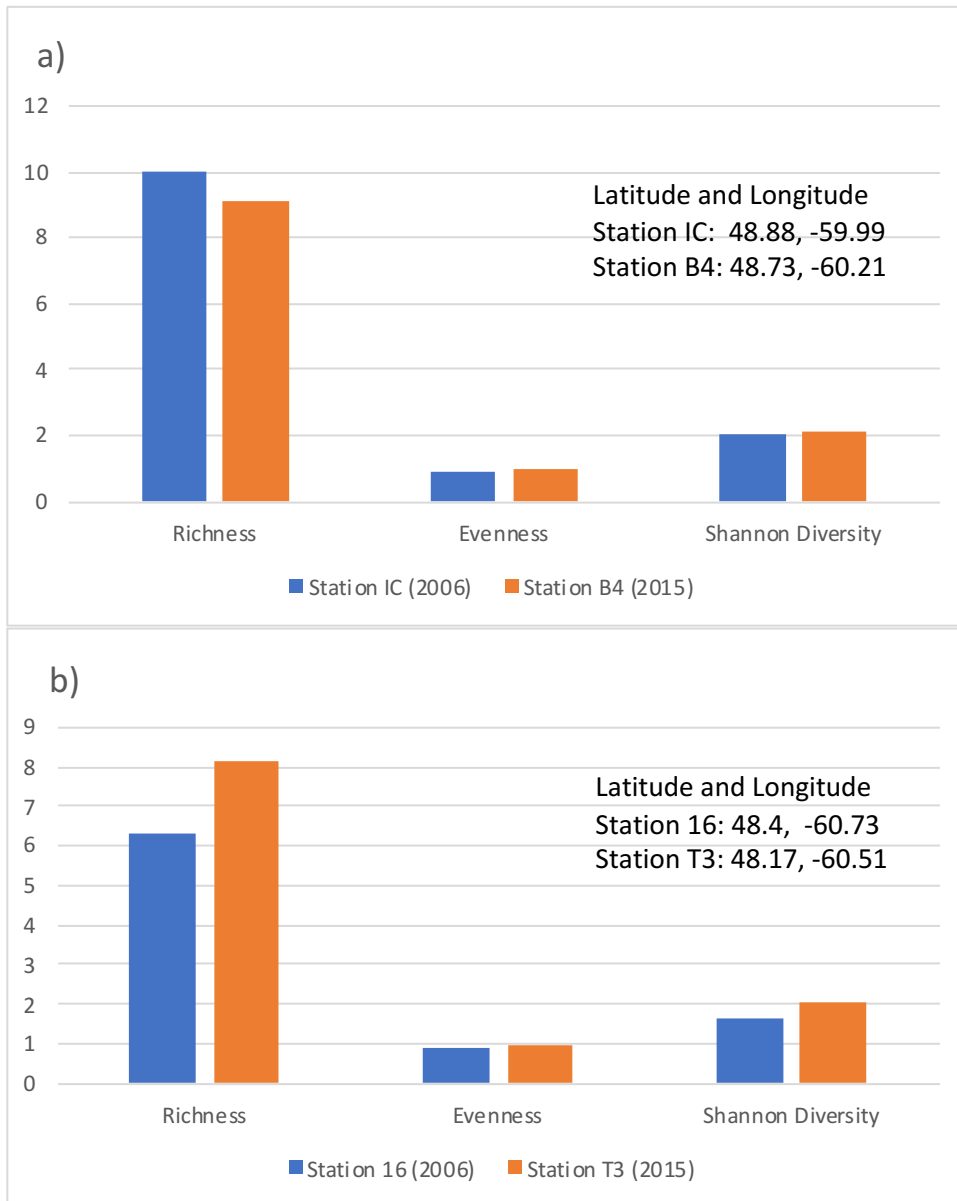


Figure 4.3. Temporal qualitative comparison of richness, evenness and Shannon diversity between a) station IC sampled in 2006 and B4 sampled in 2015, and b) station 16 sampled in 2006 and station T3 sampled in 2015. Latitude and longitude of each station is shown.

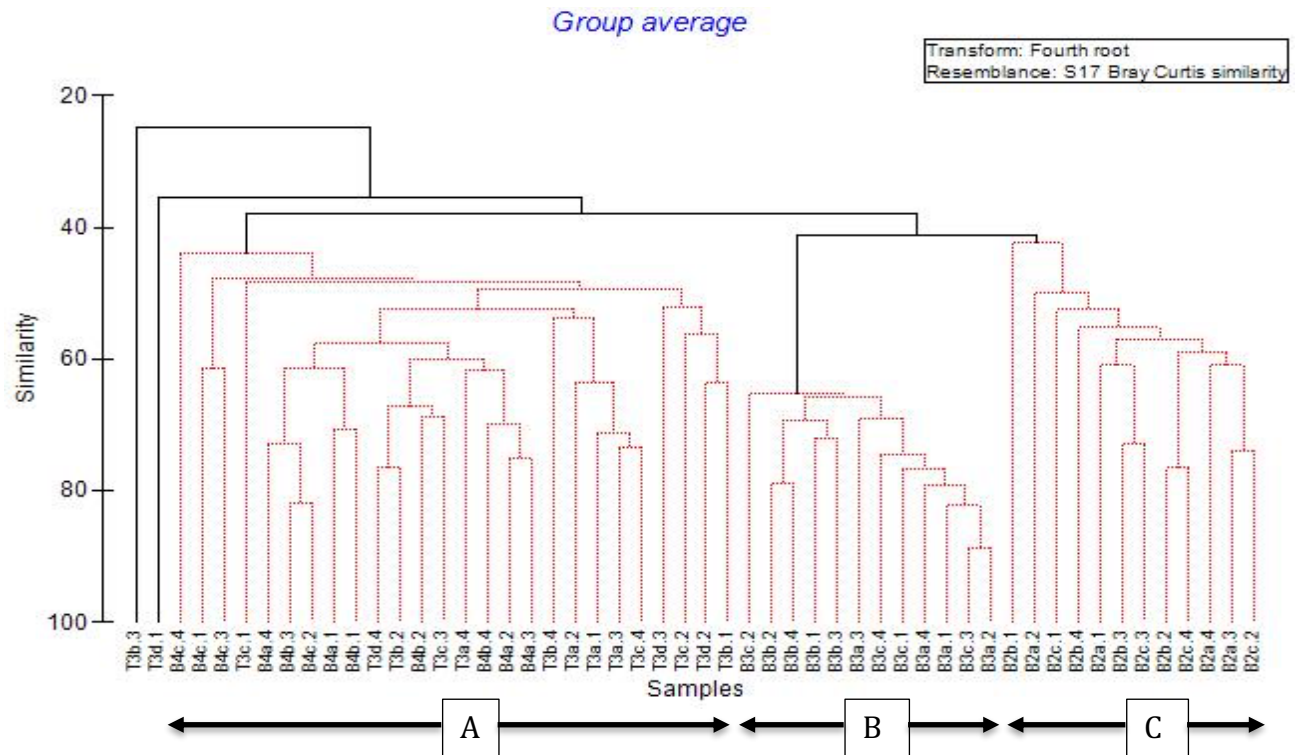


Figure 4.4. CLUSTER analysis dendrogram of stations based on similarity of family composition. Letters A, B and C refer to groups generated through the CLUSTER procedure.

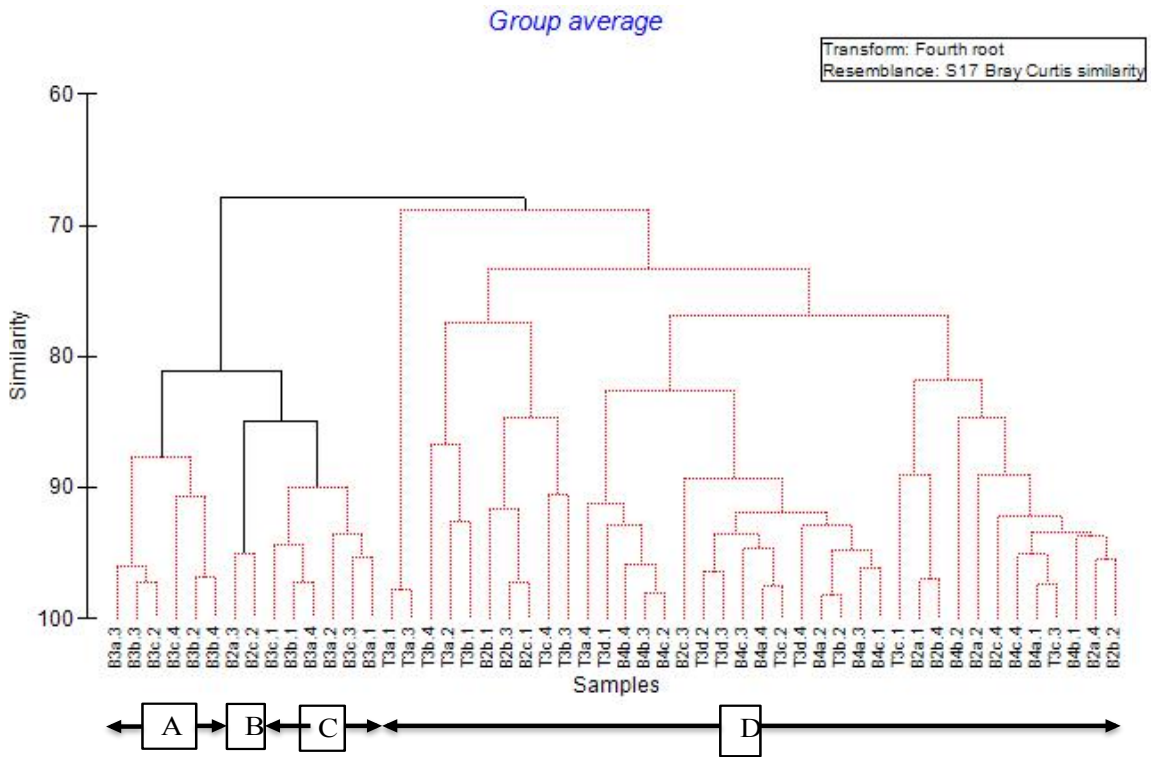


Figure 4.5. CLUSTER analysis dendrogram of 2015 cruise stations grouped together based on similarity of functional group composition. Letters A, B, C, and D refer to the groups generated through CLUSTER.

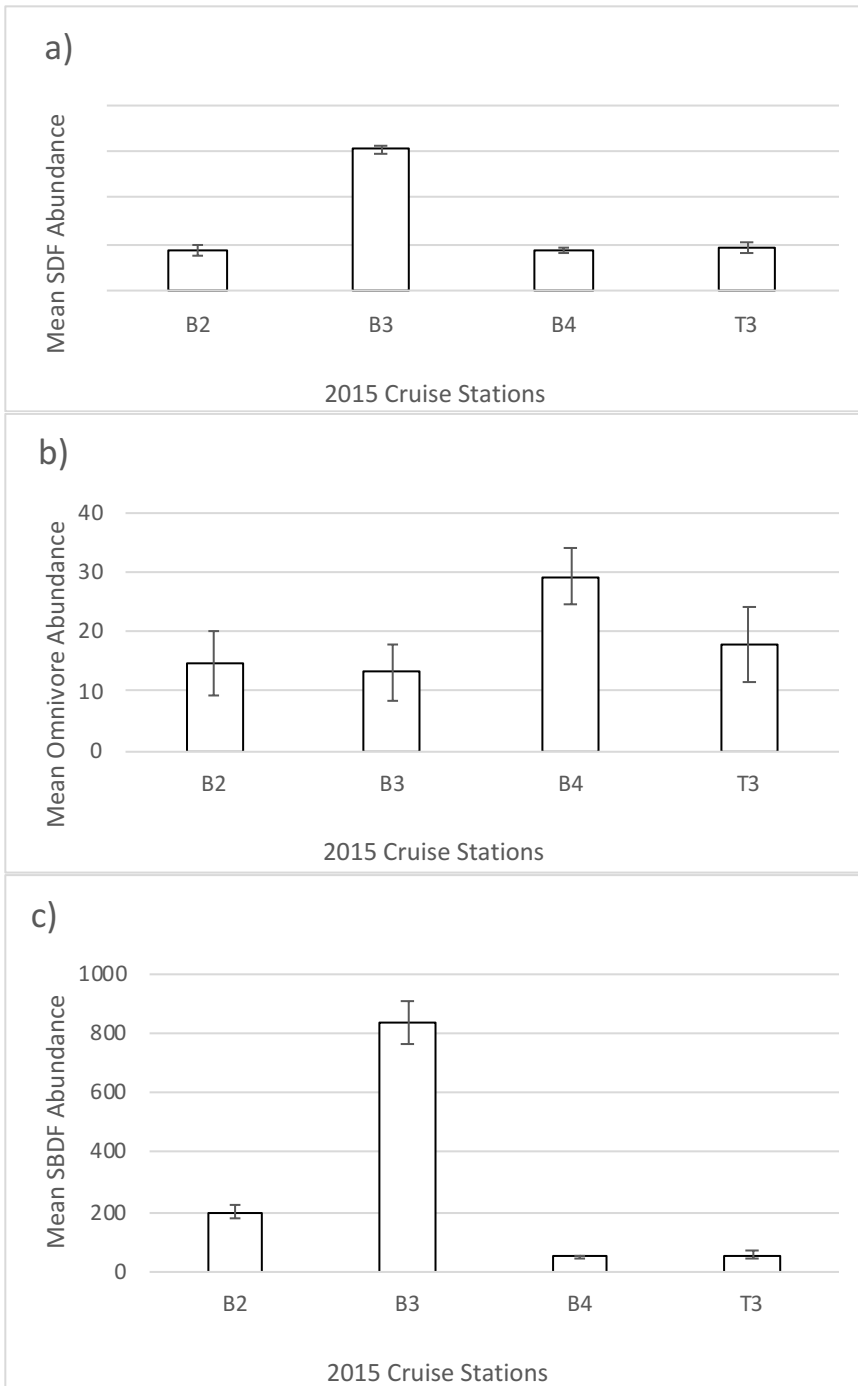


Figure 4.6. Mean a) SDF, b) omnivores and c) SBDF of the stations sampled in 2015. Error bars represent standard error of the mean.

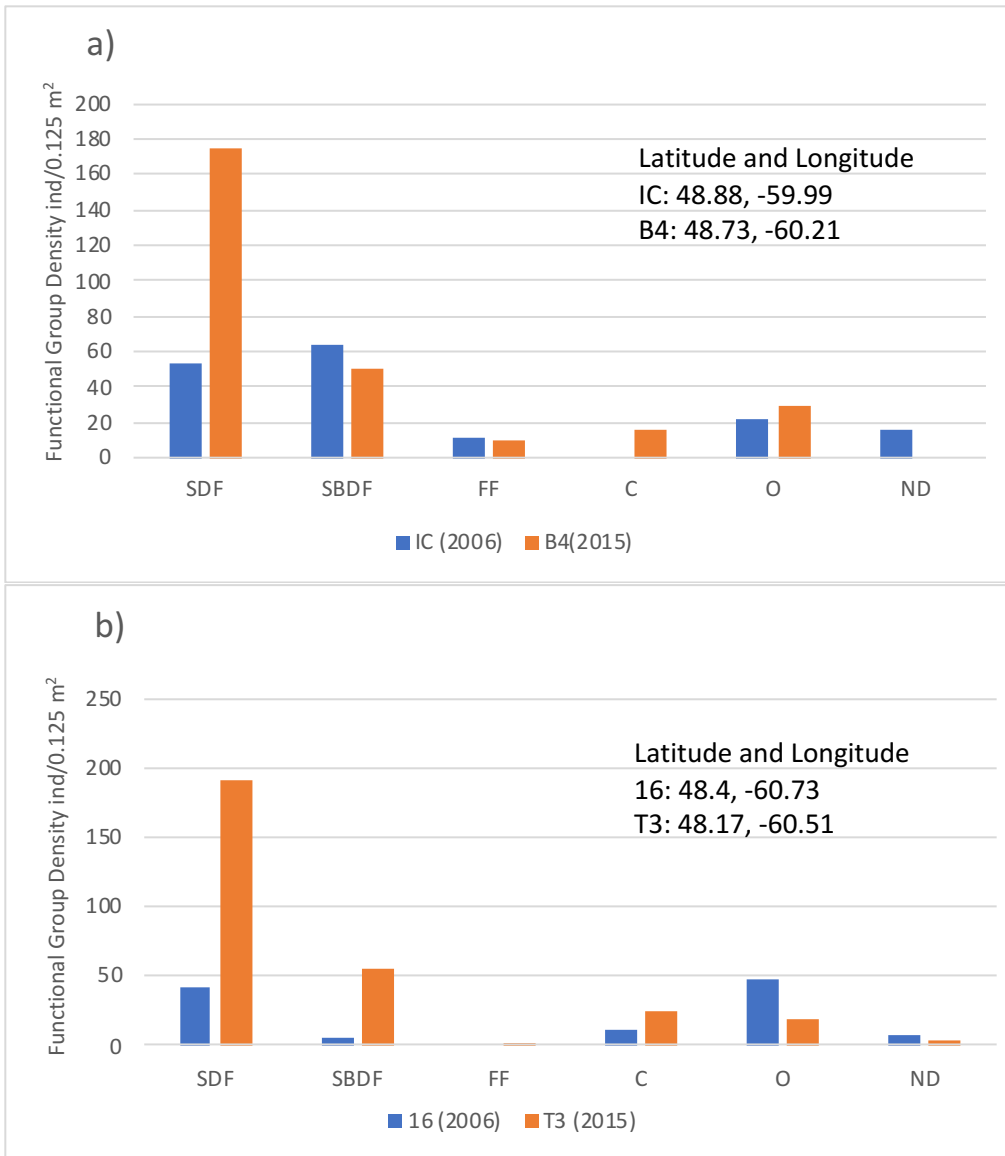


Figure 4.7. Temporal qualitative comparison of functional group density between a) station IC sampled in 2006 and station B4 sampled in 2015, and b) station 16 sampled in 2006 and station T3 sampled in 2015.

Tables

Table 4.1. Coordinates of stations sampled June 15-17, 2015, aboard the RV Coriolis II in the Gulf of St. Lawrence.

Station	Latitude	Longitude
B2a	48.426	59.323
B2b	48.415	59.279
B2c	48.422	59.259
B3a	48.656	58.718
B3b	48.638	58.748
B3c	48.644	58.724
B4a	48.763	60.066
B4b	48.725	60.303
B4c	48.717	60.251
T3a	48.023	60.404
T3b	48.276	60.539
T3c	48.197	60.431
T3d	48.182	60.661

Table 4.2. Family-level SIMPER results for 2015 cluster data indicating the three largest contributors to each group. See Figure 4.4 for the list of samples in each group. Samples were collected June 15-17 aboard the RV Coriolis II in the Gulf of St. Lawrence.

Group	Species	Av. Abund	Av. Sim	Sim/SD	Contribution %	Cumulative %
A (Channel)	Spionidae	1.43	13.43	5.15	25.51	25.51
	Paraonidae	1.16	9.72	2.02	18.48	43.99
	Capitellidae	1.05	8.82	1.73	16.76	60.75
B (Coastal)	Cossuridae	2.64	14.78	15.78	21.32	21.32
	Cirratulidae	2.21	12.34	9.48	17.79	39.11
	Nephtyidae	1.77	9.85	10.15	14.21	53.32
C (Between Coast and Channel)	Yoldiidae	1.61	11.44	3.84	21.21	21.21
	Spionidae	1.54	10.73	6.68	19.88	41.09
	Capitellidae	1.08	6.45	2.12	11.95	53.03

Table 4.3. Functional group SIMPER results for 2015 cluster data indicating the largest contributors to each group. See Figure 4.5 for the list of samples in each group. Samples were collected June 15-17 2015 aboard the RV Coriolis II in the Gulf of St. Lawrence.

Group	Functional Group	Av. Abund	Av. Sim	Sim/SD	Contribution %	Cumulative %
A (Coastal)	SBDF	2.64	33.00	16.39	36.48	36.48
	SDF	2.46	30.91	11.15	34.17	70.64
B (Between Coast and Channel)	SBDF	2.02	22.59	–	23.78	23.78
	SDF	1.92	19.00	–	19.99	43.77
	FF	1.41	15.97	–	16.81	60.58
C (Coastal)	SBDF	2.70	26.29	14.17	28.60	28.60
	SDF	2.50	24.74	15.54	26.92	55.52
D (Channel and Between Coast and Channel)	SDF	1.80	32.16	6.34	41.19	41.19
	SBDF	1.44	23.79	5.67	30.47	71.66

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CHAPTER 5: SUMMARY OF THIS THESIS

5.1 Benthic responses in a permanently hypoxic region

This thesis took advantage of research cruises in 1980, 2005 and 2006 to study the persistent, moderate levels of hypoxia and eutrophication that have gradually developed in the Lower St. Lawrence Estuary since the 1930s to analyze temporal (Chapter 2) and spatial (Chapter 3) impacts on benthic composition and diversity. A more recent research cruise in 2015 (Chapter 4) specifically addressed benthic response to hypoxic conditions and increasing temperature that were recently documented in the eastern Gulf of St. Lawrence. These different comparisons contributed distinct but complementary information about the responses of benthic communities to ongoing, sub-lethal levels of hypoxia that significantly differ from responses to brief severe hypoxic episodes. This chapter discusses the general conclusions that emerged when considering the three chapters collectively.

Increased total abundances of benthos between 1980 and 2005/6 was unexpected, given that numerous previous studies have shown that while opportunistic organisms often increase in abundance temporarily after a time-limited disturbance, benthic composition eventually returns to pre-disturbance levels (Pearson & Rosenberg, 1978). The increase in abundance and dominance of surface-dwelling polychaetes so long after hypoxia was first documented in the LSLE strongly suggests an enduring biological response to permanent hypoxia that has not been sufficiently severe to defaunate the benthic community, as occurs with many acute disturbances (Baban et al., 2016; Diaz & Rosenberg, 1995; Seitz et al., 2009). Whereas previous work reports a shift to surface deposit feeders (SDF) in disturbed environments in the LSLE (Belley et al., 2010) and elsewhere (Diaz & Rosenberg, 1995; Levin et al., 2009; Wu, 2002), the confamilial replacements that occurred between 1980 and 2005/6 suggest that this type of hypoxia has selected for relatively specific adaptations, rather than simply favouring the general

opportunistic reproductive strategies reported in most hypoxic regions. These somewhat unusual temporal changes found in the benthos help explain the unexpected findings from the spatial analysis and from the 2015 study.

The spatial study strongly complements results from the linked temporal study, by extending clear evidence of an altered benthic community within a single habitat over time to document changes in space. The highest richness and Shannon diversity in the hypoxic region sampled in the LSLE contrasted the lower richness and higher abundance typically seen in shorter-term, more severe hypoxic conditions. Evidence of adaptive and competitive species replacement from the temporal study suggests a possible mechanism for this reversal of the typical pattern of reduced richness and Shannon diversity in disturbed benthic habitats. Species replacement also accounts for at least part of the exclusion of less hypoxia-tolerant omnivores from the LSLE and the strong shift to SDF. The shift in dominance to organisms also tolerant or adapted to eutrophication likely contributed to increased richness and diversity, indicating that higher diversity may occur in hypoxic areas, especially eutrophication-induced hypoxia contributes to a shift in SDF.

Considering the spatial pattern of highest richness and Shannon diversity in the hypoxic region without temporal data could lead to the mistaken interpretation of a “healthier” benthic community, reinforcing the important role of temporal studies of biotic responses to environmental alterations. Knowledge of temporal shifts in benthos within the hypoxic LSLE region helped in interpreting the spatial results as atypical indicators of environmental disturbance and potential ecosystem functioning decreases. Importantly, the unexpectedly greater abundance, richness, and diversity of stations within the LSLE compared to the GSL might also suggest higher functional status than actually exists in the environment if considered without knowledge of temporal changes. The high diversity indices and abundance in the estuary potentially masked a significant reduction in functional richness (e.g., Belley & Snelgrove, 2016), resulting from highly

uneven functional group densities following species replacement and decreased abundance, as documented in the temporal study.

The 2015 research cruise complemented the 1980 and 2005/6 cruises, noting that the first two chapters of this thesis examined temporal and spatial biotic responses in a known region of long-term hypoxia and eutrophication, whereas the 2015 cruise assessed benthic changes in the GSL related to accelerating environmental changes. Benthic community composition from the 2015 cruise provided some clarity on benthic ecosystem health of the region, extending the findings of the first two chapters temporally and spatially.

Of the stations sampled in 2015, we observed the highest benthic abundance, family richness, Shannon diversity, and SDF/O ratio, but the lowest evenness at the B3 coastal stations. These results all mirrored both the temporal changes in the LSLE, and spatial differences between LSLE and GSL benthos in 2005/6. While the 2015 findings vary from “typical” indicators of disturbance, the atypical changes in LSLE benthos support the assertion that the eastern GSL may also be undergoing biotic changes associated with a gradual decline in oxygen to a moderate hypoxic level. The adaptations and species replacement we documented in the LSLE suggest that the similar $\sim 0.5 \mu\text{M}/\text{yr}$ reduction in oxygen in the Cabot Strait (Claret et al., 2018) that led to an oxygen level of $50 \mu\text{M}$ (Stortini et al., 2016) potentially selected for similar benthic adaptations and competition in the eastern GSL, offering a possible explanation for the benthic composition observed there.

The 2006 spatial study provided a baseline for temporal comparison with the 2015 stations that revealed a shift from less disturbance-tolerant lumbrinerids and amphirids at nearby 2006 stations to more tolerant spionids and capitellids at the Laurentian-Esquiman stations. This study facilitated interpretation of the family composition at the 2015 stations as possible adaptations to a more hypoxic and warmer

region. The greater dominance of SDF in 2015 than the shift in LSLE in 2005/6 further supports the interpretation of alteration of benthic communities to adapt to a hypoxic, warmer, and possibly eutrophic environment. Combined with the large reduction in omnivores at the eastern GSL stations, the 2015 cruise results provide evidence to extend concern for the health of the LSLE ecosystem to include the eastern GSL. We were able to use the results of the benthic changes that emerged in both the temporal and spatial studies as potential bioindicators to help interpret results where we lacked corresponding environmental data.

5.2 Biodiversity and ecosystem function changes in the LSLE and GSL

The replacement of mobile active bioturbator species by less mobile, less active bioturbators in the hypoxic regions of the LSLE has likely reduced ecosystem functioning. Belley and Snelgrove (2016) showed that functional richness, a measure of the potential functions contributed by a biotic community, provided a better predictor of benthic fluxes of multiple nutrients in the Northeast Pacific than standard measures of species richness and abundance. Bioturbation was a particularly important aspect of the linked functional richness and benthic flux differences between their study sites. However, Belley and Snelgrove (2016) also concluded that superficial modifiers of sediments in sufficiently high density had some positive effect on chemical fluxes, pointing to a need to investigate and quantify the degree of change in function from shifts to surface dwelling species in the LSLE. Maldanidae declined by 50% and *Maldane sarsi* abundance decreased by 78% during the temporal study period; presumably such reductions could significantly impact estuary functions. For example, Levin et al. (2009) reported that maldanids transfer significant amounts of nutrients into the sediment through funnel feeding.

The positive relationship between high oxygen fluxes and highest functional richness in the Pacific study (Belley & Snelgrove, 2016) supports our assertion that LSLE hypoxia and reduced bioturbation altered the important function of oxidation of elements and

compounds. Evidence of impaired oxidation of compounds emerged from Lefort's (2011) analysis of LSLE sediments sampled in 2007 that showed decreased sequestration of arsenic (As) as a result of interference from a reactive iron (Fe) compound produced during anaerobic oxidation (electron loss) of organic compounds.

As further evidence of disturbance of ecological functions in the LSLE, Thibodeau et al. (2010b) reported a nitrate deficit in the hypoxic region of the LSLE associated with nitrate flux into LSLE sediments at almost twice the rate observed in the normoxic GSL. This deficit likely resulted from sedimentary nitrate reduction occurring at depths where hypoxia-tolerant bacteria can survive but macrobenthos cannot.

An increase in bioturbation dependent functions within the GSL in 2006 may also account for the shift to omnivores. Bioturbators can act as 'ecosystem engineers' by increasing oxygen saturation, as well as porosity and penetrability of sediments (Zhang et al., 2017). In the case of a burrowing shrimp, sediment reworking improved the success of other burrowing species, but interfered with surface-dwelling species by altering the sediment-water interface (Gibson, et al., 2011). If the omnivores of the GSL affect SDF negatively or other omnivore functioning positively, the proportion of omnivores would further increase. This process represents the inverse of the synergistic interaction between hypoxia and reduction of bioturbators proposed to occur within the estuary. Importantly, the GSL area, or at least some habitats within the GSL with lower diversity indices than in the LSLE, could have had similar or even higher functional richness and levels of ecosystem functioning than the LSLE; the increase in bioturbators in the GSL compared with the LSLE described in our spatial study could increase ecosystem functioning (Belley & Snelgrove, 2016).

5.3 Taxonomic Sufficiency to Detect Hypoxic and Eutrophic Effects on Benthic Biota

Assessing the benthic ecology of the LSLE and GSL at the family level detected all major temporal changes, spatial differences in benthic composition, and most of the

significant correlations between benthos and environmental factors provided by species-level data. Multiple published studies also support the sufficiency of family-level analyses to detect assemblage change in various polluted sites (Bacci et al., 2009; Dafforn et al., 2013; Ferraro & Cole, 1990; Muniz & Pires-Vanin, 2011) and concluded that family level resolution offered the best compromise between efficacy in detecting benthic changes, and practicality and efficiency in terms of time, cost, and taxonomic expertise.

However, comparisons between species- and family-level analyses also revealed important limitations of family level taxonomic resolution. A relatively large difference in dissimilarity of 20% (56.54% at family level compared to species level dissimilarity of 79.5 %) when comparing 1980 and 2005/6 biota through SIMPER. The much smaller dissimilarity at the family level obscured many changes at the species level, potentially leading to significant underestimation of the extent of disturbance of biotic patterns. Importantly, this research distinguished between ecological changes in rapidly occurring disturbances from transformation that occurred during the very gradual and continuous development of hypoxia in the LSLE. This pattern only became evident in examining the replacements that occurred at the species level, likely through competition and differences in tolerance within families.

Similarly, SIMPER quantified 10% greater dissimilarity between species than families when comparing composition of hypoxic and maximum oxygen groups. Finally, assessing functional richness, biodiversity-ecosystem functioning (BEF) relationships, and differences in ecosystem functions both temporally and spatially critically requires information about specific species changes. Studies that demonstrate the loss of important discriminatory power with taxonomic pooling of data experimentally (Quijon & Snelgrove, 2006), and in field surveys (Musco et al., 2011) support the importance of taxonomic resolution to species.

Our study provides evidence that taxonomic comparisons at both species and family levels provides the most comprehensive information on the relationships between environment and biota. The differences in response to the environment in relation to taxonomic resolution provided further evidence that disturbed LSLE environments pushed some benthic taxa beyond their tolerance limits. We would only expect in an environment near its biological threshold that even modest differences in biological characteristics among confamilial species would lead to differences in benthic communities. Furthermore, intra-familial species substitutions (and suggesting competition) in the temporal comparison demonstrated that the permanently hypoxic LSLE selected for characteristics beyond opportunism. This process of species replacement within families is potentially quite significant, because changes in functional group richness commonly affects important ecosystem functions (Belley and Snelgrove 2016; Bonsdorff & Pearson, 1999), and species substitutions may not necessarily mean functional role substitutions. I must acknowledge that this comparison depended upon my ability to identify organisms accurately to species level, which may have introduced some errors. However the differences between taxonomic levels was large enough to be interpreted as a true result, despite errors in identification.

5.4 Limitations of this thesis

In the temporal study presented in Chapter 2, the absence of environmental data associated with the 1980 samples limited efforts to quantify the effects of environmental changes on benthic communities in 2005. No direct comparison of environmental changes at the same station to specifically link changes in benthic community composition at each station to environmental change was possible. In the spatial study detailed in Chapter 3, the absence of temporal data for most stations sampled in 2006 prevented any analysis of potential changes of either the environment or GSL benthic composition over time.

The 2015 analysis in Chapter 4 included several limitations. Weather conditions limited sampling to a small number of stations, and none of the 2006 stations could be resampled, preventing direct statistical comparisons, and limiting quantitative comparisons to only two 2006 stations. Technical problems prevented measurement of environmental data and we could there only infer any environmental relationship with benthic composition from the spatial study and published data on changes in GSL environmental conditions. Identification of benthic samples only to family level for the 2015 study limited comparisons with 2006 stations.

5.5 Areas for future research

This thesis provides evidence that the LSLE and possibly the eastern GSL have undergone sufficient ecologically significant benthic changes to warrant further investigation and possible actions to mitigate further changes. Updating knowledge of benthic community composition in the LSLE and western and eastern GSL to compare current diversity with these three studies would help in gauging the pace of climate-related changes in the benthos in this ecologically important area. Environmental measurements, particularly in the eastern GSL, would be useful in assessing the rate and degree of warming and hypoxia in this region and add significantly to our knowledge of the added effect of rising temperature, noting that hypoxia in the LSLE only occurred within the coldest near bottom water in the spatial study.

Each of the three studies showed likely adaptations to hypoxia from a slow moderate oxygen saturation decline. Recognizing the atypical benthic changes and composition in this type of hypoxic region could help in recognizing similar patterns in other disturbed geographic regions that might otherwise be misinterpreted as healthier than they really are. Given anticipated increase in future benthic community change with climate change, the findings in this thesis could help in identifying atypical responses to disturbance that could support ecological research and ocean use management in the face of climate change.

5.6 References

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APPENDICES

Appendix 1. The means of a) species-level and b) family-level diversity indices for 2005 and 2006 cruises. 2005 stations are shown in blue. 2006 stations are shown in green.

a) Species

2005 Stations	Richness	Pielou's Evenness	Shannon Index	2006 Stations	Richness	Pielou's Evenness	Shannon Index
25	13.33	0.62	1.59	23	7	0.96	1.8
24.75	14	0.67	1.76	17	12	0.93	2.3
E5	14	0.67	1.76	TCA	7	0.64	1.26
24	12.67	0.88	2.23	CA	8.333	0.89	1.86
23.6	10.67	0.64	1.51	16	2.33	0.97	0.81
23	10	0.77	1.75	IC	6.67	0.96	1.78
22	14	0.76	2	BOI	3	0.95	0.99
21	10	0.82	1.88	CE	6.67	0.77	1.46
20	9.67	0.82	1.85	SPI	6	0.77	1.36
19	6.67	0.72	1.27	TCE	5.67	0.73	1.22
18	9	0.79	1.74				

b) Families

2005 Stations	Richness	Pielou's Evenness	Shannon Index	2006 Stations	Richness	Pielou's Evenness	Shannon Index
25	14.33	0.67	1.77	23	8.67	0.94	1.98
24.75	13.33	0.6	1.55	17	14.67	0.93	2.49
E5	12.69	0.98	2.81	TCA	10.67	0.76	1.8
24	14	0.9	2.35	CA	13.67	0.86	2.25
23.6	12.33	0.68	1.7	16	6.33	0.89	1.63
23	12.33	0.8	2	IC	10	0.9	2.05
22	17	0.77	2.19	BOI	5.33	0.94	1.52
21	12.33	0.83	2.06	CE	9.33	0.81	1.79
20	11.33	0.84	2.02	SPI	9	0.83	1.81
19	7.33	0.8	1.53	TCE	8.67	0.78	1.68
18	9.33	0.84	1.87				

Appendix 2. Percent of total functional group abundance found at each station from 2005 and 2006 cruises. The highest abundance functional group of each station is bolded.

STATION	SDF	SBDF	FF	C	O	UC
25	62.7	28.3	2.8	1.5	3.4	1.2
E5	76.0	16.9	1.71	0.98	2.9	1.47
24	63.0	17.0	6.0	1.0	10.0	3.0
23_1	70.3	2.5	3.39	5.93	15.3	2.54
23_2	53.4	6.9	17.24	1.72	19.0	1.72
22	57.3	7.0	0.88	4.85	29.5	0.44
21	40.4	4.4	5.26	9.65	38.6	1.75
20	20.0	14.2	5.83	3.33	55.0	1.67
19	10.5	12.1	0.0	0.0	76.6	0.81
18	17.7	14.3	1.36	0.0	65.3	1.36
16	37.2	4.7	0.0	9.3	41.9	6.98
17	36.0	14.0	12.28	1.75	30.7	5.26
IC	32.3	38.7	6.45	0.0	12.9	9.68
BOI	7.7	7.7	15.38	11.54	57.7	0.0
CA	41.5	13.4	4.88	5.49	34.1	0.61
TCA	26.1	27.9	1.77	2.83	41.0	0.35
CE	16.3	20.2	3.85	0.0	58.7	0.96
SPI	19.6	19.6	0.93	3.74	56.1	0.0
TCE	24.7	27.4	1.61	1.08	45.2	0.0

Appendix 3. The mean abundance of six functional groups of the benthos at 13 stations sampled June 15-17 2015 aboard the RV Coriolis II in the Gulf of St. Lawrence. For definition of functional groups, see Statistical Methods, Multivariate analysis.

Station	SDF	SBDF	FF	C	O	ND
B2a	187.013	179.055	55.706	63.664	19.895	3.979
B2b	131.307	171.097	27.853	31.832	3.979	0
B2c	206.908	242.719	19.895	39.79	19.895	3.979
B3a	596.85	692.346	19.895	194.971	19.895	15.916
B3b	644.598	939.044	15.916	187.013	3.979	0
B3c	588.892	867.422	15.916	258.635	15.916	15.916
B4a	171.097	55.706	7.958	23.874	19.895	0
B4b	190.992	51.727	7.958	3.979	31.832	0
B4c	163.139	43.769	11.937	19.895	35.811	0
T3a	131.307	35.811	0	3.979	15.916	3.979
T3b	198.95	31.832	0	31.832	7.958	7.958
T3c	234.761	51.727	7.958	27.853	11.937	0
T3d	198.95	99.475	0	31.832	35.811	0