

EVALUATING ECOSYSTEM-LEVEL BENTHIC EFFECTS OF FINFISH
AQUACULTURE IN COASTAL NOVA SCOTIA

by

Stephen Finnis

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
August 2021

© Copyright by Stephen Finnis, 2021

TABLE OF CONTENTS

LIST OF TABLES	iv
LIST OF FIGURES	v
ABSTRACT.....	vii
LIST OF ABBREVIATIONS USED	viii
ACKNOWLEDGMENTS	ix
CHAPTER 1 INTRODUCTION	10
1.1 Research context.....	10
1.2 Research focus.....	12
1.3 Research goals and objectives	13
CHAPTER 2 USING MACROINFAUNA TO EVALUATE FAR-FIELD EFFECTS OF FINFISH AQUACULTURE IN PORT MOUTON BAY, NOVA SCOTIA, CANADA	14
2.1 Abstract.....	14
2.2 Introduction	14
2.3 Methods	18
2.3.1 Study site.....	18
2.3.2 Data types and collection	19
2.3.3 Environmental variables.....	21
2.3.4 Biological variables.....	21
2.3.5 Data analysis	23
2.4. Results	27
2.4.1 Environmental variables.....	27
2.4.2 Spatial variations in biodiversity indices	30
2.4.3 Relationships between biodiversity indices and environmental variables	34
2.4.4 Multivariate data analysis.....	35
2.5. Discussion.....	41
CHAPTER 3 SPATIAL AND TEMPORAL EFFECTS OF ORGANIC ENRICHMENT FROM FINFISH AQUACULTURE IN PORT MOUTON BAY, NOVA SCOTIA, CANADA	49
3.1 Abstract.....	49
3.2 Introduction	49
3.3. Methods	52

3.3.1 Study site.....	52
3.3.2 Data types and field sampling.....	53
3.3.3 Environmental variables.....	54
3.3.4 Biological variables.....	55
3.3.5 Statistical analysis.....	55
3.4 Results.....	56
3.4.1 Environmental variables.....	56
3.4.2 Biodiversity variables.....	59
3.4.3 Patterns in multivariate composition.....	63
3.4.4 Relationships with environmental variables.....	67
3.5 Discussion.....	69
CHAPTER 4 CONCLUSION.....	76
4.1 Discussion and conclusions.....	76
4.2 Research contributions.....	77
4.3 Research opportunities.....	78
REFERENCES.....	81
APPENDIX I: LIST OF TAXA AND THEIR AMBI ECOLOGICAL GROUP DESIGNATIONS.....	104

LIST OF TABLES

Table 2.1 Summary of the environmental variables collected and their associated effects on macrofaunal composition.	20
Table 2.2 Statistical parameters of environmental data and macroinfaunal diversity indices ($n = 40$).	30
Table 2.3 Spearman rank correlation coefficient matrix for environmental data ($n = 40$).	30
Table 2.4 Spearman rank correlation coefficient matrix for macroinfaunal diversity indices ($n = 40$).	31
Table 2.5 Permutational multivariate analysis of variance (PERMANOVA) on fourth root transformed Bray-Curtis dissimilarities of macroinfaunal species data for clusters identified in the hierarchical cluster analysis shown in Fig. 2.6A.....	37
Table 2.6 Similarity percentage (SIMPER) analysis showing the ten macroinfaunal species contributing most (%) to the average Bray-Curtis dissimilarities between clusters identified in the hierarchical cluster analysis shown in Fig. 2.6A.....	40
Table 3.1 Descriptive statistics of environmental variables collected within ($n = 4$) and outside of the fish farm lease ($n = 18$) in 2009, 2010 and 2011.	57
Table 3.2 Spearman rho correlation coefficient matrix for environmental data ($n = 22$) obtained in 2009, 2010 and 2011.....	59
Table 3.3 Descriptive statistics of macroinfaunal diversity collected within ($n = 4$) and outside of the fish farm lease ($n = 18$) in 2009, 2010 and 2011.	61
Table 3.4 Spearman rho correlation coefficient matrix for macroinfaunal biodiversity indices ($n = 22$) obtained in 2009, 2010 and 2011.....	63
Table 3.5 Similarity percentage (SIMPER) analysis identifying the top ten macroinfaunal taxa responsible for differentiating between the between clusters identified in the hierarchical cluster analysis in Fig. 3.4.	65
Table 3.6 Coefficient of variation (r^2) and significance by 999 permutations (P) for each environmental variable fitted to the non-metric multidimensional scaling ordination using the <i>envfit</i> function.....	69

LIST OF FIGURES

Figure 2.1	Map of the study area. Numbers refer to station names and the location of the farm lease boundary is outlined as a black rectangle.....	18
Figure 2.2	Proportional symbol maps of (A) sediment classification (Sed Class), (B) median grain size (Median), (C) percent mud (Mud), (D) organic content (Organic), (E) sediment porosity (Porosity), and (F) grain size sorting (Sorting).....	29
Figure 2.3	Maps of (A) AZTI Marine Biotic Index (AMBI), (B) Shannon diversity index (H'), (C) Pielou's evenness (J'), (D) multivariate-AMBI (M-AMBI), (E) abundance (N), and (F) species richness (S) calculated from the macroinfaunal species data.	32
Figure 2.4	Maps depicting spatial clusters and outliers based on the Local Moran's I spatial autocorrelation statistic for (A) AZTI Marine Biotic Index (AMBI), (B) Shannon diversity index (H'), (C) Pielou's evenness (J'), (D) multivariate-AMBI (M-AMBI), (E) abundance (N), and (F) species richness (S) calculated from macroinfaunal abundance data.....	34
Figure 2.5	Smoother estimates (solid line) for the generalized additive models showing the effect of organic content (%) on (A) AZTI Marine Biotic Index (AMBI), (B) Shannon diversity index (H'), (C) Pielou's evenness (J'), (D) multivariate-AMBI (M-AMBI), (E) abundance (N), and (F) species richness (S) calculated from the macroinfaunal abundance species data.....	35
Figure 2.6	Sampling stations represented as (A) a dendrogram based on hierarchical cluster analysis of fourth root transformed benthic macroinfaunal abundance and (B) a map of the resulting clusters..	36
Figure 2.7	Two-dimensional non-metric multidimensional scaling ordinations visualizing station similarity based on a Bray-Curtis dissimilarity matrix of fourth root transformed benthic macroinfaunal abundance ($n = 40$)..	38
Figure 3.1	Location map of the study area. Numbered circles represent station names, and the rectangular outline represents the fish farm lease boundary.	53
Figure 3.2	Boxplots of (A) sediment organic content and (B) sediment porosity collected in 2009, 2010 and 2011. Comparisons are shown for stations within (red; $n = 4$) and outside the farm lease boundary (blue; $n = 18$)..	58
Figure 3.3	Boxplots of (A) AMBI, (B) Shannon index (H'), (C) Pielou's evenness (J'), (D) M-AMBI, (E) abundance (N) and (F) species richness (S) from macroinfaunal species count data collected in 2009, 2010 and 2011.....	60

Figure 3.4 Dendrograms, non-metric multidimensional scaling (NMDS) ordinations and study area maps showing station similarity based on fourth root transformed macroinfaunal composition in (A) 2009, (B) 2010, and (C) 2011..... 64

Figure 3.5 Non-metric multidimensional scaling ordinations of fourth root transformed Bray-Curtis dissimilarities of macroinfaunal composition sampled in (A) 2009, (B) 2010, and (C) 2011. 68

ABSTRACT

Increased production of finfish aquaculture in coastal ecosystems is leading to greater concerns of the spatiotemporal effects of benthic organic enrichment. While the effects of organic enrichment have been studied extensively within the vicinity of cages, less is known of the dispersal hundreds of meters beyond the cage boundaries, termed far-field. The goal of this research is to examine the benthic spatial-temporal far-field effects from a net-pen finfish farm in Port Mouton Bay, Canada. Using a spatially dense sampling strategy and macroinfauna as indicators of ecosystem health, results showed the effect of organic enrichment to be restricted to within the farm lease (Chapter 2). Over a three-year fallowing period, changes in benthic conditions within the farm lease were observed, as farm stations became more similar in composition to the surrounding stations (Chapter 3). These results can provide researchers with spatially explicit insights to the potential benthic effects of finfish aquaculture.

LIST OF ABBREVIATIONS USED

AMBI	AZTI's Marine Biotic Index
BACI	Before After Control Impact
BC	British Columbia
CGS M	Alexander Graham Bell Canada Graduate Scholarships (Master's level)
CRD	Collaborative Research and Development
df	degrees of freedom
Distance	Seaway distance from the farm lease boundary
DFO	Department of Fisheries and Oceans
EAA	Ecosystem Approach to Aquaculture
EG	Ecological Group
EQS	Ecological Quality Status
F	PERMANOVA Fisher statistic
FAO	Food and Agriculture Organization
GAM	Generalized Additive Model
GCV	Generalized Cross Validation
H'	Shannon diversity index
HCA	Hierarchical Cluster Analysis
J'	Pielou's evenness
Max	Maximum
Med	Median
Min	Minimum
MS	Mean Sum of Squares
Mud	Percentage of mud in the sediment sample
n	Sample size
N	Abundance
NMDS	Non-metric Multidimensional Scaling
NSERC	Natural Sciences and Engineering Research Council
Organic	Percentage of labile matter combusted in each sample
PERMANOVA	Permutational Multivariate Analysis of Variance
Porosity	Percentage of water within the pore space of each sample
PRM	Pearson and Rosenberg Model
S	Species richness
SS	Sum of Squares
SD	Standard Deviation
SIMPER	Similarity Percentage analysis
SIMPROF	Similarity Profile analysis
Sorting	Standard deviation of the grain size distribution in each sample

ACKNOWLEDGMENTS

My sincere thanks are addressed to my supervisor, Dr. Jon Grant, for his patience, unwavering support, academic freedom, and willingness to discuss ideas. Additional thanks are addressed to my committee member, Dr. Ramón Filgueira, for his upbeat attitude, extreme attention to detail, and speedy but somehow still very detailed edits. I would also like to thank my committee member, Dr. Anna Metaxas, for her commitment to scientific excellence, helpful advice, and endless knowledge of statistics and ecology.

Great appreciation is addressed to Lin Lu, for collecting and analyzing the faunal dataset, and quick email responses. I would also like to thank the Oceanography administration, particularly Lori Lawton, for always going above and beyond with every task, and her overall thoughtfulness and kindness throughout this process.

I would like to thank the Grant and Filgueira lab members past and present including Anne McKee, Chantal Giroux, Caitlin Stockwell, Hart Koepke, Jenny Weitzman, Laura Steeves, Leigh Howarth, Marianne Parent, and Megan Rector for the motivation through research highs and lows, answering my many questions, and listening to my thousands of daily grievances. Special thanks to friend and officemate, Meredith Burke, for the crafting sessions, Aquafit lessons, pizza parties, and general support.

This thesis would not exist without the extraordinary team of doctors, pharmacists, nurses, technicians, counsellors, social workers, staff, researchers and volunteers from BC Cancer, Nova Scotia Health, Island Health, Estevan Pharmacy, and beyond. My heartfelt gratitude is addressed to Drs. Brooks, Forward, Kobiljski, MacPherson, Nette, Plourde, and Yan, and especially Drs. Freeman, How, and Prokai for their dedication, knowledge, exceptional care, and humanity. My endless appreciation is addressed to Marita and Madeleine for their guidance through the more challenging moments.

I am immensely grateful to my friends for all the fun, and advice maneuvering through both life and academia, and to my family, for pulling together every connection and resource imaginable to help get me out. Lastly, thank you to Reece, for the company, laughs, memories, and for moving from coast to coast and back again so many times.

Financial support was provided by an NSERC CRD grant to Jon Grant. I was also supported by NSERC CGS M and Dalhousie University Graduate Scholarships.

CHAPTER 1 INTRODUCTION

1.1 Research context

Aquaculture, or the cultivation of aquatic organisms, has been identified as a possible alternative to commercial fishing to provide protein for world's population (FAO, 2018). Accompanied with the rise of the net-pen finfish industry have been numerous environmental concerns, with one of the most well-studied effects being the organic enrichment of the seafloor (Edgar et al., 2005; Quero et al., 2020). Enrichment occurs when particulates from uneaten food and feces accumulate on the seafloor, causing measurable changes in sediment chemistry and biological communities (Pearson & Rosenberg, 1978; Sanz-Lázaro & Marin, 2006). These effects result from the decomposition of the settled organic matter, which can cause the upper sediment pore waters to become hypoxic or anoxic (Gray et al., 2002; Karakassis et al., 1998; Nilsson & Rosenberg, 2000). Organic enrichment of the benthos is therefore a concern for ecosystem health; however, these processes can be measured in various forms, making the benthos also a potential candidate for monitoring aquaculture impacts (Grant, 2012).

While multiple biological and geochemical variables exist to monitor the benthic effects of aquaculture, macroinfauna are often used due to the range of tolerances of different species to pollution (Dauer, 1993; Pearson & Rosenberg, 1978). These organisms also integrate longer time periods of sedimentation, while geochemical variables are more prone to fluctuations and may reflect more temporary conditions (Bilyard, 1987; Kalantzi & Karakassis, 2006). The combined effects of both oxygen stress and sulphide toxicity resulting from organic enrichment can cause quantifiable effects at individual, population and community levels in benthic fauna (Diaz & Rosenberg, 1995; Gray et al., 2002). Because of this response to organic enrichment, the macrobenthos have advantages for monitoring because they (a) are ubiquitous (Lenat et al., 1980), (b) are largely sedentary, allowing for spatial analysis of pollution (Abel, 2014; Britton & Greeson, 1989; Hellawell, 2012), (c) have relatively long lifespans, facilitating temporal analysis (Lenat et al., 1980), (d) have species-specific responses to natural and anthropogenic stress (Borja et al., 2000; Borja & Muxika, 2005), and (e) are involved in nutrient cycling (Bilyard, 1987; Dauer, 1993; Pearson & Rosenberg, 1978).

Although there are drawbacks to using macroinfauna for monitoring, such as high labor costs for species identification (Ärje et al., 2020; Nygård et al., 2016), analyzing the species present in a sample can give an indication of the ecological quality of the benthos.

Avoiding long-term benthic impacts requires an understanding of successional changes of macroinfaunal communities (Keeley et al., 2015). Rates of recovery are variable, site-specific, and dependent on multiple factors including hydrodynamics, farming size and intensity, duration of farming compared to fallowing, and the existing macroinfaunal composition (Kutti et al., 2007; Lin & Bailey-Brock, 2008; Macleod et al., 2006). Furthermore, geochemical measures are generally thought to imply that recovery has occurred sooner than biological indicators, making macroinfauna advantageous for analyzing recovery patterns (Keeley et al., 2015). Continuing farming too soon after fallowing may also decrease the amount of time before hypoxic or anoxic conditions are reached during the next production cycle (Keeley et al., 2015). Generally, studies have found that improvement in benthic conditions are observed within the first 6-12 months after farming ceases but remains incomplete for several years after (Karakassis et al., 1999; Keeley et al., 2014; Lin & Bailey-Brock, 2008; Villnäs et al., 2011).

While the benthic enrichment effects of net-pen finfish farming have been documented extensively in the near-field, less is known of the far-field effects, or those several hundred meters beyond the cage edge (Weitzman et al., 2019). These effects are of scientific and management concern, since competition for space in marine ecosystems has increased in recent decades, causing overlap with other industries and areas of public interest (Krause et al., 2020; Lester et al., 2018). The Ecosystem Approach to Aquaculture (EAA) has been proposed by the Food and Agricultural Organization of the United Nations has been identified as a framework to monitor these effects and has an ecological component focused on minimizing the degradation of ecosystem functions, services, and biodiversity (Soto et al., 2008). Monitoring far-field effects has been proposed as one such approach to implement EAA (Weitzman et al., 2019). Far-field studies in aquaculture are generally lacking and few have spatially comprehensive analyses to effectively capture the complex dispersal processes that occur in marine ecosystems (Grant, 2012).

Implementing EAA has remained challenging due to factors such as competing development objectives and a lack of common understanding of the concept (Brugère et al., 2019). However, increased sampling coverage opens new possibilities for assessing ecosystem-level variations (Grant, 2012). Data-driven approaches are often advantageous in spatial analyses to uncover trends and analyze patterns of variation (Kite et al., 2016). By visualizing results on a map, researchers allow the data to speak for themselves and can infer process from the spatial patterns (Fotheringham & Rogerson, 2008). Local indicators of spatial autocorrelation can be applied to univariate indices to determine if values are higher or lower than expected based on chance (Anselin, 1995). Consistent patterns of similar values indicate that the driving processes are not random and provide evidence for developing further hypotheses related to the environmental drivers (Anselin, 1995; Nelson & Boots, 2008; Ord & Getis, 2001). Incorporating spatial relationships into multivariate data analysis is more challenging (Anselin, 2019), although approaches such as hierarchical cluster analysis can be used to detect groupings of similar values which can then be mapped (Bridges Jr, 1966; de Vries et al., 2020). Statistical approaches with spatially explicit outputs can therefore visually assist researchers and provide visual clues of the environmental forcing behind the biological patterns (Nelson & Boots, 2008).

1.2 Research focus

Net-pen finfish aquaculture is a contentious issue in Canada, and a farm in Port Mouton Bay, Nova Scotia, has been a focal point of this conflict between the industry and local citizens (Milewski & Smith, 2019). The farm has operated intermittently since 1995 and the resulting benthic effects have been studied in numerous forms. For instance, Loucks et al. (2012) found concentrations of copper in the sediments below the farm cage at levels above the Canadian Council of Ministers of the Environment (2002) regulatory limit. Cullain et al. (2018) analyzed multiple benthic variables from eelgrass beds at ~300 m, ~700 m, and ~3000 m and attributed increased organic content, decreased eelgrass biomass and shoot density, and decreased macroinfaunal biomass in stations near the farm to the presence of the farm. Bay-scale benthic effects have also been proposed by Milewski et al. (2018) and Loucks et al. (2014) who noted decreases in Catch Per Unit Effort of lobster in years when the farm was operational in comparison to fallow years.

These two studies speculated that the lobster exhibit retreat behavior when exposed to sulfidic conditions from the farm during production periods, resulting in the lower catches. However, the catch data were aggregated within large arbitrary boundaries which can obscure the spatial patterns, since trends are averaged over distances that may not reflect the true scales of animal movement (Kite et al., 2016). As a result, the methods and analysis were critiqued by Grant et al. (2016). Explicitly identifying the spatial extent of enrichment in Port Mouton Bay is therefore an important scientific objective that has remained unstudied.

Due to the concerns of the local citizens and potential effects of the farm, a benthic dataset of macroinfaunal species counts, and environmental variables was obtained from Port Mouton Bay from three different years of study. This provides a unique opportunity to apply data-driven techniques to analyze the spatial extent and reversibility of organic enrichment from the finfish farm. Given the limited existing research on far-field effects, this study offers rare perspectives into the spatial and temporal dynamics of farm wastes which are crucial for implementing EAA.

1.3 Research goals and objectives

The overarching goal of this research is to examine the benthic spatiotemporal effects from a net-pen finfish farm in Port Mouton Bay, Nova Scotia, to implement EAA. This will be addressed by accomplishing the following objectives:

- 1) Examine the spatial variations in macroinfaunal structure and their relationships with environmental variables to characterize the far-field benthic effects from a net pen finfish aquaculture site, and
- 2) Determine how macroinfaunal composition changes spatially over a three-year fallowing period, and the environmental drivers responsible for these changes.

CHAPTER 2 USING MACROINFAUNA TO EVALUATE FAR-FIELD EFFECTS OF FINFISH AQUACULTURE IN PORT MOUTON BAY, NOVA SCOTIA, CANADA

2.1 Abstract

Organic loading to the seafloor from finfish aquaculture wastes can cause changes in sediment chemistry and macroinfaunal community structure. Despite decades of research, most studies have focused on sampling the benthos in the immediate vicinity of the cages, yet few have characterized these effects at greater distances, termed far-field. Understanding the spatial extent of these effects has important environmental and management implications and is crucial for a successful implementation of an Ecosystem Approach to Aquaculture. In this study, the far-field effects of a net pen fish farm in Port Mouton Bay (Nova Scotia, Canada) were examined using macroinfauna as indicators of benthic quality. Through a spatially comprehensive sampling strategy, bay-scale variations in biodiversity indices and multivariate assemblage structure were examined to detect if the farm imposed a detectable signature from the naturally varying benthic conditions. Results showed similar spatial zonation in biodiversity indices and multivariate assemblage structure. Correlative models showed strong associations between both biodiversity indices and multivariate structure with sediment organic content. Multivariate analyses further indicated that stations within the farm lease exhibited a distinct faunal community from the surrounding sampling locations. These results suggest organic enrichment from the farm was restricted to within the farm lease and dissipated within distances as near as 38 m from the lease edge. This study offers unique insights into bay-scale dynamics of macroinfaunal assemblages and incorporating an Ecosystem Approach to Aquaculture within benthic monitoring protocols.

2.2 Introduction

Despite the growth potential and various socioeconomic benefits of finfish aquaculture, the industry has faced negative criticism both from the public (Omundsen & Olsen, 2017; Weitzman & Bailey, 2019) and scientific community (Barrett et al., 2019; Wang et al., 2020). Organic enrichment of the benthic ecosystem from fish feces and uneaten feed is one of the most well-recognized impacts from net pen fish farming

(Carvajalino-Fernández et al., 2020; Keeley, et al., 2015). Over the past decade, emphasis has been placed on understanding these effects across broader spatial scales, such as through the promotion of an Ecosystem Approach to Aquaculture (EAA) proposed by the Food and Agricultural Organization of the United Nations (Soto et al., 2008). From an ecological perspective, an EAA seeks to evaluate the effects of aquaculture at the ecosystem level in order to minimize the degradation of ecosystem functions, services and biodiversity (Soto et al., 2008). A greater understanding of the spatial dynamics of these benthic effects is therefore of high scientific and management concern.

Although there is an extensive literature documenting benthic monitoring at finfish aquaculture sites, the majority of studies focus on localized spatial scales near the farms (i.e., near-field; Bannister et al., 2016; Weitzman et al., 2019). Studies are often limited in their spatial perspective as they routinely compare conditions below the farm to a singular reference site, or along a transect extending outward from the farm boundaries (Grant, 2012; Ottinger et al., 2016). This precludes effective spatial analysis such as delineating a zone of impact, since the organic enrichment of sediments surrounding farms is not uniform due to the complex dispersal of wastes by currents (Broch et al., 2017; Carvajalino-Fernández et al., 2020). In general, the benthos directly below the farm is known to be affected, yet these effects are thought to diminish with distance from the cage within a few hundred meters (Grant, 2012; Keeley, et al., 2013). However, several studies suggest this footprint can extend further, such as in regions with dispersive hydrodynamic regimes (Broch et al., 2017; Keeley et al., 2019). A feasible implementation of an EAA is therefore to monitor the bay-scale effects of aquaculture, termed far-field. While there is no fixed distance or definition which differentiates near- and far-field effects, the far-field can be generally be considered as hundreds of meters beyond the cage boundaries (Weitzman et al., 2019).

The effects of organic enrichment on benthic conditions are often analyzed using macroinvertebrate infauna, partly due to the range of tolerances of different species to stressors (Pearson & Rosenberg, 1978; Tomassetti et al., 2016). The generalized community response of macroinfauna to organic enrichment is commonly represented by the Pearson and Rosenberg Model (PRM), in which organisms show a reduction in average body size, a decrease in suspension feeders, a shift to dominance of deposit

feeders, and a progressive restriction of fauna to shallower depths within the sediment (Pearson & Rosenberg, 1978). Due to these responses, macroinfaunal count data can be interpreted in multiple different forms to analyze the effects of farm-related organic enrichment. Multivariate analyses allow for a detailed exploration of patterns and are widely regarded as the most sensitive means to assess changes in community structure (Austen & Warwick, 1989; Clarke, 1993; Gong et al., 2005; Gray, 2000; Schratzberger et al., 2000; Warwick & Clarke, 1991). In order to more simply communicate results and conduct statistical tests, biodiversity indices are frequently used, in which species composition data from each sample are summarized into a single number (Bilkovic et al., 2006; Dauer et al., 2000; Hale et al., 2004). Importantly, these indices are spatially explicit and can be directly mapped in order to evaluate the extent of effects from aquaculture activities (Keeley et al., 2012, 2014). While biologically informative, these approaches on their own do not directly provide information on the benthic conditions or pollution status. Therefore, indices with an associated qualitative disturbance classification have been developed for easier interpretation by management officials and policy makers (Diaz et al., 2004; Pinto et al., 2009). Among the most popular are the AZTI Marine Biotic Index (AMBI; Borja et al., 2000) and multivariate-AMBI (M-AMBI; Muxika et al., 2007) which have both been successfully applied to aquaculture (Keeley et al., 2012, 2015, 2014; Nickell et al., 2009; Urbina, 2016).

Multiple environmental variables are known to affect the distributions of benthic macroinfauna and require consideration when developing hypotheses related to the effects of aquaculture (Lu et al., 2008). For example, organic content, a fundamental component of the PRM, occurs naturally in coastal environments and acts as a food source for macrofauna (Gray, 1974; Lopez & Levinton, 1987). Sediment grain size has also been identified as an influential factor of macrofaunal distributions (Gray, 1974; Lu, 2005; Lu et al., 2008; Snelgrove & Butman, 1994). While the mechanistic link is still in question, factors related to specific grain size distributions such as food availability, predator activity, microbial abundance and composition, and pore water chemistry are thought to be the causative mechanisms (Snelgrove & Butman, 1994). For example, grain-size sorting, a measure of the similarity of grain sizes, has also been identified as a potential driver, as it can affect potential niche space (Coleman et al., 1997; Etter &

Grassle, 1992; Gray, 1974; Nichols, 1970). Examining the relationships between macroinfaunal communities and these drivers may therefore help tease apart the effects of each variable within an ecosystem and develop more nuanced hypotheses related to the spatial effects of fish farm wastes.

Following EAA recommendations, spatially dense sampling strategies at the bay-scale can yield new insights into the benthic effects of fish farms. In particular, data-driven approaches with spatially explicit outputs can be used to examine inherent properties of the data and detect unusual trends which may be associated with a deviation from background conditions (Nelson & Boots, 2008). For a quantitative spatial analysis of biodiversity indices, local indicators of spatial autocorrelation allow for patterns of variation over the study area to be examined (Nelson & Boots, 2008; van der Wal et al., 2017). For multivariate datasets, cluster analysis has been used to group samples with similar macrofaunal composition and examine potential spatial dynamics (de Vries et al., 2020; Jun et al., 2016). Notably, these approaches do not require analysis within *a-priori* boundaries, thereby obviating arbitrary regions or subjective distance thresholds in which to study spatial patterns (de Vries et al., 2020; Openshaw & Taylor, 1979). Although spatial trends do not prove causation, these patterns may help delineate a zone or areas affected by fish farming activities.

The goal of this study was to examine the spatial variations in macroinfaunal structure to characterize the far-field benthic effects from a net pen finfish aquaculture site in Port Mouton Bay, Nova Scotia. The effects of the fish farm were determined by evaluating spatial trends and analyzing relationships with environmental variables to discern if the farm imposed a detectable signature on the background conditions within the bay. Specifically, the objectives were to (1) evaluate spatial patterns in the benthic community composition using various measures of macroinfaunal diversity, (2) determine which environmental variables best explain the patterns in assemblage structure, and (3) examine whether the community composition was significantly different within the farm lease than at other sites within the bay. This study offers new perspectives for bay-scale monitoring protocols and the growing knowledge of potential far-field effects of marine fish farming activities.

2.3 Methods

2.3.1 Study site

Port Mouton Bay is a semi-protected bay (~56 km²) situated on the South Shore of Nova Scotia (Fig. 2.1). Depths within the bay range from 8-18 m, with bathymetry characterized by shallow sills, including a ridge separating Spectacle Island from the mainland (DFO, 2007; Gregory et al., 1993). Tidal currents are generally low (2-3 cm s⁻¹; Gregory et al., 1993) and surface currents are primarily wind-driven (DFO, 2007, 2009). Tides are semi-diurnal with average amplitudes of 1.5 m.

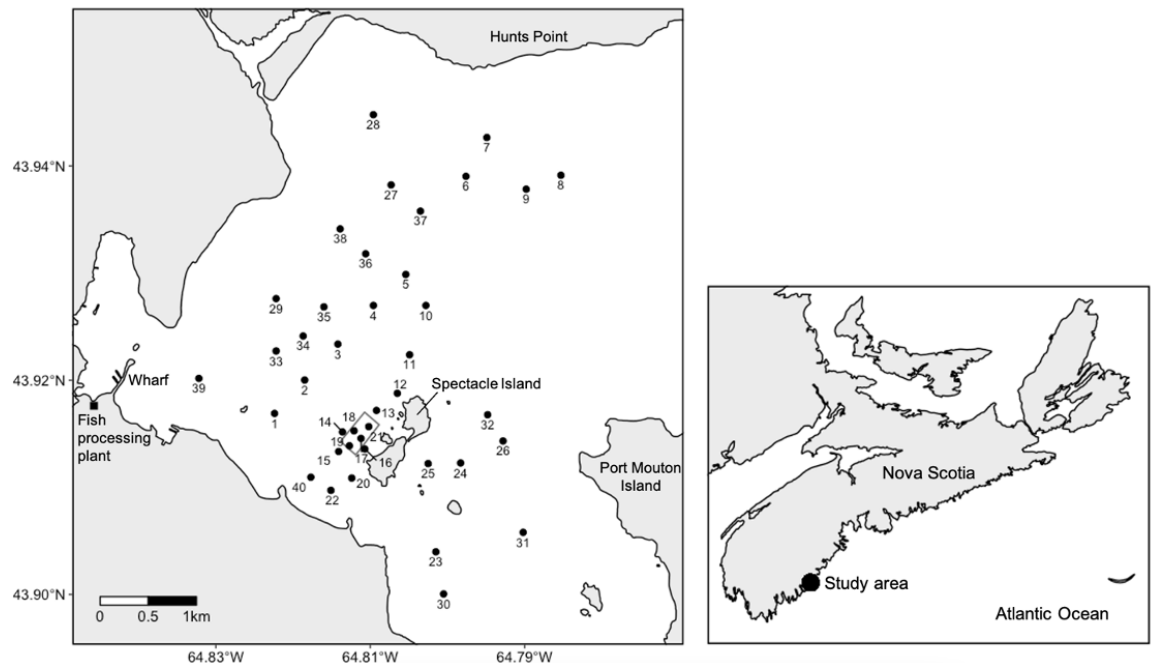


Figure 2.1 Map of the study area. Numbers refer to station names and the location of the farm lease boundary is outlined as a black rectangle.

The first finfish farm in the bay was established in 1995 on the western side of Spectacle Island. The fish farm was initially stocked with steelhead trout (*Oncorhynchus mykiss*) which were then replaced by Atlantic salmon (*Salmo salar*) in 2000 until 2009. The fish were harvested in July 2009 and the site remained fallow until August 2012. Prior to the present study (October and November 2009), cages were of a rectangular grid design, and were positioned within the farm lease of 8.03 ha. The depth within the lease ranges from 10-12 m and the cages typically occupy the upper 8-10 m of the water

column. Additionally, a large wharf and fish processing plant operated in the northwest of the bay until 2013 (Fig. 2.1). These could potentially serve as additional stressors to the benthos, although the effects of plant effluent and industrial vessel activity in this region of the bay are unknown.

2.3.2 Data types and collection

Field measurements were obtained during October and November 2009 after ~ 3 months of fallow ($n = 40$ stations) (Fig. 2.1). Sampling stations were selected to span the entirety of the bay to allow for the examination of both near- and far-field effects from the farm. The selected environmental variables were median grain size (μm), grain size sorting (μm), organic content (%), percent mud (%; i.e., amount of the sediment $<63 \mu\text{m}$), porosity (%), and seaway distance from the farm (m) (Table 2.1). Samples for grain size analysis were collected in October 2010 as part of a separate field campaign and were used in this analysis as they were assumed to not change substantially during the study period. In Port Mouton Bay, the rate of sediment supply by is low, although wave transport and storm resuspension may affect sediment distributions (Gregory et al., 1993; Piper et al. 1986). However, similar spatial patterns of surficial sediments were observed by Piper et al. (1986) suggesting the same general pattern remains constant over time.

Table 2.1 Summary of the environmental variables collected and their associated effects on macrofaunal composition.

Variable	Summary	Rationale
Distance	Seaway distance from the farm lease boundary (m)	May account for unmeasured variables that affect macrofaunal composition such as sulfides (Diaz & Rosenberg, 1995) or heavy metals (Sanz-Lázaro & Marín, 2011) resulting from the fish farm.
Median	Median grain size of the sediment sample (μm)	Specific grain size distributions may be associated with biogeochemical factors (e.g., pore water chemistry) that can affect macrofaunal composition (Snelgrove & Butman, 1994).
Mud	Percentage of mud (grains $<63 \mu\text{m}$) in the sediment sample (%)	May constrain the relative abundances (Holland, 1985) or maximum densities (Thrush et al., 2003, 2005) of macrofauna. Higher mud content may also be more favourable for deposit feeders while coarser grains are often associated with suspension feeders (Gray, 1974).
Organic	Percentage of labile matter combusted in each sample (%)	Acts as a food source (Gray, 1974; Lopez & Levinton, 1987) and can cause changes in sediment pore water chemistry at high levels of enrichment (Diaz & Rosenberg, 1995; Gray et al., 2002; Pearson & Rosenberg, 1978).
Porosity	Percentage of water within the pore space of each sample (%)	Affects pore water chemistry (Ahmerkamp et al., 2020), potential niches (McArthur et al., 2010) and can be used to detect changes in sediment consistency from flocculation of feces, feed and fine-grained sediments (Milligan & Law, 2005).
Sorting	Geometric standard deviation of the grain size distribution in each sample (μm)	Poorly sorted sediments have a larger range of grain sizes and more interstitial spaces than well sorted sediments, which may provide greater niches for macrofauna (Etter & Grassle, 1992; Gray, 1974; Nichols, 1970).

Benthic samples were retrieved using an Ekman grab (237.16 cm² surface area, 15 cm maximum depth) for both environmental and biological measurements. Prior to sampling, water was removed from the top of the grab with a siphon, without disturbing the surface sediments. Two sediment subsamples were obtained for organic content and porosity measurements from the grab using plastic, truncated syringes (5 ml volume, 1.4 cm diameter). For each subsample, ~1-2 ml was extracted from a sediment depth of 0-2 cm at three different locations in the grab. The sediment was injected into pre-weighed glass vials (20 ml) and tightly capped to minimize dehydration. A separate grab was obtained for biological analysis, where all contents from the grab were washed with seawater through a 500 µm sieve. The remaining macroinfauna were preserved in a 10% buffered formalin solution and transferred to the laboratory for taxonomic identification to species where possible. In 2010, two small sediment samples (~200 g) were collected from a grab with a metal trowel and placed in a sealed plastic bag for grain size analysis.

2.3.3 Environmental variables

Sediment grain size analysis was performed by wet sieving the sample through stainless steel sieves with decreasing mesh size (2000 µm, 1000 µm, 500 µm, 250 µm, 125 µm, and 63 µm) according to Buchanan (1984). The portion remaining on each sieve was dried at 60 °C and then weighed. The silt-clay fraction (<63 µm) was filtered onto a pre-weighed Whatman GF/F filter (2.5 cm diameter, 0.7 µm pore size) and dried at 60 °C. Grain size distributions were calculated using GRADISTAT Version 4.0 (Blott & Pye, 2001) in conformance with the Folk and Ward classification system (Folk & Ward, 1957).

Porosity (water content) was evaluated as the change in mass upon drying the sample at 60 °C for 24 h. Organic matter content was then determined as the loss on ignition of the dried sediment sample at 500 °C for 4 h (Buchanan, 1984).

2.3.4 Biological variables

In the laboratory, benthic macroinfauna were identified and counted under a dissecting microscope to the lowest possible taxon (in most cases, species level). The following biodiversity indices were calculated for each station including:

- a) Abundance (N), the total number of individuals per sample,
- b) Richness (S), the total number of species per sample,
- c) Shannon diversity index (Shannon, 1948),

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

where p_i is the proportion of the i_{th} species of the total abundance, and

- d) Pielou's evenness (Pielou, 1966),

$$J' = \frac{H'}{\ln S}$$

In the present study, AMBI and M-AMBI indices were also calculated. The AMBI approach involves classifying macrofaunal species into one of five ecological groups (EG) according to their response to organic enrichment including EG I: disturbance-sensitive species, EG II: disturbance-indifferent species, EG III disturbance-tolerant species, EG IV: second-order opportunistic species, EG V: first-order opportunistic species (Borja et al., 2000).

Species were first assigned to an EG from the library already included in the AZTI software v. 5.0 (<http://ambi.azti.es>; Borja et al., 2000; Borja et al., 2003; Borja et al., 2011). Species not included in this list were then assigned to groups based on literature sources (e.g., Fauchald, 1977; Gosner, 1971; Meinkoth, 1981; Pollock, 1998) or expert opinion by the authors of this paper. Species with insufficient information to designate a group, were listed as “not assigned” (n.a.). Other taxa such as non-soft sediment taxa (e.g., Nudibranchia), and epifaunal or planktonic taxa, were removed from the analysis (“ignored”) following Borja & Muxika (2005) or the expert decision of the taxonomist of this study. Neither designation (“not assigned” or “ignored”) affect the final AMBI score in the following calculation.

Following these designations, AMBI values were calculated based on the proportion of species within each EG and the sensitivity coefficients for each group (Borja et al. 2000):

$$AMBI = [(0 \times \%EG I) + (1.5 \times \%EG II) + (3 \times \%EG III) + (4.5 \times \%EG IV) + (6 \times \%EG V)]/100$$

This results in AMBI values ranging from 0 to 6, with a value of 7 used to represent azoic conditions. The following ranges represent the ecological quality status (EQS; Muxika et al. 2005): “high” ecological status, 0-1.2; “good”, 1.2-3.3; “moderate”, 3.3-4.3; “poor”, 4.3-5.5; and “bad” 5.5-7.

More recently, M-AMBI, which incorporates AMBI, richness, and Shannon diversity in a factor analysis, was introduced to overcome observed biases of AMBI such as in low-salinity environments which have naturally low species richness (Borja et al., 2004; Muxika et al., 2007). The M-AMBI calculation requires reference conditions that correspond to undisturbed and totally disturbed or impacted endpoints (Muxika et al., 2007). In this study, the totally disturbed reference conditions were assigned as $AMBI = 6$, $H' = 0$, $S = 0$ (Muxika et al., 2007). The undisturbed reference conditions were assigned as the lowest AMBI value in the dataset, and the highest values for H' and S . Using these conditions, M-AMBI values were calculated using the AZTI software with resulting values ranging from 0 to 1. Briefly, the program performs a factor analysis on a matrix of the standardized metrics for all stations. The factor scores are then orthogonally projected on a line between the reference conditions, where the reference conditions are normalized to range between 0 (bad) and 1 (high). The Euclidean distance between 0 and the score projection represents the M-AMBI value (for full details, see Muxika et al., 2007). These were divided into five categories to describe the EQS using the thresholds: “high” quality, 0.77-1; “good”, 0.53-0.77; “moderate”, 0.38-0.53; “poor”, 0.20-0.38; and “bad”, 0-0.20 (Borja et al., 2007; Borja et al., 2009).

2.3.5 Data analysis

2.3.5.1 Spatial trends in biodiversity indices

Local Moran’s I was calculated for each station to identify significant spatial patterns for each index. Local Moran’s I quantifies the degree of local spatial autocorrelation in the study area by comparing the value of each site to its neighbouring samples to test the statistical hypothesis that the observed patterns were generated by a random process. Rejecting the null hypothesis can therefore be used as a threshold to identify sites with significantly high or low values (Anselin, 1995; Nelson & Boots,

2008). Local Moran's I has been shown previously as an effective measure for identifying both pollution hotspots (e.g., Li et al., 2014; Liu et al., 2013; Zhang et al., 2008) and detecting significant spatial patterns of macrofaunal biodiversity (van der Wal et al., 2017).

Local Moran's I (I_i) is calculated according to the formula (Anselin, 1995):

$$I_i = \frac{Z_i - \bar{Z}}{\sigma^2} \sum_{j=1, j \neq i}^n [W_{ij}(Z_j - \bar{Z})]$$

Where Z_i is the attribute value at sampling station i , Z_j is the value at all other stations (where $j \neq i$), \bar{Z} is the mean value of Z within the sample number of n , σ^2 is the variance of Z , and W_{ij} is a distance weighting between Z_i and Z_j . Spatial relationships (W_{ij}) were defined using the inverse seaway distance between two stations, with no maximum distance threshold set. Seaway distance was used instead of Euclidean distance as a more accurate reflection of proximity between stations due to the presence of islands. Despite the small gap (~15 m) in the high-water line between the two main islands comprising Spectacle Island (Fig. 2.1), depths are shallow (<1 m) and assumed to have only minor water exchange across the boundary between the islands. This was therefore treated as one connected boundary for the seaway distance calculations.

A high positive local Moran's I value refers to a location which has a similar value as its surrounding neighbors, termed a spatial cluster. This includes high-high clusters (high values surrounded by high values, also known as hotspots) and low-low clusters (low values surrounded by low values, also known as coldspots). In contrast, spatial outliers refer to a high negative local Moran's I, including high-low (high value surrounded by low value) and low-high (low value surrounded by high value) outliers. Tests for Local Moran's I were performed in ArcMap v. 10.5 (ESRI, 2018). Significance was determined using 9,999 randomizations of the input data and a critical value of 0.05 (Anselin, 1995; Boots & Tiefelsdorf, 2000; Sokal et al., 1998).

2.3.5.2 Modeling biodiversity indices

Multiple environmental variables are known to affect macrofaunal distributions, and regression-based approaches are often used to model these relationships (Borja et al., 2011). In particular, Generalized Additive Models (GAMs; Austin, 2007; Yuan &

Norton, 2003) have been used in numerous studies to relate indices of macrofaunal composition to environmental variables (e.g., Castella et al., 2001; Maloney et al., 2011; Milner et al., 2001; Yuan & Norton, 2003). In this study, GAMs were used to account for potential non-monotonic and non-linear relationships between the environmental variables and biological indices (Guisan et al., 2002; Hastie & Tibshirani, 1990; Wood, 2006). Thin plate regression splines were used as the smoothing function and the degree of smoothing was estimated using generalized cross-validation (GCV). To control for overfitting, the effective degrees of freedom for each model were adjusted from the default gamma value of 1 to 1.4 (Kim & Gu, 2004). In addition, the model was constrained to be at maximum a quartic relationship by reducing the basis dimension to 4 (i.e., $k = 5$ in the GAM notation) from the default of 10. Models were created using the *mgcv* package in R v. 4.0.3 (R Core Team, 2020; Wood, 2003).

Continuous variables (AMBI, M-AMBI, Pielou's evenness and Shannon diversity) were modelled using GAMs with a Gaussian error distribution and the identity-link function. Indices expressed as integer values (abundance and species richness) were modelled using a quasi-Poisson error distribution and a log-link identity function. The quasi-Poisson family was selected over the Poisson model due to overdispersion, which was detected when the ratio of the residual deviance to the residual degrees of freedom was higher than 1 (Zuur et al., 2007).

GAMs with highly correlated variables can lead to multicollinearity issues such as large variances of estimates and overall reductions in model performance (Kuhn & Johnson, 2013). Prior to running GAMs, environmental predictors were assessed for multicollinearity using a Spearman correlation coefficient threshold of 0.70 ($P < 0.05$), keeping the most ecologically relevant variable for the final model (Dormann et al., 2013). Due to the well-documented relationship between organic content and macrofaunal communities (e.g., Pearson & Rosenberg, 1978; Snelgrove & Butman, 1994), organic content was selected as an explanatory variable for model fitting. Grain size sorting was also included as it was the only variable not correlated with organic content above the selected threshold.

The most parsimonious model was selected as the model with the lowest GCV score (Wood & Augustin, 2002; Wood et al., 2008). This was chosen over other model-

selection criteria (e.g., Akaike Information Criterion) as the preferred estimator for GAMs when an overdispersion term is used (Wood et al., 2008). Following this approach, the initial model was fit using all explanatory variables and the predictor with the highest P value was then removed. If the GCV score decreased, the reduced model became the candidate model. Overall goodness of fit of the models was assessed based on the percentage of deviance explained. The presence of significant residual spatial autocorrelation for each GAM model was tested using Local Moran's I (Elith & Leathwick, 2009).

2.3.5.6 Multivariate data analysis

Hierarchical cluster analysis (HCA) was used to characterize stations based on their macroinfaunal composition. The HCA was based on the fourth root transformed Bray-Curtis dissimilarity matrix using Ward's minimum variance algorithm (Ward, 1963) to group stations into clusters. Macroinfaunal data were fourth root transformed in order to emphasize the importance of rare species (Thorne et al., 1999). The HCA results are displayed as a dendrogram, with the length of the branches representing the dissimilarity between adjacent clusters (Jain et al., 1999). Dendrograms provide a visual basis for selecting the number of clusters in which to categorize the data (Jain et al., 1999; Langfelder et al., 2008). Since the choice of the number of clusters to include is a somewhat subjective decision (Hardy, 1996), the linkage height at which to cut the dendrogram was visually selected to minimize inter-group differences relative to intra-group differences. The final groupings were checked for geographic consistency by mapping the clusters and Permutational Multivariate Analysis of Variance (PERMANOVA) was used to assess the significance of the final selected groupings (i.e., H_0 : the centroids of all clusters are equivalent) (Anderson & Walsh, 2013). The similarity percentage (SIMPER) analysis was then applied to identify the macroinfaunal species which contributed most to the dissimilarities between the clusters (Clarke & Warwick, 1994). Dendrograms were produced using the *dendextend* package in R (Galili, 2015), and the SIMPER analysis was conducted using the *simper* function within the R *vegan* package (Oksanen et al., 2020).

As an additional method to visually assess station similarity, ordinations of the fourth root transformed macroinfaunal data were created using two-dimensional non-metric multidimensional scaling (NMDS) based on a Bray-Curtis dissimilarity matrix. The overall goodness of fit of the ordination is given by Kruskal's stress function (1964), which is a measure of how well the dissimilarities can be graphically displayed in 2-dimensional space. Stress values range from 0 to 1, with lower values indicating a better fit (Clarke, 1993). Ordinations were created using the *metaMDS* function in R within the *vegan* package (Oksanen et al., 2020).

The relationship between the environmental variables and multivariate assemblage structure was assessed using BIO-ENV (Clarke & Ainsworth, 1993). This method seeks to identify the proportion of variation in the faunal dataset explained by the environmental variables by selecting the subset of environmental variables that maximize the Spearman rank correlations between the environmental (Euclidean distance matrix) and fourth root transformed macroinfaunal (Bray-Curtis dissimilarity matrix) data (Clarke & Ainsworth, 1993). To overcome issues of multicollinearity (Clarke & Ainsworth, 1993), the same variables from the GAM analyses were used. Bubble plots of the NMDS ordination were then used to visualize the effect of the variables from the best model identified in BIO-ENV on the macroinfaunal structure. BIO-ENV was run using the *bioenv* function in the *vegan* package (Oksanen et al., 2020) in R.

2.4. Results

2.4.1 Environmental variables

The environmental variables at the sampling stations exhibited spatial variability throughout the bay (Fig. 2.2). Sediment type consisted of sandy mud at sampling stations on the farm lease, and to the east and west of Spectacle Island (Fig. 2.2A). Muddy sand sediments were observed at distances greater than 1 km from the farm lease, and sediments were sand at four of the northernmost sampling stations and at one station in the southeastern region of the study area (Fig. 2.2A-C). Median grain size in the bay ranged from 46.56 μm to 143.00 μm , and grain size sorting varied between 1.33 μm to 2.30 μm (Table 2.2). The sediments at two stations within the farm lease were classified

as poorly sorted, while the remainder of the sediments in the bay were classified as moderately sorted, moderately well sorted, or well sorted (Fig. 2.2D). Organic content in the sediment ranged from 0.73% to 11.85% (Table 2.2), with the highest values at stations within the farm lease boundary, and high values also extending outwards by several kilometers to the north and south of the farm (Fig. 2.2E). The lowest organic content was sampled in the northern and southeastern regions of the bay where the sediments were sand (Fig. 2.2E). Sediment porosity ranged from 22.75% to 69.82% (Table 2.2) with lowest values in sandy sediments and following similar spatial patterns to organic content (Fig. 2.2F). All environmental variables were significantly correlated with each other at a Spearman rho correlation threshold of 0.70 ($P < 0.05$) except for grain size sorting, which was not significantly correlated with any environmental variables above this value (Table 2.3).

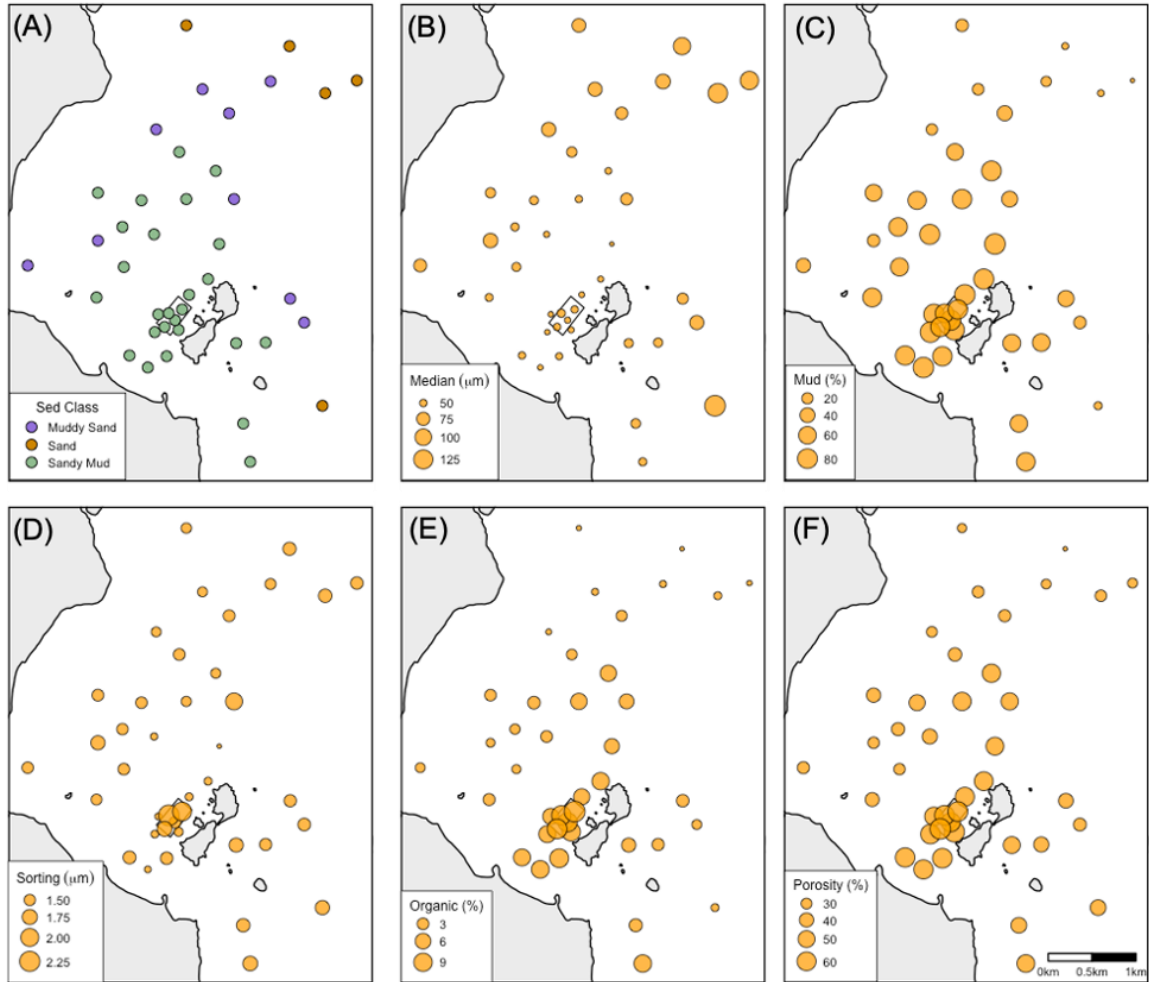


Figure 2.2 Proportional symbol maps of (A) sediment classification (Sed Class), (B) median grain size (Median), (C) percent mud (Mud), (D) organic content (Organic), (E) sediment porosity (Porosity), and (F) grain size sorting (Sorting). The location of the farm lease boundary is outlined as a black rectangle.

Table 2.2 Statistical parameters of environmental data and macroinfaunal diversity indices ($n = 40$).

Variable	Units	Mean	SD	Min	Max
Environment					
Distance	m	1207	937	0	3194
Median	μm	64.58	23.49	46.56	143.00
Mud	%	56.79	26.61	5.30	89.30
Organic	%	4.69	3.27	0.73	11.85
Porosity	%	46.77	13.69	22.75	69.82
Sorting	μm	1.56	0.19	1.33	2.30
Macrofauna					
AMBI	grab^{-1}	1.15	0.80	0.17	3.27
H'	grab^{-1}	1.91	0.44	0.83	2.58
J'	grab^{-1}	0.62	0.13	0.37	0.95
M-AMBI	grab^{-1}	0.71	0.10	0.48	0.89
N	ind grab^{-1}	375	244	21	1125
S	species grab^{-1}	23	7	8	37

Environmental variables represent seaway distance from the farm lease (Distance), median grain size (Median), percent mud (Mud), organic content (Organic), sediment porosity (Porosity), and grain size sorting (Sorting). Macrofaunal abbreviations are the AZTI Marine Biotic Index (AMBI), Shannon diversity index (H'), Pielou's evenness (J'), multivariate-AMBI (M-AMBI), abundance (N), and species richness (S).

Table 2.3 Spearman rank correlation coefficient matrix for environmental data ($n = 40$).

	Distance	Median	Mud	Organic	Porosity	Sorting
Distance	-					
Median	0.81***	-				
Mud	-0.82***	-1.00***	-			
Organic	-0.83***	-0.80***	0.80***	-		
Porosity	-0.83***	-0.78***	0.79***	0.96***	-	
Sorting	0.12	0.50***	-0.48**	0.03	0.00	-

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Variable names represent seaway distance from the farm lease (Distance), median grain size (Median), percent mud (Mud), organic content (Organic), sediment porosity (Porosity), and grain size sorting (Sorting).

2.4.2 Spatial variations in biodiversity indices

A total of 15,016 individual organisms were identified from the 40 benthic macroinfaunal samples. Total macroinfauna abundance ranged from 21 ind grab^{-1} to 1125 ind grab^{-1} and species richness ranged 8 species grab^{-1} to 37 species grab^{-1} (Table 2.2). Evenness ranged from 0.37 grab^{-1} to 0.95 grab^{-1} and Shannon diversity varied from 0.83 grab^{-1} to 2.58 grab^{-1} (Table 2.2). All AMBI values were classified as having good or high

ecological quality status (EQS), with values ranging from 0.17 to 3.27 (Table 2.2). M-AMBI values ranged from 0.48 to 0.89, with three of the sampling stations within the farm lease boundaries having M-AMBI classifications of moderate EQS, while the remainder of the sites in the bay were classified as good or high EQS (Table 2.2). Overall, 88 taxa were recorded, and the three most abundant taxa were *Aricidea catherinae*, *Diastylis sculpta* and *Ninoe nigripes* (Appendix 1).

Significant correlations existed between multiple biodiversity variables (Table 2.4). AMBI was significantly positively correlated with Shannon diversity ($\rho = 0.75$, $P < 0.001$) and Pielou’s evenness ($\rho = 0.84$, $P < 0.001$), and Shannon index and Pielou’s evenness were also significantly positively correlated ($\rho = 0.90$, $P < 0.001$). M-AMBI was significantly positively correlated with abundance ($\rho = 0.66$, $P < 0.001$) and richness ($\rho = 0.95$, $P < 0.001$), while abundance and richness were also significantly positively correlated ($\rho = 0.79$, $P < 0.001$). Other significant correlations were observed between Shannon diversity and M-AMBI ($\rho = 0.38$, $P < 0.05$), and Pielou’s evenness and abundance ($\rho = -0.33$, $P < 0.05$).

Table 2.4 Spearman rank correlation coefficient matrix for macroinfaunal diversity indices ($n = 40$).

	AMBI	H'	J'	M-AMBI	N	S
AMBI	-					
H'	0.75***	-				
J'	0.84***	0.90***	-			
M-AMBI	-0.08	0.38*	0.06	-		
N	-0.19	-0.07	-0.33*	0.66***	-	
S	-0.16	0.23	-0.11	0.95***	0.79***	-

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Abbreviations are the AZTI Marine Biotic Index (AMBI), Shannon diversity index (H'), Pielou’s evenness (J'), multivariate-AMBI (M-AMBI), abundance (N), and species richness (S).

Visualization of the biodiversity indices showed spatially variable trends throughout the bay (Fig. 2.3). Generally speaking, AMBI, Pielou’s evenness and Shannon diversity had low values at stations within the farm lease and in the northernmost sampling stations (Fig. 2.3A-C). M-AMBI, species richness and abundance all showed similar spatial trends, with predominantly low values within the farm and at sampling

stations to the west of Spectacle Island (Fig. 2.3D-F). Although all indices exhibited low values within the farm lease boundaries, trends were variable and stations with the lowest and highest values were not consistent between indices within the lease. Furthermore, in the immediate vicinity of the farm, all indices exhibited variable spatial patterns, with both high and low values surrounding the farm.

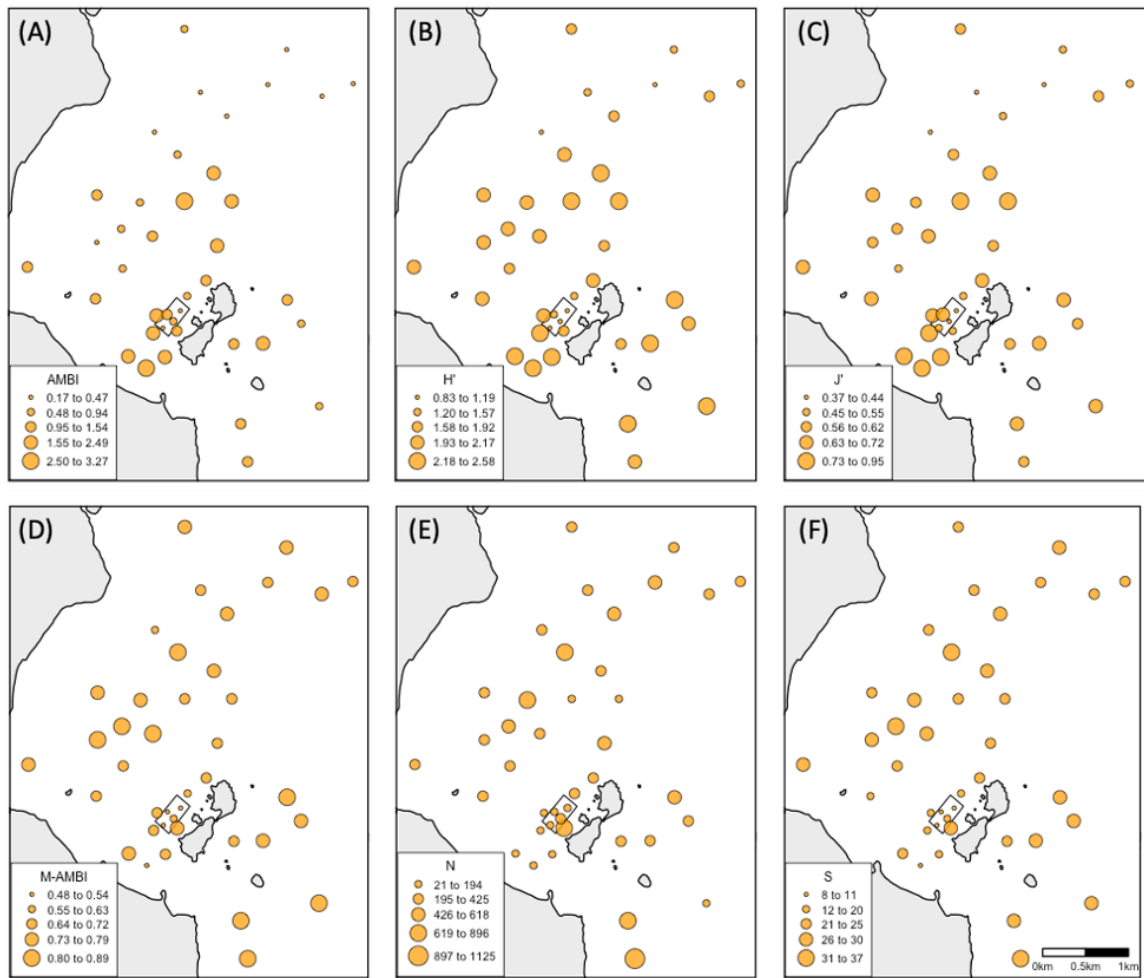


Figure 2.3 Maps of (A) AZTI Marine Biotic Index (AMBI), (B) Shannon diversity index (H'), (C) Pielou's evenness (J'), (D) multivariate-AMBI (M-AMBI), (E) abundance (N), and (F) species richness (S) calculated from the macroinfaunal species data. Data for each index are grouped into five classes using Jenks Natural Breaks; a clustering method used to minimize the variance within classes (Jenks, 1967). The location of the farm lease boundary is outlined as a black rectangle.

Local Moran's I showed significant spatial trends for each biodiversity index, which were consistent with the areas of high and low values identified in the maps

described above. Coldspots (low-low spatial clusters) were observed in the northernmost region of the bay for AMBI, Shannon diversity and Pielou's evenness (Fig. 2.4A-C). Coldspots were also observed at stations within the farm lease and along the western side of Spectacle Island for Shannon diversity, M-AMBI, abundance and species richness (Fig. 2.4D-F). Hotspots (high-high clusters) were observed several kilometers to the north, south and east of the farm site for AMBI (Fig. 2.4A), in the southeastern most corner of the study area for Shannon Index (Fig. 2.4B) and south of the lease boundaries for Pielou's evenness (Fig. 2.4C). Few outliers were observed in the analysis including two high-low outliers within the regions of low-low clusters of Shannon diversity, M-AMBI, abundance and species richness, although the locations of these outliers varied between indices. A low-high outlier for species richness was present in northern region of the bay, and two low-high outliers for Pielou's evenness were identified at sampling stations to the east of Spectacle Island.

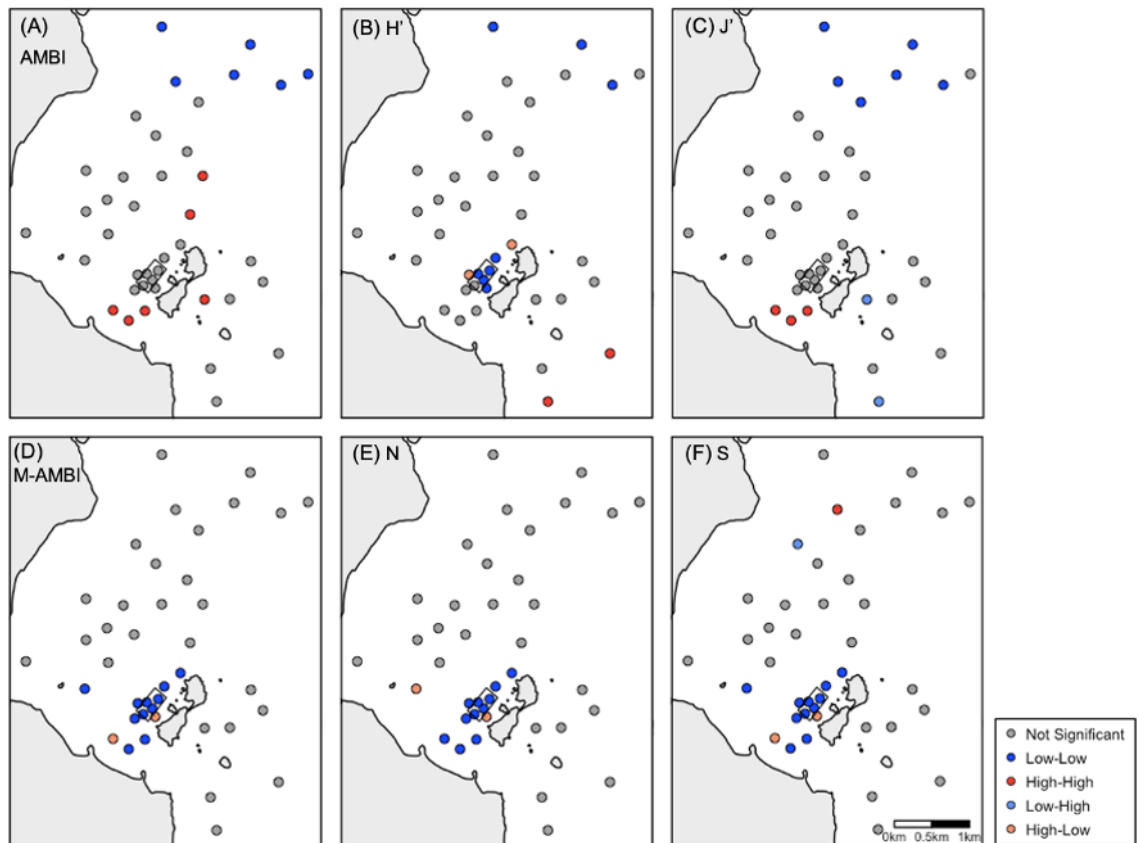


Figure 2.4 Maps depicting spatial clusters and outliers based on the Local Moran's I spatial autocorrelation statistic for (A) AZTI Marine Biotic Index (AMBI), (B) Shannon diversity index (H'), (C) Pielou's evenness (J'), (D) multivariate-AMBI (M-AMBI), (E) abundance (N), and (F) species richness (S) calculated from macroinfaunal abundance data. Statistically significant values are shown in color denoting low values surrounded by low values (dark blue circles), high values surrounded by high values (red), low values surrounded by high values (light blue), and high values surrounded by low values (pink). Non-significant sampling locations are shown in grey. Significance was evaluated using 9999 permutations of the original data values and a critical value of 0.05. The location of the farm lease boundary is outlined as a black rectangle.

2.4.3 Relationships between biodiversity indices and environmental variables

For all generalized additive models, variation in each index was best explained with organic content as the sole predictor. The explained deviance for each top model ranged from 13.44% to 64.97%, with AMBI having the highest explained deviance. Non-linear responses were observed for all biological indices in relation to organic content (Fig. 2.5). AMBI, Shannon diversity and Pielou's evenness showed similar unimodal responses, with peaks at an organic content of $\sim 6.5\%$ (Fig. 2.5A-C). Abundance also exhibited a unimodal response, with a maximum value at $\sim 4\%$ organic content (Fig.

2.5E). M-AMBI and species richness showed similar bimodal responses, with peaks in value at $\sim 2.5\%$ and $\sim 8\%$ and large declines in value at organic content exceeding $\sim 8.5\%$ (Fig. 2.5D, F).

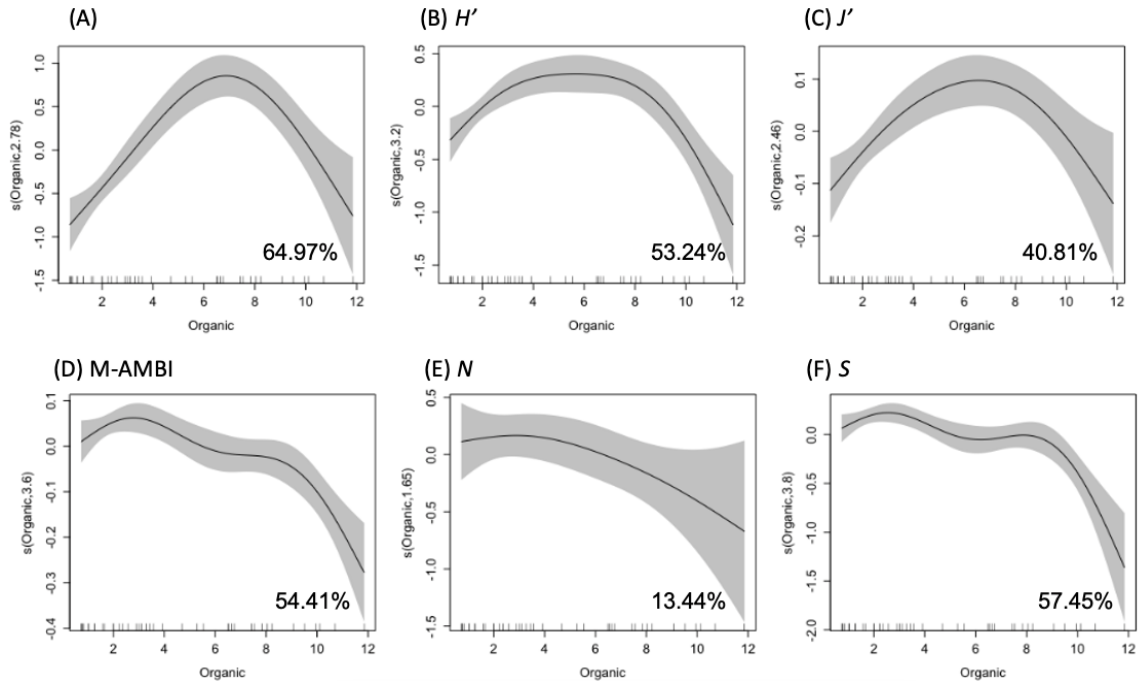


Figure 2.5 Smoother estimates (solid line) for the generalized additive models showing the effect of organic content (%) on (A) AZTI Marine Biotic Index (AMBI), (B) Shannon diversity index (H'), (C) Pielou's evenness (J'), (D) multivariate-AMBI (M-AMBI), (E) abundance (N), and (F) species richness (S) calculated from the macroinfaunal abundance species data. Zero on the y-axis represents the mean model estimates and the degree of smoothing is indicated in the y-axis label. The approximate 95% confidence intervals are shown in grey shading. The tick marks along the top of the x-axis of each plot indicate the sampled data points. Percentages represent the deviance explained for each model.

2.4.4 Multivariate data analysis

The dendrogram obtained from the HCA suggested a partitioning into four classes (Fig. 2.6A) and PERMANOVA confirmed that all four selected clusters were significantly different in assemblage structure ($P < 0.001$; Table 2.5). When mapped, these final clusters showed distinct spatial patterns (Fig. 2.6B). Cluster A ($n = 4$) included the stations within the farm lease boundaries, and Cluster B ($n = 15$) included stations surrounding the farm lease and extending several kilometers north and south of Spectacle Island. Stations within Cluster C ($n = 12$) were located in the eastern and western margins of the study area, adjacent to those in Cluster B. Stations within Cluster D ($n = 9$)

included those in the northernmost region of the bay and one site in the southeast region of the study area where the sediments were sand.

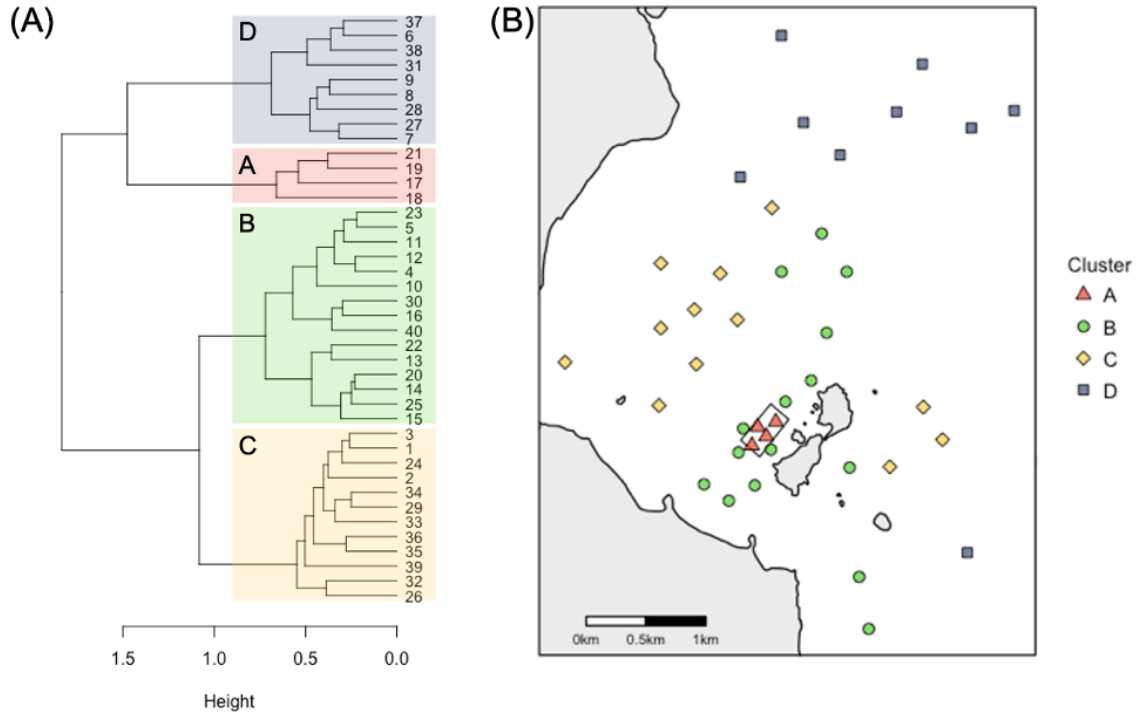


Figure 2.6 Sampling stations represented as (A) a dendrogram based on hierarchical cluster analysis of fourth root transformed benthic macroinfaunal abundance and (B) a map of the resulting clusters. The dendrogram was derived from a Bray-Curtis dissimilarity matrix using the Ward linkage algorithm. The length of the branches represents the dissimilarity between adjacent clusters, and numbers along the *x*-axis refer to station names. The clusters are shaded in colour and labelled A-D. The location of the farm lease boundary is outlined as a black rectangle on the map.

Table 2.5 Permutational multivariate analysis of variance (PERMANOVA) on fourth root transformed Bray-Curtis dissimilarities of macroinfaunal species data for clusters identified in the hierarchical cluster analysis shown in Fig. 2.6A. The PERMANOVA was followed by *a posteriori* tests among individual clusters. Significance was evaluated using 9999 permutations of the input data.

Source	df	SS	MS	F	P
Class	3	3.362	1.121	13.036	<0.001
Residual	36	3.095	0.086		
Total	39	6.457			
Comparison					
A vs. B				12.729	0.001
A vs. C				13.951	0.004
A vs. D				9.782	0.002
B vs. C				7.842	0.001
B vs. D				22.814	0.001
C vs. D				9.819	0.001

df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; F-value by permutation; P: significance by permutation

Superimposition of the clusters from the HCA onto the NMDS ordination showed similar groupings by both techniques (Fig. 2.7A). The NMDS ordination revealed a distinct separation of the four sampling stations within the farm lease along the *y*-axis (Cluster A; Fig 2.7A). A change in macroinfaunal composition was also observed along the *x*-axis (left to right), which corresponded to a transition from Cluster B to C to D (Fig. 2.7A). The BIO-ENV analysis showed that organic content alone had the highest correlation with the macroinfaunal data ($\rho = 0.662$, $P < 0.001$). For the non-farm stations, this influence of organic content on community structure was observed along NMDS axis 1 (Fig 2.7B). In the ordination, a gradual increase along the *x*-axis (left to right) corresponded to a decrease in organic content. As previously mentioned, the highest organic content was found in stations within the farm lease boundaries (Fig. 2.7B).

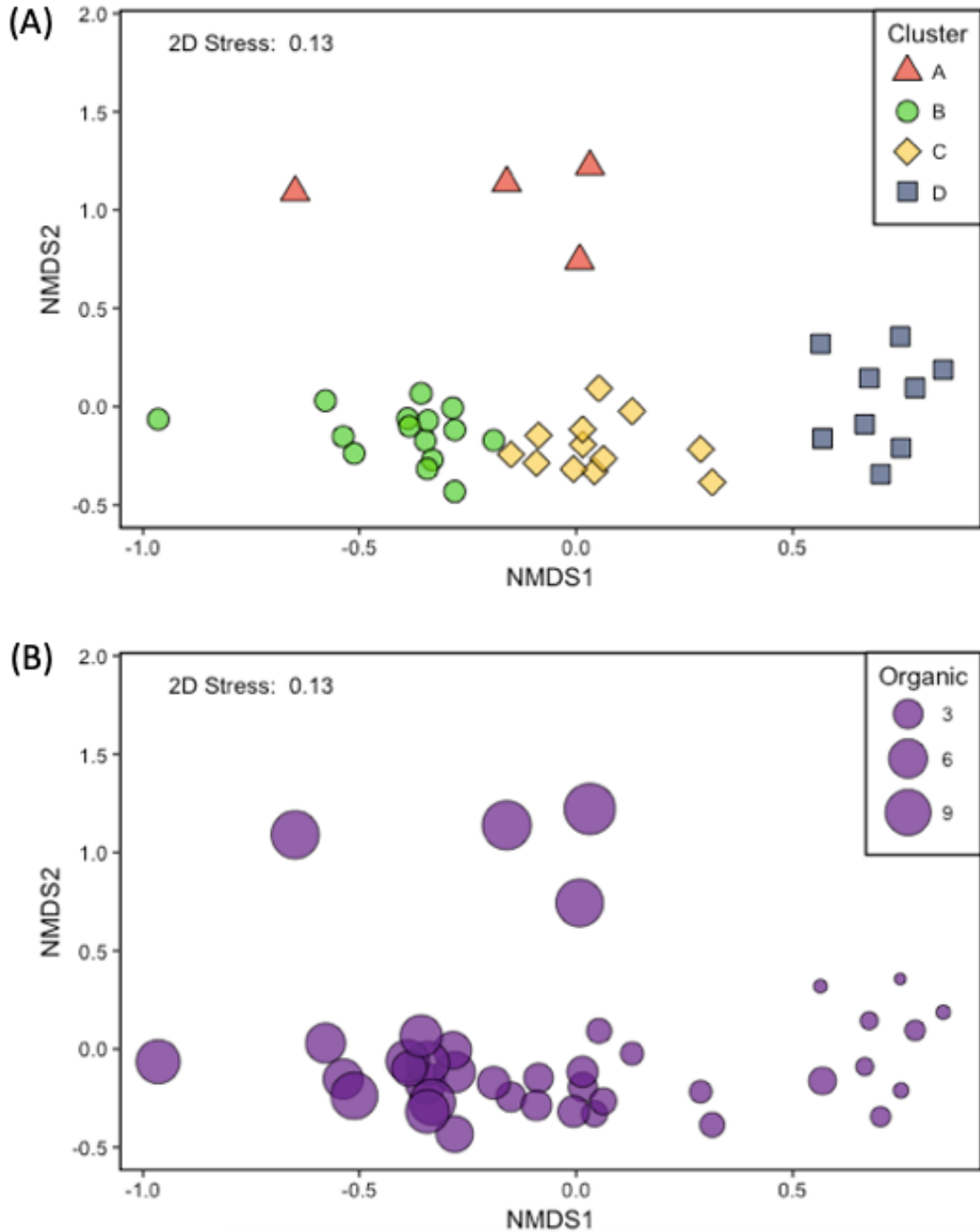


Figure 2.7 Two-dimensional non-metric multidimensional scaling ordinations visualizing station similarity based on a Bray-Curtis dissimilarity matrix of fourth root transformed benthic macroinfaunal abundance ($n = 40$). The ordinations show (A) clusters from the hierarchical cluster analysis in Fig. 2.6A and (B) the organic matter content (%) of each sampling location, with the size of the circles are proportional to the variable magnitude. Stress values between 0.1 and 0.2 correspond to acceptable representations of the data in two dimensions (Clarke, 1993).

The SIMPER analysis identified the species contributing most to the differences in composition between clusters identified in the HCA (Table 2.6). For brevity, results are reported for clusters that are geographically adjacent (Clusters A and B; Fig. 2.6B), or contiguous in the NMDS ordination (Clusters B and C, Clusters C and D; Fig. 2.7A). The macroinfauna species responsible for the divisions between Clusters A and B were primarily polychaetes, which all had higher abundances in Cluster B. These included species from EG IV (*Leitoscoloplos robustus*, *Prionospio steenstrupi*, *Aphelochaeta marioni* and *Schisteromeringos rudolphi*) and two species from EG III (*Ninoe nigripes*, *Mediomastus californiensis*). Two non-polychaete species had higher abundances within Cluster A including the cumacean *D. sculpta* (EG II) and the isopod *Edotia montosa* (II). The key species responsible for differentiating between Clusters B and C were generally more abundant in Cluster C, including *D. sculpta* (EG II), *A. catherinae* (EG II) and *Cythere* sp. (ignored). However, species classified into higher EGs were more abundant in Cluster B such as the polychaetes *Aphelochaeta marioni* (EG IV) and *Eteone longa* (EG III). Species of polychaetes were primarily responsible for the differences in assemblage structure between Clusters C and D. This included *Nephyts incisa* (EG II), *Prionospio steenstrupi* (EG IV) and *Leitoscoloplos robustus* (EG IV). However, the bivalves *Trachardium* sp. (n.a.) and *Nucula proxima* (EG I), and the polychaete *Clymenella torquata* (EG I) were more abundant in Cluster D.

Table 2.6 Similarity percentage (SIMPER) analysis showing the ten macroinfaunal species contributing most (%) to the average Bray-Curtis dissimilarities between clusters identified in the hierarchical cluster analysis shown in Fig. 2.6A. The second and third columns correspond to the mean abundances (fourth root transformed) within each cluster in the order the clusters are mentioned in column one. Explanations of the AMBI Ecological Groups (EG) are included in the main text. Results are shown only for comparisons of clusters that are geographically adjacent (Clusters A and B; Fig. 2.6B), or contiguous in the NMDS ordination (Clusters B and C, Clusters C and D; Fig. 2.7A).

Species	Mean abundance (I)	Mean abundance (II)	Contribution (%)	AMBI EG
Cluster A vs B				
<i>Leitoscoloplos robustus</i>	0.00	2.27	6.55	IV
<i>Ninoe nigripes</i>	0.00	1.87	5.51	III
<i>Mediomastus californiensis</i>	0.00	1.88	5.51	III
<i>Diastylis sculpta</i>	2.83	1.80	4.77	II
<i>Aricidea catherinae</i>	1.04	2.64	4.74	II
<i>Nephtys incisa</i>	0.50	2.09	4.61	II
<i>Prionospio steenstrupi</i>	0.00	1.60	4.61	IV
<i>Aphelochaeta marioni</i>	0.00	1.59	4.60	IV
<i>Schistomeringos rudolphi</i>	0.00	1.32	3.92	IV
<i>Edotia montosa</i>	1.41	0.18	3.88	II
Cluster B vs C				
<i>Diastylis sculpta</i>	1.80	3.02	4.66	II
<i>Aphelochaeta marioni</i>	1.59	0.40	3.51	IV
<i>Aricidea catherinae</i>	2.64	3.33	3.50	II
<i>Cythere</i> sp.	0.15	1.34	3.44	ignored
<i>Harpinia propinqua</i>	0.31	1.36	3.28	I
<i>Eteone longa</i>	1.27	0.10	3.27	III
<i>Diplodonta</i> sp.	0.30	1.16	3.00	n.a.
<i>Cytherura</i> sp.	0.15	1.11	2.93	ignored
<i>Leucon americanus</i>	1.53	2.07	2.65	II
<i>Ampharete oculata</i>	0.76	1.51	2.63	II
Cluster C vs D				
<i>Nephtys incisa</i>	2.13	0.40	3.89	II
<i>Prionospio steenstrupi</i>	1.75	0.22	3.48	IV
<i>Trachycardium</i> sp.	0.33	1.63	3.17	n.a.
<i>Leitoscoloplos robustus</i>	1.58	0.28	3.09	IV
<i>Euchone</i> sp.	1.48	0.22	2.91	n.a.
<i>Ampharete oculata</i>	1.51	0.29	2.90	II
<i>Mediomastus californiensis</i>	1.36	0.11	2.82	III
<i>Nucula proxima</i>	0.29	1.46	2.81	I
<i>Clymenella torquata</i>	0.39	1.44	2.72	I
<i>Ninoe nigripes</i>	2.16	1.08	2.52	III

2.5. Discussion

This study aimed to identify far-field effects of a net pen finfish farm using macroinfauna as indicators of ecosystem quality. In an effort to implement EAA, a bay-scale sampling approach was employed from which the spatial trends of biodiversity indices and multivariate composition were analyzed. The biodiversity indices exhibited spatially variable trends, and all variables showed low values within the farm lease. The multivariate analyses showed spatial zonation in assemblage structure, with similar groupings to the biodiversity indices. However, the multivariate analyses showed a further distinction of the stations sampled within the lease boundaries, suggesting an effect of the farm could be distinguished from the naturally varying spatial patterns in the bay. Models of both the biodiversity indices and multivariate composition showed an association with sediment organic content, although other variables correlated with organic content may also be influential. These findings suggest that the effects of enrichment from the fish farm were limited to within the farm lease boundaries.

In this study, the spatial patterns for each biodiversity index were variable, yet general trends were identified. An area west of Spectacle Island exhibited coldspots of abundance, M-AMBI and species richness. This region coincided with sediments comprised of high organic content and percent mud, which is consistent with research suggesting reduced macrofaunal diversity in low-energy depositional environments (Borja et al., 2000; Pearson & Rosenberg, 1978; Robertson et al., 2015). A region of low AMBI, Pielou's evenness and Shannon diversity was observed in the northernmost portion of the study area which was comprised of sandy sediments. This indicates that relatively few species of low EG dominated the area and may be related to physiological and behavioral adaptations to higher-energy environments (Sanders, 1958; Sanders et al., 1962). The locations of outliers (high-low and low-high) for each index were sporadic and did not show consistent trends. This highlights the patchy nature of macroinfaunal distributions (David et al., 1997) and emphasizes that monitoring strategies with a low sample size, such as those along a single transect, may lead to inconclusive or misleading results (Fairweather, 1991; Grant, 2012).

The multivariate analyses served as additional data-driven approaches to visualize spatial trends by examining inherent similarity in the faunal dataset. When mapped, the

four clusters identified from the HCA exhibited spatial zonation throughout the bay, and this change in composition was further reflected in the NMDS and SIMPER results. Contrary to expectations, species typically associated with organic enrichment were absent from stations within the farm lease (Cluster A). For instance, *D. sculpta* (EG II; Borja et al., 2000) was the most abundant species within the lease boundaries, yet were generally lower in abundance than at stations outside of the farm lease. Similar patterns have also been observed at a fish waste disposal site in Newfoundland and Labrador (Tay et al., 2009). In addition, higher-EG organisms such as *Leitoscoloplos robustus* (EG IV; Borja et al., 2000) and *Aphelochaeta marioni* (EG IV; Borja et al., 2000) were responsible for differentiating Cluster B from Cluster A, yet were not present in any farm samples (Cluster A). The polychaete *Capitella capitata* (EG V; Borja et al., 2000; McCall, 1977), a well-known pollution indicator had only one individual counted within the lease boundaries and instead had highest abundances at stations to the east of Spectacle Island. Contradictions in species presence are not unusual in environmental monitoring (Dean, 2008; Martinez-Garcia et al., 2019; Quintino et al., 2012) and may be partially responsible for the unexpected low AMBI values (i.e., less affected than expected) at stations within the farm lease. Dean (2008) noted there may be regional differences in how macrofaunal populations respond to pollutants, which can result in differences in the EG classifications among regions (Gillett et al., 2015; Robertson, et al., 2016; Rodil et al., 2013). As well, the co-occurrence of multiple types of pollutants (e.g., organic enrichment, heavy metal contamination) may cause unpredictable or confounding responses (Dean, 2008). However, these high-EG organisms had relatively low overall abundances and therefore had little effect on the ecological quality classification of each station.

The SIMPER results emphasized that bivalve suspension feeders were greater in number in Cluster D and were responsible for its differentiation from Cluster C. For instance, the bivalves *Diplodonta* sp. (n.a.) and *Trachycardium* sp. (n.a.), were observed in greater abundance within the sandy, low organic content sediments in the northernmost region of the study area (Cluster D). This is consistent with research suggesting that filter-feeders dominate sandy sediment environments since stronger currents may aid the ability of these organisms to capture food (e.g., Arrighetti & Penchaszadeh, 2010;

Sanders et al., 1962; Shimeta & Jumars, 1991). This study observed the co-existence of deposit and suspension feeders which can occur due to species switching between feeding mode in response to hydrodynamic flow conditions and organic flux (Snelgrove & Butman, 1994).

Overall, similar spatial patterns between the biodiversity indices and multivariate analyses were observed. The region of significantly low abundance, M-AMBI and species richness partially aligned with the stations belonging to Clusters A and B. As well, the northernmost stations exhibiting significantly low AMBI, Pielou's evenness and Shannon diversity overlapped with sampling locations belonging to Cluster D. In general, stations which were not significant in the spatial autocorrelation analysis of any index overlapped with those of Cluster C. Discrepancies between the analysis types occur because biodiversity indices represent summarized components of the multivariate data, which results in a loss of information. As well, different combinations of species and their abundances can often yield same the biodiversity index despite having distinct composition. For example, stations below the farm and those in the northernmost region of the study area both exhibited low values of AMBI, Pielou's evenness and Shannon diversity, yet these were differentiated in the multivariate analyses. Increasingly, due to these issues, the use of indices to inform management decisions has been criticized (e.g., Green & Chapman, 2011). However, in many cases indices are required by regulatory initiatives (Pinto et al., 2009; Salas et al., 2006); therefore, in these instances, pairing them with multivariate data is often recommended for a more informative analysis (Fonseca & Gallucci, 2016).

Organic content was considered the primary determinant of macroinfaunal distributions for all biodiversity indices and the multivariate structure. The GAM analyses showed non-linear associations with organic content; however, these relationships did not fully coincide with the Pearson & Rosenberg (1978) model (PRM). This study found no peak of opportunistic species and no corresponding increase in abundance or Shannon diversity at moderate to high levels of organic content (Sanz-Lázaro & Marín, 2011). Numerous studies on the benthic effects of aquaculture have also found a lack of agreement with the PRM (Aguado-Giménez et al., 2007; Carroll et al., 2003; Grant et al., 1995; Mayor et al., 2010; Sanz-Lázaro & Marín, 2011; Vita & Marin,

2007). These deviations from the PRM generally occur when there are too few sampling points to characterize the gradient, or enrichment is too low to show the full range of macrofaunal succession (Sanz-Lázaro & Marín, 2011). Due to the greater number of samples and high sampling resolution in this study, it is likely that organic enrichment was not extreme nor widespread enough for this increase in opportunistic species to be observed.

Despite the discrepancies with the PRM, an effect of organic content on the biodiversity indices and multivariate composition was observed. In the northernmost region, low organic content was associated with low values of AMBI, Pielou's evenness, and Shannon diversity, which is potentially related to adaptations to food availability and higher-energy environments (Gray, 1974; Lopez & Levinton, 1987; Pearson & Rosenberg, 1978; Sanders, 1958; Snelgrove & Butman, 1994). Within the farm lease, where the highest organic content values were observed, all indices exhibited low values which is likely a response to organic enrichment (Diaz & Rosenberg, 1995; Gray et al., 2002; Pearson & Rosenberg, 1978). A distinct separation of multivariate composition of the farm sites was also observed on the y -axis of the NMDS ordination, suggesting enrichment from the farm (Cluster A). The high multivariate dispersion of stations within Cluster A are likely indicative of patchy disturbances and different recovery trajectories as variability in sites below cages are generally attributed to the heterogeneous deposition of wastes from currents and cage rotation (Brooks et al., 2003; Fonseca & Gallucci, 2016; Keeley et al., 2014; Warwick & Clarke, 1993).

Potentially, other environmental variables correlated with organic content may be responsible for the observed macroinfaunal distributions. For example, organic content was highly correlated with percent mud, which has been identified as a top predictor of macroinfaunal distributions (Ellingsen, 2002; Lu, 2005; Sousa et al., 2006). High organic content can also be associated with increased adsorption of chemical contaminants which may produce changes in the structure of benthic communities (Lamberson et al., 1992; Landrum & Robbins, 1990; Thompson & Lowe, 2004; Sanz-Lázaro & Marín, 2011). In October and November 2009, Loucks et al. (2012) found elevated copper concentrations within sediments below the Port Mouton Bay farm site, which may be an additional mechanism to explain the altered species composition within the farm lease. Further,

since the source of the organic matter was not identified, delineating the extent of enrichment caused by the finfish farm is complicated by the naturally varying spatial patterns of the environmental variables. Sediment grain size is considered the primary determinant of organic content in marine ecosystems (Hedges & Keil, 1995; Mayer, 1994); therefore, the high organic content to the west of Spectacle Island is likely largely influenced by grain size characteristics. The smaller grains in this region are expected to be the result of reduced current speeds caused by the obstruction of Port Mouton Island and Spectacle Island, while the more exposed region in the north likely has larger grains due to reduced deposition of fine-grained sediments. This had been observed before the establishment of the farm by Piper (1986).

Deciphering the zone or area influenced by the farm activities is further complicated by the varying responses from the multiple indices and analysis methods. For instance, coldspots of abundance, species richness and M-AMBI were observed to the west of Spectacle Island, including the stations within the farm lease boundaries. However, the multivariate analyses showed a further distinction of the farm stations. These latter stations had the highest organic content, and the separation in assemblage structure on the *y*-axis of the NMDS ordination likely shows a response to farm-related enrichment. Although the non-farm sites show a gradual change in composition with varying levels of organic content (*x*-axis of the NMDS ordination), for the reasons stated above, this is not expected to be driven by aquaculture-derived organic content. Cluster B remained distinct from farm samples (Cluster A), despite their separation of only 38 meters, suggesting the effect of is restricted to within the lease boundaries. These results are in agreement with the majority of salmon aquaculture studies which have found the effects of organic enrichment to be constrained to within approximately 150 m or less from the cage boundaries (Brooks et al., 2002; Carroll et al., 2003; Hargrave, 2005; Pitta et al., 2006; Vezzulli et al., 2008; Welch et al., 2019).

Despite the advantages of multivariate analyses, they do not explicitly specify the level of response or provide a disturbance classification (Warwick & Clarke, 1991). The sediments below the farm site did not reach azoic conditions, and this was reflected in the biotic indices. Both AMBI and M-AMBI values were generally higher in ecological status (i.e., less affected) in comparison to other salmon aquaculture studies (e.g., Callier

et al., 2013; Keeley et al., 2014). In this study, high agreement was observed between the NMDS ordination and M-AMBI values, since three of the farm stations were classified as “moderate” EQS. The fourth farm station was classified as “good” M-AMBI EQS and was closer to the non-farm stations on the NMDS ordination which likely suggests reduced impact during farming or that some recovery had occurred. These indices serve as a qualitative means to assess the magnitude of impact, and in this study, they show that the benthos within Port Mouton was not severely degraded.

While the data suggest that farm-related organic enrichment was restricted to the lease boundaries, samples were collected after the farm had been fallow for approximately three months. Documented rates of benthic recovery from fish farming operations vary widely, ranging from several months (Brooks et al., 2003; Ritz et al., 1989) to years (Brooks et al., 2004; Hussin et al., 2012; Keeley et al., 2014). In most medium-length studies (<3 years), rapid improvement in conditions are seen in the first 6-12 months, yet complete recovery is not observed in this time frame (Karakassis et al., 1999; Lin & Bailey-Brock, 2008; Villnäs et al., 2011). Benthic conditions below the farm may therefore have improved since farming operations ceased. However, biological remediation is generally slower than geochemical remediation (Brooks et al., 2004) and low-energy sites with muddy sediments such as Port Mouton Bay typically recover slower than high-energy sites (Borja et al., 2000). Future work should incorporate monitoring across larger spatial scales during production periods to delineate potential bay-scale effects of organic enrichment.

In contrast to this study, several instances of benthic far-field effects have been reported. For example, organic enrichment has been documented at distances of 200-500 m from fish farming activities (Holmer et al., 2008; Keeley et al., 2013a, b; Pohle et al., 2001). At a shallow (31-40 m depth) site in Norway, Keeley et al. (2019) found biological effects up to 600-1000 m away from the farm and attributed the large footprint to the physically dynamic and dispersive hydrodynamic conditions. As well, studies using fatty acids and stable isotopes as tracers have recorded farm wastes up to 500 m (White et al., 2017) and 1000 m (Sarà, et al., 2004; Woodcock et al., 2018) from the farm boundaries, respectively. However, tracers themselves are not necessarily indicative of negative effects, as the presence of organic matter from the farm does not necessarily

imply a negative impact on the ecosystem. In Port Mouton Bay, Loucks et al. (2014) suggested potential bay-wide effects after observing reductions in catch per unit effort of lobster between feed and fallow years. However, the methods and results have been questioned due the spatial design of the study, the form of regression analysis used, and lack of clear mechanism attributable to the farm (Grant et al., 2016). Further, Cullain et al. (2018) evaluated the effects of the fish farm in Port Mouton Bay and noted some nutrient and organic enrichment, higher epiphyte loads, lower eelgrass cover and biomass, and lower macroinfauna biomass were observed at sites nearest to the farm. Contrary to these findings, the present study suggests enrichment from the farm was restricted to within the lease.

Investigating far-field effects from aquaculture wastes may require adjustments in monitoring approaches. Studies with few samples, particularly those along a single transect, may not effectively capture dispersal patterns, while increasing the number of sampling locations, particularly across different sediment types, may become hindered by a lack of adequate reference stations. This study instead sought to infer waste dispersal processes from spatial patterns and these approaches are generally more informative with a greater sample size (McIntire & Fajardo, 2009; Webster & Oliver, 2007). Using detailed sampling regimes and geostatistics is common in pollution literature to analyze spatial patterns and delineate affected regions including interpolating surface grids of heavy metals in marine sediments (Gu & Gao, 2019), analyzing the spatial scales of variation in pollutants (Alary & Demougeot-Renard, 2010; Lin et al., 2002; Zhou et al., 2016) and detecting contaminant hotspots (Li et al., 2014; Liu et al., 2013; Zhang et al., 2008). Similar sampling approaches and spatial statistics are less often applied in aquaculture research, although examples do exist (Dowd et al., 2014; Howarth et al., 2019). Casual inference to directly attribute the patterns to aquaculture is typically not the goal, yet these methods instead offer a first glance at the potential spatial distribution of wastes (McIntire & Fajardo, 2009). In contrast to most benthic aquaculture studies, this study is unique, not only in the extent of spatial coverage, but in the number of samples close to the farm.

Although EAA is an aspirational goal in aquaculture management, successful implementation has remained challenging. This research provides approaches for

integrating EAA within benthic monitoring through detailed bay-scale spatial coverage of Port Mouton Bay. These results indicate that stations within the fish farm lease had unique macroinfaunal assemblages (Cluster A), while a separate distinct fauna (Cluster B) occurred as close as 38 m to the lease boundaries. Consequently, elevated organic content within the lease suggests that the fauna in Cluster A responded to those conditions. Despite the presence of a fish farm since 1995, neither AMBI nor M-AMBI suggested bay-scale degradation of the benthos. M-AMBI had high agreement with the multivariate analyses as three stations within the lease were classified as “moderate” EQS, which is likely attributed to localized enrichment from the farm. This work highlights the value in using multivariate techniques to delineate the area of enrichment with biotic indices to determine the magnitude of the effects. Following this approach would allow for more spatially comprehensive studies of aquaculture wastes to be analyzed across different hydrodynamic regimes. As aquaculture competes for space in marine environments, understanding the complex relationship between finfish wastes and the benthos is crucial to monitor and mitigate negative effects.

CHAPTER 3 SPATIAL AND TEMPORAL EFFECTS OF ORGANIC ENRICHMENT FROM FINFISH AQUACULTURE IN PORT MOUTON BAY, NOVA SCOTIA, CANADA

3.1 Abstract

For decades, researchers have studied the effects of benthic organic enrichment from finfish farming, yet little is known of the persistence and effects of these wastes far beyond the cage boundaries. The Ecosystem Approach to Aquaculture (EAA) was developed, in part, as a management approach to evaluate the sustainability of the industry and monitor these effects on the wider ecosystem. The goal of this study was to implement EAA to examine the spatial and temporal effects of enrichment from a net-pen finfish farm located in Port Mouton Bay, Nova Scotia, Canada. Macroinfauna were used as indicators of benthic quality and were sampled at 22 stations throughout the bay in 2009, 2010 and 2011, to delineate the effects of enrichment and analyze changes in benthic conditions over time. Results showed spatial zonation of macroinfaunal distributions, primarily driven by organic content and sediment grain size. Multivariate analyses and biotic indices suggest the effect of the farm was restricted to within the lease boundaries. Further, unique assemblage structure was observed in the four stations within the lease in 2009, three in 2010 and one in 2011, suggesting an improvement in benthic conditions, yet compositional differences to the surrounding stations remained at the end of the study period. This research identifies data-driven approaches to determine the extent and recovery dynamics of far-field benthic effects from net-pen aquaculture.

3.2 Introduction

The global expansion of the finfish aquaculture industry has led to competition for space in marine ecosystems, driving increased attention to the dispersal and remediation of waste products (Keeley et al., 2015; Weitzman et al., 2019). As part of these concerns, the ecosystem approach to aquaculture (EAA) was developed as a holistic management approach with an environmental component focused on monitoring ecosystem-level impacts (Soto et al., 2008). For net-pen finfish farming, a primary concern is the organic enrichment of the seafloor, whereby the decomposition of fish feces and uneaten feed results in potential hypoxia and harmful changes in sediment pore water chemistry

(Cranford et al., 2017; Kalantzi & Karakassis, 2006). Although this is well-documented in the immediate vicinity of the cages, less is known of the far-field effects, or those hundreds of meters beyond the cage boundaries (Keeley et al., 2019; Weitzman et al., 2019). Understanding how these effects persist over time is crucial for the sustainable development of the industry (Armstrong et al., 2020; Zhulay et al., 2015). A tangible implementation of EAA is therefore to monitor the effects of organic enrichment over time across large geographic areas.

Macroinfauna are frequently used to measure the spatial and temporal dynamics of benthic organic enrichment, since certain species have different tolerances to pollutants (Bilyard, 1987; Diaz et al., 2004; Pinna et al., 2013). These data are expressed in various forms, including univariate biodiversity indices where macroinfaunal communities are summarized to a single value for simpler statistical comparisons over space and time. Biotic indices such as the AZTI Marine Biotic Index (AMBI; Borja et al., 2000) and multivariate-AMBI (M-AMBI; Muxika et al., 2007), have also been developed to exploit the inherent species-specific responses to enrichment and assign a qualitative ecological classification for each sample. Lastly, multivariate methods are used to analyze the entire community structure and are regarded as the most sensitive means to assess subtle variations in benthic conditions (Austen & Warwick, 1989; Warwick & Clarke, 1991). By applying data-driven analytical methods to quantify similarity in these metrics, researchers can infer the spatial footprint of enrichment on the benthic ecosystem. Specifically, approaches with spatially explicit outputs allow the data to be explored in the context of multiple driving forces, establishing a visual association between patterns in macroinfaunal distributions and the underlying environmental processes. Using these methods to define macroinfaunal response to enrichment across broader geographic areas can therefore help operationalize aquaculture management at the ecosystem level.

Multiple factors other than aquaculture wastes affect macroinfaunal distributions and require consideration when conducting monitoring studies. For example, while high inputs of organic content from aquaculture effluents can trigger successional shifts in macroinfaunal communities (Pearson & Rosenberg, 1978), organic content also occurs naturally in sediments and acts as a food source for these organisms (Gray, 1974; Lopez

& Levinton, 1987). Macroinfaunal communities therefore often show a predictable response along a continuum of increasing organic content in marine ecosystems (Pearson & Rosenberg, 1978). Decades of research have also shown relationships with sediment grain size distributions, yet the strength of this relationship often varies, and further, the causative mechanisms are not considered to be directly related to the grain sizes (Snelgrove & Butman, 1994). For instance, silt-clay fraction and median grain size have been recognized as important drivers in numerous studies (Ellingsen, 2002; Lu, 2005), yet these are understood to instead act as proxies for the underlying mechanisms such as pore water chemistry, food levels, and microbial activity (Snelgrove & Butman, 1994). Establishing these relationships with environmental variables allows researchers to disentangle the effects of multiple forces with the goal of isolating the role of aquaculture wastes in structuring faunal communities.

Fallowing, or periods where no fish are stocked, is a management strategy largely used to reverse the effects of benthic organic enrichment (Keeley et al., 2015). To balance sustainability with an optimized harvest, decision-makers need to understand the length of time required for the benthos to recover (Brooks & Mahnken, 2003; Lin & Bailey-Brock, 2008). Documented rates of recovery from finfish enrichment vary widely, ranging from several months (Brooks et al., 2003; Ritz et al., 1989) to years (Brooks et al., 2004; Hussin et al., 2012; Keeley et al., 2014). These variations are primarily attributed to differences in environmental conditions such as faunal colonization and bioturbation, hydrodynamics, and farming intensity (Carroll et al., 2003; Keeley et al., 2015; Kutti et al., 2007). Recovery is often assessed by testing for statistical differences in benthic conditions between affected regions and reference stations; however, there is no universal definition of recovery which complicates analysis as different indicators may suggest recovery at different times (Keeley et al., 2014). Keeley et al. (2014) examined six different methods to assess benthic recovery at a salmon farm and noted that biotic indices which incorporated multiple variables (e.g., M-AMBI) were the most robust, and emphasized the use of multiple indices, and employing data-visualization methods in the analysis. For long-term viability of the industry, understanding these rates of recovery are essential for determining when to restock without the potential for

cumulative impacts (Keeley et al., 2015; Macleod et al., 2007) or compromising the resilience of the ecosystem (Borja et al., 2010).

As the focus of aquaculture monitoring broadens to incorporate far-field effects, defining the outer limits of enrichment and determining the lasting influence on the benthos require greater attention. To address these growing concerns, this study aims to implement EAA to examine the spatiotemporal effects of enrichment from a net-pen finfish farm located in Port Mouton Bay, Nova Scotia. Specifically, the objectives of this study are to (1) investigate if sampling stations within the farm site lease exhibited unique macroinfaunal composition in comparison to non-farm sites and if this changed over time, and (2) identify the environmental variables associated with patterns in macroinfaunal composition and if these varied among study years. By applying a spatially comprehensive sampling strategy throughout the bay, these results provide unique insights into potential far-field effects of aquaculture and patterns of benthic remediation.

3.3. Methods

3.3.1 Study site

Port Mouton Bay is located on the South Shore of Nova Scotia, Canada (Fig. 3.1). The bay consists of a heterogeneous coastline, comprises a surface area of approximately 56 km², and has a bathymetry of multiple shallow sills (Gregory et al., 1993). Water flow is affected by two main islands including Port Mouton Island (~3.95 km²) which separates the bay from open ocean, and Spectacle Island (~0.19 km²), which is located to the east of the fish farm (Fig. 3.1). Within the bay, tides are semi-diurnal with an average amplitude 1.5 m and maximum depth is 21.9 m (Gregory et al., 1993).

A fish farm operated intermittently in the bay since 1995. The farm was stocked with steelhead trout (*Oncorhynchus mykiss*) from 1995 until 2000, Atlantic salmon (*Salmo salar*) from 2000 until July 2009, and then fallowed from July 2009 to August 2012. Prior to the study (October 2009 to October 2011), cages were of a rectangular grid system, and were positioned at varying locations within the lease which had an area of 8.03 ha.

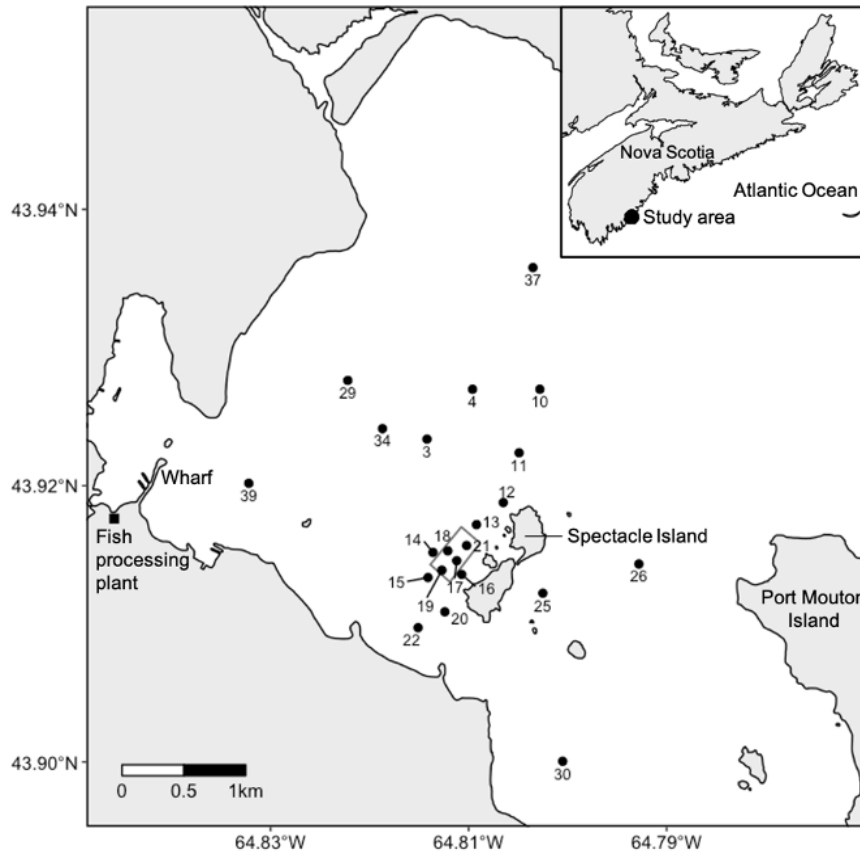


Figure 3.1 Location map of the study area. Numbered circles represent station names, and the rectangular outline represents the fish farm lease boundary.

3.3.2 Data types and field sampling

Samples were collected during three time periods: October and November 2009 (~3 months fallow), October 2010 (~15 months fallow), and October 2011 (~27 months fallow). Forty stations were sampled in 2009 for a comprehensive spatial analysis of near- and far-field effects (Finnis et al., in prep.), but in subsequent years this was reduced to a subset of 22 locations due to logistical and time constraints. For consistency in analysis, only the data from the 22 stations were included in this study. In all years, samples for analysis of organic content (%) and porosity (i.e., water content; %) were obtained. Samples for grain size analysis were obtained in 2010 and were treated as static variables and therefore representative of all years of study. Although wave transport and storms may affect sediment composition within the bay (Piper, 1986), river input is low (Gregory et al., 1993). Furthermore, the low range of grain sizes and a similar spatial pattern of sediment types reported by Piper (1986) suggests the patterns have remained

relatively consistent over several decades and likely would not change dramatically during the study period. Derived measurements from the grain size distributions included median grain size (μm), percent mud (%; i.e., silt-clay fraction or amount of the sediment $<63 \mu\text{m}$), and grain size sorting (μm). Seaway distance from the farm lease (m) was also calculated in R v. 4.0.3 (R Core Team, 2020) and used as an explanatory variable in all years to account for potentially unmeasured variables from the farm that could affect macroinfaunal composition.

Benthic samples were collected using a hand-operated Ekman grab sampler (15.4 cm width, 15.4 cm length, 23.0 cm depth). Upon retrieval, water was siphoned off from the surface of the grab. Two replicates were obtained for analysis of porosity and organic content, which are measured sequentially from the same sediment sample. For these analyses, 1-2 ml of sediment from 0-2 cm sediment depth were extracted using 5 ml truncated syringes at three different locations in the grab. The sediment was then emptied into pre-weighed 20 ml glass vials and capped. In 2010, a 200 g sediment sample for grain size analysis was obtained with a trowel and transferred into a plastic bag.

A single separate grab was retrieved at each station for macroinfaunal analyses. The contents were washed through a 0.5 mm sieve using seawater, and the remaining fauna were preserved in a 10% buffered formalin solution. In the laboratory, fauna were identified and enumerated under a dissecting microscope to the lowest possible taxonomic level (in most cases, to species).

3.3.3 Environmental variables

In the laboratory, porosity was evaluated as the percent change in mass after drying the sample in a gravity convection oven at 60 °C for 24 h. The sample was then transferred from the glass vials to pre-weighed aluminum trays and organic content was measured as the percent change in mass after burning the sample in a muffle furnace at 500 °C for 4 h (Buchanan, 1984). One organic content sample at one farm station in 2010 was lost and therefore removed from all further analyses.

Sediment grain size was determined by wet sieving the samples across a series of stainless-steel sieves (2000 μm , 1000 μm , 500 μm , 250 μm , 125 μm , and 63 μm) following Buchanan (1984). The remaining filtrate was vacuum filtered through a pre-

weighed 0.7 μm Whatman GF/F filter for the determination of the silt-clay fraction ($<63 \mu\text{m}$). The mass retained on each sieve was determined after drying the samples in a gravity convection oven $60 \text{ }^\circ\text{C}$ for 24 h. These masses were entered into the GRADISTAT Version 4.0 Excel package (Blott & Pye, 2001) in order to calculate grain size distributions including median grain size (μm), percent mud (%), and grain size sorting (i.e., geometric standard deviation of the grain sizes, μm ; Folk and Ward, 1957).

3.3.4 Biological variables

A variety of variables were calculated for each station from the faunal dataset including number of individuals (abundance), number of species (richness), Pielou's evenness and the Shannon diversity index (base e). In addition, the AZTI Marine Biotic Index (AMBI; Borja et al., 2000) and multivariate-AMBI (M-AMBI; Muxika et al., 2007) were derived using the AMBI software (<http://ambi.azti.es>). For AMBI, taxa were classified into Ecological Groups (EG) based on their response to disturbance including EG I: disturbance-sensitive species, EG II: disturbance-indifferent species, EG III disturbance-tolerant species, EG IV: second-order opportunistic species, EG V: first-order opportunistic species (Borja et al., 2000; Finnis et al., in prep.; <http://ambi.azti.es>). For the M-AMBI calculations, the minimum reference values were assigned AMBI = 6, Shannon diversity = 0, richness = 0. High reference conditions were designated as the highest values within each year of study following Muxika et al. (2007). As a descriptive means to evaluate the distribution of each biodiversity index and how they varied between farm- and non-farm stations, boxplots were constructed for each index in each year.

3.3.5 Statistical analysis

To detect groupings of stations with similar macroinfaunal composition, hierarchical cluster analysis (HCA) was used, based on a Bray-Curtis dissimilarity matrix of fourth root transformed abundance data with the average linkage clustering algorithm. Data for each year were clustered separately to reduce the influence of compositional variations between sampling years. Similarity profile analysis (SIMPROF; Clarke et al., 2008) was used to detect significant ($P < 10^{-7}$; Baldwin et al., 2018; Clarke et al., 2008)

groupings in assemblage structure using the *simprof* function in the *clustsig* package in R (Whitaker & Christman, 2014). The clusters identified from the SIMPROF analysis were then mapped to detect spatial trends in assemblage structure. Station similarity was also assessed using two-dimensional non-metric multidimensional scaling (NMDS) ordinations based on a fourth root transformed Bray-Curtis dissimilarity matrix. Stations in the ordinations were visualized with symbols to represent clusters identified from the SIMPROF analysis. Ordinations were constructed using the *metaMDS* function within the R *vegan* package (Oksanen et al., 2020). The similarity percentage (SIMPER) algorithm was then used to identify which macroinfaunal species contributed most to differences between farm- and non-farm stations (Clarke & Warwick, 1994). Specifically, SIMPER was run between the SIMPROF clusters composed entirely of farm sites and any geographically adjacent clusters. SIMPER was run separately for each year of study using the *simper* function within the *vegan* package (Oksanen et al., 2020).

Lastly, the *envfit* function was used to determine the correlation between the environmental variables and the ordination space in each year of study, and significant ($P < 0.05$) variables were fit to the NMDS ordinations. Goodness-of-fit was evaluated using squared correlation coefficients (r^2) and significance was tested using 999 permutations in the *vegan* package (Oksanen et al., 2020).

3.4 Results

3.4.1 Environmental variables

The sediments in most of the study area, including beneath the farm lease (Fig. 3.1), consisted of sandy mud, while the eastern, western, and northern margins of the study area sediments consisted of muddy sands. Within the farm, the median grain size ranged from 47.92 to 52.32 μm , the percentage of mud ranged from 68.10 to 82.90% and grain size sorting ranged from 1.53 to 2.30 μm (Table 3.1). Outside of the farm, the median grain size ranged from 46.56 to 81.10 μm , the percentage of mud ranged from 26.00 to 89.30% and grain size sorting ranged from 1.33 to 1.90 μm . Two of the stations within the lease were classified as poorly sorted, while all other stations within the study area were classified as moderately sorted, moderately well sorted, or well sorted.

Table 3.1 Descriptive statistics of environmental variables collected within ($n = 4$) and outside of the fish farm lease ($n = 18$) in 2009, 2010 and 2011.

Variable	Farm				Non-Farm			
	Med	SD	Min	Max	Med	SD	Min	Max
2009								
Distance	0	0	0	0	869	663	38	2164
Median	49.89	1.80	47.92	52.32	49.40	10.68	46.56	81.10
Mud	75.35	6.04	68.10	82.90	77.10	20.06	26.00	89.30
Organic	10.41	0.86	9.95	11.85	6.02	2.58	2.00	9.51
Porosity	64.65	3.55	61.22	69.82	54.94	11.58	33.43	67.87
Sorting	1.92	0.34	1.53	2.30	1.48	0.15	1.33	1.90
2010								
Distance	0	0	0	0	869	663	38	2164
Median	49.89	1.80	47.92	52.32	49.40	10.68	46.56	81.10
Mud	75.35	6.04	68.10	82.90	77.10	20.06	26.00	89.30
Organic	8.59	2.01	7.08	11.07	6.03	3.01	1.51	10.08
Porosity	63.40	3.16	58.10	65.40	56.80	13.18	28.60	64.30
Sorting	1.92	0.34	1.53	2.30	1.48	0.15	1.33	1.90
2011								
Distance	0	0	0	0	869	663	38	2164
Median	49.89	1.80	47.92	52.32	49.40	10.68	46.56	81.10
Mud	75.35	6.04	68.10	82.90	77.10	20.06	26.00	89.30
Organic	9.00	0.76	8.05	9.88	5.81	2.80	1.48	10.49
Porosity	63.05	1.01	61.44	63.79	52.89	11.84	31.77	66.84
Sorting	1.92	0.34	1.53	2.30	1.48	0.15	1.33	1.90

Environmental variables represent seaway distance from the farm lease (Distance, m), median grain size (Median, μm); percent mud (Mud, %), organic content (Organic, %), sediment porosity (Porosity, %), and grain size sorting (Sorting, μm).

Throughout the study period, organic content showed the highest values within the lease in 2009 (median of 10.41%, range of 9.95 to 11.85%), decreasing in 2010 (8.59%, 7.08 to 11.07%) and showing less change in 2011 (9.00%, 8.05 to 9.88%) (Fig. 3.2A; Table 3.1). Stations outside of the lease boundary exhibited a greater range in organic content than farm stations but showed less change in median value between years. In 2009, median organic content in non-farm stations was 6.02% (range of 2.00 to 9.51%), 6.03% in 2010 (1.51 to 10.08%) and 5.81% in 2011 (1.48 to 10.49%). Porosity showed similar patterns to organic content, with the highest values measured within the farm lease in 2009 (median: 64.65%, range: 61.22 to 69.82%) (Fig 3.2B). Similarly, this

decreased slightly in 2010 (63.40%, 58.1 to 65.4%) and showed little change in 2011 (63.05%, 61.44 to 63.79%). Stations outside of the farm lease generally had lower porosity than stations within the lease and showed little change in median value between years.

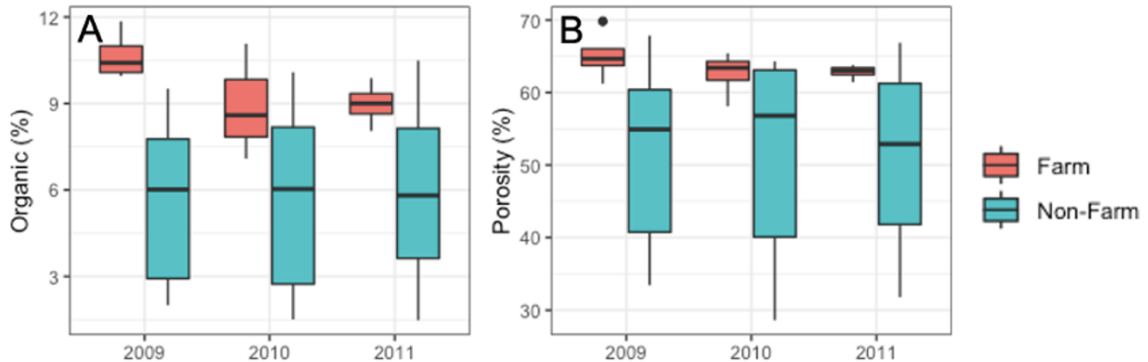


Figure 3.2 Boxplots of (A) sediment organic content and (B) sediment porosity collected in 2009, 2010 and 2011. Comparisons are shown for stations within (red; $n = 4$) and outside the farm lease boundary (blue; $n = 18$). The boxes show the interquartile range and the horizontal line within the box represents the median value. Vertical lines extending from the boxes indicate the minimum and maximum values up to 1.5 times the interquartile range. Dots above and below the lines represent observations extending beyond this range.

In each year of study, many environmental variables were significantly correlated (Table 3.2). In 2009, organic matter was significantly positively correlated with porosity ($\rho = 0.93$, $P < 0.001$) and percent mud ($\rho = 0.43$, $P < 0.05$), and significantly negatively correlated with seaway distance from the farm ($\rho = -0.79$, $P < 0.001$) and median grain size ($\rho = -0.43$, $P < 0.05$). In 2010, organic matter was significantly positively correlated with porosity ($\rho = 0.92$, $P < 0.001$), and significantly negatively correlated with seaway distance ($\rho = -0.52$, $P < 0.05$). In 2011, organic matter significantly positively correlated with porosity ($\rho = 0.93$, $P < 0.001$), and significantly negatively correlated with seaway distance ($\rho = -0.59$, $P < 0.01$). The static environmental variables (grain size measurements and seaway distance) were also highly intercorrelated. Median grain size was significantly positively correlated with grain size sorting ($\rho = 0.72$, $P < 0.001$) and seaway distance ($\rho = 0.55$, $P < 0.01$), and significantly negatively correlated with percent mud ($\rho = -1.00$, $P < 0.01$).

Table 3.2 Spearman rho correlation coefficient matrix for environmental data ($n = 22$) obtained in 2009, 2010 and 2011.

	Distance	Median	Mud	Organic	Porosity	Sorting
2009						
Distance	-					
Median	0.55**	-				
Mud	-0.55**	-1.00***	-			
Organic	-0.79***	-0.43*	0.43*	-		
Porosity	-0.88***	-0.54**	0.54**	0.93***	-	
Sorting	0.00	0.72***	-0.72***	0.24	0.09	-
2010						
Distance	-					
Median	0.55**	-				
Mud	-0.55*	-1.00***	-			
Organic	-0.52*	-0.40	0.40	-		
Porosity	-0.63**	-0.47*	0.47*	0.92***	-	
Sorting	0.00	0.72***	-0.72***	0.03	0.02	-
2011						
Distance	-					
Median	0.55**	-				
Mud	-0.55**	-1.00***	-			
Organic	-0.59**	-0.27	0.27	-		
Porosity	-0.70***	-0.49*	0.49*	0.93***	-	
Sorting	0.00	0.72***	-0.72***	0.10	-0.06	-

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Abbreviations are seaway distance from the farm lease (Distance), median grain size (Median); percent mud (Mud), organic content (Organic), sediment porosity (Porosity), and grain size sorting (Sorting).

3.4.2 Biodiversity variables

A total of 11933, 7247 and 13446 individual organisms were identified in 2009, 2010 and 2011, respectively. Seventy taxa were identified in 2009, the most abundant taxa being (in descending order) *Aricidea catherinae*, *Diastylis sculpta* and *Ninoe nigripes*. In 2010 and 2011, 88 and 89 taxa were identified, respectively, and in both years the most abundant taxa were *D. sculpta*, *A. catherinae* and *Eudorella truncatula* (Appendix 1).

Generally, biodiversity indices measured from within the farm lease exhibited lower values in comparison to non-farm stations (Fig. 3.3; Table 3.3). Stations within the lease exhibited large increases in biodiversity measures from 2009 to 2010. In general,

non-farm stations in 2009 and all variables (farm- or non-farm) remained relatively constant throughout 2010 and 2011. Shannon diversity increased in median value from within the farm increased from 1.10 grab⁻¹ (2.10 grab⁻¹ outside of the farm) in 2009 to 2.17 grab⁻¹ (2.14 grab⁻¹) in 2010 and decreased to 1.91 grab⁻¹ (2.10 grab⁻¹) in 2011. Pielou's evenness within the farm increased from 0.46 grab⁻¹ (0.64 grab⁻¹) in 2009 to 0.68 grab⁻¹ (0.62 grab⁻¹) in 2010 and decreased to 0.60 grab⁻¹ (0.61 grab⁻¹) in 2011. Abundance increased from 87 ind. grab⁻¹ within the farm (328.5 ind. grab⁻¹) in 2009 to 335.5 ind. grab⁻¹ (600.5 ind. grab⁻¹) in 2010 and 475.5 ind. grab⁻¹ (722.5 ind. grab⁻¹) in 2011. Richness within the farm was 8.5 species grab⁻¹ (21.5 species grab⁻¹) in 2009 which increased to 21.5 species grab⁻¹ (30 grab⁻¹) in 2010 and to 26.5 species grab⁻¹ (30.5 species grab⁻¹) in 2011.

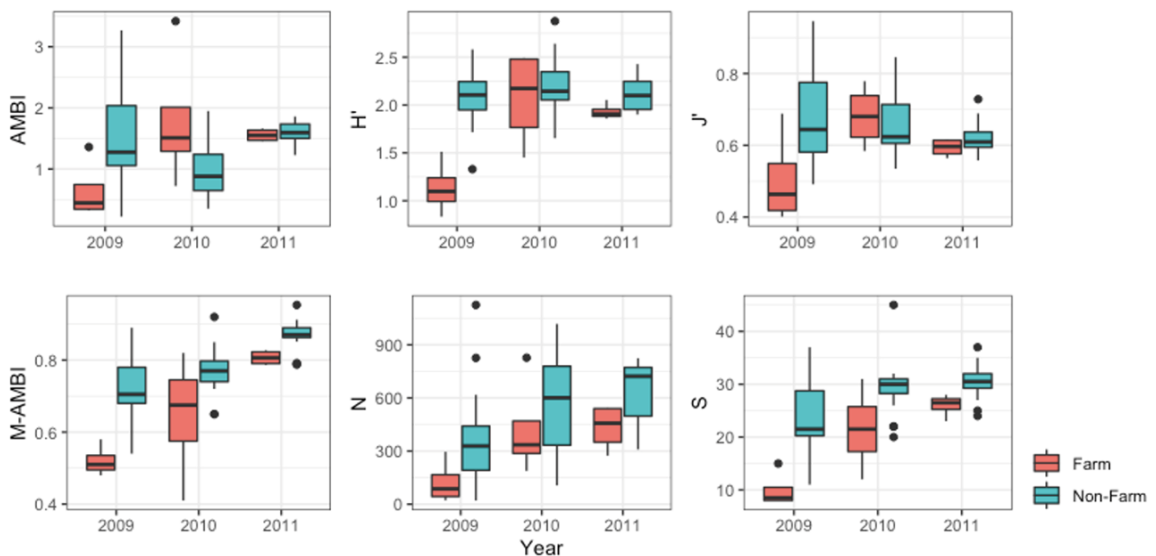


Figure 3.3 Boxplots of (A) AMBI, (B) Shannon index (H'), (C) Pielou's evenness (J'), (D) M-AMBI, (E) abundance (N) and (F) species richness (S) from macroinfaunal species count data collected in 2009, 2010 and 2011. Comparisons are shown for stations within ($n = 4$) and outside the farm lease boundary ($n = 18$). The boxes show the interquartile range and the horizontal line within the box represents the median value. Vertical lines extending from the boxes indicate the minimum and maximum values up to 1.5 times the interquartile range. Dots above and below the lines represent observations extending beyond this range.

Table 3.3 Descriptive statistics of macroinfaunal diversity collected within ($n = 4$) and outside of the fish farm lease ($n = 18$) in 2009, 2010 and 2011.

Variable	Farm				Non-Farm			
	Med	SD	Min	Max	Med	SD	Min	Max
2009								
AMBI	0.44	0.49	0.32	1.36	1.27	0.83	0.22	3.27
H'	1.10	0.28	0.83	1.51	2.10	0.30	1.33	2.58
J'	0.46	0.13	0.40	0.69	0.64	0.13	0.49	0.95
M-AMBI	0.51	0.04	0.48	0.58	0.70	0.09	0.54	0.89
N	87	123	22	296	329	273	21	1125
S	9	3	8	15	22	7	11	37
2010								
AMBI	1.51	1.15	0.72	3.42	0.88	0.45	0.35	1.95
H'	2.17	0.50	1.45	2.49	2.14	0.30	1.65	2.88
J'	0.68	0.09	0.58	0.78	0.62	0.09	0.53	0.85
M-AMBI	0.68	0.17	0.41	0.82	0.77	0.06	0.65	0.92
N	336	279	188	827	601	280	106	1018
S	22	8	12	31	30	5	20	45
2011								
AMBI	1.55	0.10	1.46	1.66	1.59	0.18	1.23	1.86
H'	1.91	0.09	1.86	2.05	2.10	0.16	1.90	2.43
J'	0.60	0.03	0.56	0.62	0.61	0.04	0.56	0.73
M-AMBI	0.81	0.02	0.79	0.83	0.87	0.04	0.79	0.95
N	458	131	274	542	723	163	309	824
S	27	2	23	28	31	3	24	37

Abbreviations are the AZTI Marine Biotic Index (AMBI), Shannon diversity index (H'), Pielou's evenness (J'), multivariate-AMBI (M-AMBI), abundance (N), and species richness (S).

In 2009, all stations within the farm were classified as having good or high AMBI ecological status (EQS), with values ranging from 0.32 (high EQS) to 1.36 (good EQS) while outside the farm lease, stations ranged from 0.22 (high) to 3.27 (good) (Table 3.1). In 2010, AMBI ranged from 0.72 (high) to 3.42 (moderate) within the lease, while stations outside the lease ranged from 0.35 (high) to 1.95 (good). In 2011, all AMBI values were classified as high ES. Values ranged from 1.46 to 1.63 within the farm and 1.23 to 1.86 in the non-farm stations. In 2009, M-AMBI ranged from 0.48 (moderate) to 0.58 (good) within the farm lease, while non-farm stations ranged from 0.54 (good) to 0.89 (high). In 2010, M-AMBI values ranged from 0.41 (moderate) to 0.82 (high) within

the farm lease, while those outside of the farm ranged from 0.65 (good) to 0.92 (high). In 2011, M-AMBI ranged from 0.79 to 0.83 within the farm (high) and 0.79 to 0.95 (high) outside of the farm lease.

In each year, many of the biodiversity indices were highly correlated (Table 3.4). In 2009, AMBI was highly correlated with Pielou's evenness ($\rho = 0.89$, $P < 0.001$) and Shannon diversity ($\rho = 0.77$, $P < 0.001$) (Table 3.3). Other highly correlated variables included Shannon diversity and Pielou's evenness ($\rho = 0.88$, $P < 0.001$), richness and abundance ($\rho = 0.88$, $P < 0.001$), M-AMBI and abundance ($\rho = 0.79$, $P < 0.001$), and M-AMBI and richness ($\rho = 0.96$, $P < 0.001$). In 2010, the most highly correlated variables were M-AMBI and richness ($\rho = 0.88$, $P < 0.001$), and abundance and richness ($\rho = 0.65$, $P < 0.001$). In 2011, Shannon diversity was significantly positively correlated with Pielou's evenness ($\rho = 0.88$, $P < 0.001$) and M-AMBI ($\rho = 0.85$, $P < 0.001$). M-AMBI was also significantly correlated with richness ($\rho = 0.69$, $P < 0.001$).

Table 3.4 Spearman rho correlation coefficient matrix for macroinfaunal biodiversity indices ($n = 22$) obtained in 2009, 2010 and 2011.

	AMBI	H'	J'	M-AMBI	N	S
2009						
AMBI	-					
H'	0.77***	-				
J'	0.89***	0.88***	-			
M-AMBI	-0.12	0.33	0.00	-		
N	-0.34	-0.10	-0.40	0.79***	-	
S	-0.12	0.25	-0.06	0.96***	0.88***	-
2010						
AMBI	-					
H'	0.25	-				
J'	0.53*	0.87***	-			
M-AMBI	-0.47*	0.54**	0.18	-		
N	-0.49*	-0.25	-0.52	0.36	-	
S	-0.49*	0.34	-0.06	0.88***	0.65***	-
2011						
AMBI						
H'	0.15	-				
J'	0.15	0.88***	-			
M-AMBI	-0.01	0.85***	0.65**			
N	0.08	0.29	0.16	0.52*	-	
S	0.09	0.51	0.12	0.69***	0.55**	-

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Abbreviations are the AZTI Marine Biotic Index (AMBI), Shannon diversity index (H'), Pielou's evenness (J'), multivariate-AMBI (M-AMBI), abundance (N), and species richness (S).

3.4.3 Patterns in multivariate composition

In 2009, the SIMPROF analysis revealed five significant clusters in assemblage structure (Fig. 3.4A). The four stations within the farm lease comprised a unique cluster (Cluster A), which was separated from non-farm stations along the x -axis of the NMDS ordination. The four remaining clusters identified from the SIMPROF analysis varied along the y -axis of NMDS ordination, including Cluster F ($n = 2$), which comprised stations to the north and south of the farm lease and Cluster D ($n = 10$) which included stations surrounding the farm lease, stations extending northward from the farm by >1 km, and those to the east of Spectacle Island. Cluster B ($n = 4$) included stations in the westernmost region of the bay, and Cluster E ($n = 2$) included the northernmost and

easternmost stations. In general, for non-farm stations (Clusters B to F), increasing values on the y-axis of the NMDS ordination corresponded to a decrease in organic content, increase in median grain size and decrease in percent mud.

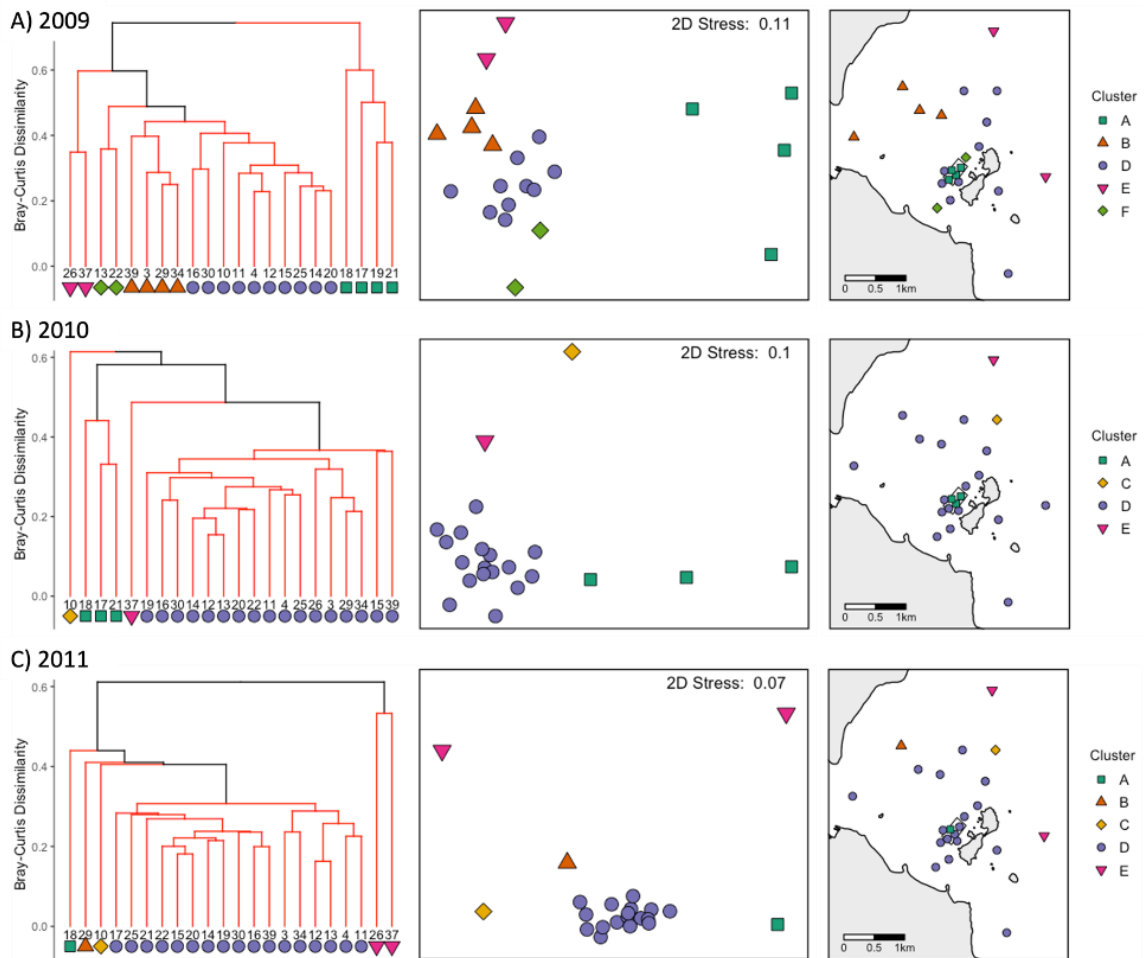


Figure 3.4 Dendrograms, non-metric multidimensional scaling (NMDS) ordinations and study area maps showing station similarity based on fourth root transformed macroinfaunal composition in (A) 2009, (B) 2010, and (C) 2011. Stations with no significant differences in assemblage structure ($P < 10^{-7}$), as indicated from a similarity profile analysis (SIMPROF) are highlighted in red on the dendrograms and assigned a unique symbol. These clusters were visualized on the NMDS ordinations and mapped. Dendrograms were constructed using the Bray-Curtis dissimilarity metric and the average linkage algorithm. Numbers along the x-axis of the dendrograms represent station names.

In 2010, the SIMPROF analysis identified four significant clusters (Fig. 3.4B). Three of the stations within the farm lease were identified as a separate cluster (Cluster A), which extended linearly along the x-axis of the NMDS ordination. One station

sampled within the farm lease was incorporated within Cluster D ($n = 17$) which also included stations surrounding the farm, all stations which belonged to Clusters B and F in 2009 and the easternmost station from Cluster E. Two other clusters were identified including Cluster C ($n = 1$) in the north and Cluster E ($n = 1$) which included the northernmost station.

One station within the farm lease remained as separate cluster in 2011 (Cluster A; $n = 1$), while the other three stations were incorporated within Cluster D ($n = 17$), which remained mostly unchanged from 2010. Three other clusters were identified including Cluster B ($n = 1$) located in the northwestern region of the study area, Cluster C ($n = 1$) which remained unchanged from 2010, and Cluster E ($n = 2$) which, similar to 2009, included the northernmost and easternmost stations.

The SIMPER analysis identified macroinfaunal species responsible for differentiating between clusters comprising only farm stations (Cluster A) from those in geographically contiguous clusters (D and F in 2009, and D in 2010 and 2011) (Table 3.4). In 2009, the main species responsible for differentiating between Clusters A and D were polychaetes, which were all more abundant in Cluster D including *Leitoscoloplos robustus* (EG IV), *Mediomastus californiensis* (EG III) and *Ninoe nigripes* (EG III). The only non-polychaete species identified was the cumacean *D. sculpta* (EG II), which was also the only listed species more abundant within the farm lease. Similarly, polychaetes were primarily responsible for differentiating between Clusters A and F in 2009, including *N. nigripes* (EG III), *L. robustus* (EG IV) and *A. catherinae* (EG II), all of which were more abundant in Cluster F. *D. sculpta* (EG II), the isopod *Edotia montosa* (EG II) and the amphipod *Orchomenella minuta* (EG II) were more abundant in Cluster A and were responsible for its differentiation from Cluster F.

Table 3.5 Similarity percentage (SIMPER) analysis identifying the top ten macroinfaunal taxa responsible for differentiating between the between clusters identified in the hierarchical cluster analysis in Fig. 3.4. Results show comparisons between the clusters containing only stations within the fish farm lease (Cluster A) and geographically adjacent clusters (Clusters D or F) for each year of study. The second column shows the percent dissimilarity explained by that taxon, and the third column shows the cumulative Bray-Curtis dissimilarity metric explained tallied in the order listed in the table. The fourth and fifth columns represent the fourth root transformed abundances of the clusters

in the order referred to in the first column. Explanations of the AMBI Ecological Groups (EG) are included in the main text.

Species	Contribution (%)	Cumulative contribution (%)	Mean abundance (I)	Mean abundance (II)	AMBI EG
2009					
Cluster A vs D					
<i>Leitoscoloplos robustus</i>	6.38	6.38	0.00	2.26	IV
<i>Mediomastus californiensis</i>	5.47	11.85	0.00	1.91	III
<i>Ninoe nigripes</i>	5.20	17.04	0.00	1.83	III
<i>Aricidea catherinae</i>	4.99	22.04	1.04	2.85	II
<i>Nephtys incisa</i>	4.82	26.85	0.50	2.19	II
<i>Prionospio steenstrupi</i>	4.73	31.58	0.00	1.69	IV
<i>Diastylis sculpta</i>	4.52	36.10	2.83	1.64	II
<i>Aphelochaeta marioni</i>	4.44	40.53	0.00	1.60	IV
<i>Schistomeringos rudolphi</i>	4.11	44.64	0.00	1.43	IV
<i>Euchone</i> sp.	4.05	48.69	0.00	1.45	n.a.
Cluster A vs F					
<i>Diastylis sculpta</i>	9.74	9.74	2.83	0.66	II
<i>Ninoe nigripes</i>	7.29	17.03	0.00	1.72	III
<i>Leitoscoloplos robustus</i>	7.22	24.25	0.00	1.67	IV
<i>Aricidea catherinae</i>	6.20	30.45	1.04	2.40	II
<i>Mediomastus californiensis</i>	6.16	36.61	0.00	1.41	III
<i>Edotia montosa</i>	5.84	42.45	1.41	0.00	II
<i>Prionospio steenstrupi</i>	5.68	48.14	0.00	1.30	IV
<i>Aphelochaeta marioni</i>	5.46	53.60	0.00	1.28	IV
<i>Schistomeringos rudolphi</i>	5.19	58.79	0.00	1.21	IV
<i>Orchomenella minuta</i>	4.81	63.60	1.16	0.00	II
2010					
Cluster A vs D					
<i>Edotia montosa</i>	5.43	5.43	2.74	0.42	II
<i>Eudorella truncatula</i>	4.67	10.10	0.58	2.62	I
<i>Aricidea catherinae</i>	4.52	14.62	1.54	3.42	II
<i>Euchone incolor</i>	4.48	19.10	0.00	1.98	II
<i>Orchomenella minuta</i>	4.36	23.46	2.27	0.44	II
<i>Leitoscoloplos fragilis</i>	4.07	27.53	0.00	1.79	III
<i>Capitella capitata</i>	3.83	31.36	2.62	0.98	V
<i>Nephtys incisa</i>	3.13	34.48	0.91	2.22	II
<i>Prionospio steenstrupi</i>	3.05	37.53	0.94	2.23	IV
<i>Asabellides oculata</i>	2.93	40.45	0.00	1.35	II
2011					
Cluster A vs D					
<i>Euchone incolor</i>	6.02	6.02	0.00	2.14	II
<i>Ninoe nigripes</i>	5.99	12.01	0.00	2.11	III
<i>Edotia montosa</i>	5.72	17.72	2.17	0.14	II
<i>Bathymedon</i> sp.	5.05	22.77	0.00	1.77	ignored
<i>Dyopodos porrectus</i>	4.39	27.16	0.00	1.55	III
<i>Cytheridea</i> sp.	3.25	30.41	0.00	1.15	ignored
<i>Leitoscoloplos fragilis</i>	3.03	33.44	1.00	2.08	III
<i>Thracia</i> sp.	3.00	36.45	0.00	1.05	ignored
<i>Yoldia limatula</i>	2.84	39.29	1.00	0.00	I
<i>Spiophanes bombyx</i>	2.75	42.04	1.32	0.35	III

In 2010, the species responsible for differentiation from the geographically adjacent Cluster D included the isopod *E. montosa* (EG II), amphipod *O. minuta* (EG II), and polychaete *Capitella capitata* (EG V) (Table 3.4). These were more abundant within farm lease (Cluster A). The SIMPER analysis also identified the polychaetes *Eudorella truncatula* (EG I), *A. catherinae* (EG II), *Euchone incolor* (EG II) and *Leitoscoloplos fragilis* (EG III) which were more abundant in Cluster D.

In 2011, polychaetes *E. incolor* (EG II), and *N. nigripes* (EG III) were more abundant in Cluster D and were responsible for its differentiation from Cluster A. The SIMPER analysis also identified the amphipods *Bathymedon* sp. (ignored) and *Dyopetos porrectus* (EG III) which were more abundant in Cluster D. The isopod *E. montosa* (EG II) and polychaete *Spiophanes bombyx* (EG III) were more common in A.

3.4.4 Relationships with environmental variables

The envfit analysis identified the environmental variables correlated with NMDS structure which were fitted onto the ordination (Fig. 3.5). In 2009, all tested variables were significantly correlated to macroinfaunal structure ($P < 0.05$) and the most highly correlated variables were organic content ($r^2 = 0.69$, $P = 0.001$), porosity ($r^2 = 0.68$, $P = 0.001$) and seaway distance from farm ($r^2 = 0.60$, $P = 0.001$) (Table 3.6). In 2010, grain size sorting ($r^2 = 0.69$, $P = 0.001$), and porosity ($r^2 = 0.69$, $P = 0.001$) were the most highly correlated variables with NMDS structure. In 2011, community composition was most strongly related to median grain size ($r^2 = 0.61$, $P = 0.004$), porosity ($r^2 = 0.58$, $P < 0.001$), percent mud ($r^2 = 0.54$, $P = 0.002$) and organic content ($r^2 = 0.54$, $P = 0.001$).

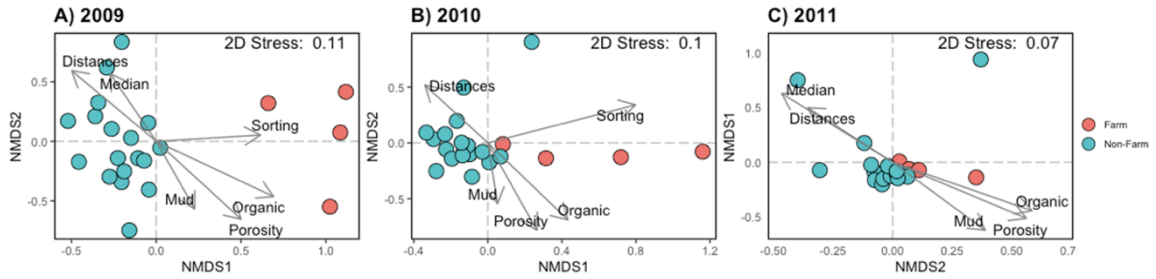


Figure 3.5 Non-metric multidimensional scaling ordinations of fourth root transformed Bray-Curtis dissimilarities of macroinfaunal composition sampled in (A) 2009, (B) 2010, and (C) 2011. Stations are shown as circles and coloured to represent those within the farm lease (red) compared to those outside the lease boundaries (blue). Arrows represent the significant ($P < 0.05$) environmental vectors fitted to the ordinations using the *envfit* function and the arrow length denotes the strength of the correlation. Abbreviations are seaway distance from the farm lease (Distance), median grain size (Median); percent mud (Mud), organic content (Organic), sediment porosity (Porosity), and grain size sorting (Sorting).

Table 3.6 Coefficient of variation (r^2) and significance by 999 permutations (P) for each environmental variable fitted to the non-metric multidimensional scaling ordination using the *envfit* function. Ordinations were constructed using fourth root transformed Bray-Curtis dissimilarities of macroinfaunal composition in 2009, 2010 and 2011.

Environmental Variable	r^2	P
2009		
distance	0.60	0.001
median	0.40	0.012
mud	0.37	0.011
organic	0.69	0.001
porosity	0.68	0.001
sorting	0.38	0.015
2010		
distance	0.42	0.003
median	0.31	0.051
mud	0.30	0.030
organic	0.66	0.001
porosity	0.69	0.001
sorting	0.79	0.001
2011		
distance	0.38	0.006
median	0.61	0.004
mud	0.54	0.002
organic	0.54	0.001
porosity	0.58	0.001
sorting	0.07	0.445

Abbreviations are seaway distance from the farm lease (Distance), median grain size (Median); percent mud (Mud), organic content (Organic), sediment porosity (Porosity), and grain size sorting (Sorting).

3.5 Discussion

This study assessed spatial and temporal changes in benthic macroinfaunal composition in coastal Nova Scotia during a fallowing period to detect potential far-field effects of a net-pen fish farm. Benthic health was characterized using AMBI and M-AMBI, which showed localized effects within the farm lease. Several farm stations were characterized as “moderate” EQS in 2009 and 2010, and by 2011, all stations within the bay were designated as “high” EQS. Multivariate analysis was used to identify clusters of stations with similar macroinfaunal composition and delineate the spatial area affected by enrichment. SIMPROF analysis showed a statistical separation of all four farm stations

from all others in 2009, three stations in 2010 and one in 2011, suggesting the effects of the farm were localized within the farm lease and an improvement in conditions was observed over time. Correlative models showed strong associations between community composition and organic content, likely reflecting faunal food requirements in non-farm stations, and enrichment-related effects within the lease.

Within the farm lease, most biodiversity indices showed the greatest change from 2009 to 2010 and less pronounced changes from 2010 to 2011. These results are consistent with research showing rapid changes in benthic conditions after farming ceases, and more variable improvements in years following (Karakassis et al., 1999; Keeley et al., 2014; Lin & Bailey-Brock, 2008; Villnäs et al., 2011). Despite their popularity in aquaculture studies, biodiversity indices are generally less sensitive for detecting change than more complex measures (Austen & Warwick, 1989; Clarke, 1993). For example, Keeley et al. (2014) noted that indices such as richness and abundance have variable responses after multiple years of fallowing and these measures also suggested recovery occurred earlier than indicated from more complex indices (e.g., M-AMBI). Due to the difficulties of interpreting univariate biodiversity indices, this study used AMBI and M-AMBI as additional descriptive measures to characterize benthic conditions. Stations were never found to be in an early successional stage (i.e., “poor” or “bad” EQS). In 2009, all AMBI values indicated “good” or “high” EQS but interestingly, in 2010, one station within the farm increased in value (i.e., became more affected) to “moderate” EQS. In contrast, in 2009, three of the farm stations were classified as “moderate” M-AMBI EQS while one station in 2010 was classified as “moderate” M-AMBI EQS. By 2011, all stations had “high” AMBI and M-AMBI EQS. The M-AMBI classifications were more closely aligned with the NMDS ordinations, suggesting M-AMBI may be a more reliable measure than AMBI for interpreting benthic health. Poor agreement between AMBI and M-AMBI such as in this dataset in 2009 has been documented previously (Quintino et al., 2012; Van Hoey et al., 2007) and is generally attributed to M-AMBI showing greater influence of richness or Shannon diversity in its calculation (in this case, richness). Despite these discrepancies, these indices suggest enrichment from the farm was concentrated within the lease and bay-wide benthic degradation was not observed.

SIMPROF analysis was applied as a data-driven approach to detect if farm stations had statistically distinct assemblage structure from non-farm stations. Similar to the biodiversity indices, the multivariate composition suggested the effects of enrichment were localized within the lease boundaries, since farm stations were initially identified as a unique cluster in the SIMPROF analysis and were distinctly separated along the x -axis of the NMDS ordination. This is consistent with the majority of studies showing that wastes dissipate quickly from the cages within a few hundred meters (Grant, 2012; Keeley, et al., 2013). In particular, this is expected in lower-energy hydrodynamic environments which often have more concentrated but less dispersed wastes (Broch et al., 2017; Keeley et al., 2019), such as Port Mouton Bay (Gregory et al., 1993). In addition, the separation of farm stations and subsequent incorporation of these stations within non-farm clusters over each year suggests improvements in the benthic conditions of the farm stations occurred, since the composition became statistically similar to non-farm stations. However, these results suggest benthic effects were still measurable, since one farm station remained as a separate distinct cluster in 2011. While recovery from fish farm wastes can occur on time scales as short as 7-14 weeks in some locations (Brooks et al., 2003; Ritz et al., 1989), others have found recovery can take multiple years to achieve (Brooks, 2004; Keeley et al., 2014). Furthermore, despite the statistical incorporation of farm stations within non-farm clusters (Fig. 3.4), these farm stations still showed intra-cluster separation from the non-farm stations according to the NMDS ordinations (Fig. 3.5). Keeley et al. (2014) noted similar results in which macroinfaunal compositional differences were observed between cage and reference sites even after multiple years of fallow. This was attributed to the possibility that multiple stable states can occur (Beisner et al., 2003) or the absence of late successional equilibrium species (Whitlatch et al., 2001; Connell & Slatyer, 1977), leading to compositional discrepancies in the two locations.

Non-farm stations exhibited spatial zonation through the bay which was generally reflective of both sediment conditions and changes in organic content. Sediments surrounding the farm were sandy mud and the dominant taxa within these clusters (Clusters B, D, and F) were primarily deposit-feeding polychaetes and cumaceans. Stations in the northern region of the study area (Clusters C and E) were composed of

muddy sands and had corresponding less organic matter. Numerically dominant organisms were also primarily polychaetes and cumaceans, although with different relative abundances, making them unique clusters. While the spatial distribution of clusters remained relatively similar between years, the species composition within each cluster varied between years. Seasonal and inter-annual variations of macroinfaunal communities are known to occur due to reproduction and recruitment dynamics (Kuklinski et al., 2013; McArthur et al., 2010; Reiss & Kröncke, 2005; Zhulay et al., 2015), food availability (Cisneros et al., 2011), or water temperatures (Reiss et al., 2006). Factors such as these may be responsible for the change in composition between each year of study.

Throughout the study period, organisms associated with organic enrichment (i.e., high EG) were present in locations not expected to be affected by farm-related enrichment and vice versa. For instance, in 2009, SIMPER analysis identified *D. sculpta* and *A. catherinae* (both EG II) as the numerically dominant species within the farm lease. Similarly, Tay et al. (2009) observed *D. sculpta* as an abundant species within a fish waste disposal site in Newfoundland, and Martinez-Garcia et al., (2019) found *A. claudiae* (EG I) present in fish farm sampling locations. Further, higher-EG organisms including *Prionospio steenstrupi*, *Aphelochaeta marioni* and *Schisteomeringos rudolphii* (all EG IV) were present outside of the lease in 2009 but absent within farm stations. The unexpected presence of contradictory EG-types is known to occur and has generally been attributed to different geographic populations having differing responses to enrichment (Bustos-Baez & Frid, 2003; Dean, 2008; Martinez-Garcia et al., 2019; Quintino et al., 2012). This is in part the reason for low AMBI values within the farm lease. Despite the unusual species presence, known indicators of enrichment were observed within the farm in 2010. A spike of *Capitella capitata* (EG V), a frequently-referenced indicator of pollution (Borja et al., 2000; McCall, 1977), was observed and increased from one individual in 2009 to the second most dominant taxa within the lease in 2010. This lag in the response is possible if they do not have established populations within the region (Grémare et al., 1989). In 2011, only one individual of *C. capitata* was observed within the lease which is likely associated with a successional response to enrichment (Pearson & Rosenberg, 1978). These findings highlight the need for the inclusion of multivariate

analyses since specific organisms may not be universal indicators of enrichment (Dean, 2008; Martinez-Garcia et al., 2019; Quintino et al., 2012).

Macroinfaunal composition showed a strong association with organic content in each year of study. Non-farm stations generally showed a gradual change in composition with increasing values of organic content along the *y*-axis axis of the NMDS ordinations, likely associated with species-specific responses to food requirements (Gray, 1974; Lopez & Levinton, 1987). Farm stations showed further changes on the *x*-axis of the NMDS ordinations suggesting enrichment-related effects, as these were typically also stations with the highest levels of organic content. While not measured here, the incorporation of other geochemical variables such as total free sulfides may provide more direct evidence of enrichment and corresponding changes in pore water chemistry (Hyland et al., 2005). Although the fit was generally highest with organic content, other correlated variables may be influential in structuring the macroinfaunal distributions. For instance, multiple studies have found a high association between macroinfaunal composition and variables such as silt-clay fraction (Ellingsen, 2002; Lu, 2005; Sousa et al., 2006). The high correlation between organic content and sediment grain size characteristics observed in this study is a common feature in marine ecosystems since organic content is adsorbed onto clay mineral particles (Hedges & Keil, 1995; Mayer, 1994). Spatial patterns of organic content in the bay are therefore largely driven by grain size dynamics and hydrodynamics and highlight the need for the inclusion of grain size characteristics in aquaculture monitoring studies (Martinez-Garcia et al., 2015). Grain size sorting has also been previously identified as a potential driver of macroinfaunal distributions as it is thought to be related to increased potential niche space (Coleman et al., 1997; Etter & Grassle, 1992; Gray, 1974; Nichols, 1970). However, in this study the stations with the highest sorting occurred at the farm site, which may be associated with the flocculation of wastes from the farm (Law et al., 2014). Overall, due to the complex mix of highly correlated variables, organic content is likely largely influential, yet determining the exact underlying mechanism structuring the macroinfaunal patterns remains challenging.

As EAA expands, new methods are needed to study spatial and temporal effects in the far-field. Monitoring approaches such as Before-After Control-Impact (BACI) or

beyond-BACI designs (Underwood, 1991, 1992) are often preferred for environmental recovery, since they can partition the sources of variability and therefore could attribute an effect specifically to aquaculture (Smokorowski & Randall, 2017; Wauchope et al., 2020). To increase spatial coverage, this study instead applied an exploratory multivariate approach to analyze recovery from macroinfaunal distributions. AMBI and M-AMBI were then used to assess if wastes were detrimental to the ecosystem. EAA has been applied to waste dispersal from aquaculture in Port Mouton Bay in various forms. For instance, ammonia dispersal was modelled throughout the bay by Filgueira et al. (2021) who found increased nitrogen levels were not high enough to negatively affect eelgrass beds. Cullain et al. (2018) analyzed a suite of benthic variables from eelgrass beds at ~300 m, ~700 m and ~3000 m and attributed increased organic enrichment, decreased eelgrass biomass and shoot density, and decreased macroinfaunal biomass in stations near the farm to aquaculture. This study adds greater sampling coverage to Cullain et al. (2018) in assessing the role of the farm and explicitly delineating spatial patterns of macroinfaunal distributions to understand dispersal processes. The results of this study provide supplemental findings to Loucks et al. (2014) and Milewski et al. (2018) which suggested bay-wide effects from the farm. Both studies found reduced lobster catches during production versus fallowing periods and suggested sulfidic plumes caused by the farm resulted in retreat behavior of the lobster. This work found the effects of the farm be restricted to within the lease. Future work could look at monitoring enrichment during production periods or including spatially explicit mapping methods to track sulfidic plumes to further substantiate these claims. Other factors from the farm such as noise and lights proposed by Milewski et al. (2021) that may affect lobster distributions could also be further investigated. This study sought to explore patterns of enrichment in multiple cardinal directions from the farm and bay-wide enrichment effects were ultimately not observed.

As aquaculture expands globally in a variety of hydrodynamic regimes, delineating the footprint of wastes and monitoring changes in the benthos over time remain active areas of research. This study used multivariate analyses to map the affected area and track changes in these benthic effects during a fallowing period. AMBI and M-AMBI indices were then used to assign a qualitative ecological status, which at worst,

showed “moderate” EQS below the farm. Although far-field effects remain a concern in aquaculture management, this study shows the effects were localized to within the lease boundaries. Multivariate methods suggest one station below the farm had distinct assemblage structure, suggesting recovery remained incomplete, despite biotic indices suggesting “high” EQS in the final year of study. Future research may examine how effects change based on hydrodynamics and during production periods in a spatially comprehensive manner. This research provides highly detailed information of bay-scale dynamics of macroinfaunal assemblages and their changes over time in relation to benthic finfish aquaculture wastes.

CHAPTER 4 CONCLUSION

4.1 Discussion and conclusions

Net-pen finfish aquaculture is known to result in localized organic enrichment of the seafloor, yet these effects at the ecosystem level remain understudied (Keeley et al., 2019; Weitzman et al., 2019). While several studies have suggested that bay-wide effects can occur (e.g., Loucks et al., 2014; Milewski et al., 2018), few have explicitly measured this or had the sampling coverage necessary to support these conclusions. Benthic aquaculture studies are typically conducted using transects and there exists a need for multi-directional sampling at kilometer distances away from the cages (Grant, 2012; Hargrave et al., 2008). The Ecosystem Approach to Aquaculture (EAA) has been developed as an approach to monitor the sustainability of the industry while considering these effects on the wider ecosystem (Soto et al., 2008). This thesis therefore sought to address these research gaps by implementing EAA to provide detailed spatial and temporal data on the benthic effects from aquaculture.

In Chapter 2, macroinfaunal species composition was analyzed with the specific aim of characterizing the far-field benthic effects of a net-pen finfish aquaculture site in Port Mouton Bay. Using a spatially comprehensive sampling strategy, the spatial patterns of macroinfaunal distributions were analyzed using biodiversity indices, qualitative indices of benthic ecological status, and multivariate statistics. Consistent with previous research, this study found multivariate statistical techniques to be the most sensitive in detecting change (Austen & Warwick, 1989; Clarke, 1993; Gong et al., 2005; Gray, 2000; Schratzberger et al., 2000; Warwick & Clarke, 1991) as they showed a distinct separation of farm stations. This corroborates multiple studies which show localized enrichment within non-dispersive hydrodynamic regimes (Brooks et al., 2002; Carroll et al., 2003; Hargrave, 2005; Pitta et al., 2006; Vezzulli et al., 2008; Welch et al., 2019). Macroinfaunal composition within the bay showed a strong association with organic content, and enrichment was likely responsible for the altered composition of stations within the farm lease. Biotic indices showed all non-farm stations were of “good” or “high” ecological quality status (EQS), while three of the farm stations were of “moderate” EQS. These analyses were critical in documenting the spatial effects of enrichment.

In Chapter 3, macroinfaunal distributions in Port Mouton Bay were analyzed over three years to address how composition changed during a following period. Data-driven approaches were used to detect statistically unique groupings of stations. SIMPROF analysis showed a separation of the four farm stations from all others in the first year of study and a gradual improvement in benthic conditions occurred in the years following. However, one station within the lease had a distinct composition after three years, suggesting that remediation processes remained incomplete. These results were consistent with research elsewhere showing both a localized spatial footprint (Keeley et al., 2019) and a rapid improvement in conditions within the first year after farming ceases (Karakassis et al., 1999; Keeley et al., 2014; Lin & Bailey-Brock, 2008; Villnäs et al., 2011).

4.2 Research contributions

A major contribution of this research was that it provided spatially dense benthic data in both the near- and far-field. Bay-scale waste dispersal from aquaculture has been conducted through field sampling by Howarth et al. (2019) and modelled by Filgueira et al. (2021). However, these approaches have been lacking in benthic studies, despite a repeated call for far-field sampling (e.g., Grant, 2012; Keeley et al., 2019). This work therefore helps fill a research gap and addresses claims where harmful far-field effects have been speculated without the underlying mechanism being directly studied (Loucks et al., 2014; Milewski et al., 2018). Previous benthic studies have been conducted with sampling along singular transects, which may not fully capture the dispersal patterns from farms (Grant, 2012; Hargrave et al., 2008). This study provided multidirectional sampling and found the consensus that wastes do not extend far beyond the cage boundaries to hold true. Furthermore, the observed spatial heterogeneity of macroinfaunal distributions reinforces the notion that study designs with a low sample size may result in misleading interpretations, particularly when summarized using biodiversity indices (Aguado-Giménez et al., 2012).

This work also contributes to the methodological and technical challenges of implementing EAA. This thesis used an exploratory sampling approach and analyses including spatial statistics, multivariate statistics, and biotic indices to assess benthic

quality. These data-driven approaches allow data to speak for themselves to find trends and visualize the outputs on a map. While these approaches cannot achieve causal inference (Wauchope et al., 2020), spatially explicit outputs allow researchers to create a visual link between macroinfaunal patterns and the underlying environmental processes. AMBI and M-AMBI were also applied to assess if the conditions were of an altered ecological status. This exploratory approach can act as a hypothesis-generating study from which further questions can be refined in future work.

Lastly, this study provided unique insight into the benthic ecology of macroinfauna in coastal environments. For example, this research included improved methods for statistical analysis of macroinfaunal patterns using generalized additive models and multivariate statistics. Despite the well-known non-linear responses to covariates such as organic content (Pearson & Rosenberg, 1978), aquaculture studies commonly use Pearson correlations to relate biodiversity indices to environmental variables (e.g., Han et al., 2013; Karakassis et al., 2002; Lu et al., 2008; Nickell et al., 2009). This research therefore provides information on the ecology of macroinfauna and their relationships to environmental variables in Port Mouton Bay which may be useful as baseline data for future work of environmental perturbations.

4.3 Research opportunities

Reflections on this thesis reveal new areas for future research. A primary limitation of this work is that visual identification of macroinfaunal organisms is time-consuming with high labor costs (Ärje et al., 2020; Nygård et al., 2016). Methods to reduce the identification time could greatly aid in the speed of analysis and number of samples gathered. Increasingly, genetic methods show great potential to monitor effects of aquaculture and even provide a classification status of ecological pollution at lower costs and higher speed (Stoeck et al., 2018; Verhoeven et al., 2018). However, these methods are not yet standardized (Ärje et al., 2020) and some government initiatives require abundance data such as the Water Framework Directive, a management strategy used by the European Union to characterize benthic health (Hering et al., 2010). While genetic tools are advancing, image recognition software could reduce identification time in the interim, and it has been suggested that automatic identification of macroinfauna

will replace human experts in most cases (Ärje et al., 2020). As these technologies progress, similar sampling designs to those used in this study could be performed with greatly reduced labor costs.

For a more informative temporal analysis, sampling prior to the development of a farm, and during production periods would be beneficial. For example, beyond-BACI designs which are generally considered an optimal approach for analyzing recovery patterns (Aguado-Giménez et al., 2012; Verdonschot et al., 2013), require before and after data with respect to an impact or intervention (Underwood, 1991, 1992). For instance, Aguado-Giménez et al. (2012) used a conventional beyond-BACI approach to assess benthic recovery of a fish farm using biological (polychaete abundance) and geochemical variables. However, in the research for this thesis, the farm was established in 1995, and these data were not collected. These non-ideal impact assessment designs are common in environmental monitoring (Aguado-Giménez et al., 2012) but would be valuable for future planned farms.

Lastly, this study only assessed structural metrics, or those that focus on the number of species and their relative abundance, to determine gradients of impact and rates of fallowing (Keeley et al., 2012; Keeley et al., 2014; Starr et al., 2014). However, EAA requires assessment of ecosystem functions and services, since structural metrics alone may not encompass all aspects of benthic health (Bremner, 2008; Villnäs et al., 2011; Villnäs et al., 2018). This includes quantifying processes such as productivity, and energy and nutrient transfer to characterize anthropogenic forcing (Bremner, 2008; Villnäs et al., 2011; Villnäs et al., 2018). Recently, an approach termed Biological Traits Analysis (BTA) has been developed which uses faunal traits as proxies of ecosystem functioning (Bremner et al., 2003; Bremner, 2008; Paganelli et al., 2012). For example, organism size, depth within the sediment, and reworking mode represent traits used to characterize bioturbation, which in turn influences sediment oxygen concentrations, organic matter cycling, and nutrient levels (Johnson et al., 1999; Kristensen, 2000; Solan et al., 2004; Witte et al., 2003). This method has shown potential for understanding how functioning varies spatially and in response to organic enrichment (Gusmao et al., 2016). The analysis of both structural and functional characteristics of macroinvertebrate communities may therefore provide complementary information for characterizing the

effects of finfish aquaculture (Culhane et al., 2014; Kalantzi et al., 2014; Kalantzi & Karakassis, 2006).

REFERENCES

- Abel, P. D. (2014). *Water pollution biology*. CRC Press.
- Aguado-Giménez, F., Marín, A., Montoya, S., Marín-Guirao, L., Piedecausa, A., & García-García, B. (2007). Comparison between some procedures for monitoring offshore cage culture in western Mediterranean Sea: Sampling methods and impact indicators in soft substrata. *Aquaculture*, *271*(1), 357–370. <https://doi.org/10.1016/j.aquaculture.2007.03.026>
- Aguado-Giménez, F., Piedecausa, M. A., Gutiérrez, J. M., García-Charton, J. A., Belmonte, A., & García-García, B. (2012). Benthic recovery after fish farming cessation: a “beyond-BACI” approach. *Marine Pollution Bulletin*, *64*(4), 729–738. <https://doi.org/10.1016/j.marpolbul.2012.02.012>
- Ahmerkamp, S., Marchant, H. K., Peng, C., Probandt, D., Littmann, S., Kuypers, M. M. M., & Holtappels, M. (2020). The effect of sediment grain properties and porewater flow on microbial abundance and respiration in permeable sediments. *Scientific Reports*, *10*(1), 1–12. <https://doi.org/10.1038/s41598-020-60557-7>
- Alary, C., & Demougeot-Renard, H. (2010). Factorial kriging analysis as a tool for explaining the complex spatial distribution of metals in sediments. *Environmental Science & Technology*, *44*(2), 593–599. <https://doi.org/10.1021/es9022305>
- Anderson, M. J., & Walsh, D. C. I. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? *Ecological Monographs*, *83*(4), 557–574. <https://doi.org/10.1890/12-2010.1>
- Anselin, L. (1995). Local Indicators of Spatial Association—LISA. *Geographical Analysis*, *27*(2), 93–115. <https://doi.org/10.1111/j.1538-4632.1995.tb00338.x>
- Ärje, J., Raitoharju, J., Iosifidis, A., Tirronen, V., Meissner, K., Gabbouj, M., ... Kärkkäinen, S. (2020). Human experts vs. machines in taxa recognition. *Signal Processing: Image Communication*, *87*, 115917. <https://doi.org/10.1016/j.image.2020.115917>
- Armstrong, E. G., Mersereau, J., Salvo, F., Hamoutene, D., & Dufour, S. C. (2020). Temporal change in the spatial distribution of visual organic enrichment indicators at aquaculture sites in Newfoundland, Canada. *Aquaculture International*, *28*(2), 569–586. <https://doi.org/10.1007/s10499-019-00478-z>
- Arrighetti, F., & Penchaszadeh, P. E. (2010). Macrobenthos-sediment relationships in a sandy bottom community off Mar del Plata, Argentina. *Journal of the Marine Biological Association of the United Kingdom*, *90*(5), 933. <https://doi.org/10.1017/S0025315409991524>

- Austen, M. C., & Warwick, R. M. (1989). Comparison of univariate and multivariate aspects of estuarine meiobenthic community structure. *Estuarine, Coastal and Shelf Science*, 29(1), 23–42. [https://doi.org/10.1016/0272-7714\(89\)90071-1](https://doi.org/10.1016/0272-7714(89)90071-1)
- Austin, P. C. (2007). A comparison of regression trees, logistic regression, generalized additive models, and multivariate adaptive regression splines for predicting AMI mortality. *Statistics in Medicine*, 26(15), 2937–2957. <https://doi.org/10.1002/sim.2770>
- Baldwin, C. C., Tornabene, L., & Robertson, D. R. (2018). Below the mesophotic. *Scientific Reports*, 8(1), 4920. <https://doi.org/10.1038/s41598-018-23067-1>
- Bannister, R. J., Johnsen, I. A., Hansen, P. K., Kutti, T., & Asplin, L. (2016). Near- and far-field dispersal modelling of organic waste from Atlantic salmon aquaculture in fjord systems. *ICES Journal of Marine Science*, 73(9), 2408–2419. <https://doi.org/10.1093/icesjms/fsw027>
- Barrett, L. T., Swearer, S. E., & Dempster, T. (2019). Impacts of marine and freshwater aquaculture on wildlife: a global meta-analysis. *Reviews in Aquaculture*, 11(4), 1022–1044. <https://doi.org/10.1111/raq.12277>
- Beisner, B. E., Haydon, D. T., & Cuddington, K. (2003). Alternative stable states in ecology. *Frontiers in Ecology and the Environment*, 1(7), 376–382. [https://doi.org/10.1890/1540-9295\(2003\)001\[0376:ASSIE\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2003)001[0376:ASSIE]2.0.CO;2)
- Bilkovic, D. M., Roggero, M., Hershner, C. H., & Havens, K. H. (2006). Influence of land use on macrobenthic communities in nearshore estuarine habitats. *Estuaries and Coasts*, 29(6), 1185–1195. <https://doi.org/10.1007/BF02781819>
- Bilyard, G. R. (1987). The value of benthic infauna in marine pollution monitoring studies. *Marine Pollution Bulletin*, 18(11), 581–585. [https://doi.org/10.1016/0025-326X\(87\)90277-3](https://doi.org/10.1016/0025-326X(87)90277-3)
- Blott, S. J., & Pye, K. (2001). Gradstat: A Grain Size Distribution and Statistics Package for the Analysis of Unconsolidated Sediments. *Earth Surface Processes and Landforms*, 26, 1237–1248. <https://doi.org/10.1002/esp.261>
- Boots, B., & Tiefelsdorf, M. (2000). Global and local spatial autocorrelation in bounded regular tessellations. *Journal of Geographical Systems*, 2(4), 319–348. <https://doi.org/10.1007/PL00011461>
- Borja, Á., Barbone, E., Basset, A., Borgersen, G., Brkljacic, M., Elliott, M., ... Trayanova, A. (2011). Response of single benthic metrics and multi-metric methods to anthropogenic pressure gradients, in five distinct European coastal and transitional ecosystems. *Marine Pollution Bulletin*, 62(3), 499–513. <https://doi.org/10.1016/j.marpolbul.2010.12.009>

- Borja, Á., Dauer, D. M., Elliott, M., & Simenstad, C. A. (2010). Medium- and Long-term Recovery of Estuarine and Coastal Ecosystems: Patterns, Rates and Restoration Effectiveness. *Estuaries and Coasts*, 33(6), 1249–1260. <https://doi.org/10.1007/s12237-010-9347-5>
- Borja, Á., Franco, J., & Pérez, V. (2000). A marine Biotic Index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin*, 40(12), 1100–1114. [https://doi.org/10.1016/S0025-326X\(00\)00061-8](https://doi.org/10.1016/S0025-326X(00)00061-8)
- Borja, Á., Franco, J., Valencia, V., Bald, J., Muxika, I., Belzunce, M. J., & Solaun, O. (2004). Implementation of the European water framework directive from the Basque country (northern Spain): A methodological approach. *Marine Pollution Bulletin*, 48(3–4), 209–218. <https://doi.org/10.1016/j.marpolbul.2003.12.001>
- Borja, Á., Josefson, A. B., Miles, A., Muxika, I., Olsgard, F., Phillips, G., ... Rygg, B. (2007). An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. *Marine Pollution Bulletin*, 55(1–6), 42–52. <https://doi.org/10.1016/j.marpolbul.2006.08.018>
- Borja, Á., Miles, A., Occhipinti-Ambrogi, A., & Berg, T. (2009). Current status of macroinvertebrate methods used for assessing the quality of European marine waters: Implementing the Water Framework Directive. *Hydrobiologia*, 633(1), 181–196. <https://doi.org/10.1007/s10750-009-9881-y>
- Borja, Á., & Muxika, I. (2005). Guidelines for the use of AMBI (AZTI's Marine Biotic Index) in the assessment of the benthic ecological quality. *Marine Pollution Bulletin*, 50(7), 787–789. <https://doi.org/10.1016/j.marpolbul.2005.04.040>
- Borja, Á., Muxika, I., & Franco, J. (2003). The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts. *Marine Pollution Bulletin*, 46(7), 835–845. [https://doi.org/10.1016/S0025-326X\(03\)00090-0](https://doi.org/10.1016/S0025-326X(03)00090-0)
- Bremner, J. (2008). Species' traits and ecological functioning in marine conservation and management. *Journal of Experimental Marine Biology and Ecology*, 366(1–2), 37–47. <https://doi.org/10.1016/j.jembe.2008.07.007>
- Bremner, J., Rogers, S. I., & Frid, C. L. J. (2003). Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. *Marine Ecology Progress Series*, 254, 11–25. <https://doi.org/10.3354/meps254011>
- Bridges Jr, C. C. (1966). Hierarchical cluster analysis. *Psychological Reports*, 18(3), 851–854. <https://doi.org/10.2466/pr0.1966.18.3.851>

- Britton, L. J., & Greeson, P. E. (1989). *Methods for collection and analysis of aquatic biological and microbiological samples*. US Government Printing Office.
- Broch, O. J., Daae, R. L., Ellingsen, I. H., Nepstad, R., Bendiksen, E. Å., Reed, J. L., & Senneset, G. (2017). Spatiotemporal dispersal and deposition of fish farm wastes: a model study from central Norway. *Frontiers in Marine Science*, 4, 199. <https://doi.org/10.3389/fmars.2017.00199>
- Brooks, K. M., Mahnken, C., & Nash, C. (2002). Environmental effects associated with marine netpen waste with emphasis on salmon farming in the Pacific Northwest. *Responsible Marine Aquaculture*, 159–203.
- Brooks, K. M., & Mahnken, C. V. W. (2003). Interactions of Atlantic salmon in the Pacific northwest environment: II. Organic wastes. *Fisheries Research*, 62(3), 255–293. [https://doi.org/10.1016/S0165-7836\(03\)00064-X](https://doi.org/10.1016/S0165-7836(03)00064-X)
- Brooks, K. M., Stierns, A. R., & Backman, C. (2004). Seven year remediation study at the Carrie Bay Atlantic salmon (*Salmo salar*) farm in the Broughton Archipelago, British Columbia, Canada. *Aquaculture*, 239(1–4), 81–123. <https://doi.org/10.1016/j.aquaculture.2003.12.024>
- Brooks, K. M., Stierns, A. R., Mahnken, C. V. W., & Blackburn, D. B. (2003). Chemical and biological remediation of the benthos near Atlantic salmon farms. *Aquaculture*, 219(1), 355–377. [https://doi.org/10.1016/S0044-8486\(02\)00528-8](https://doi.org/10.1016/S0044-8486(02)00528-8)
- Brugère, C., Aguilar-Manjarrez, J., Beveridge, M. C. M., & Soto, D. (2019). The ecosystem approach to aquaculture 10 years on—a critical review and consideration of its future role in blue growth. *Reviews in Aquaculture*, 11(3), 493–514. <https://doi.org/10.1111/raq.12242>
- Buchanan, J. B. (1984). *Sediment analysis*. (N. A. Holme & A. D. McIntyre, Eds.), *Methods for the Study of Marine Benthos* (2nd ed.). Blackwell Scientific Publications.
- Bustos-Baez, S., & Frid, C. (2003). Using indicator species to assess the state of macrobenthic communities. *Hydrobiologia*, 496, 299–309. <https://doi.org/10.1023/A:1026169520547>
- Callier, M., Lefebvre, S., MK, D., MP, B., Coughlan, J., & P, C. T. (2013). Shift in benthic assemblages and organisms' diet at salmon farms: community structure and stable isotope analyses. *Marine Ecology Progress Series*, 483, 153–167. <https://doi.org/10.3354/meps10251>
- Carroll, M. L., Cochrane, S., Fieler, R., Velvin, R., & White, P. (2003). Organic enrichment of sediments from salmon farming in Norway: environmental factors, management practices, and monitoring techniques. *Aquaculture*, 226(1), 165–180. [https://doi.org/10.1016/S0044-8486\(03\)00475-7](https://doi.org/10.1016/S0044-8486(03)00475-7)

- Carvajalino-Fernández, M. A., Sævik, P. N., Johnsen, I. A., Albretsen, J., & Keeley, N. B. (2020). Simulating particle organic matter dispersal beneath Atlantic salmon fish farms using different resuspension approaches. *Marine Pollution Bulletin*, *161*, 111685. <https://doi.org/10.1016/j.marpolbul.2020.111685>
- Castella, E., Adalsteinsson, H., Brittain, J. E., Gislason, G. M., Lehmann, A., Lencioni, V., ... Olafsson, J. S. (2001). Macrobenthic invertebrate richness and composition along a latitudinal gradient of European glacier-fed streams. *Freshwater Biology*, *46*(12), 1811–1831. <https://doi.org/10.1046/j.1365-2427.2001.00860.x>
- Cisneros, K. O., Smit, A. J., Laudien, J., & Schoeman, D. S. (2011). Complex, dynamic combination of physical, chemical and nutritional variables controls spatio-temporal variation of sandy beach community structure. *PLoS One*, *6*(8), e23724. <https://doi.org/10.1371/journal.pone.0023724>
- Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, *18*(1988), 117–143. <https://doi.org/10.1111/j.1442-9993.1993.tb00438.x>
- Clarke, K. R., & Ainsworth, M. (1993). A method of linking multivariate community structure to environmental variables. *Marine Ecology-Progress Series*, *92*, 205.
- Clarke, K. R., Somerfield, P. J., & Gorley, R. N. (2008). Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage. *Journal of Experimental Marine Biology and Ecology*, *366*(1–2), 56–69. <https://doi.org/10.1016/j.jembe.2008.07.009>
- Clarke, K. R., & Warwick, R. M. (1994). *Change in marine communities: an approach to statistical analysis and interpretation*. *Change in marine communities* (2nd ed.). PRIMER-E: Plymouth.
- Coleman, N., Gason, A. S. H., & Poore, G. C. B. (1997). High species richness in the shallow marine waters of south-east Australia. *Marine Ecology Progress Series*, *154*, 17–26. <https://doi.org/10.3354/meps154017>
- Connell, J. H., & Slatyer, R. O. (1977). Mechanisms of succession in natural communities and their role in community stability and organization. *The American Naturalist*, *111*(982), 1119–1144. <https://doi.org/10.1086/283241>
- Cranford, P. J., Brager, L., & Wong, D. (2017). A dual indicator approach for monitoring benthic impacts from organic enrichment with test application near Atlantic salmon farms. *Marine Pollution Bulletin*, *124*(1), 258–265. <https://doi.org/10.1016/j.marpolbul.2017.07.049>
- Culhane, F. E., Briers, R. A., Tett, P., & Fernandes, T. F. (2014). Structural and functional indices show similar performance in marine ecosystem quality assessment. *Ecological Indicators*, *43*, 271–280. <https://doi.org/10.1016/j.ecolind.2014.03.009>

- Cullain, N., McIver, R., Schmidt, A. L., Milewski, I., & Lotze, H. K. (2018). Potential impacts of finfish aquaculture on eelgrass (*Zostera marina*) beds and possible monitoring metrics for management: a case study in Atlantic Canada. *PeerJ*, 6:e5630. <https://doi.org/10.7717/peerj.5630>
- Dauer, D. M. (1993). Biological criteria, environmental health and estuarine macrobenthic community structure. *Marine Pollution Bulletin*, 26(5), 249–257. [https://doi.org/10.1016/0025-326X\(93\)90063-P](https://doi.org/10.1016/0025-326X(93)90063-P)
- Dauer, Daniel M, Ranasinghe, J. A., & Weisberg, S. B. (2000). Relationships between benthic community condition, water quality, sediment quality, nutrient loads, and land use patterns in Chesapeake Bay. *Estuaries*, 23(1), 80–96. <https://doi.org/10.2307/1353227>
- David, P., Berthou, P., Noel, P., & Jarne, P. (1997). Patchy recruitment patterns in marine invertebrates: a spatial test of the density-dependent hypothesis in the bivalve *Spisula ovalis*. *Oecologia*, 111(3), 331–340. <https://doi.org/10.1007/s004420050243>
- de Vries, J., Kraak, M. H. S., Verdonschot, R. C. M., & Verdonschot, P. F. M. (2020). Species composition drives macroinvertebrate community classification. *Ecological Indicators*, 119, 106780. <https://doi.org/10.1016/j.ecolind.2020.106780>
- Dean, H. K. (2008). The use of polychaetes (Annelida) as indicator species of marine pollution: a review. *Revista de Biología Tropical*, 56(4), 11–38.
- DFO. (2007). *Port Mouton Aquaculture Site Review*. Retrieved from <https://waves-vagues.dfo-mpo.gc.ca/Library/345509.pdf>
- DFO. (2009). *Review of cumulative effects report for Port Mouton Bay, Nova Scotia*. Retrieved from <http://www.dfo-mpo.gc.ca/Library/319225.pdf>
- Diaz, R. J., & Rosenberg, R. (1995). Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanography and Marine Biology. An Annual Review*, 33, 203–245.
- Diaz, R. J., Solan, M., & Valente, R. M. (2004). A review of approaches for classifying benthic habitats and evaluating habitat quality. *Journal of Environmental Management*, 73(3), 165–181. <https://doi.org/10.1016/j.jenvman.2004.06.004>
- Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., ... Leitão, P. J. (2013). Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, 36(1), 27–46. <https://doi.org/10.1111/j.1600-0587.2012.07348.x>
- Dowd, M., Grant, J., & Lu, L. (2014). Predictive modeling of marine benthic macrofauna and its use to inform spatial monitoring design. *Ecological Applications*, 24(4), 862–876. <https://doi.org/10.1890/12-2081.1>

- Edgar, G. J., Macleod, C. K., Mawbey, R. B., & Shields, D. (2005). Broad-scale effects of marine salmonid aquaculture on macrobenthos and the sediment environment in southeastern Tasmania. *Journal of Experimental Marine Biology and Ecology*, 327(1), 70–90. <https://doi.org/https://doi.org/10.1016/j.jembe.2005.06.003>
- Elith, J., & Leathwick, J. R. (2009). Species Distribution Models: Ecological Explanation and Prediction Across Space and Time. *Annual Review of Ecology, Evolution, and Systematics*, 40(1), 677–697. <https://doi.org/10.1146/annurev.ecolsys.110308.120159>
- Ellingsen, K. E. (2002). Soft-sediment benthic biodiversity on the continental shelf in relation to environmental variability. *Marine Ecology Progress Series*, 232, 15–27. <https://doi.org/doi:10.3354/meps232015>
- Etter, R. J., & Grassle, J. F. (1992). Patterns of species diversity in the deep sea as a function of sediment particle size diversity. *Nature*, 360, 576. <https://doi.org/10.1038/360576a0>
- Fairweather, P. G. (1991). Statistical power and design requirements for environmental monitoring. *Marine and Freshwater Research*, 42(5), 555–567. <https://doi.org/10.1071/MF9910555>
- Fauchald, K. (1977). *The polychaete worms. Definitions and keys to the orders, families and genera. Natural History Museum of Los Angeles County, Science Series.*
- Filgueira, R., Guyondet, T., Thupaki, P., Reid, G. K., Howarth, L. M., & Grant, J. (2021). Inferring the potential for nitrogen toxicity on seagrass in the vicinity of an aquaculture site using mathematical models. *Journal of Environmental Management*, 282, 111921. <https://doi.org/10.1016/j.jenvman.2020.111921>
- Folk, R. L., & Ward, W. C. (1957). Brazos River bar [Texas]; a study in the significance of grain size parameters. *Journal of Sedimentary Research*, 27(1), 3–26. <https://doi.org/10.1306/74D70646-2B21-11D7-8648000102C1865D>
- Fonseca, G., & Gallucci, F. (2016). The need of hypothesis-driven designs and conceptual models in impact assessment studies: An example from the free-living marine nematodes. *Ecological Indicators*, 71, 79–86. <https://doi.org/10.1016/j.ecolind.2016.06.051>
- Fotheringham, A. S., & Rogerson, P. A. (2008). *The SAGE handbook of spatial analysis.* Sage.
- Getis, A., & Ord, J. K. (1996). Local spatial statistics: an overview. *Spatial Analysis: Modelling in a GIS Environment*, 374, 261–277.

- Gillett, D. J., Weisberg, S. B., Grayson, T., Hamilton, A., Hansen, V., Leppo, E. W., ... Dauer, D. (2015). Effect of ecological group classification schemes on performance of the AMBI benthic index in US coastal waters. *Ecological Indicators*, *50*, 99–107. <https://doi.org/10.1016/j.ecolind.2014.11.005>
- Gong, J., Song, W., & Warren, A. (2005). Periphytic ciliate colonization: annual cycle and responses to environmental conditions. *Aquatic Microbial Ecology*, *39*(2), 159–170. <https://doi.org/10.3354/ame039159>
- Gosner, K. L. (1971). *Guide to identification of marine and estuarine invertebrates: Cape Hatteras to the Bay of Fundy*. New York: Wiley-Interscience.
- Grant, J. (2012). Coastal communities, participatory research, and far-field effects of aquaculture. *Aquaculture Environment Interactions*, *1*(2), 85–93. <https://doi.org/10.3354/aei00009>
- Grant, J., Filgueira, R., & Barrell, J. (2016). Lack of interaction between finfish aquaculture and lobster catch in coastal Nova Scotia. *Marine Pollution Bulletin*, *110*(1), 613–615. <https://doi.org/10.1016/j.marpolbul.2016.06.043>
- Grant, J., Hatcher, A., Scott, D. B., Pocklington, P., Schafer, C. T., & Winters, G. V. (1995). A multidisciplinary approach to evaluating impacts of shellfish aquaculture on benthic communities. *Estuaries*, *18*(1), 124–144. <https://doi.org/10.2307/1352288>
- Gray, J. S. (1974). Animal-sediment relationships. *Marine Biology Annual Review*, *12*, 223–261.
- Gray, J. S. (2000). The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. *Journal of Experimental Marine Biology and Ecology*, *250*(1–2), 23–49. [https://doi.org/10.1016/S0022-0981\(00\)00178-7](https://doi.org/10.1016/S0022-0981(00)00178-7)
- Gray, J. S., Wu, R. S., & Or, Y. Y. (2002). Effects of hypoxia and organic enrichment on the coastal marine environment. *Marine Ecology Progress Series*, *238*, 249–279. <https://doi.org/10.3354/meps238249>
- Green, R., & Chapman, P. (2011). The problem with indices. *Marine Pollution Bulletin*, *62*(7), 1377–1380. <https://doi.org/10.1016/j.marpolbul.2011.02.016>
- Gregory, D., Petrie, B., Jordan, F., & Langille, P. (1993). *Oceanographic, geographic and hydrological parameters of Scotia-Fundy and Southern Gulf of St. Lawrence Inlets*. *Canadian Technical Report of Fisheries and Aquatic Sciences* (Vol. 143).
- Grémare, A., Marsh, A. G., & Tenore, K. R. (1989). Secondary production and reproduction of *Capitella capitata* type I (Annelida: Polychaeta) during a population cycle. *Marine Ecology Progress Series*, *51*(1), 99–105.

- Gu, Y.-G., & Gao, Y.-P. (2019). An unconstrained ordination-and GIS-based approach for identifying anthropogenic sources of heavy metal pollution in marine sediments. *Marine Pollution Bulletin*, *146*, 100–105. <https://doi.org/10.1016/j.marpolbul.2019.06.008>
- Guisan, A., Edwards Jr, T. C., & Hastie, T. (2002). Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecological Modelling*, *157*(2–3), 89–100. [https://doi.org/10.1016/S0304-3800\(02\)00204-1](https://doi.org/10.1016/S0304-3800(02)00204-1)
- Gusmao, J. B., Brauko, K. M., Eriksson, B. K., & Lana, P. C. (2016). Functional diversity of macrobenthic assemblages decreases in response to sewage discharges. *Ecological Indicators*, *66*, 65–75. <https://doi.org/10.1016/j.ecolind.2016.01.003>
- Hale, S. S., Paul, J. F., & Heltshe, J. F. (2004). Watershed landscape indicators of estuarine benthic condition. *Estuaries*, *27*(2), 283–295. <https://doi.org/10.1007/BF02803385>
- Han, Q., Wang, Y., Zhang, Y., Keesing, J., & Liu, D. (2013). Effects of intensive scallop mariculture on macrobenthic assemblages in Sishili Bay, the northern Yellow Sea of China. *Hydrobiologia*, *718*. <https://doi.org/10.1007/s10750-013-1590-x>
- Hardy, A. (1996). On the number of clusters. *Computational Statistics & Data Analysis*, *23*(1), 83–96. [https://doi.org/10.1016/S0167-9473\(96\)00022-9](https://doi.org/10.1016/S0167-9473(96)00022-9)
- Hargrave, B. T. (2005). *Environmental effects of marine finfish aquaculture* (Vol. 5). Springer Science & Business Media.
- Hargrave, B. T., Holmer, M., & Newcombe, C. P. (2008). Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. *Marine Pollution Bulletin*, *56*(5), 810–824. <https://doi.org/10.1016/j.marpolbul.2008.02.006>
- Hastie, T., & Tibshirani, R. (1987). Generalized additive models: some applications. *Journal of the American Statistical Association*, *82*(398), 371–386.
- Hedges, J. I., & Keil, R. G. (1995). Sedimentary organic matter preservation: an assessment and speculative synthesis. *Marine Chemistry*, *49*(2–3), 81–115. [https://doi.org/10.1016/0304-4203\(95\)00008-F](https://doi.org/10.1016/0304-4203(95)00008-F)
- Hellawell, J. M. (2012). *Biological indicators of freshwater pollution and environmental management*. Springer Science & Business Media.
- Hering, D., Borja, Á., Carstensen, J., Carvalho, L., Elliott, M., Feld, C. K., ... de Bund, W. van. (2010). The European Water Framework Directive at the age of 10: A critical review of the achievements with recommendations for the future. *Science of The Total Environment*, *408*(19), 4007–4019. <https://doi.org/10.1016/j.scitotenv.2010.05.031>

- Holland, A. F. (1985). Long-term variation of macrobenthos in a mesohaline region of Chesapeake Bay. *Estuaries*, 8(2), 93. <https://doi.org/10.2307/1351860>
- Holmer, M., Argyrou, M., Dalsgaard, T., Danovaro, R., Diaz-Almela, E., Duarte, C. M., ... Tsapakis, M. (2008). Effects of fish farm waste on *Posidonia oceanica* meadows: Synthesis and provision of monitoring and management tools. *Marine Pollution Bulletin*, 56(9), 1618–1629. <https://doi.org/10.1016/j.marpolbul.2008.05.020>
- Howarth, L. M., Filgueira, R., Jiang, D., Koepke, H., Frame, M. K., Buchwald, C., ... Grant, J. (2019). Using macroalgal bioindicators to map nutrient plumes from fish farms and other sources at a bay-wide scale. *Aquaculture Environment Interactions*, 11, 671–684. <https://doi.org/10.3354/aei00340>
- Hussin, W. M. R. W., Cooper, K. M., Froján, C. R. S. B., Defew, E. C., & Paterson, D. M. (2012). Impacts of physical disturbance on the recovery of a macrofaunal community: a comparative analysis using traditional and novel approaches. *Ecological Indicators*, 12(1), 37–45. <https://doi.org/10.1016/j.ecolind.2011.03.016>
- Hyland, J., Balthis, L., Karakassis, I., Magni, P., Petrov, A., Shine, J., ... Warwick, R. (2005). Organic carbon content of sediments as an indicator of stress in the marine benthos. *Marine Ecology Progress Series*, 295, 91–103. <https://doi.org/10.3354/meps295091>
- Jain, A. K., Murty, M. N., & Flynn, P. J. (1999). Data clustering: a review. *ACM Computing Surveys (CSUR)*, 31(3), 264–323. <https://doi.org/10.1145/331499.331504>
- Jenks, G. F. (1967). The data model concept in statistical mapping. *International Yearbook of Cartography*, 7, 186–190.
- Johnson, K. S., Chavez, F. P., & Friederich, G. E. (1999). Continental-shelf sediment as a primary source of iron for coastal phytoplankton. *Nature*, 398(6729), 697. <https://doi.org/10.1038/19511>
- Julian, D., Chang, M., Judd, J., & Arp, A. (2001). Influence of environmental factors on burrow irrigation and oxygen consumption in the mudflat invertebrate *Urechis caupo*. *Marine Biology*, 139(1), 163–173. <https://doi.org/10.1007/s002270100570>
- Jun, Y.-C., Kim, N.-Y., Kim, S.-H., Park, Y.-S., Kong, D.-S., & Hwang, S.-J. (2016). Spatial Distribution of Benthic Macroinvertebrate Assemblages in Relation to Environmental Variables in Korean Nationwide Streams. *Water*, 8(1), 27. <https://doi.org/10.3390/w8010027>
- Kalantzi, I., & Karakassis, I. (2006). Benthic impacts of fish farming: Meta-analysis of community and geochemical data. *Marine Pollution Bulletin*, 52(5), 484–493. <https://doi.org/https://doi.org/10.1016/j.marpolbul.2005.09.034>

- Kalantzi, I., Papageorgiou, N., Sevastou, K., Black, K. D., Pergantis, S. A., & Karakassis, I. (2014). Metals in benthic macrofauna and biogeochemical factors affecting their trophic transfer to wild fish around fish farm cages. *Science of The Total Environment*, 470–471, 742–753. <https://doi.org/10.1016/j.scitotenv.2013.10.020>
- Karakassis, I., Hatziyanni, E., & Tsapakis, M. (1999). Benthic recovery following cessation of fish farming: a series of successes and catastrophes. *Marine Ecology Progress Series*, 184, 205–218. <https://doi.org/10.3354/meps184205>
- Karakassis, I., Tsapakis, M., & Hatziyanni, E. (1998). Seasonal variability in sediment profiles beneath fish farm cages in the Mediterranean. *Marine Ecology Progress Series*, 162, 243–252. <https://doi.org/10.3354/meps162243>
- Karakassis, I., Tsapakis, M., Smith, C. J., & Rumohr, H. (2002). Fish farming impacts in the Mediterranean studied through sediment profiling imagery. *Marine Ecology Progress Series*, 227, 125–133. <https://doi.org/https://doi.org/10.3354/meps227125>
- Keeley, N. B., Crome, C. J., Goodwin, E. O., Gibbs, M. T., & Macleod, C. M. (2013). Predictive depositional modelling (DEPOMOD) of the interactive effect of current flow and resuspension on ecological impacts beneath salmon farms. *Aquaculture Environment Interactions*, 3(3), 275–291. <https://doi.org/10.3354/aei00068>
- Keeley, N. B., Forrest, B. M., Crawford, C., & Macleod, C. K. (2012). Exploiting salmon farm benthic enrichment gradients to evaluate the regional performance of biotic indices and environmental indicators. *Ecological Indicators*, 23, 453–466. <https://doi.org/10.1016/j.ecolind.2012.04.028>
- Keeley, N. B., Forrest, B. M., & Macleod, C. K. (2013). Novel observations of benthic enrichment in contrasting flow regimes with implications for marine farm monitoring and management. *Marine Pollution Bulletin*, 66(1–2), 105–116. <https://doi.org/10.1016/j.marpolbul.2012.10.024>
- Keeley, N. B., Forrest, B. M., & Macleod, C. K. (2015). Benthic recovery and re-impact responses from salmon farm enrichment: Implications for farm management. *Aquaculture*, 435, 412–423. <https://doi.org/10.1016/j.aquaculture.2014.10.007>
- Keeley, N. B., Macleod, C. K., Hopkins, G. A., & Forrest, B. M. (2014). Spatial and temporal dynamics in macrobenthos during recovery from salmon farm induced organic enrichment: When is recovery complete? *Marine Pollution Bulletin*, 80(1–2), 250–262. <https://doi.org/10.1016/j.marpolbul.2013.12.008>
- Keeley, N. B., Valdemarsen, T., Woodcock, S., Holmer, M., Husa, V., & Bannister, R. (2019). Resilience of dynamic coastal benthic ecosystems in response to large-scale finfish farming. *Aquaculture Environment Interactions*, 11, 161–179. <https://doi.org/10.3354/aei00301>

- Kim, Y., & Gu, C. (2004). Smoothing spline Gaussian regression: more scalable computation via efficient approximation. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *66*(2), 337–356. <https://doi.org/10.1046/j.1369-7412.2003.05316.x>
- Kite, R., Nelson, T., Stenhouse, G., & Darimont, C. (2016). A movement-driven approach to quantifying grizzly bear (*Ursus arctos*) near-road movement patterns in west-central Alberta, Canada. *Biological Conservation*, *195*, 24–32. <https://doi.org/10.1016/j.biocon.2015.12.020>
- Krause, G., Billing, S.-L., Dennis, J., Grant, J., Fanning, L., Filgueira, R., ... Wawrzynski, W. (2020). Visualizing the social in aquaculture: How social dimension components illustrate the effects of aquaculture across geographic scales. *Marine Policy*, *118*, 103985. <https://doi.org/10.1016/j.marpol.2020.103985>
- Kristensen, E. (2000). Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. In *Life at interfaces and under extreme conditions* (pp. 1–24). Springer.
- Kruskal, J. B. (1964). Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*, *29*(1), 1–27. <https://doi.org/10.1007/BF02289565>
- Kuhn, M., & Johnson, K. (2013). Data pre-processing. In *Applied Predictive Modeling* (pp. 27–59). Springer.
- Kuklinski, P., Berge, J., McFadden, L., Dmoch, K., Zajaczkowski, M., Nygård, H., ... Tatarek, A. (2013). Seasonality of occurrence and recruitment of Arctic marine benthic invertebrate larvae in relation to environmental variables. *Polar Biology*, *36*(4), 549–560. <https://doi.org/10.1007/s00300-012-1283-3>
- Kutti, T., Hansen, P. K., Ervik, A., Høisæter, T., & Johannessen, P. (2007). Effects of organic effluents from a salmon farm on a fjord system. II. Temporal and spatial patterns in infauna community composition. *Aquaculture*, *262*(2–4), 355–366. <https://doi.org/10.1016/j.aquaculture.2006.10.008>
- Lamberson, J. O., DeWitt, T. H., & Swartz, R. C. (1992). Assessment of sediment toxicity to marine benthos. *Sediment Toxicity Assessment*, *9*, 183–211.
- Landrum, P. F., & Robbins, J. A. (1990). Bioavailability of Sediment-Associated Contaminants to Benthic Invertebrates. In *Sediments: chemistry and toxicity of in-place pollutants* (1st ed., pp. 237–263). CRC Press.
- Langfelder, P., Zhang, B., & Horvath, S. (2008). Defining clusters from a hierarchical cluster tree: the Dynamic Tree Cut package for R. *Bioinformatics*, *24*(5), 719–720. <https://doi.org/10.1093/bioinformatics/btm563>

- Law, B. A., Hill, P. S., Maier, I., Milligan, T. G., & Page, F. (2014). Size, settling velocity and density of small suspended particles at an active salmon aquaculture site. *Aquaculture Environment Interactions*, 6(1), 29–42.
<https://doi.org/10.3354/aei00116>
- Lenat, D. R., Smock, L. A., & Penrose, D. L. (1980). Use of benthic macroinvertebrates as indicators of environmental quality. In D. L. Worf (Ed.), *Biological monitoring for environmental effects* (pp. 97–112). Toronto, Canada: Lexington Books,.
- Lester, S. E., Stevens, J. M., Gentry, R. R., Kappel, C. V., Bell, T. W., Costello, C. J., ... White, C. (2018). Marine spatial planning makes room for offshore aquaculture in crowded coastal waters. *Nature Communications*, 9(1), 945.
<https://doi.org/10.1038/s41467-018-03249-1>
- Li, W., Xu, B., Song, Q., Liu, X., Xu, J., & Brookes, P. C. (2014). The identification of ‘hotspots’ of heavy metal pollution in soil–rice systems at a regional scale in eastern China. *Science of The Total Environment*, 472, 407–420.
<https://doi.org/10.1016/j.scitotenv.2013.11.046>
- Lin, D. T., & Bailey-Brock, J. H. (2008). Partial recovery of infaunal communities during a fallow period at an open-ocean aquaculture. *Marine Ecology Progress Series*, 371, 65–72. <https://doi.org/10.3354/meps07675>
- Lin, Y.-P., Chang, T.-K., Shih, C.-W., & Tseng, C.-H. (2002). Factorial and indicator kriging methods using a geographic information system to delineate spatial variation and pollution sources of soil heavy metals. *Environmental Geology*, 42(8), 900–909.
<https://doi.org/10.1007/s00254-002-0600-5>
- Liu, G., Bi, R., Wang, S., Li, F., & Guo, G. (2013). The use of spatial autocorrelation analysis to identify PAHs pollution hotspots at an industrially contaminated site. *Environmental Monitoring and Assessment*, 185(11), 9549–9558.
<https://doi.org/10.1007/s10661-013-3272-6>
- Lopez, G. R., & Levinton, J. S. (1987). Ecology of deposit-feeding animals in marine sediments. *The Quarterly Review of Biology*, 62(3), 235–260.
<https://doi.org/10.1086/415511>
- Loucks, R. H., Smith, R. E., & Fisher, E. B. (2014). Interactions between finfish aquaculture and lobster catches in a sheltered bay. *Marine Pollution Bulletin*, 88(1–2), 255–259. <https://doi.org/10.1016/j.marpolbul.2014.08.035>
- Loucks, R. H., Smith, R. E., Fisher, C. V., & Brian Fisher, E. (2012). Copper in the sediment and sea surface microlayer near a fallowed, open-net fish farm. *Marine Pollution Bulletin*, 64(9), 1970–1973.
<https://doi.org/10.1016/j.marpolbul.2012.05.025>

- Lu, L. (2005). The relationship between soft-bottom macrobenthic communities and environmental variables in Singaporean waters. *Marine Pollution Bulletin*, 51(8), 1034–1040. <https://doi.org/10.1016/j.marpolbul.2005.02.013>
- Lu, L., Grant, J., & Barrell, J. (2008). Macrofaunal spatial patterns in relationship to environmental variables in the Richibucto estuary, New Brunswick, Canada. *Estuaries and Coasts*, 31(5), 994–1005. <https://doi.org/10.1007/s12237-008-9097-9>
- Macleod, C. K., Moltschaniwskyj, N. A., & Crawford, C. M. (2006). Evaluation of short-term fallowing as a strategy for the management of recurring organic enrichment under salmon cages. *Marine Pollution Bulletin*, 52(11), 1458–1466. <https://doi.org/10.1016/j.marpolbul.2006.05.007>
- Macleod, C. K., Moltschaniwskyj, N. A., Crawford, C. M., & Forbes, S. E. (2007). Biological recovery from organic enrichment: some systems cope better than others. *Marine Ecology Progress Series*, 342, 41–53. <https://doi.org/10.3354/meps342041>
- Maloney, K. O., Munguia, P., & Mitchell, R. M. (2011). Anthropogenic disturbance and landscape patterns affect diversity patterns of aquatic benthic macroinvertebrates. *Journal of the North American Benthological Society*, 30(1), 284–295. <https://doi.org/10.1899/09-112.1>
- Martinez-Garcia, E., Capaccioni-Azzati, R., Sánchez-Lizaso, J. L., & Sanchez-Jerez, P. (2019). Application of a new protocol to evaluate the benthic impacts of aquaculture: Colonization of experimental units for monitoring by polychaeta. *Ecological Indicators*, 101, 50–61. <https://doi.org/10.1016/j.ecolind.2019.01.006>
- Martinez-Garcia, E., Carlsson, M. S., Sanchez-Jerez, P., Sánchez-Lizaso, J. L., Sanz-Lazaro, C., & Holmer, M. (2015). Effect of sediment grain size and bioturbation on decomposition of organic matter from aquaculture. *Biogeochemistry*, 125(1), 133–148. <https://doi.org/10.1007/s10533-015-0119-y>
- Mayer, L. M. (1994). Relationships between mineral surfaces and organic carbon concentrations in soils and sediments. *Chemical Geology*, 114(3–4), 347–363. [https://doi.org/10.1016/0009-2541\(94\)90063-9](https://doi.org/10.1016/0009-2541(94)90063-9)
- Mayor, D. J., Zuur, A. F., Solan, M., Paton, G. I., & Killham, K. (2010). Factors affecting benthic impacts at Scottish fish farms. *Environmental Science & Technology*, 44(6), 2079–2084. <https://doi.org/10.1021/es903073h>
- McArthur, M. A., Brooke, B. P., Przeslawski, R., Ryan, D. A., Lucieer, V. L., Nichol, S., ... Radke, L. C. (2010). On the use of abiotic surrogates to describe marine benthic biodiversity. *Estuarine, Coastal and Shelf Science*, 88(1), 21–32. <https://doi.org/10.1016/j.ecss.2010.03.003>
- McCall, P. L. (1977). Community patterns and adaptive strategies of the infaunal benthos of Long Island Sound. *Journal of Marine Research*, 35, 221–265.

- McIntire, E. J. B., & Fajardo, A. (2009). Beyond description: the active and effective way to infer processes from spatial patterns. *Ecology*, *90*(1), 46–56. <https://doi.org/10.1890/07-2096.1>
- Meinkoth, N. A. (1981). *The Audubon Society field guide to North American seashore creatures*. Knopf.
- Milewski, I, Loucks, R., Fisher, B., Smith, R., McCain, J., & Lotze, H. (2018). Sea-cage aquaculture impacts market and berried lobster (*Homarus americanus*) catches. *Marine Ecology Progress Series*, *598*, 85–97. <https://doi.org/10.3354/meps12623>
- Milewski, Inka, & Smith, R. E. (2019). Sustainable aquaculture in Canada: Lost in translation. *Marine Policy*, *107*, 103571. <https://doi.org/10.1016/j.marpol.2019.103571>
- Milligan, T. G., & Law, B. A. (2005). The effect of marine aquaculture on fine sediment dynamics in coastal inlets. In *Environmental Effects of Marine Finfish Aquaculture* (pp. 239–251). Springer.
- Milner, A. M., Brittain, J. E., Castella, E., & Petts, G. E. (2001). Trends of macroinvertebrate community structure in glacier-fed rivers in relation to environmental conditions: a synthesis. *Freshwater Biology*, *46*(12), 1833–1847. <https://doi.org/10.1046/j.1365-2427.2001.00861.x>
- Miron, G., & Kristensen, E. (1993a). Behavioural response of three nereid polychaetes to injection of sulfide inside burrows. *Marine Ecology-Progress Series*, *101*, 147.
- Miron, G., & Kristensen, E. (1993b). Factors influencing the distribution of nereid polychaetes: the sulfide aspect. *Marine Ecology-Progress Series*, *93*, 143.
- Muxika, I., Borja, Á., & Bald, J. (2007). Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. *Marine Pollution Bulletin*, *55*(1), 16–29. <https://doi.org/10.1016/j.marpolbul.2006.05.025>
- Muxika, I., Borja, Á., & Bonne, W. (2005). The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. *Ecological Indicators*, *5*(1), 19–31. <https://doi.org/10.1016/j.ecolind.2004.08.004>
- Nelson, T. A., & Boots, B. (2008). Detecting spatial hot spots in landscape ecology. *Ecography*, *31*(5), 556–566. <https://doi.org/10.1111/j.0906-7590.2008.05548.x>
- Nichols, F. H. (1970). Benthic polychaete assemblages and their relationship to the sediment in Port Madison, Washington. *Marine Biology*, *6*(1), 48–57. <https://doi.org/10.1007/BF00352607>

- Nickell, T. D., Cromeey, C. J., Borja, Á., & Black, K. D. (2009). The benthic impacts of a large cod farm — Are there indicators for environmental sustainability? *Aquaculture*, 295(3), 226–237. <https://doi.org/10.1016/j.aquaculture.2009.07.011>
- Nilsson, H. C., & Rosenberg, R. (2000). Succession in marine benthic habitats and fauna in response to oxygen deficiency: analysed by sediment profile-imaging and by grab samples. *Marine Ecology Progress Series*, 197, 139–149. <https://doi.org/10.3354/meps197139>
- Nygård, H., Oinonen, S., Hällfors, H. A., Lehtiniemi, M., Rantajärvi, E., & Uusitalo, L. (2016). Price vs. value of marine monitoring. *Frontiers in Marine Science*, 3, 205. <https://doi.org/10.3389/fmars.2016.00205>
- Openshaw, S., & Taylor, P. J. (1979). A million or so correlation coefficients: three experiments on the modifiable areal unit problem. *Statistical Applications in the Spatial Sciences*, 127–144.
- Ord, J. K., & Getis, A. (2001). Testing for local spatial autocorrelation in the presence of global autocorrelation. *Journal of Regional Science*, 41(3), 411–432. <https://doi.org/10.1111/0022-4146.00224>
- Osmundsen, T. C., & Olsen, M. S. (2017). The imperishable controversy over aquaculture. *Marine Policy*, 76, 136–142. <https://doi.org/10.1016/j.marpol.2016.11.022>
- Ottinger, M., Clauss, K., & Kuenzer, C. (2016). Aquaculture: Relevance, distribution, impacts and spatial assessments—A review. *Ocean & Coastal Management*, 119, 244–266. <https://doi.org/10.1016/j.ocecoaman.2015.10.015>
- Paganelli, D., Marchini, A., & Occhipinti-Ambrogi, A. (2012). Functional structure of marine benthic assemblages using Biological Traits Analysis (BTA): a study along the Emilia-Romagna coastline (Italy, North-West Adriatic Sea). *Estuarine, Coastal and Shelf Science*, 96, 245–256. <https://doi.org/10.1016/j.ecss.2011.11.014>
- Pearson, T. H., & Rosenberg, R. (1978). Macrobenthic Succession in relation to Organic Enrichment and Pollution of the Marine Environment. *Oceanography and Marine Biology: An Annual Review*, 16, 229–311.
- Pielou, E. C. (1966). Species-diversity and pattern-diversity in the study of ecological succession. *Journal of Theoretical Biology*, 10(2), 370–383. [https://doi.org/10.1016/0022-5193\(66\)90133-0](https://doi.org/10.1016/0022-5193(66)90133-0)
- Pinna, M., Marini, G., Rosati, I., Neto, J. M., Patricio, J., Marques, J. C., & Basset, A. (2013). The usefulness of large body-size macroinvertebrates in the rapid ecological assessment of Mediterranean lagoons. *Ecological Indicators*, 29, 48–61. <https://doi.org/10.1016/j.ecolind.2012.12.011>

- Pinto, R., Patrício, J., Baeta, A., Fath, B. D., Neto, J. M., & Marques, J. C. (2009). Review and evaluation of estuarine biotic indices to assess benthic condition. *Ecological Indicators*, 9(1), 1–25. <https://doi.org/10.1016/j.ecolind.2008.01.005>
- Piper, D. J. W. (1986). *The marine geology of the inner Scotian Shelf off the South Shore, Nova Scotia*. Ottawa: Geological Survey of Canada.
- Pitta, P., Apostolaki, E. T., Tsagaraki, T., Tsapakis, M., & Karakassis, I. (2006). Fish farming effects on chemical and microbial variables of the water column: a spatio-temporal study along the Mediterranean Sea. *Hydrobiologia*, 563(1), 99–108. <https://doi.org/10.1007/s10750-005-1593-3>
- Pohle, G., Frost, B., & Findlay, R. (2001). Assessment of regional benthic impact of salmon mariculture within the Letang Inlet, Bay of Fundy. *ICES Journal of Marine Science*, 58(2), 417–426. <https://doi.org/10.1006/jmsc.2000.1039>
- Pollock, L. W. (1998). *A practical guide to the marine animals of northeastern North America*. Rutgers University Press.
- Quero, G. M., Ape, F., Manini, E., Mirto, S., & Luna, G. M. (2020). Temporal changes in microbial communities beneath fish farm sediments are related to organic enrichment and fish biomass over a production cycle. *Frontiers in Marine Science*, 7, 524. <https://doi.org/10.3389/fmars.2020.00524>
- Quintino, V., Azevedo, A., Magalhães, L., Sampaio, L., Freitas, R., Rodrigues, A. M., & Elliott, M. (2012). Indices, multispecies and synthesis descriptors in benthic assessments: Intertidal organic enrichment from oyster farming. *Estuarine, Coastal and Shelf Science*, 110, 190–201. <https://doi.org/10.1016/j.ecss.2012.05.028>
- Reiss, H., & Kröncke, I. (2005). Seasonal variability of infaunal community structures in three areas of the North Sea under different environmental conditions. *Estuarine, Coastal and Shelf Science*, 65(1–2), 253–274. <https://doi.org/10.1016/j.ecss.2005.06.008>
- Reiss, H., Meybohm, K., & Kröncke, I. (2006). Cold winter effects on benthic macrofauna communities in near-and offshore regions of the North Sea. *Helgoland Marine Research*, 60(3), 224–238. <https://doi.org/10.1007/s10152-006-0038-3>
- Ritz, D. A., Lewis, M. E., & Shen, M. (1989). Response to organic enrichment of infaunal macrobenthic communities under salmonid seacages. *Marine Biology*, 103(2), 211–214. <https://doi.org/10.1007/BF00543349>
- Robertson, B. P., Gardner, J. P. A., & Savage, C. (2015). Macrobenthic–mud relations strengthen the foundation for benthic index development: a case study from shallow, temperate New Zealand estuaries. *Ecological Indicators*, 58, 161–174. <https://doi.org/10.1016/j.ecolind.2015.05.039>

- Robertson, B. P., Savage, C., Gardner, J. P. A., Robertson, B. M., & Stevens, L. M. (2016). Optimising a widely-used coastal health index through quantitative ecological group classifications and associated thresholds. *Ecological Indicators*, *69*, 595–605.
- Rodil, I. F., Lohrer, A. M., Hewitt, J. E., Townsend, M., Thrush, S. F., & Carbines, M. (2013). Tracking environmental stress gradients using three biotic integrity indices: advantages of a locally-developed traits-based approach. *Ecological Indicators*, *34*, 560–570. <https://doi.org/10.1016/j.ecolind.2013.06.023>
- Salas, F., Marcos, C., Neto, J. M., Patrício, J., Pérez-Ruzafa, A., & Marques, J. C. (2006). User-friendly guide for using benthic ecological indicators in coastal and marine quality assessment. *Ocean & Coastal Management*, *49*(5), 308–331. <https://doi.org/10.1016/j.ocecoaman.2006.03.001>
- Sanders, H. L. (1958). Benthic Studies in Buzzards Bay. I. Animal-Sediment Relationships1. *Limnology and Oceanography*, *3*(3), 245–258. <https://doi.org/10.4319/lo.1958.3.3.0245>
- Sanders, H. L., Goudsmit, E. M., Mills, E. L., & Hampson, G. E. (1962). A Study of the Intertidal Fauna of Barnstable Harbor, Massachusetts. *Limnology and Oceanography*, *7*(1), 63–79. <https://doi.org/10.4319/lo.1962.7.1.0063>
- Sanz-Lázaro, C., & Marín, A. (2006). Benthic recovery during open sea fish farming abatement in Western Mediterranean, Spain. *Marine Environmental Research*, *62*(5), 374–387. <https://doi.org/10.1016/j.marenvres.2006.05.006>
- Sanz-Lázaro, C., & Marín, A. (2011). Diversity patterns of benthic macrofauna caused by marine fish farming. *Diversity*, *3*(2), 176–199. <https://doi.org/10.3390/d3020176>
- Sarà, G., Scilipoti, D., Mazzola, A., & Modica, A. (2004). Effects of fish farming waste to sedimentary and particulate organic matter in a southern Mediterranean area (Gulf of Castellammare, Sicily): a multiple stable isotope study ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). *Aquaculture*, *234*(1), 199–213. <https://doi.org/10.1016/j.aquaculture.2003.11.020>
- Schratzberger, M., Gee, J. M., Rees, H. L., Boyd, S. E., & Wall, C. M. (2000). The structure and taxonomic composition of sublittoral meiofauna assemblages as an indicator of the status of marine environments. *Journal of the Marine Biological Association of the United Kingdom*, *80*(6), 969–980. <https://doi.org/10.1017/S0025315400003039>
- Shannon, C. E. (1948). A mathematical theory of communication. *Bell System Technical Journal*, *27*(3), 379–423.
- Shimeta, J., & Jumars, P. A. (1991). Physical mechanisms and rates of particle capture by suspension feeders. *Oceanography and Marine Biology: An Annual Review*, *29*(19), 197–257.

- Smokorowski, K. E., & Randall, R. G. (2017). Cautions on using the Before-After-Control-Impact design in environmental effects monitoring programs. *FACETS*, 2, 212–232. <https://doi.org/10.1139/facets-2016-0058>
- Snelgrove, P V R, & Butman, C. A. (1994). Animal-sediment relationships revisited: cause versus effect. *Oceanography and Marine Biology: An Annual Review*, 8(42), 668.
- Snelgrove, Paul V R. (1998). The biodiversity of macrofaunal organisms in marine sediments. *Biodiversity & Conservation*, 7(9), 1123–1132. <https://doi.org/10.1023/A:1008867313340>
- Sokal, R. R., Oden, N. L., & Thomson, B. A. (1998). Local spatial autocorrelation in a biological variables. *Geographical Analysis*, 65(1), 41–62. <https://doi.org/10.1111/j.1095-8312.1998.tb00350.x>
- Solan, M., Cardinale, B. J., Downing, A. L., Engelhardt, K. A. M., Ruesink, J. L., & Srivastava, D. S. (2004). Extinction and ecosystem function in the marine benthos. *Science*, 306(5699), 1177–1180. <https://doi.org/10.1126/science.1103960>
- Soto, D., Aguilar-Manjarrez, J., Brugère, C., Angel, D., Bailey, C., Black, K., ... Deudero, S. (2008). Applying an ecosystem-based approach to aquaculture: principles, scales and some management measures. *Building an Ecosystem Approach to Aquaculture*, 14.
- Sousa, R., Dias, S., & Antunes, J. C. (2006). Spatial subtidal macrobenthic distribution in relation to abiotic conditions in the Lima estuary, NW of Portugal. *Hydrobiologia*, 559(1), 135–148. <https://doi.org/10.1007/s10750-005-1371-2>
- Starr, C., Taggart, R., Evers, C., & Starr, L. (2014). *Biology: The unity and diversity of life*. Nelson Education.
- Stoeck, T., Kochems, R., Forster, D., Lejzerowicz, F., & Pawlowski, J. (2018). Metabarcoding of benthic ciliate communities shows high potential for environmental monitoring in salmon aquaculture. *Ecological Indicators*, 85, 153–164. <https://doi.org/10.1016/j.ecolind.2017.10.041>
- Tay, K.-L., Worthman, G., Wadman, R., & Stewart, P. (2009). Environmental monitoring of two fish waste ocean disposal sites in Newfoundland and Labrador, Canada. *International Journal of Environmental Engineering*, 1(4), 352–368. <https://doi.org/10.1504/IJEE.2009.027981>
- Theede, H., Ponat, A., Hiroki, K., & Schlieper, C. (1969). Studies on the resistance of marine bottom invertebrates to oxygen-deficiency and hydrogen sulphide. *Marine Biology*, 2(4), 325–337. <https://doi.org/10.1007/BF00355712>

- Thompson, B., & Lowe, S. (2004). Assessment of macrobenthos response to sediment contamination in the San Francisco Estuary, California, USA. *Environmental Toxicology and Chemistry*, 23(9), 2178–2187. <https://doi.org/10.1897/03-233>
- Thorne, R. S. J., Williams, W. P., & Cao, Y. (1999). The influence of data transformations on biological monitoring studies using macroinvertebrates. *Water Research*, 33(2), 343–350. [https://doi.org/10.1016/S0043-1354\(98\)00247-4](https://doi.org/10.1016/S0043-1354(98)00247-4)
- Thrush, S. F., Hewitt, J. E., Herman, P. M. J., & Ysebaert, T. (2005). Multi-scale analysis of species–environment relationships. *Marine Ecology Progress Series*, 302, 13–26. <https://doi.org/10.3354/Meps302013>
- Thrush, S. F., Hewitt, J. E., Norkko, A., Nicholls, P. E., Funnell, G. A., & Ellis, J. I. (2003). Habitat change in estuaries: predicting broad-scale responses of intertidal macrofauna to sediment mud content. *Marine Ecology Progress Series*, 263, 101–112. <https://doi.org/10.3354/meps263101>
- Tomassetti, P., Gennaro, P., Lattanzi, L., Mercatali, I., Persia, E., Vani, D., & Porrello, S. (2016). Benthic community response to sediment organic enrichment by Mediterranean fish farms: Case studies. *Aquaculture*, 450, 262–272. <https://doi.org/10.1016/j.aquaculture.2015.07.019>
- Underwood, A. J. (1991). Beyond BACI: Experimental designs for detecting human environmental impacts on temporal variations in natural populations. *Marine and Freshwater Research*, 42(5), 569–587. <https://doi.org/10.1071/MF9910569>
- Underwood, A. J. (1992). Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. *Journal of Experimental Marine Biology and Ecology*, 161(2), 145–178. [https://doi.org/10.1016/0022-0981\(92\)90094-Q](https://doi.org/10.1016/0022-0981(92)90094-Q)
- Urbina, M. A. (2016). Temporal variation on environmental variables and pollution indicators in marine sediments under sea Salmon farming cages in protected and exposed zones in the Chilean inland Southern Sea. *Science of The Total Environment*, 573, 841–853. <https://doi.org/10.1016/j.scitotenv.2016.08.166>
- van der Wal, D., Lambert, G. I., Ysebaert, T., Plancke, Y. M. G., & Herman, P. M. J. (2017). Hydrodynamic conditioning of diversity and functional traits in subtidal estuarine macrozoobenthic communities. *Estuarine, Coastal and Shelf Science*, 197, 80–92. <https://doi.org/10.1016/j.ecss.2017.08.012>
- Van Hoey, G., Rees, H., & Berghe, E. Vanden. (2007). A Comparison of Indicators Reflecting the Status of the North Sea benthos.
- Verdonschot, P. F. M., Spears, B. M., Feld, C. K., Brucet, S., Keizer-Vlek, H., Borja, A., ... Johnson, R. K. (2013). A comparative review of recovery processes in rivers, lakes, estuarine and coastal waters. *Hydrobiologia*, 704(1), 453–474. <https://doi.org/10.1007/s10750-012-1294-7>

- Verhoeven, J. T. P., Salvo, F., Knight, R., Hamoutene, D., & Dufour, S. C. (2018). Temporal bacterial surveillance of salmon aquaculture sites indicates a long lasting benthic impact with minimal recovery. *Frontiers in Microbiology*, *9*, 3054. <https://doi.org/10.3389/fmicb.2018.03054>
- Vezzulli, L., Moreno, M., Marin, V., Pezzati, E., Bartoli, M., & Fabiano, M. (2008). Organic waste impact of capture-based Atlantic bluefin tuna aquaculture at an exposed site in the Mediterranean Sea. *Estuarine, Coastal and Shelf Science*, *78*(2), 369–384. <https://doi.org/10.1016/j.ecss.2008.01.002>
- Villnäs, A., Perus, J., & Bonsdorff, E. (2011). Structural and functional shifts in zoobenthos induced by organic enrichment—implications for community recovery potential. *Journal of Sea Research*, *65*(1), 8–18. <https://doi.org/10.1016/j.seares.2010.06.004>
- Villnäs, Anna, Hewitt, J., Snickars, M., Westerbom, M., & Norkko, A. (2018). Template for using biological trait groupings when exploring large-scale variation in seafloor multifunctionality: *Ecological Applications*, *28*(1), 78–94. <https://doi.org/10.1002/eap.1630>
- Vita, R., & Marin, A. (2007). Environmental impact of capture-based bluefin tuna aquaculture on benthic communities in the western Mediterranean. *Aquaculture Research*, *38*(4), 331–339. <https://doi.org/10.1111/j.1365-2109.2007.01649.x>
- Wang, X., Cuthbertson, A., Gualtieri, C., & Shao, D. (2020). A Review on Mariculture Effluent: Characterization and Management Tools. *Water*, *12*(11), 2991. <https://doi.org/10.3390/w12112991>
- Ward Jr, J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association*, *58*(301), 236–244.
- Warwick, R., & Clarke, K. (1991). A Comparison of some methods for analysing changes in benthic community structure. *Journal of the Marine Biological Association of the United Kingdom*, *71*(01), 225–244. <https://doi.org/10.1017/S0025315400037528>
- Warwick, R. M., & Clarke, K. R. (1993). Increased variability as a symptom of stress in marine communities. *Journal of Experimental Marine Biology and Ecology*, *172*(1–2), 215–226. [https://doi.org/10.1016/0022-0981\(93\)90098-9](https://doi.org/10.1016/0022-0981(93)90098-9)
- Wauchope, H. S., Amano, T., Geldmann, J., Johnston, A., Simmons, B. I., Sutherland, W. J., & Jones, J. P. G. (2020). Evaluating impact using time-series data. *Trends in Ecology & Evolution*, *36*(3), 1–10. <https://doi.org/10.1016/j.tree.2020.11.001>
- Weitzman, J., & Bailey, M. (2019). Communicating a risk-controversy: Exploring the public discourse on net-pen aquaculture within the Canadian media. *Aquaculture*, *507*(2), 172–182. <https://doi.org/10.1016/j.aquaculture.2019.04.025>

- Weitzman, J., Steeves, L., Bradford, J., & Filgueira, R. (2019). Chapter 11 - Far-Field and Near-Field Effects of Marine Aquaculture. In C. B. T.-W. S. and E. E. (Second E. Sheppard (Ed.) (pp. 197–220). Academic Press. <https://doi.org/10.1016/B978-0-12-805052-1.00011-5>
- Welch, A. W., Knapp, A. N., El Tourky, S., Daughtery, Z., Hitchcock, G., & Benetti, D. (2019). The nutrient footprint of a submerged-cage offshore aquaculture facility located in the tropical Caribbean. *Journal of the World Aquaculture Society*, 50(2), 299–316. <https://doi.org/10.1111/jwas.12593>
- Whilatch, R. B., Lohrer, A. M., & Thrush, S. F. (2001). Scale-dependent recovery of the benthos: Effects of larval and post-larval life stages. *Organism-Sediment Interactions*, 181–198.
- Whitaker, D., & Christman, M. (2014). Clustsig: Significant cluster analysis. *R Package Version, 1*, 2–4.
- White, C. A., Nichols, P. D., Ross, D. J., & Dempster, T. (2017). Dispersal and assimilation of an aquaculture waste subsidy in a low productivity coastal environment. *Marine Pollution Bulletin*, 120(1–2), 309–321. <https://doi.org/10.1016/j.marpolbul.2017.05.042>
- Whitlatch, R. B., Lohrer, A. M., & Thrush, S. F. (2001). Scale-dependent recovery of the benthos: effects of larval and post-larval stages. *Organism–Sediment–Interaction*, 21, 181–197.
- Witte, U., Wenzhöfer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., ... Jørgensen, B. B. (2003). In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. *Nature*, 424(6950), 763. <https://doi.org/10.1038/nature01799>
- Wood, S. N. (2003). Thin plate regression splines. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, 65(1), 95–114. <https://doi.org/10.1111/1467-9868.00374>
- Wood, S. N. (2006). *Generalized additive models: an introduction with R*. Chapman and Hall/CRC.
- Wood, S. N., & Augustin, N. H. (2002). GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. *Ecological Modelling*, 157(2–3), 157–177. [https://doi.org/10.1016/S0304-3800\(02\)00193-X](https://doi.org/10.1016/S0304-3800(02)00193-X)
- Wood, S. N., Bravington, M. V., & Hedley, S. L. (2008). Soap film smoothing. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, 70(5), 931–955.

- Woodcock, S. H., Strohmeier, T., Strand, Ø., Olsen, S. A., & Bannister, R. J. (2018). Mobile epibenthic fauna consume organic waste from coastal fin-fish aquaculture. *Marine Environmental Research*, 137, 16–23. <https://doi.org/10.1016/j.marenvres.2018.02.017>
- Yuan, L. L., & Norton, S. B. (2003). Comparing responses of macroinvertebrate metrics to increasing stress. *Journal of the North American Benthological Society*, 22(2), 308–322. <https://doi.org/10.2307/1468000>
- Zhang, C., Luo, L., Xu, W., & Ledwith, V. (2008). Use of local Moran's I and GIS to identify pollution hotspots of Pb in urban soils of Galway, Ireland. *Science of the Total Environment*, 398(1–3), 212–221. <https://doi.org/10.1016/j.scitotenv.2008.03.011>
- Zhou, J., Feng, K., Li, Y., & Zhou, Y. (2016). Factorial Kriging analysis and sources of heavy metals in soils of different land-use types in the Yangtze River Delta of Eastern China. *Environmental Science and Pollution Research*, 23(15), 14957–14967. <https://doi.org/10.1007/s11356-016-6619-z>
- Zhulay, I., Reiss, K., & Reiss, H. (2015). Effects of aquaculture fallowing on the recovery of macrofauna communities. *Marine Pollution Bulletin*, 97(1), 381–390. <https://doi.org/https://doi.org/10.1016/j.marpolbul.2015.05.064>
- Zuur, A., Ieno, E. N., & Smith, G. M. (2007). *Analyzing ecological data*. Springer Science & Business Media.

APPENDIX I: LIST OF TAXA AND THEIR AMBI ECOLOGICAL GROUP DESIGNATIONS

Alphabetized list of taxa and their respective AZTI Marine Biotic Index Ecological Group (AMBI EG) designations observed in Port Mouton Bay in 2009, 2010 and 2011. Explanations of the AMBI EGs are included in the main text.

Taxa	AMBI EG	Year(s) present		
<i>Aceroides</i> sp.	ignored	2010		
Actinian	ignored	2009	2010	
<i>Agriopoma morrhuanum</i>	I	2009		
<i>Alitta virens</i>	III	2009	2010	
<i>Ameritella agilis</i>	II	2009	2010	2011
<i>Ampelisca macrocephala</i>	I	2009	2010	
<i>Ampharete oculata</i>	II	2009	2010	2011
<i>Amphipoda</i> sp.	ignored	2010		
<i>Anonyx sarsi</i>	II	2009		
<i>Aphelochaeta marioni</i>	IV	2009		
<i>Argissa hamatipes</i>	II	2009	2010	2011
<i>Aricidea catherinae</i>	II	2009	2010	2011
<i>Aricidea wassi</i>	I	2011		
<i>Astarte</i> sp.	I	2010		
<i>Astarte subaequilatera</i>	I	2011		
<i>Baffinicythere emarginata</i>	ignored	2011		
<i>Bathymedon</i> sp.	ignored	2011		
<i>Bopyridae</i> sp.	ignored	2010		
<i>Calanoida</i> sp.	ignored	2011		
<i>Campylaspis</i> sp.	ignored	2010		
<i>Cancer pagarus</i>	III	2010		
<i>Capitella capitata</i>	V	2009	2010	2011
<i>Caprella</i> sp.	ignored	2010		
<i>Caulleriella</i> sp.	III	2011		
<i>Cerebratulus lacteus</i>	II	2009	2010	2011
<i>Cerianthidae</i> sp.	I	2011		
<i>Chaetozone</i> sp.	ignored	2010	2011	
<i>Chiridotea almyra</i>	II	2010		
<i>Chiridotea coeca</i>	II	2009		
<i>Clymenella torquata</i>	I	2009	2010	2011
<i>Coryphella</i> sp.	ignored	2010		
<i>Cossura longocirrata</i>	IV	2009	2010	2011
<i>Crangon septemspinosa</i>	I	2009	2010	2011
<i>Crenella decussata</i>	I	2010	2011	
<i>Cyathura carinata</i>	III	2009		
<i>Cythere</i> sp.	ignored	2009		
<i>Cytheridea</i> sp.	ignored	2011		

Taxa	AMBI EG	Year(s) present		
<i>Cytherura</i> sp.	ignored	2009		
<i>Dexamine thea</i>	III	2010		
<i>Diastylis sculpta</i>	II	2009	2010	2011
<i>Diplodonta</i> sp.	not assigned	2009		
<i>Dipolydora quadrilobata</i>	IV	2009		
<i>Dosinia</i> sp.	not assigned	2009		
<i>Dyopedos porrectus</i>	III	2009	2010	2011
<i>Echinarachnius parma</i>	I	2009	2010	
<i>Echinoidea</i> sp.	I	2009		
<i>Edotia montosa</i>	II	2009	2010	2011
<i>Edotia triloba</i>	II	2009		
<i>Edwardsiidae</i> sp.	II	2011		
<i>Ensis</i> sp.	ignored	2011		
<i>Eteone flava</i>	III	2011		
<i>Eteone longa</i>	III	2009	2010	2011
<i>Euchone elegans</i>	II	2010	2011	
<i>Euchone incolor</i>	II	2010	2011	
<i>Euchone rubrocincta</i>	II	2009		
<i>Euchone</i> sp.	not assigned	2009		
<i>Euclymene zonalis</i>	I	2009		2011
<i>Eudorella truncatula</i>	I		2010	2011
<i>Galathowenia oculata</i>	III	2011		
<i>Gammaropsis</i> sp.	not assigned	2010		
<i>Gitanopsis bispinosa</i>	not assigned	2010		
<i>Gitanopsis</i> sp.	ignored	2011		
<i>Glycera capitata</i>	II	2009		
<i>Glycera dibranchiata</i>	II	2009	2010	2011
<i>Goniada maculata</i>	II	2009		
<i>Goniada</i> sp.	ignored	2011		
<i>Halacaridae</i> sp. (sea mites)	I	2011		
<i>Harmothoe imbricata</i>	II	2010		
<i>Harpacticoida</i> sp.	ignored	2011		
<i>Harpinia propinqua</i>	I	2009	2010	2011
<i>Hartmania moorei</i>	ignored	2010		
<i>Heteromastus filiformis</i>	IV	2009	2010	
<i>Hiatella arctica</i>	I	2011		
<i>Hippomedon serratus</i>	I	2009		
<i>Hirudinea</i> sp.	IV	2011		
<i>Hypereteone lactea</i>	III	2009		
<i>Ischyrocerus anguipes</i>	II	2010		
<i>Kirkegaardia annulosa</i>	III	2011		
<i>Lacuna</i> sp.	not assigned		2010	2011
<i>Lanassa venusta</i>	I	2011		
<i>Laonice cirrata</i>	II	2009		

Taxa	AMBI EG	Year(s) present		
<i>Leitoscoloplos fragilis</i>	III		2010	2011
<i>Leitoscoloplos robustus</i>	IV	2009		
<i>Leptocythere</i> sp.	III	2009	2010	2011
<i>Leucon americanus</i>	II	2009		
<i>Limecola balthica</i>	III	2009	2010	
<i>Macoma</i> sp.	ignored	2011		
<i>Mediomastus ambiseta</i>	III		2010	2011
<i>Mediomastus californiensis</i>	III	2009		
<i>Metopella angusta</i>	II	2009	2010	2011
<i>Micronephthys neotena</i>	II	2011		
<i>Micronephthys neotena</i>	II	2010		
<i>Micronephthys</i> sp.	ignored	2011		
<i>Microphthalmus aberrans</i>	II	2009	2010	2011
<i>Microspio mecznikowiana</i>	III	2010		
<i>Modiolus modiolus</i>	II	2009	2010	2011
<i>Monocorophium sextonae</i>	III	2009	2010	
<i>Monoculodes</i> sp.	ignored	2009		
<i>Mya truncata</i>	II	2011		
<i>Mysis mixta</i>	II	2010		
<i>Nemertean</i> sp.	III	2010		
<i>Nephtys caeca</i>	II	2009	2010	
<i>Nephtys incisa</i>	II	2009	2010	2011
<i>Nephtys longosetosa</i>	II	2011		
<i>Ninoe nigripes</i>	III	2009	2010	2011
<i>Notoproctus oculatus</i>	II	2010		
<i>Nucula proxima</i>	I	2009	2010	2011
<i>Odostomia</i> sp.	ignored	2009	2010	
<i>Oligochaeta</i> sp.	ignored	2009		
<i>Oniscidae</i> sp.	not assigned	2011		
<i>Ophelina acuminata</i>	III	2009	2010	2011
<i>Ophiuroidea</i> sp. (laevae)	II	2011		
<i>Opisthobranchia</i> sp.	ignored	2009		
<i>Orchomenella minuta</i>	II	2009	2010	2011
<i>Ostracoda</i> sp.1	ignored	2009		
<i>Ostracoda</i> sp.1 (hard)	ignored	2010		
<i>Ostracoda</i> sp.2	ignored	2009		
<i>Ostracoda</i> sp.2 (soft)	ignored	2010		
<i>Owenia fusiformis</i>	II	2009	2010	
<i>Parapleustes gracilis</i>	not assigned	2010		
<i>Parapleustes</i> sp.	ignored	2010		
<i>Parexogone hebes</i>	II	2009		
<i>Parougia caeca</i>	IV		2010	2011
<i>Parvicardium pinnulatum</i>	I	2011		
<i>Parvicardium</i> sp.	ignored	2009	2010	

Taxa	AMBI EG	Year(s) present		
<i>Pectinaria gouldii</i>	I	2010		
<i>Petaloproctus tenuis</i>	II	2010		
<i>Pherusa affinis</i>	I	2009	2010	2011
<i>Pherusa plumosa</i>	III	2011		
<i>Pholoe minuta</i>	II	2009	2010	2011
<i>Phoronis</i> sp.	ignored	2009		
<i>Photis pollex</i>	I	2009	2010	2011
<i>Phoxocephalus holbolli</i>	I	2009	2010	2011
<i>Phyllodoce groenlandica</i>	IV	2009	2010	2011
<i>Pitar</i> sp.	II		2010	2011
<i>Pleurogonium</i> sp.	not assigned		2010	2011
<i>Polycirrus medusa</i>	IV	2009	2010	2011
<i>Polycopidae</i> sp.	ignored	2011		
<i>Polydora cornuta</i>	IV	2010		
<i>Prionospio steenstrupi</i>	IV	2009	2010	2011
<i>Pseudoleptocuma minus</i>	ignored	2011		
<i>Pygospio elegans</i>	III	2009		
<i>Retusa obtusa</i>	II	2009	2010	2011
<i>Schistomeringos rudolphi</i>	IV	2009		
<i>Scoletoma fragilis</i>	II	2011		
<i>Scoletoma laurentiana</i>	II	2011		
<i>Scoletoma tenuis</i>	II	2009	2010	
<i>Sipunculida</i> sp.	ignored	2009		
<i>Solemya</i> sp.	I	2009	2010	2011
<i>Sphaerodoropsis minutum</i>	II	2009	2010	2011
<i>Sphaerosyllis</i> sp.	ignored	2010		
<i>Spio filicornis</i>	III	2009	2010	2011
<i>Spiophanes bombyx</i>	III	2009	2010	2011
<i>Stenopleustes</i> sp.	ignored	2009		
<i>Sthenelais limicola</i>	II	2009	2010	2011
<i>Streblosoma</i> sp.	ignored	2010		
<i>Terebellidae</i> (fragment)	I	2011		
<i>Thracia</i> sp.	ignored	2009	2010	2011
<i>Thyasira gouldi</i>	I	2011		
<i>Thyasira</i> sp.	ignored	2010		
<i>Trachycardium</i> sp.	not assigned	2009		
<i>Typhlotanais</i> sp.	ignored	2010		
<i>Unciola inermis</i>	ignored	2009		
<i>Unciola irrorata</i>	I	2009		2011
<i>Yoldia limatula</i>	I	2009		2011
<i>Yoldiella lucida</i>	I	2011		