Spatio-temporal changes in the distribution of benthic organisms at finfish farm sites on the south coast of Newfoundland

by

© Joseph Montague Mersereau

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Abstract

In Newfoundland, assessing benthic community changes associated with organic matter enrichment near finfish farms is challenging because the majority of aquaculture sites are in deep waters, over hard, patchy substrata, where soft sediment sampling methods are ineffective. A promising approach is the use of video surveys to analyze epibenthic characteristics, including taxa that are indicative of organic matter enrichment, *Beggiatoa spp.* and OPC. Here, I compare seafloor biotic and abiotic characteristics on temporal and spatial scales over 2 years and at six locations. Benthic organisms and indicators were identified and evaluated in relation to distance to cage, aquaculture production status and environmental conditions. Abundance and percent cover of the benthos, with the exception of bio-indicators, was higher in non-production sites compared to the production and fallow sites, with minimal changes over time. Organic enrichment via aquaculture can lead to long term benthic changes (> 1 year) on hard substrata.

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"Determine never to be idle. No person will have occasion to complain of the want of time, who never loses any. It is wonderful how much may be done, if we are always doing."

— Thomas Jefferson

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List of Abbreviations and Symbols

ADCP – Acoustic Doppler Current Profiler

BCMOE – British Columbia Ministry of Environment

DELG – Department of Environment and Local Government

FAO – Food and Agriculture Organization of the United Nations

IOE – Indicators of Organic Enrichment

NL – Newfoundland

NLDFA – Newfoundland and Labrador Department of Fisheries and Aquaculture

NSDFA – Nova Scotia Department of Fisheries and Aquaculture

OM – Organic Matter

OPC – Opportunistic Polychaete Complex

R – Spearman Correlation Coefficient

Chapter 1. Introduction and overview

1.1 Salmonid aquaculture in Newfoundland

Aquaculture, the farming of aquatic flora and fauna, currently accounts for ~ 50% of the seafood produced for human consumption (FAO, 2011). Aquaculture is conducted on every continent, excluding Antarctica, and is currently the fastest growing animal food sector globally (FAO, 2011). The substantial growth of the aquaculture industry can be related to the increase in demand for seafood and the stagnant production of the capture fisheries (FAO, 2011). Aquaculture is needed in order to supply this demand both globally and locally; it is predicted that by 2025 an additional 416 000 tonnes of seafood is needed to supply North America's demand (FAO, 2011). Aquaculture production in Canada has steadily increased in the last twenty years and as of 2011 had an approximate production value of ~ $845 000 (DFO, 2013). In Newfoundland and Labrador (NL) the aquaculture industry has also shown progressive expansion especially in terms of salmonid production. From 2006 to 2012, the NL industry has grown ~116% in terms of total export value (reaching $113 million) and ~130% in terms of salmonid production (NLDFA, 2013). In 2012, there were 145 licenses leasing an approximate water area of ~6089 hectares (NLDFA, 2013) though not all of them are active at a given time.

In Newfoundland, salmonid farming is mainly located in the Coast of Bays region, on the south shore of the island (NLDFA, 2013) (Figure 1). This area is characterized by fjords of over 300 m in depth and protected bays, with benthic substrates predominated by rock and cobble along with patchy depositional areas (Anderson et al., 2005). Salmonids are raised in cages and the typical biomass harvested from one site can range

from 250 000 kg – 1 000 000 kg and depending on farming practices and size of site. The production cycle for Atlantic salmon is generally 16-24 months but on occasion is extended for logistical or fish health purposes (Boghen, 1995). After a production cycle, for biosecurity (i.e., fish health) reasons, farms are required to remain fallow for at least one year (Aquaculture Management Directorate, 2010). Aquaculture sites can be and are consistently utilized for more than one production cycle as a long as they abide by the mandatory fallow period.

1.2 Impacts of aquaculture on the benthos

Although the Newfoundland and Labrador government has supported the aquaculture industry due to economic implications of job creation and access to domestic and international markets (NLDFA, 2013). There are concerns over the potential impact to the benthos from the accumulation of feed and feces originating from salmonid farms (NLDFA, 2014). Consultations by Newfoundland and Labrador Fisheries and Aquaculture department (NLDFA) with industry, community organizations, government organizations, and academia revealed that the effects of feed and feces on the environment were one of the top five sustainable management issues (NLDFA, 2014). Feed and feces can accumulate and cause organic matter (OM) enrichment of the benthic environment (Crawford, 2003). OM enrichment is known to cause geochemical changes in the sediment (Holmer et al., 2005) and when severe, can change benthic community composition (Pearson, 1975). The geochemical changes include a reduction in oxygen

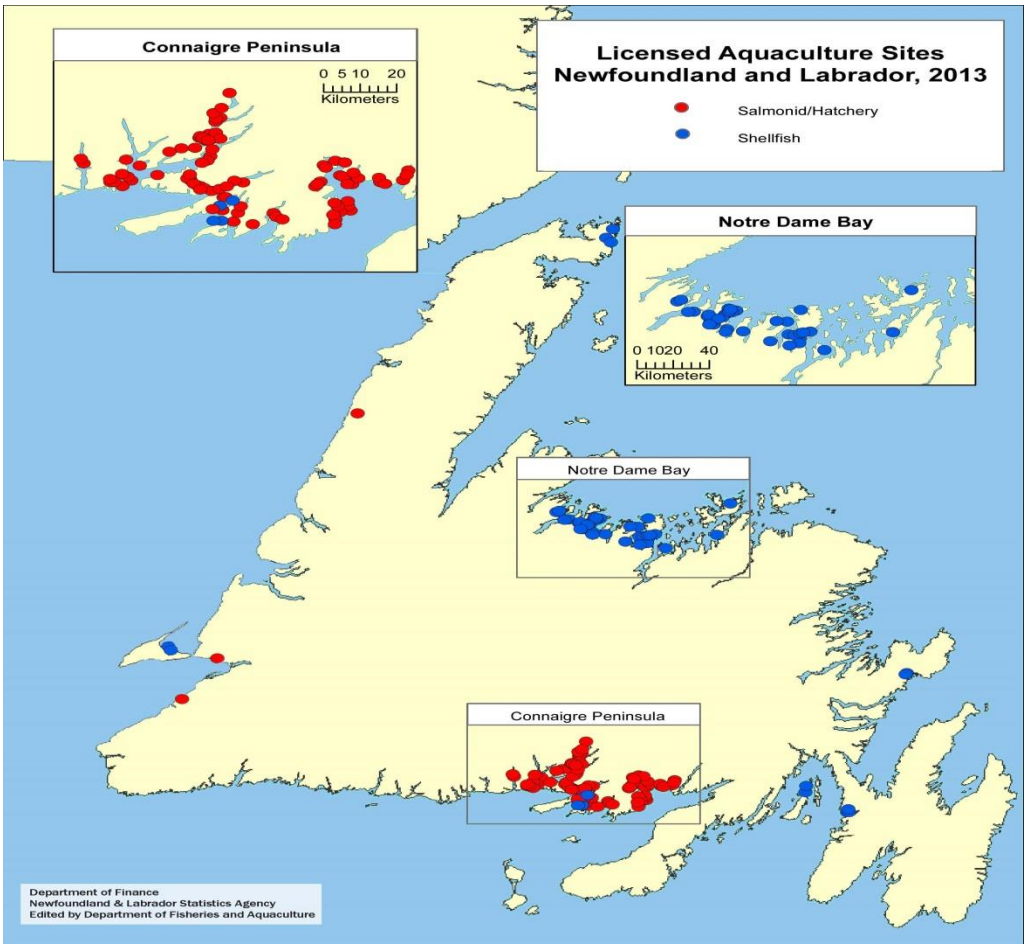

Figure 1. Licensed aquaculture sites in Newfoundland in 2013. Source: NLDFA (2013).

(Mazzola et al., 2000) and increase in sulphides (Tomassetti & Porrello, 2005) due to microbial activity in benthic substrates (Brooks et al., 2003). The biotic changes caused by OM enrichment are changes in abundance, community structure, diversity, a decrease in the size of species and increases in opportunistic species (Pearson & Rosenberg, 1978; Huang et al., 2012).

1.3 Environmental monitoring approaches

Environmental monitoring of the benthic habitat at finfish aquaculture sites is common practice worldwide. The variables monitored differ between countries and even regions due to habitat differences and government policies (Crawford et al., 2002). Kalantzi and Karakassis (2006) revealed that over 120 geochemical and biological variables were used to determine the benthic impact of fish farming (Kalantzi & Ioannis, 2006). These techniques typically measure community structure or chemical characteristics of sediments (Wildish et al., 2001). Measuring sediment geochemistry is the preferred method because of its relatively lower cost and time required for analysis (Wildish et al., 2001). Measuring sediment redox potential and sulphide concentrations are the favoured methods as they are associated with microbial activity that implies a change from aerobic to anaerobic respiration which is commonly found in organically enriched areas (Wildish et al., 1999; Hargrave et al., 2008). Diver cores or sediment grabs are typically used to obtain samples that can be further analyzed to determine sulphide and redox levels. Alternatively, environmental monitoring can be undertaken by measuring benthic community composition using soft sediment techniques. These

techniques can be costly due to the extensive training and time required to properly identify and classify organisms. However, measuring benthic community composition gives direct biological evidence of changes in benthic composition.

In Canada, each jurisdiction has specific thresholds for sulphide concentrations and if exceeded aquaculturists are required to implement best management practices, mitigation measures, additional monitoring, and/or increase the fallow period to decrease the potential impacts on the benthic habitat (BCMOE, 2002; NLDFA, 2010; NSDFA, 2011; DELG, 2012). In addition to sediment sampling, many jurisdictions also include video surveys as supplemental information that can be added to environmental monitoring requirements.

Seafloor imaging is an easy, cost effective and non-destructive technique for monitoring marine environments (Crawford et al., 2002). Video or still images form a permanent record that can be used and interpreted at any point. In the last decade, video techniques have been used more frequently to observe underwater macrofauna and habitat (Pelletier et al., 2012) and can provide sufficient taxonomic resolution to detect changes (Houk & Woesik, 2006). Video recordings are widely used in combination with other approaches to monitor the environmental impact of aquaculture, as this technique can provide data on the presence, absence, abundance or percent coverage of identifiable taxa including indicator species such as *Beggiatoa spp.* and OPC (Crawford et al., 2002; Angel & Spanier, 2002; Crawford, 2003; Brooks et al., 2003; Kamp et al., 2008). The use of video surveys as an environmental monitoring tool is of importance in the context of the Newfoundland aquaculture industry. The majority of aquaculture sites in the south coast of Newfoundland are located over areas with hard and patchy substrates, where sampling

using sediment grabs, diver cores and sediment imaging profiling is inefficient and often impossible to do. Sediment grabs often fail to collect samples due to the rocky substrate and diver cores are dangerous to obtain due to the depth of the sites. Visual surveys of the seafloor have been proposed and used as an alternative to sediment-based techniques in the challenging Newfoundland environment (DFO, 2012; Hamoutene et al., 2013).

1.4 Bio-indicators of organic enrichment

Changes to community structure can take place in highly organically enriched areas (Pearson & Rosenberg, 1978). The presence and/or dominance of opportunistic species can be used to determine the degree or intensity of organic enrichment (Bustos-Baez & Frid, 2003). Two common biotic indicators of organic enrichment are *Beggiatoa spp.* and opportunistic polychaete complexes (OPC). These indicators have also been identified in proximity to aquaculture sites in NL (Bungay, 2012; Hamoutene et al., 2013; Hamoutene, 2014). Bacteria belonging to the genus *Beggiatoa* are commonly found at the oxygen/sulphide interface where they form filamentous white mats (Preisler et al., 2007) and are typically associated with elevated sulphide levels (Hargrave et al., 2008). Mats of *Beggiatoa* have been considered as indicators of organic enrichment (Gray & Elliot, 2009) including in benthic habitats that have been degraded by aquaculture organic input (Crawford, 2001). OPC is a conglomerate of polychaetes frequently observed in areas of organic enrichment and reduced conditions (Tomassetti & Porrellp, 2005). Although the species of worms forming OPC may vary, they are generally utilized as indicators because of their tolerance to severe hypoxia and opportunistic responses to organic

enrichment: they exhibit rapid exponential growth and become sexually mature within four weeks (AMR Ltd., 2008). The prevalence of these indicator species along with the abundances of other epibenthic species can aid in the understanding of benthic habitat quality.

1.5 Thesis objectives

Aquaculture sites are continually monitored and are required to be in compliance with environmental thresholds set by regulatory bodies (DFO, 2010). However, the thresholds set in Canada are designed for soft sediment sampling and have yet to be adapted to hard bottom substrates. In order to properly regulate the Newfoundland aquaculture industry, which is located over hard and patchy substrates, we must be able to understand the extent of the potential impact from OM enrichment on benthic assemblages over temporal and spatial scales.

To understand the influence of OM enrichment originating from aquaculture sites on benthic biota, we must first characterize the seasonal or temporal variation in abiotic factors, and in the biota, in this area. The second chapter of this thesis aims to determine, with the use video surveys, the abiotic and biotic characteristics of subtidal benthic habitats on the south coast of Newfoundland. Specifically, the survey data are used to determine how benthic taxa are distributed according to depth, temperature, dissolved oxygen, substrate type, sampling date, and aquaculture production status (i.e., sites with no production, with production, and undergoing fallow are compared). By analyzing these

datasets, we can start to identify trends regarding the influence of organic enrichment on benthic organisms in this region.

The third chapter aims to understand how the relative abundance of visible epibenthic taxa is affected both spatially and temporally by organic enrichment. The presence and abundance of taxa are examined in areas that undergo a continuous source of OM enrichment, in areas that are recovering from OM enrichment, and in areas not experiencing such enrichment. Relative abundances of taxa are related to distance from the source of OM enrichment (aquaculture cages) and to the recovery processes during the fallow period.

The fourth chapter focuses on indicator species (*Beggiatoa spp.* and OPC): their spatial distribution and percent cover are investigated to determine relationships with bathymetric properties and distance to cages. In addition, temporal changes in these indicator species and potential relationships between them are investigated.

Finally, fifth chapter draws general conclusions from all chapters and suggests areas for further research.

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Chapter 2. Biotic and abiotic characteristics of the seafloor in proximity to aquaculture sites on the south coast of Newfoundland

2.1. Introduction

2.1.1 Monitoring of the environmental impacts of aquaculture in Newfoundland

Salmonid aquaculture is a growing industry in NL, and associated environmental impacts must be considered to ensure its sustainability and long term viability (NLDFA, 2014). At aquaculture sites, organic enrichment of the benthos can occur when fish feces, excess feed, and biofouling organisms are deposited and accumulate onto the ocean bottom (Macleod et al., 2004). Organic enrichment from aquaculture sites has been shown to cause a reduction in species richness, biodiversity and body size, changes in community structure, the disappearance of suspension feeders, and an increase in deposit feeders (Ritz et al., 1989; Hall-Spencer et al., 2006; Hargrave et al., 2008). There is limited information available on impacts of organic enrichment on hard and patchy substrates, such as those that characterize aquaculture sites on the south coast of NL, as most studies have focused on changes to infaunal communities and not to the epibenthos.

Changes to community structure can take place in highly organically enriched areas (Pearson & Rosenberg, 1978), and the presence and/or dominance of opportunistic species can be used to indicate the degree or intensity of organic enrichment (Bustos-Baez & Frid, 2003). Two common biotic indicators of organic enrichment are *Beggiatoa spp.* and opportunistic polychaete complexes (OPC). These indicators have been identified in proximity to aquaculture sites in NL (Hamoutene et al., 2013; Hamoutene,

2014). Bacteria belonging to the genus *Beggiatoa* are commonly found at the oxygen/sulphide interface where they form filamentous white mats (Preisler et al., 2007); they can occur both within sediments and at the surface of various types of substrates. *Beggiatoa spp.* initially forms a thin white mat that increases in thickness as it expands upwards to remain at the oxygen/sulphide interface (Kamp et al., 2008). *Beggiatoa spp.* is present in a wide temperature range and growth is not constrained at low temperatures (Jorgensen et al., 2010). OPC is a term referring to a conglomerate of either a single polychaete species, or multiple species that have been frequently observed in areas of organic enrichment and reduced conditions (Tomassetti & Porrellp, 2005). Such polychaetes have been found to colonize reduced sediments in proximity to aquaculture operations (Kunihiro et al., 2008) and have been utilized as indicator species in both tropical and temperate regions (Brooks et al., 2003; Lee et al., 2006).

Benthic changes due to aquaculture can depend on a number of factors including farming practices, oceanographic conditions, environmental conditions and the assimilative capacity of the local ecosystem for organic matter (OM) (Corner et al., 2006). There are established methods of sampling benthic habitats to determine the level of impact from OM enrichment that differ depending on region, habitat type, or government policy (Crawford et al., 2002). In Canada, soft sediment sampling is commonly used in environmental monitoring programs (BCMOE, 2002; NLDFA, 2010; NSDFA, 2011; DELG, 2012) and focus on the measurement of sediment sulphide and redox after collecting sediment grabs. Regulatory bodies have developed sulphide and redox thresholds for sediment samples that aquaculture operators must abide by (BCMOE, 2002; NLDFA, 2010; NSDFA, 2011; DELG, 2012). However, in NL the

majority of sites are located in deep waters with hard substrates where soft sediment techniques are ineffective and often impossible. Fortunately, video surveys have been shown to be useful for long term monitoring of epibiotic communities (Houk & Woesik, 2006). Current NL regulatory policies use video surveys in aquaculture environmental monitoring reports. It is pertinent to use video surveys in the south coast of NL to analyze the impacts of OM enrichment on the benthos from aquaculture operations (Bungay, 2012). Although there has been extensive research in other temperate regions on the impacts of salmon aquaculture on the benthic environment (Pohle et al., 2001; Brooks et al., 2003), there have only been limited studies conducted on the south coast of NL (Anderson et al., 2005).

Current regulations from the Newfoundland Department of Fisheries and Aquaculture (NLDFA) require applicants to submit a baseline report, with monitoring and reporting conducted by a third party, upon initial license application (NLDFA, 2013). Site owners must also submit two additional reports during fallowing: sampling for "Part 1" must be conducted within a four week period when harvesting is completed and sampling for "Part 2" must be conducted 4-8 weeks prior to restocking. These reports outline the condition of the seafloor at all dates sampled, and require collecting sediment grabs and describing sediment condition (colour and consistency of the substrate). When obtainable, sediment grabs are used to determine sulphide and redox concentrations. Additionally, photos and videos, accompanied by observations of each sample, are required at locations with depths <100 m.

2.1.2 The benthic environment at Newfoundland aquaculture sites

NL coastal benthic substrates are primarily rock and cobble (Anderson et al., 2005). Only a few studies of the composition of the NL near shore benthic biota have been completed (Barrie, 1979; LGL Limited, 2001; Christian et al., 2010; Bungay, 2012), and the aquaculture industry has identified a lack of knowledge concerning the biotic and abiotic characteristics of the seafloor (e.g. seawater temperature, oxygen content, community composition) in proximity to currently operating salmon farms (AMEC, 2002). Limited information on such biotic and abiotic characteristics is available from baseline and fallowing reports which are submitted by environmental monitoring companies to regulatory bodies for compliance purposes. Although the depth measurements in these reports are quantitative, the biological and substrate type data are often qualitative and do not include abundance counts, percent cover of substrates by organisms or calculations of biodiversity. A more thorough benthic sampling is required to document and evaluate temporal changes in physical parameters at the seafloor, as well as in the abundance and habitat preference of benthic biota in this region.

The communities inhabiting hard bottom substrates are commonly comprised of sessile invertebrates such as sponges, cnidarians, ascidians and bryozans (Wenner et al., 1983) with sponges exhibiting the highest biomass among all groups (Wenner et al., 1983; Bungay, 2012). Epifaunal species attached to the substratum are frequently filter feeders (Osman, 1977). Benthic flora and fauna are extremely patchy in their distribution due to competition, predation, patterns of recruitment, distribution of substrata, and physical disturbances (Osman, 1977; Wenner, et al., 1983; Gray & Elliot, 2009; Eleftheriou, 2013). Benthic macrofaunal communites show a direct relationship with

substrate type (Barrie, 1979; Warwick & Uncles, 1980). Wenner et al. (1983) showed that changes in benthic composition in hard bottom communities were related more to differences in depth than to seasonal changes. These authors hypothesized that the difference was due to bottom temperature, as deeper habitats exhibited less temperature variability. However, other studies have shown that natural seasonal environmental changes can contribute to variations in benthic species composition on a temporal scale (Morrisey et al., 1992; Pacheco et al., 2010). Sampling programs should account for these spatial and temporal changes while noting habitat type and environmental conditions to truly understand changes in benthic communities.

2.1.3 Objectives

The objective of this chapter is to describe the biotic and abiotic characteristics of subtidal benthic habitats on the south coast of NL with the use of video surveys in combination with oceanographic recorders. The study was conducted at six locations: two sites with aquaculture cages, two sites undergoing fallow, and two sites not currently experiencing aquaculture production. Physical characteristics investigated include depth, temperature, dissolved oxygen (DO) and substrate type. Biological characteristics studied include the presence, abundance or percent cover of identifiable taxa. The biotic and abiotic characteristics of the seafloor along transects at each site are described, and species associations with habitat types, environmental conditions and/or seasonal changes are considered.

Two species associated with organic enrichment, *Beggiatoa spp.* and OPC, are also evaluated; both can be readily observed with video. As these organisms are small and too numerous to count, their presence is quantified as % cover (as for macroalgae). The baseline report and the Part 1- Part 2 reports differ in sampling design from our study, however data from these reports are drawn upon in this study.

2.2. Materials and methods

2.2.1 Study sites

Underwater video sampling occurred at six different sites in two bays on the south coast of Newfoundland: Hermitage Bay and Fortune Bay (Figure 2). Sampling sites represented different habitat conditions (e.g. depth ranges, substrate types) and levels or stages of Atlantic Salmon (*Salmo salar*) production.

Sites were grouped into three classes: Production (P), Non-Production (NP), and Fallow (F) (Table 1). The production sites are coded as P1 and P2. Over the course of this study, P1 produced approximately 490 000 Atlantic Salmon in six cages and P2 produced approximately 354 000 Atlantic Salmon in 11 cages. Non-production sites are coded NP1 and NP2. F1 and F2 refer to two fallow sites located in the same bay. Due to confidentiality agreements with aquaculture producers, the exact coordinates of the sites cannot be displayed.

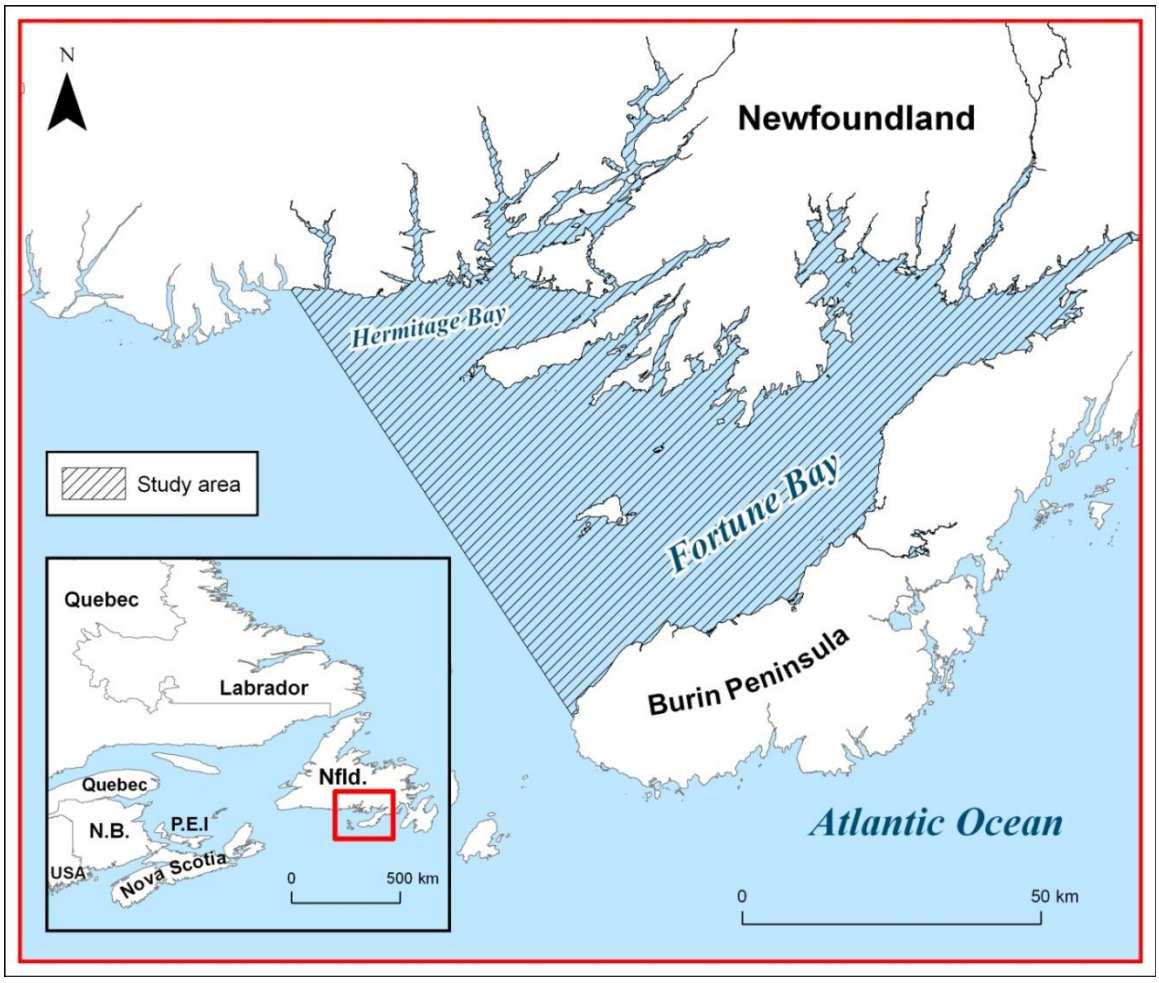

Figure 2. Map of the aquaculture portion of the South Coast of Newfoundland, Canada. Shaded area represents approximate area where sampling of the six sites occurred. Inset shows location relative to Eastern Canada.

Table 1. Details of production, sampling and location of the six sites.

Site	Location	Sampling periods	Number of stations	Number of images analyzed
P1	Hermitage Bay	July 2011	21	21
		Aug 2011	20	20
		Sept 2011	20	20
		Oct 2011	19	19
		June 2012	20	83
		Aug 2012	19	76
		Oct/Nov 2012	18	72
P2	Fortune Bay	Aug 2011	49	49
		Sept 2011	27	27
		Oct 2011	27	27
		June 2012	32	130
		Aug 2012	36	128
		Oct/Nov 2012	32	128
NP1	Hermitage Bay	July 2011	26	26
		Aug 2011	29	29
		Sept 2011	26	26
		June 2012	34	131
		Aug 2012	36	144
		Oct/Nov 2012	35	140
NP2	Fortune Bay	Sept 2011	44	44
		Oct 2011	40	40
		June 2012	42	168
		Aug 2012	43	172
		Oct/Nov 2012	43	172
F1	Hermitage Bay	July 2011	30	30
		Aug 2011	29	29
		Sept 2011	27	27
		June 2012	31	124
		Aug 2012	31	124
F2	Hermitage Bay	Sept 2011	42	42
		Oct 2011	43	43
		June 2012	39	156
		Aug 2012	38	152
		Oct/Nov 2012	40	160

For sites in production, video sampling was initiated during the month when cages were initially stocked, and repeated at various times throughout the production cycle. Sites were sampled on a monthly basis in 2011 (July – October) and on a bimonthly basis in 2012 (June – Oct/Nov). Both sites were in production at all times sampled, and records indicated that this is the first time that aquaculture production has been carried out at these leased bodies of water.

Fallow sites had previously experienced aquaculture production. Prior to video sampling, fish were harvested from the site and cages were left empty for the duration of this study. Video sampling began immediately after fish had been harvested, in July 2011 (F1) and September 2011 (F2). Nets from the cages were removed from the site once harvesting was complete, but some derelict gear, cages (polar circles), ropes and PVC piping, cage floats and frames remained on site. Based on production records, F1 was initially stocked in July 2009. At site F2, several lease holders managed aquaculture production, and there is no information available on this site prior to 2006. F2 was stocked in 2006, harvested in November 2007, and lay fallow until 2009; fish were stocked again in 2009 and were harvested from November 2010 – September 2011.

The production sites were chosen because initial surveys showed that they displayed different depth ranges and habitat conditions. The non-production sites (NP1 and NP2) were selected because their depth profiles resembled those of P1 and P2, respectively, and were within close proximity (~2 km) of the latter sites. Fallowed sites, F1 and F2, had comparable habitat conditions.

2.2.2 Data collection

2.2.2.1 Underwater video camera system

An underwater video camera system was used for imaging the seafloor, following a procedure standardized by DFO for regulatory purposes (DFO 2012). The procedure was specifically designed for the south coast of NL by the Aquaculture Section, Department of Fisheries and Oceans, Canada, 2012.

The camera used was a SV-16HR colour underwater camera with a resolution of 550 TVL, mounted on a stainless steel frame (the frame served as a measurement tool as the camera had two grids within its field of view, measuring 25 X 25 cm and 50 X 50 cm, respectively (Figure 3). The frame was equipped with two 150 watts SV – Q10K underwater lights w, pointing downwards to illuminate the frame and the benthos. The power supply line and 150 m long video cables connected the camera and the lights to a video recording system on board the boat. Depths greater than 150 m were not sampled due to the cable length constraints.

The video camera and lights were connected and powered by the on-board deck box that included a Datavideo digital video recorder, geostamp, glare resistant monitor, and GPS (Garmin GPS map CSX). The GPS had a precision of approximately 10 m. Controls on the deck box allowed for adjustment of light intensity, and for recording of the video to digital storage units. Date, time and GPS coordinates were overlain onto the video.

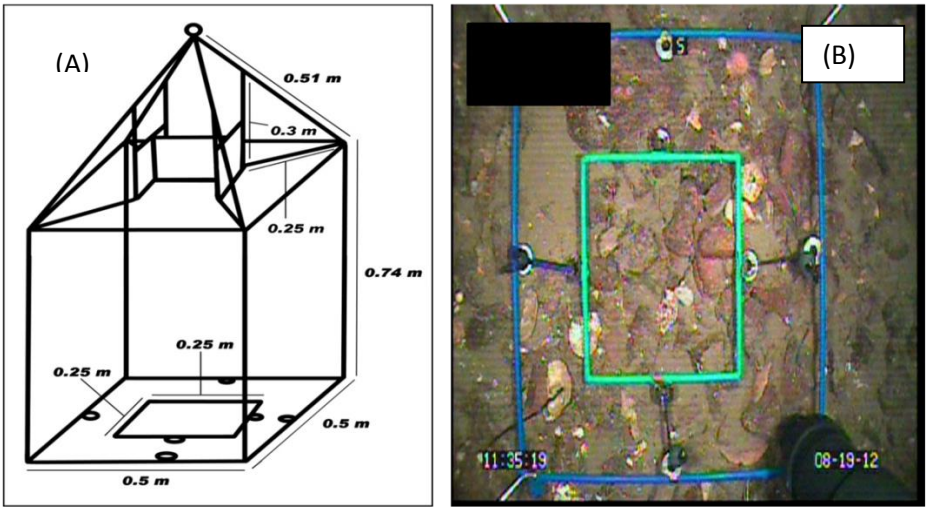

Figure 3. Blueprint for underwater video cage (A) and picture of frame and benthic environment from underwater video camera (B). The blue frame measures 50 x 50 cm and green frame measures 25 cm x 25 cm. The blacked out section shows coordinates for site (DFO 2012).

2.2.2.2 Environmental monitoring equipment

Logging instruments were attached to the cage frame. The Vemco Minilog-II-T submersible data logger recorded temperature and depth in 2011 and 2012. An RBR DO meter recorded depth, temperature and DO in 2012 only. The two instruments were used together to determine the reliability of depth measurements compared to those obtained using a depth sounder. The Vemco and RBR instruments recorded similar results, but the depth sounder measurements were not included in analyses. The Vemco and RBR recorders were deployed concurrently to provide back-up temperature and depth measurements in case of equipment malfunction. Measurements from the RBR DO meter were used primarily, with data extracted from the second device if recording failed. The frame rested on the bottom for at least thirty seconds and the measurements from logging instruments were obtained during this time period.

2.2.3 Sampling protocol

2.2.3.1 Transects

At all sites, video recording was performed at stations situated at 20 m intervals along three transects, each extending to a minimum of 160 m unless water depth was greater than 150 m or an obstruction, cage, shoreline or gear made further sampling impossible. Two transects ran parallel to each other, while the third ran perpendicular to the shoreline (Figure 4). At production sites, transects began at the cage edge and continued outwards. At non-production sites, two transects ran parallel and another

transect was perpendicular. Due to the risk of entanglement with gear, sampling was not conducted on a transect perpendicular to the shore. At fallow sites, the design was the same as for non-production sites but the transects overlapped where cages had existed for aquaculture production. Coordinates were recorded for each station, and given a unique ID based on location and time. All stations were geo-referenced using handheld GPS.

At production sites, the distance of each station from the aquaculture cage edge (representing 0 m) was recorded to the nearest 20 m increment. At fallow sites, any stations located underneath pre-existing cages or on cage edge were designated as 0 m and other stations extended outwards in 20 m increments. Non-production sites did not have a measurable distance from cages.

2.2.3.2 Video recording

At each station, the camera was lowered to the ocean bottom, and recording began when the seafloor was visible. The camera was lowered until it rested on the benthos, and was left on the bottom for at least ten seconds until a clear image of the benthic environment was obtained. In 2011, the camera rested at one area (quadrat) at each station. After reviewing the video and determining that the majority of benthic environments were highly patchy, we decided that the camera would image four quadrats per station for the 2012 field season (Table 1). The camera continually recorded throughout this period until all the four quadrats were viewed.

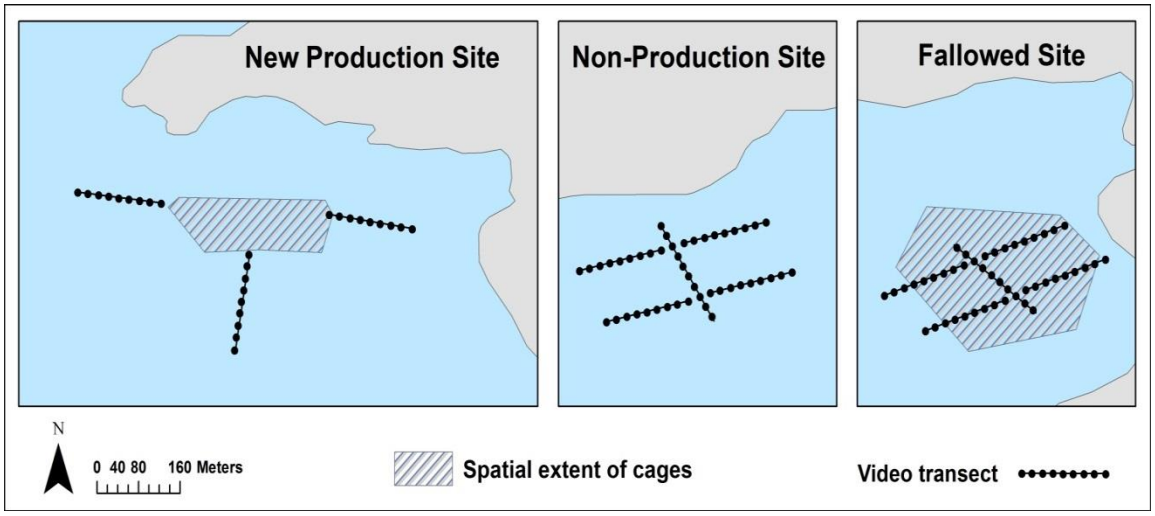

Figure 4. Sampling design for production, control and fallow sites. Dots represent locations where underwater video was recorded.

2.2.4 Video and image analysis

After video recording was completed, all files were converted to the ".avi" format. Video files were viewed on Image grab and VLC Media Player software, so that frames from video could be selected, extracted and converted into image files (jpeg, png). Only frames that corresponded to the cage resting flat on the bottom and displayed a clear image were selected for extraction.

To properly identify organisms and substrate types the Photographic Guide to Benthic Species of Hard Bottom Communities in Southwest Newfoundland was used (DFO 2012). On each image epifaunal, macroalgal and indicator species (*Beggiatoa spp.* and OPC) were identified, along with substrate type (Table 2). Organisms were identified to the lowest possible taxonomic group, and organisms that could not be readily identified due to a combination of video resolution and small size were omitted from the data. For each identifiable taxon, presence, abundance, or percent cover were recorded. Percent cover (in two dimensions, relative to the 50 cm x 50 cm frame area) was calculated for OPC, *Beggiatoa spp.* and macroalgae. Any fast moving organisms that continuously moved in and out of the frame were excluded as counts could be inaccurate (e.g. fish, zooplankton, etc). Substrate type was classified based on a modified Wentworth scale (Table 2). Flocculent matter looks like a black organic non-consolidated layer and separates by clumps when distributed. Flocculent matter appears after organic enrichment and covers up the natural substrate type. Identifying the natural substrate in the presence of flocculent matter was difficult. Therefore, although flocculent matter is not a naturally occurring substrate, it was used as a substrate type. Within each station, the percent cover

of each identified substrate type was determined. The substrate type with the highest percent cover was deemed the dominant substrate type and was utilized for the analysis. To determine substrate composition per site, the substrates were divided by # of images taken for each site and represented as relative proportions. Additional observations, such as presence of feed pellets, shell debris and black sediment, were recorded. Each image was time stamped which allowed temperature, depth and dissolved oxygen to be matched to biotic and abiotic data with the same GPS coordinates.

2.2.5 Environmental monitoring reports from consultants

Provincial and federal regulatory policies require environmental monitoring and reporting when bodies of water are leased to aquaculture companies for the farming of salmon. The environmental monitoring reports include video monitoring performed using comparable procedures and analytical approaches, except for the spatial coverage of sampling. Data from baseline reports were gathered for P1, F1, and F2 in 2009 and for P2 in 2010 and were used to determine pre-site conditions (termed ‘baseline’). As the sampling design and the quantification of species of baseline differed, only stations that were in close proximity (< 20 m) to the cage locations and substrate types were considered.

Table 2. Substrate classification guide use in this study, modified from Wentworth scale (Wentworth 1922).

Class	Description
Bedrock	Bedrock (continuous solid bedrock)
Coarse	Boulder and Rubble (>130mm)
Medium	Cobble and gravel (2-130 mm)
Fine	Sand and mud (<1 mm)
Flocculent matter	Flocculent matter (covered by organic matter, detritus)

2.2.6 Statistics

2.2.6.1 Box and Whisker Plots

Box and whisker plots were used to graphically display differences in data sets on temporal and spatial scales. The line within boxes represents the median, while the upper and lower edges of the box correspond to the 25th and 75th percentiles. The lower whisker extends to lowest data point within 1.5 inter-quartile range and the upper whisker extends to the highest data point within 1.5 inter-quartile range. Outliers are represented by dots. Box and whisker plots were created with R statistical software.

2.2.6.2 Statistical Analysis

The Pearson product-moment correlation coefficient (r) was used to determine correlations between; depth and temperature, DO and depth, abundance and temperature, and abundance and depth. Tukey HSD was used to determine significant pairwise differences of DO concentrations over sampling periods. All statistical analysis was done with R statistical software (R. Development Core Team, 2005). Furthermore, data was tested for normality and homoscedasticity.

2.3. Results

2.3.1 Environmental parameters

Data was recorded from 2829 images, not including baseline data received from environmental monitoring reports.

The depth range for all sites was of 5.6 – 145.7 m with an average depth of 64.2 m; depth varied considerably within each site (Figure 5, Table 3). Sites P1, NP1, and F1 had maximum depths greater than 100 m and depth averages of 79.4 m, 89.8 m and 76.8 m, respectively. All stations at P2, NP2 and F2 had depths < 100 m, and respective average depths of 45.5 m, 50.8 m, and 52.6 m. The P1, NP1 and F1 depth distributions have a bell shape while the P2 and F2 depth distributions are skewed to the right.

When considering all sampling dates and stations, water temperature at the seafloor ranged from 1.4 – 16.1°C with an average of 3.6°C. Temperatures were generally lower and less variable at P1, NP1 and F1 than at P2, NP2 and F2 (Figure 6). Seasonal

and depth patterns in temperature at the seafloor were apparent (Table 3, Figures 6, 7). Temperature showed a significant negative correlation (R= - 0.479, $p < 0.001$) with depth and was lowest during the beginning of each sampling year (July 2011 and June 2012), increasing over the next few months (Figure 6).

DO recordings were obtained during the June 2012, August 2012 and Oct/Nov sampling periods. Overall, DO values recorded ranged from 2.8 – 8.0 $mL.L^{-1}$ with an average of 5.7 $mL.L^{-1}$ (Table 3).

The lowest DO recordings (3.1 and 2.8 $mL.L^{-1}$) were taken at P2 and F2, and highest DO values (8.1 $mL.L^{-1}$) were recorded at NP2 (Figure 8), with no correlation with depth observed. All median values among sites are similar, between 5.5 to 6 $mL.L^{-1}$, except for P2 (5.3 $mL.L^{-1}$) (Figure 9). In 2012, a distinct seasonal pattern is discerned with a decrease in median DO over time (Figure 10). A Tukey HSD test revealed that DO values differed significantly ($p < 0.01$, for all pairwise comparisons) between sampling times: June 2012 – Aug 2012, June 2012 – Oct/Nov 2012, and Aug 2012 – Oct/Nov 2012.

Substrate composition varied among sites and with sampling period (Figure 11); however, the variability with time was relatively low at NP sites. As expected, flocculent matter was not observed at the NP sites (similar results as in baseline reports). At P1, flocculent matter was most dominant in October 2011, occurring in 42.1% of the stations. P2 was mainly comprised of fine sediments which accounted for 79.6% of all the stations at all times considered. At P2 flocculent matter occurred in the highest number of stations

(16 %) in August 2011. F2 had substrates mainly comprising of fine and/or flocculent matter at all times sampled. Considering all sampling dates, 88.7 % of the stations at F1 and 97.7 % of the stations at F2 were classified as fine or flocculent matter substrates (Figure 11). At F1 and F2, the percentage of stations dominated by flocculent matter matter declined with time.

Table 3. Characteristics of the six sites surveyed off the south coast of Newfoundland in 2011 and 2012. Data from all sampling periods are considered. DO: dissolved oxygen.

Site	Depth range (m)	Average depth ± SD (m)	Temperature range (°C)	Temperature average (°C) ± SD	DO range ($mL.L^{-1}$)	DO average ± SD ($mL.L^{-1}$)
P1	47 – 143	79.4 ± 18.8	1.8 – 7.5	3.2 ± 1.5	5.1 – 6.7	5.9 ± 0.3
P2	22 – 56	45.5 ± 9.5	1.8 – 13.6	4.1 ± 2.4	3.1 – 6.6	5.3 ± 0.64
NP1	14 –146	89.8 ± 24.8	1.8 – 15.2	3.0 ± 1.4	4.8 – 6.7	5.6 ± 0.35
NP2	8 – 96	50.8 ± 21.2	1.4 – 16.1	4.3 ± 3.3	4.3 – 8.1	5.8 ± 0.46
F1	40 – 111	76.8 ± 16.4	1.9 – 6.1	2.6 ± 0.6	5.4 – 6.9	6.0 ± 0.48
F2	6 – 63	52.6 ± 12.2	1.9 – 14.8	4.0 ± 2.1	2.8 – 7.2	5.7 ± 0.76

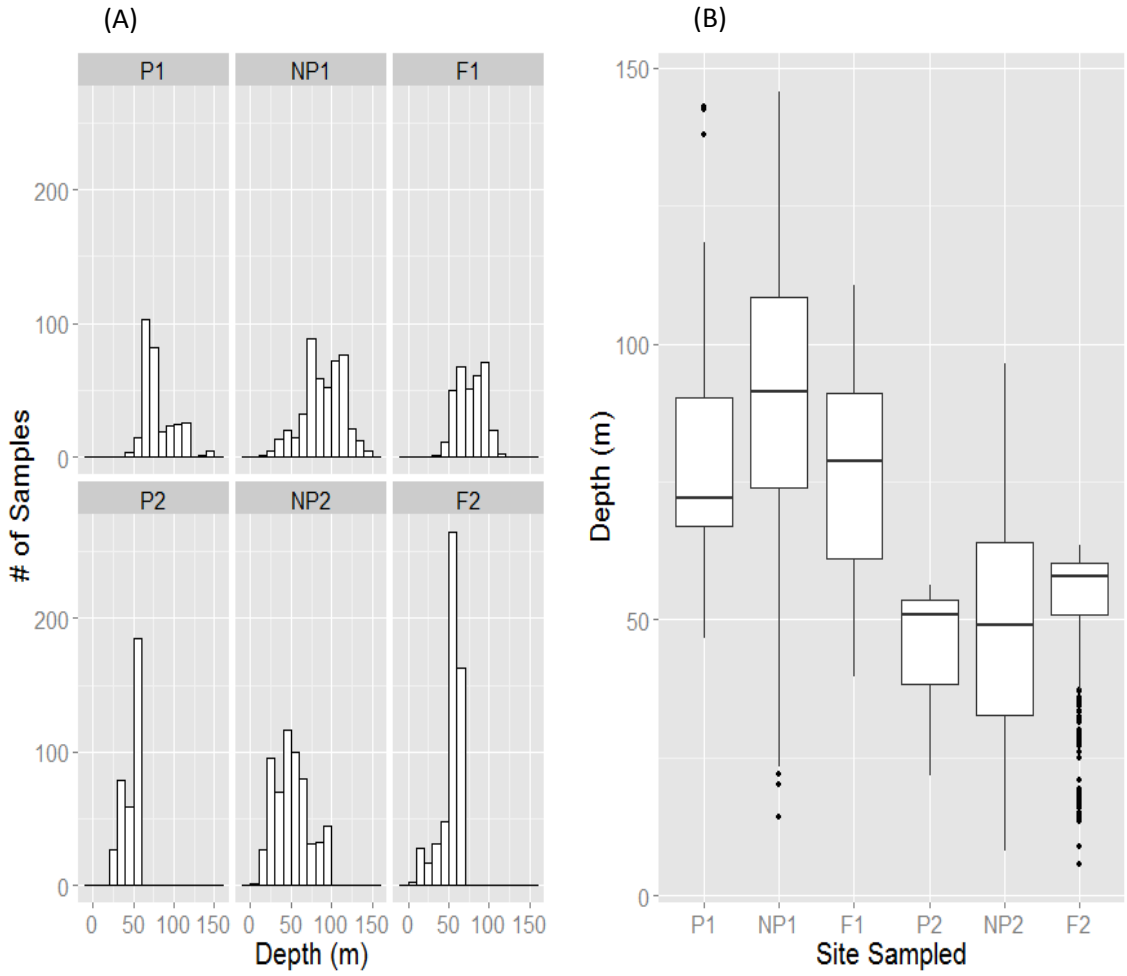

Figure 5. (A) Distribution of station depths at each site surveyed during 2011 and 2012. (B) Boxplots of depths at each site.

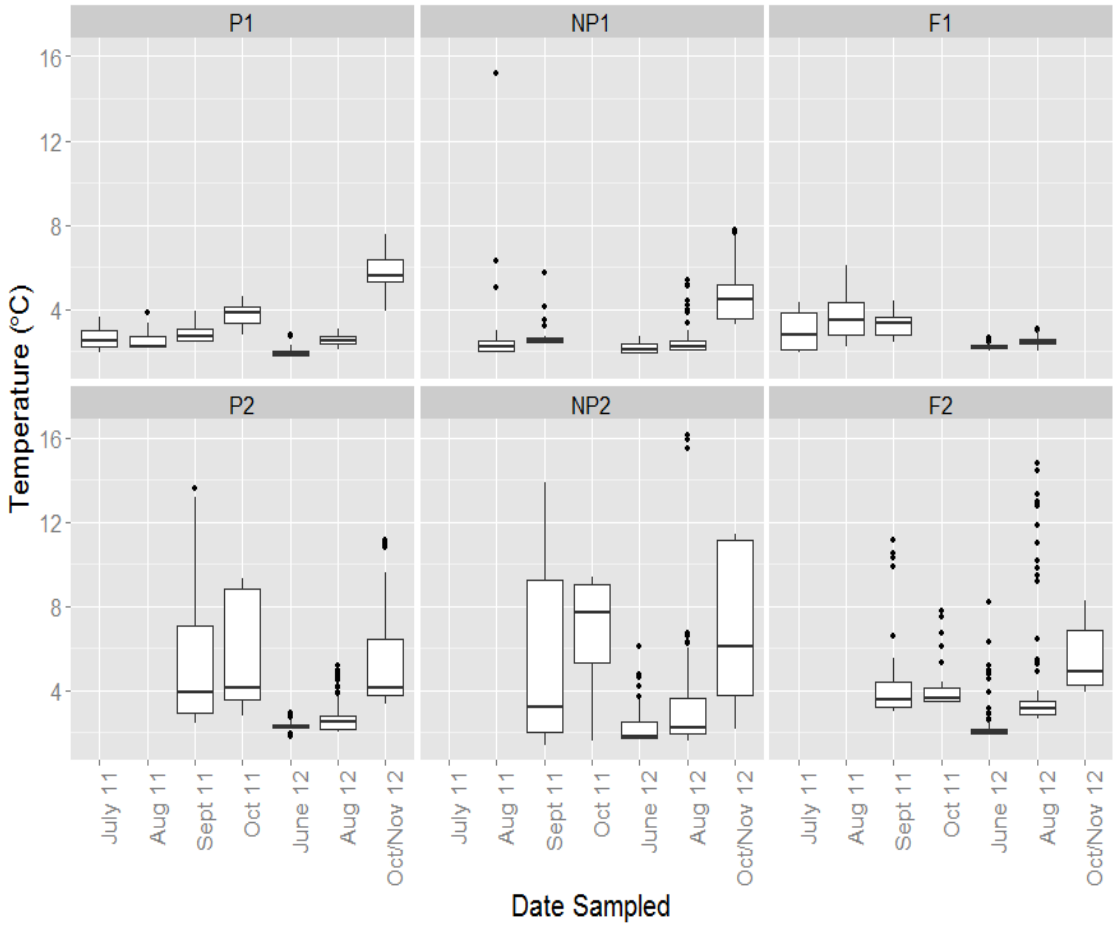

Figure 6. Boxplots of temperature of the seafloor at different sampling dates, for each study site.

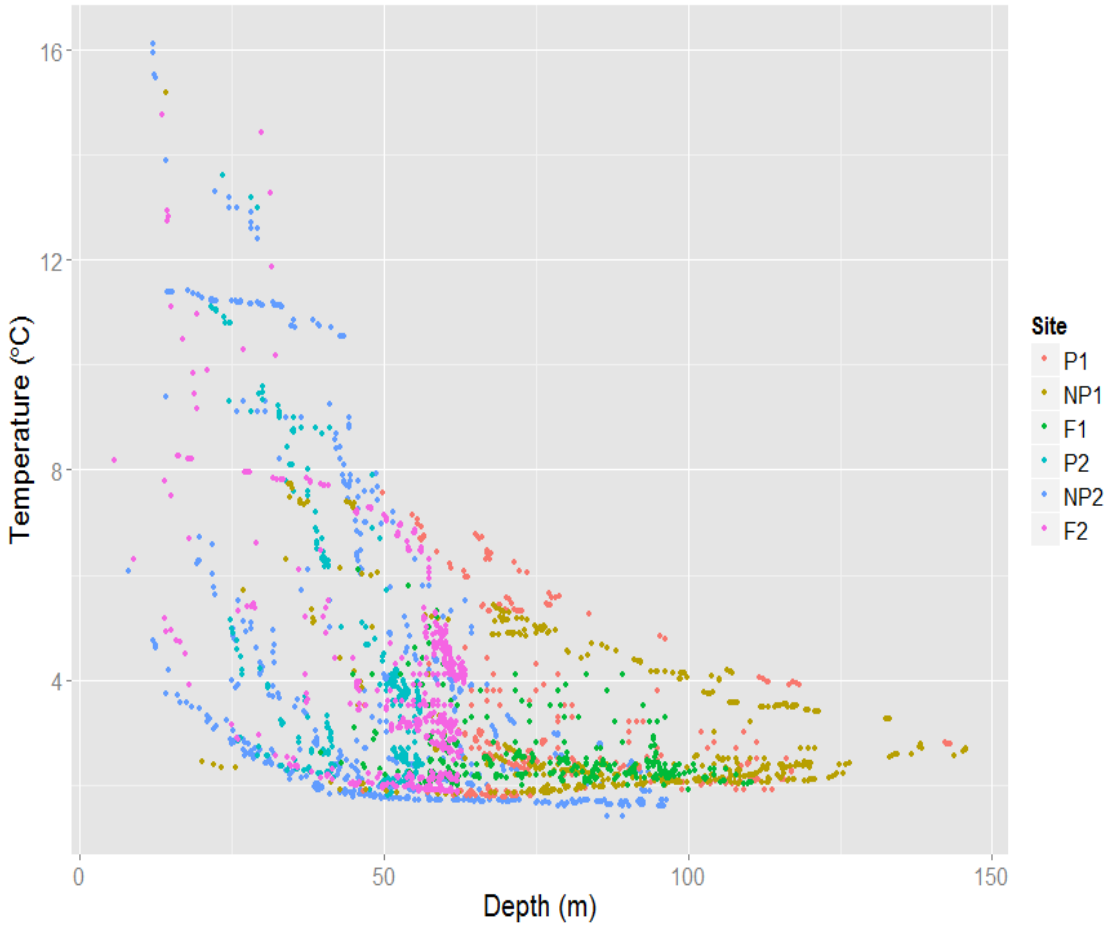

Figure 7. Temperatures recorded at the seafloor according to depth, at all stations and dates surveyed.

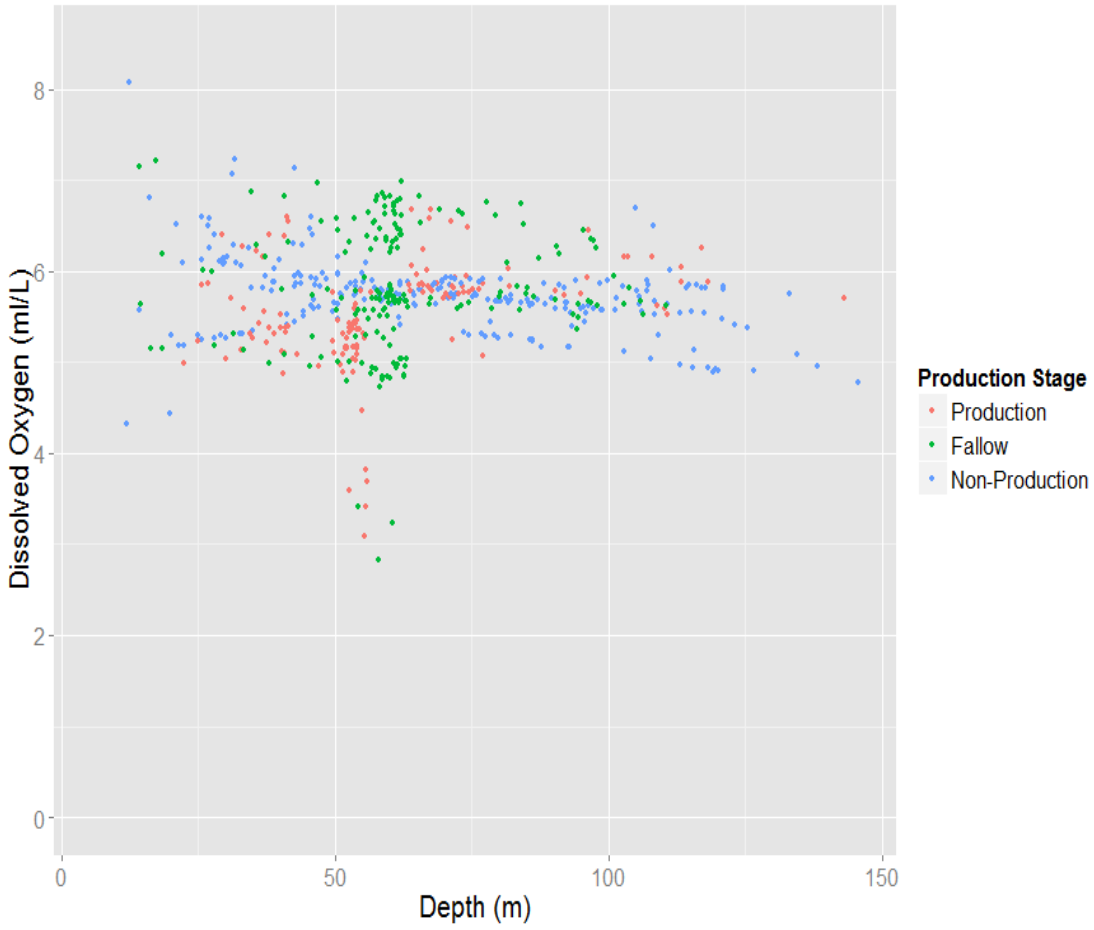

Figure 8. Dissolved oxygen concentration at the seafloor according to depth, for June 2012, August 2012 and Oct/Nov 2012 sampling periods.

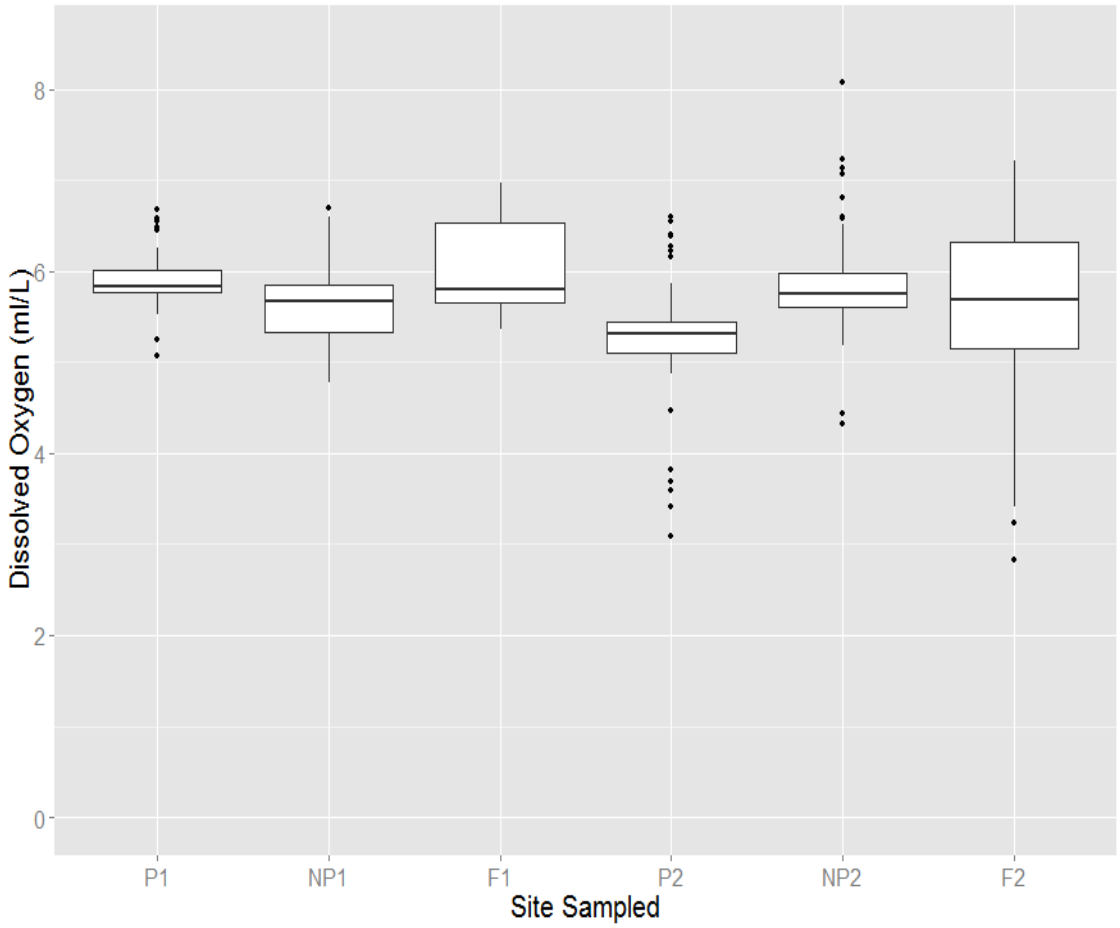

Figure 9. Boxplots of dissolved oxygen concentration at the seafloor at each site for June 2012, August 2012 and Oct/Nov 2012 sampling periods.

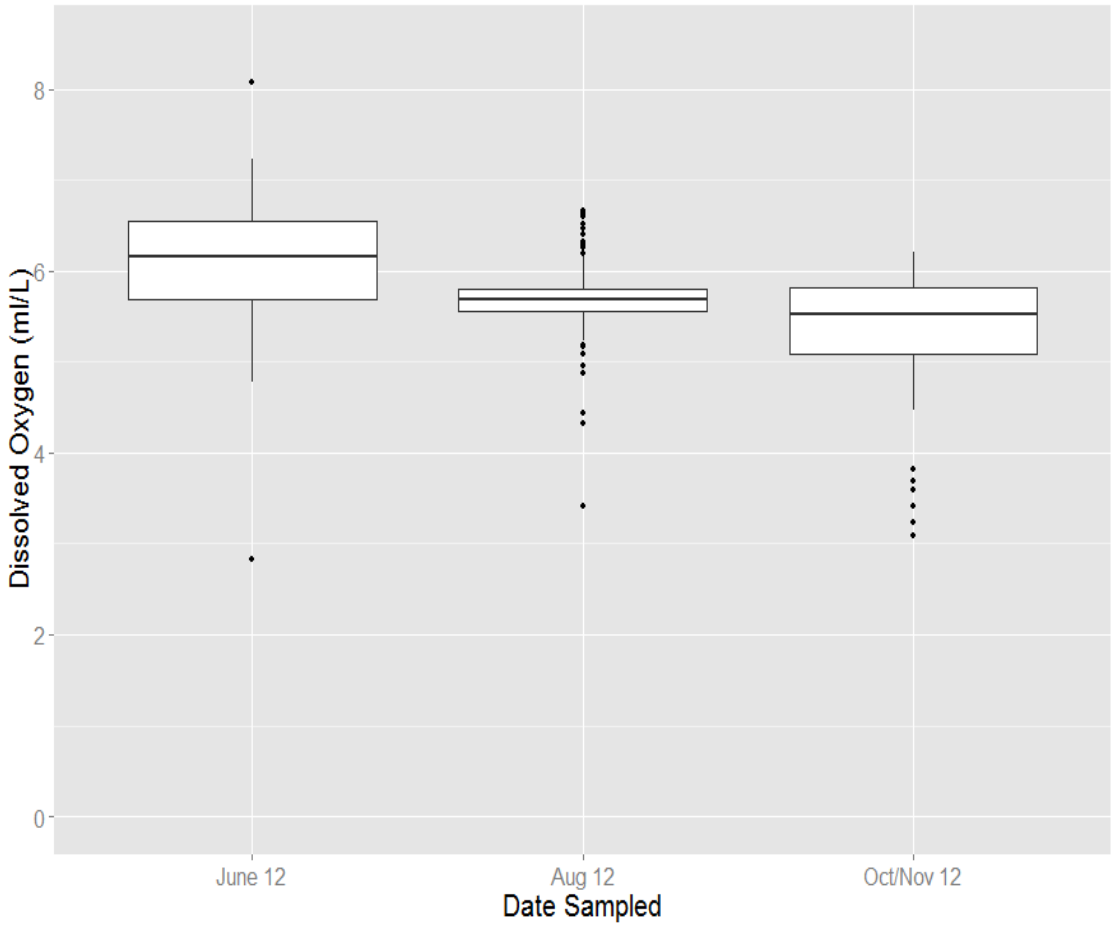

Figure 10. Boxplots of dissolved oxygen concentration at the seafloor, all sites combined, during the June 2012, August 2012 and Oct/Nov 2012 sampling periods.

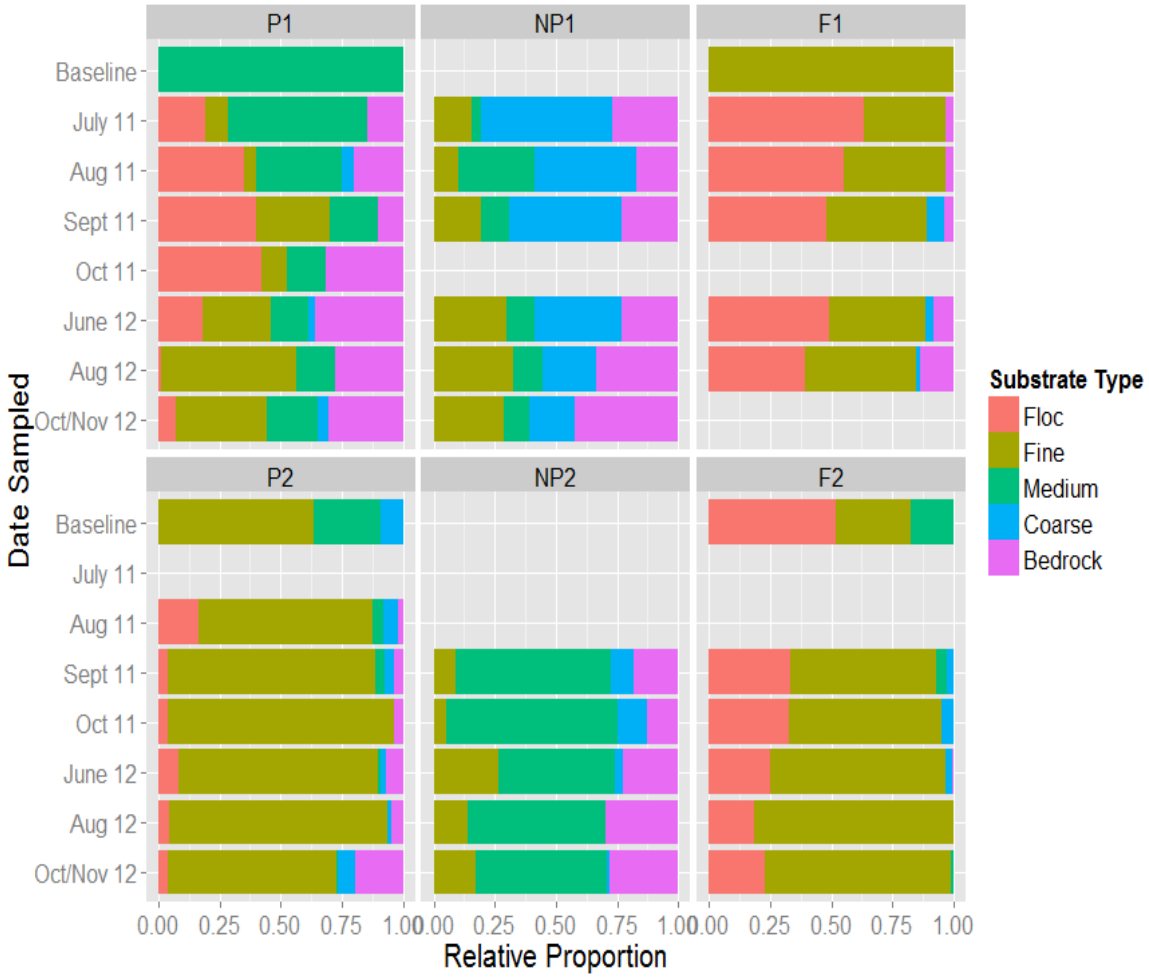

Figure 11. Proportion of substrate types at all sites surveyed. Blank areas are periods where sampling did not occur.

2.3.2 Benthic communities

Over 10 500 individuals, excluding *Beggiatoa spp.*, OPC and algal species, were identified, comprising at least 44 distinct taxa. Because 25 taxa were considered rare (i.e., fewer than 5 occurrences, considering all stations and times), only the most common taxa were used for data analysis (Table 4).

Many faunal groups had wide depth distributions. Macrofauna were recorded at the shallowest depth (2 individuals at a depth of 5 m) as well as the greatest depth (1 individual at 146 m) (Figure 12). However, some species seemed to be restricted by depth. The genus *Strongylocentrotus* existed in a relatively shallow depth range: 5 – 62 m. Some of the photosynthetic species also appeared restricted by depth: *Laminaria* sp. were present at depths of 8 – 96 m and *Hildenbrandia* sp. occurred, with the exception of one point, at shallower depths ranging between 8 – 77 m. Alternatively, OPC and the family Comasteridae were found at greater depths, from 48 – 138 m and 35 – 117 m, respectively.

Organisms generally occupied a wide range of temperatures (Figure 13). However, some species only occurred in a limited temperature range: OPC were found at cold temperatures ranging from 1.8 – 4.7 °C, with the exception of one data point with a temperature of 7.9°C. The family Comasteridae and *Polymastia* sp. had similar temperature ranges of 1.8 – 7.5°C and 1.8°C – 7.6°C, respectively.

Most organisms were observed at a range of DO values between 4.5 – 7 $mL.L^{-1}$. Only indicator species, OPC and *Beggiatoa spp.*, were observed at the lowest DO concentrations (Figure 14).

Most taxa were observed living on a variety of substrate classes, although several taxa were not found on flocculent matter: *Hildenbrandia* sp., *Agarum cribosum, Laminaria* sp., F. Comasteridae, *Gersemia* sp., *Halichondria* sp., and F. Pectinidae, while others showed only few occurrences: *Asterias vulgaris*, F. Strongylocentrotidae, and *Polymastia* sp. (Figures 15, 16). Certain relationships between taxon presence and production stage were observed: *Beggiatoa spp.*, OPC, and urchins (Strongylocentotidae) were only present in the fallow and/or production sites (Figures 12 – 16).

The majority of taxa encountered had an abundance of <10 individuals per station at each sampling time. Mussels (*Mytilus* sp.), *Serpula* sp. and the sponges (*Melonanchora* sp., *Halichondria* sp. and *Polymastia)* had abundances of >10 per sample. The most abundant taxonomic group was the sponges. *Melonanchora* sp. was the most common taxon (3977 individuals) among all sites. The greatest observed number of individuals recorded within a station was 68 *Mytilus* sp. at a fallow site; no other taxa were seen at this station. Macroalgal species, in general, had more occurrences and higher % cover at shallower depths. The percent cover of *Beggiatoa spp.* seemed unaffected by depth (Figure 16). The abundance of organisms showed a significant positive correlation with depth ($R = 0.325$, $p < 0.001$) and a significant negative correlation with temperature ($R = -0.0891$, $p < 0.001$).

Table 4. Most common taxa identified during video surveys and means of quantification.

Identified Organisms	Classification	Biological Measurement
Asterias vulgaris	Macrofauna	Abundance
F. Strongylocentotidae	Macrofauna	Abundance
F. Comasteridae	Macrofauna	Abundance
Gersemia sp.	Macrofauna	Abundance
Stomphia sp.	Macrofauna	Abundance
Serpula sp.	Macrofauna	Abundance
Melonanchora sp.	Macrofauna	Abundance
Halichondria sp.	Macrofauna	Abundance
Polymastia sp.	Macrofauna	Abundance
Mytilus sp.	Macrofauna	Abundance
F Pectinidae	Macrofauna	Abundance
Cancer sp.	Macrofauna	Abundance
Beggiatoa spp.	Indicator	% Cover
OPC	Indicator	% Cover
Hildenbrandia sp.	Macroflora	% Cover
Lithothamnium sp.	Macroflora	% Cover
Chondrus sp.	Macroflora	% Cover
Agarum cribosum	Macroflora	% Cover
Laminaria sp.	Macroflora	% Cover

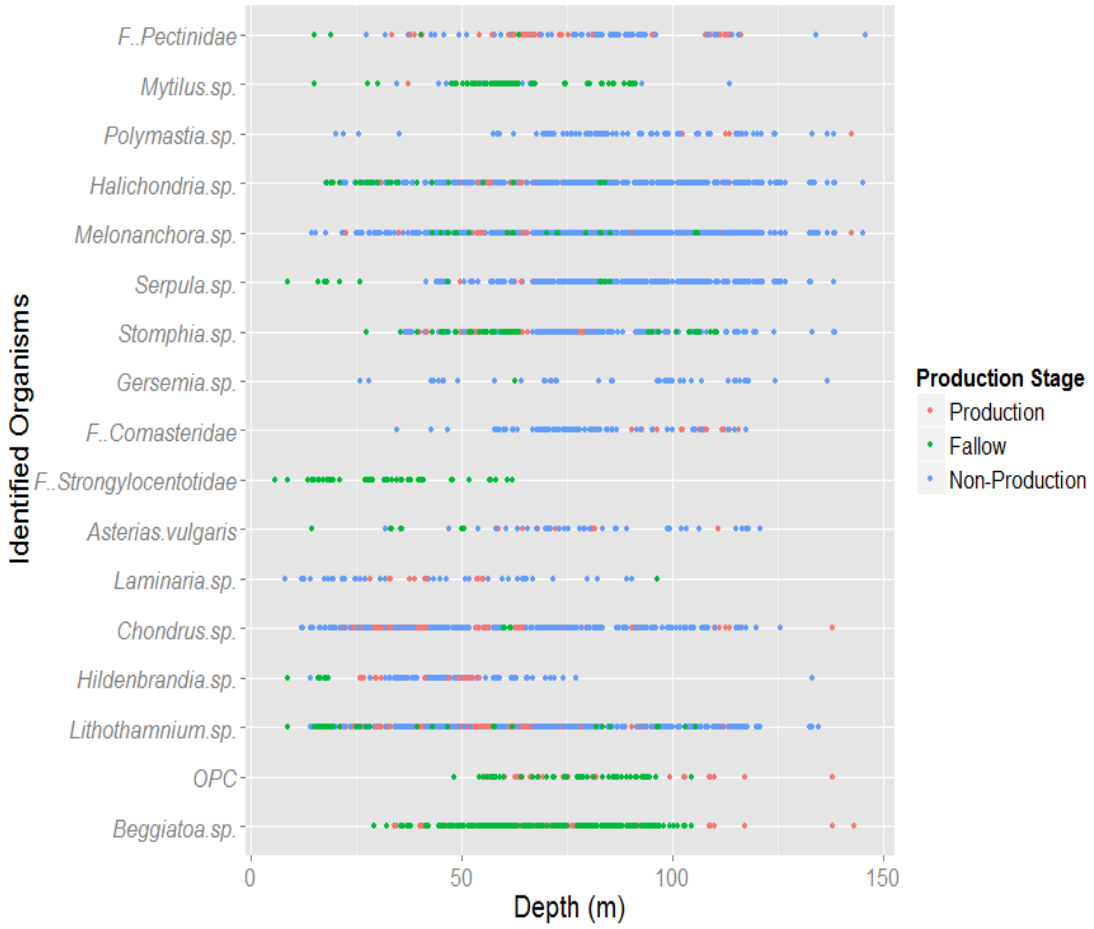

Figure 12. Distribution of observed taxa according to depth, considering all sampling periods.

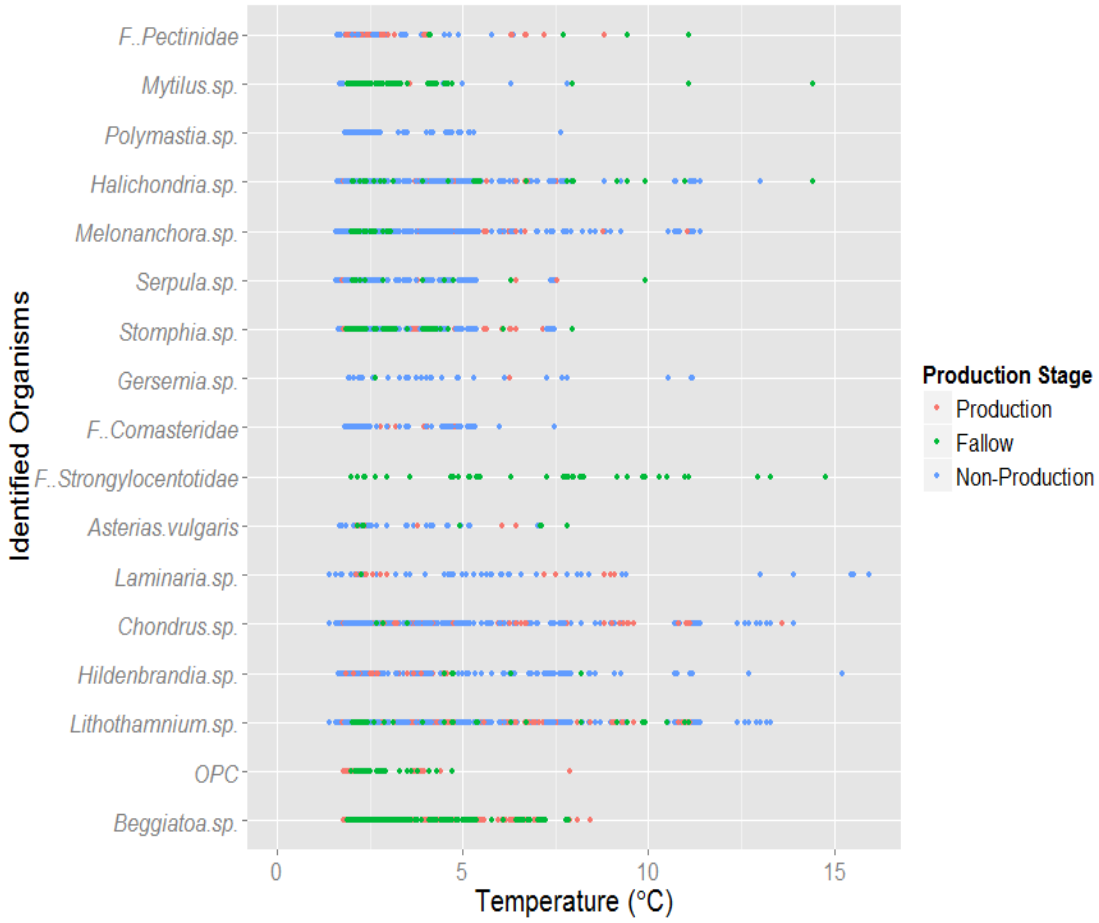

Figure 13. Relationship between taxon occurrence and temperature at the seafloor, considering all sampling periods.

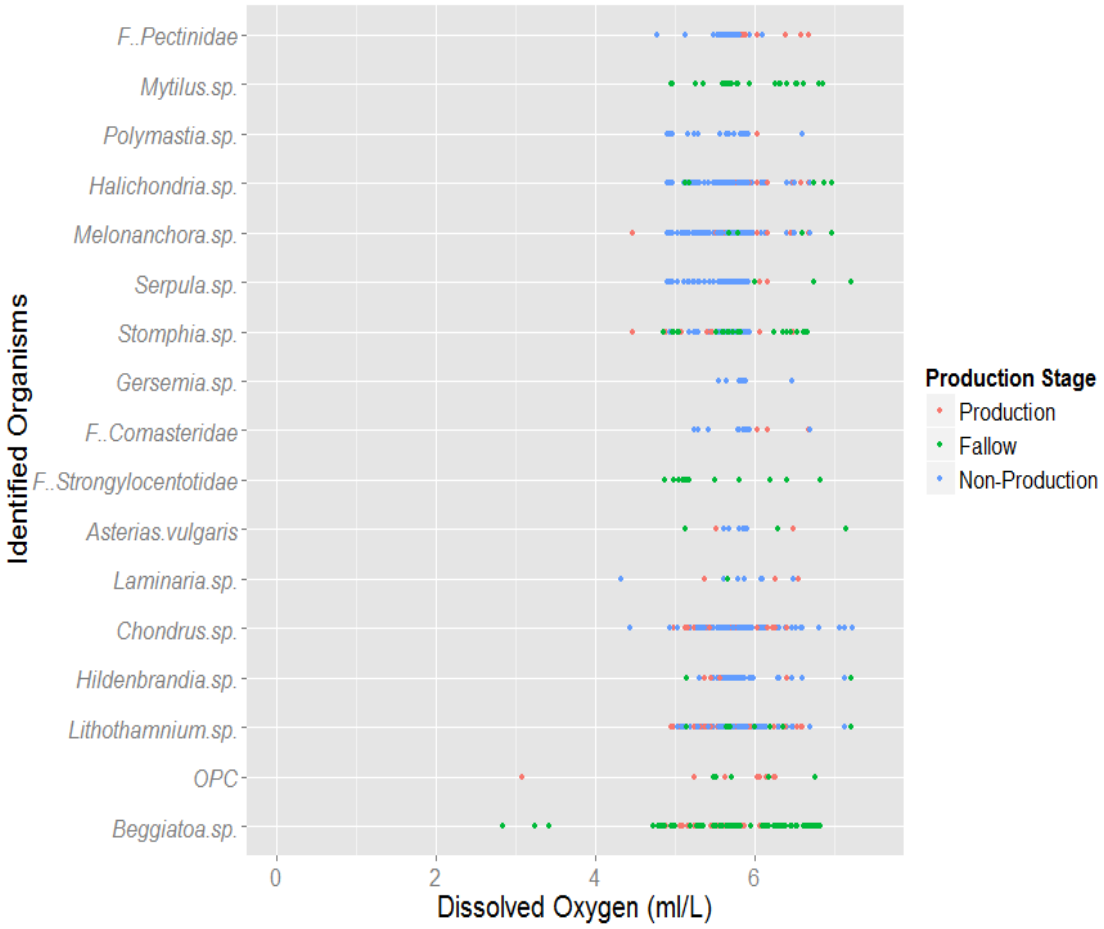

Figure 14. Relationship between occurrence of taxa and associated dissolved oxygen concentrations at all sampling periods.

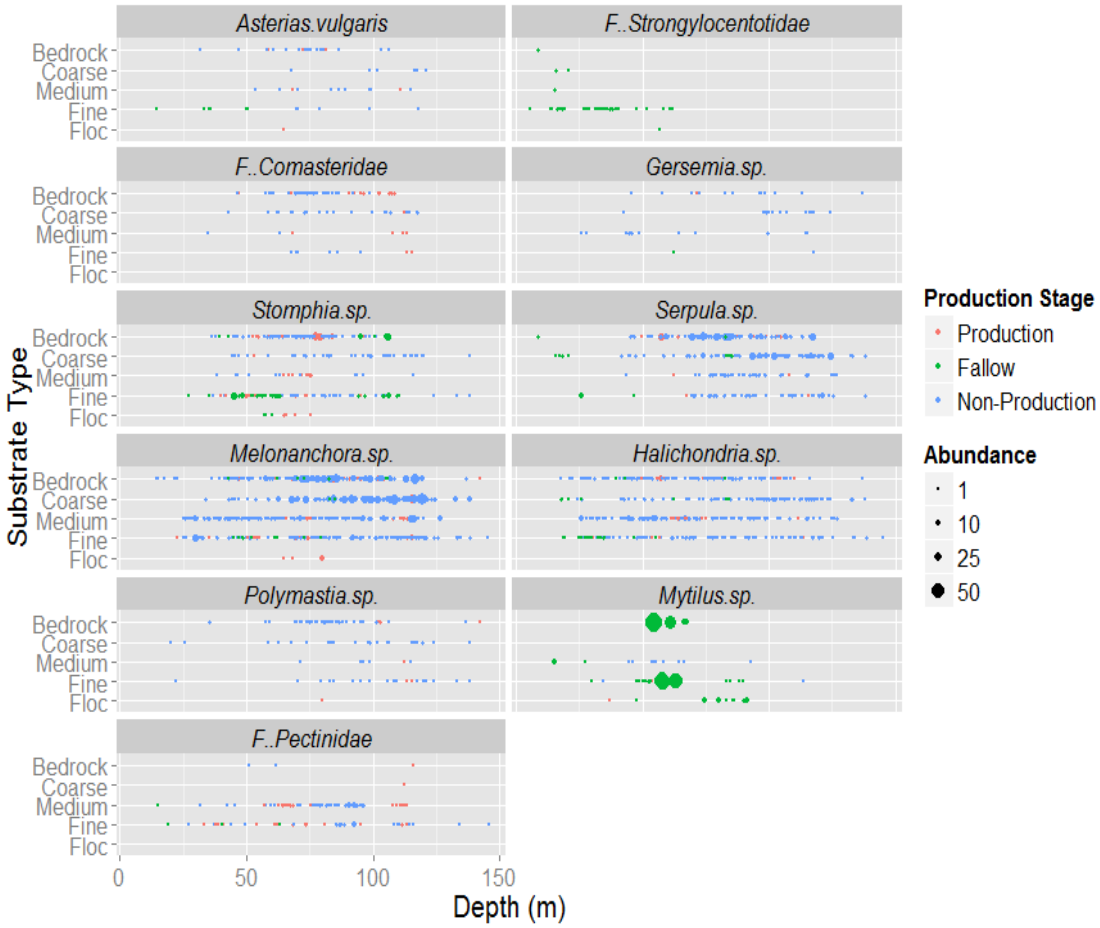

Figure 15. Taxon abundance (# of individuals) in relation to substrate and depth, at all sampling periods. Floc: flocculent matter.

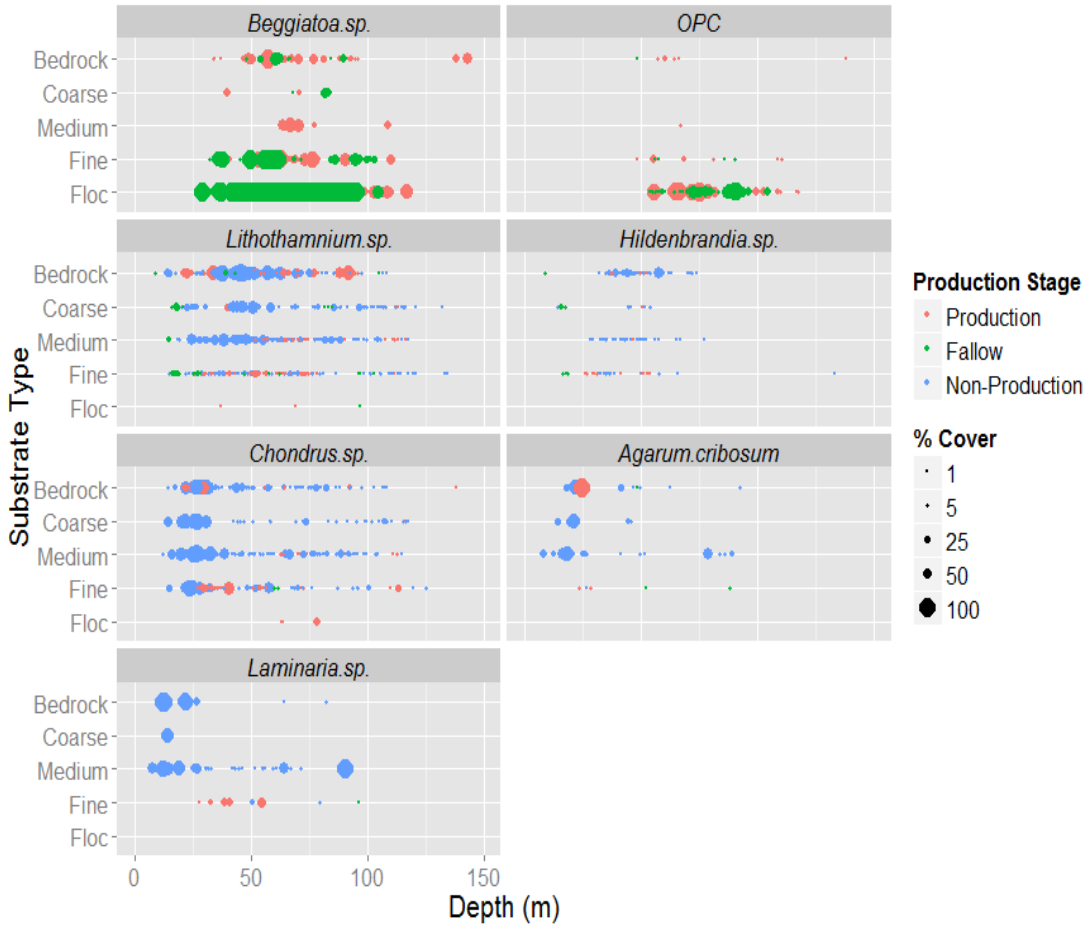

Figure 16. Percent cover of taxa in relation to depth and substrate type, considering all sampling periods. Floc: flocculent matter.

2.4. Discussion

The use of underwater video in combination with environmental data recorders allowed biological data (taxon presence and abundance) to be associated with physical data (depth, temperature, DO and substrate type) at sites experiencing different stages of aquaculture production.

All of the sites were initially chosen based on depth attributes extracted from navigational charts, such that two main groups with similar intra-group conditions could be formed: [P1, NP1 and F1] and [P2, NP2, and F2]. Our data suggests that the two sets of sites had similar depth characteristics and frequency distributions (Figure 5). P1, NP1 and F1 had stations deeper than 100 m and average depths of 80, 90 and 80 m, respectively (Table 3). All stations at P2, NP2 and F2 were at < 100 m depth, with average depths being around 50 m respectively (Table 3).

Temperature changes in the water column and at the seafloor can result from increases in depth and seasonal changes (Gray & Elliot, 2009). The data supported this pattern by a negative correlation (R = -0.479) showing a decrease in temperature with increasing depth as expected (Figure 7). In addition, temperature changed with each sampling period although the range of mean temperatures for all sampling periods varied between 2.2 – 5.6°C (Figure 6).

DO data showed a slight tendency to decrease with increasing depth; however, no significant correlation was found (Figure 8). DO changed significantly with time and Tukey HSD revealed that all dates differed significantly from each other after pairwise comparisons. Changes in DO could be related to multiple factors including seasonal temperature changes, seasonal stratification of the water column or long term climatic

variability (Anderson et al., 2005). The mean dissolved oxygen concentration was however comparable at all sites, ranging from 5.6 – 6.0 $mL.L^{-1}$ The lowest DO values were observed at P2 and F2 and might be due to organic enrichment at those sites. OM enrichment has been shown to create hypoxic zones, and DO concentrations at P2 and F2 were nearly hypoxic (1.4 $mL.L^{-1}$) as previously observed by other authors (e.g. Axler et al., 1998; Hamoutene et al., 2013). However it should be noted that sites in NL can experience low oxygen levels even prior to aquaculture set-up (Hamoutene et al., 2013; Hamoutene, 2014).

Substrate composition differed between sites and displayed variation over time within sites (Figure 11). However, the patchiness of the area and the design of the video surveys might explain some of the variation of substrate type over time. It is likely that the video does not survey the exact same location which could lead to differences in substrate types at identical stations over time. It is also important to note that the baseline reports are not covering the same surface or number of stations as we do, and that aquaculture sites contain both stations receiving settled OM from cages, and stations that do not (depending on local hydrodynamic conditions). As expected, substrate composition at NP1 and NP2 varied little over time and was mostly composed of medium sediment, with no flocculent matter. For P1, initially mostly covered with medium substrates, sampling taking place during the 2011 production cycle showed a relative increase in flocculent matter and fine substrates. Over 50% of stations were classified as fine substrate and flocculent matter from September 2011 onwards. F1 and F2 showed a consistent pattern in relative composition as well, with the majority of substrates at these sites comprised of flocculent matter and fine substrates. Throughout time flocculent

matter and fine sediments comprised 88% of the substrates at F1 and 97% at F2. The increasing presence of fine and flocculent matter likely results from organic enrichment deriving from the aquaculture sites (Wood & Armitage 1997; Gray et al., 2002; Cromey et al., 2002; Giles 2008; Huang et al., 2012).

2.4.1 Benthic composition

Most of the invertebrates identified were sessile, as expected due to the nature of hard bottom substrata (Wenner et al., 1983) and the sampling methodology: highly motile species were not considered in this study.

Depth and temperature have been shown to affect benthic species distributions (Bergen et al., 2001). The overall abundance of species showed a significant positive correlation with depth ($R = 0.325$, $p < 0.001$) and a significant negative correlation with temperature ($R = -0.0891$, $p < 0.001$). Some species appeared to be restricted by temperature and/or depth (i.e. OPC, family Comasteridae). Other taxa existed at the minimum and maximum values of depth and temperature (i.e. *Halichondria* sp., *Melonanchora* sp.). As expected, the prevalence of photosynthetic macroalgae is affected by depth considering that light may not be able to reach greater depths (Markager & Sand-Jensen, 1992). *Mytilus* sp. occurred at 40 – 75 m depth but was concentrated in a few stations at the fallow site. Mussels are usually attached to derelict gear and cages and it is assumed that they fell off the gear and accumulated on the seafloor or could have been detached during net cleaning or cage removal. It is possible that mussels could have

always inhabited this area, however by analyzing temporal trends (Chapter 3), we can investigate this further.

DO appeared to impact species distribution, with most species being present at the highest DO levels whereas only *Beggiatoa spp.* and OPC were observed at low oxygen values. Low oxygen levels due to organic enrichment could impact macrobenthic community structure and favor opportunistic species (Pearson & Rosenberg, 1978; Lu & Wu, 1997; Nilsson & Rosenberg, 2000) which is most likely why only *Beggiatoa spp.* and OPC, which are tolerant of reduced conditions (Tomassetti & Porrellp, 2005; Preisler et al., 2007) were present at the lowest recorded DO concentrations.

Some species seem to prefer particular substrate types and/or flocculent matter: the indicator species and sea urchins were found in high concentration on flocculent matter and fine substrates. *Beggiatoa spp.* and OPC were only present at the production and fallow stages and their presence was likely triggered by the organic enrichment in that area (Crawford, 2003; Brooks et al., 2004; Hall-Spencer et al., 2006; Hamoutene et al., 2013; Hamoutene 2014). The family Strongylocentotidae (sea urchins) was only present during the fallow stage and could be attracted to abundant OM as a food source (Vetter & Dayton, 1999).

In conclusion, two sets of sites were comparable in terms of depth range. OM sedimentation resulting in the accumulation of flocculent matter occurs only at production and fallow sites. Most species showed no site preference except for indicator species which were absent from non-production sites and were the only taxa observed at lower

oxygen levels. Most species showed a pattern with depth which may be related to temperature patterns, both being correlated.

As no clear differences in temperature, depth, DO ranges and macrobenthic organisms (excluding OPC and *Beggiatoa spp.*) were observed between sites in each set, we can assume that sites are comparable and can be used to determine aquaculture impact on macrofaunal communities. This will be further investigated in the next chapter.

2.5 References

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Chapter 3. Spatio-temporal changes in epibenthic communities at finfish farm sites

3.1. Introduction

3.1.1 Overview of environmental impacts from aquaculture operations

Environmental impacts of aquaculture can be biological [e.g., interactions with wild stocks, causing genetic changes (Hindar et al., 1991; Crozier, 2000), disease transfer (Heggberget et al., 1993; Bridger et al., 2001) and/or competition (McGinnity et al., 2003; Mazur & Curtis, 2008)], or physico-chemical [e.g. changes in water chemistry (Alongi et al., 2003), nutrient loading (Angel et al., 2002; Gao et al., 2005), and organic enrichment of the benthos (Carrol et al. 2003; Sutherland et al., 2007)]. Organic matter (OM) enrichment of the benthos has been extensively researched and is possibly the most direct effect aquaculture can have on its local ecosystem (AMEC, 2002; Kalantzi & Ioannis 2006). OM enrichment can occur at aquaculture sites when feed, feces and other fish by-products flow through the cage and accumulate on the seafloor (Crawford, 2003). If the sediment OM is not diluted or assimilated by the natural processes, the local habitat may experience nutrient loading. Organic enrichment can lead to the smothering of benthic organisms and reduced oxygen concentrations, along with changes in biodiversity, abundances, biomass and assemblage composition (Gray & Elliot, 2009). The effects of various types of OM enrichment on benthic infaunal communities have been well studied (Pearson & Rosenberg, 1978; Nilsson & Rosenberg, 2000; Gray et al., 2002) and modelled (Pearson & Rosenberg, 1978). In general, OM enrichment causes a decrease in the size and diversity of species, and an increase in the abundance of smaller opportunistic species (Pearson & Rosenberg, 1978; Huang et al., 2012).

3.1.2 Potential impacts of organic matter enrichment

In sedimentary benthic environments, OM enrichment can result in ecological successional patterns over both temporal and spatial scales (Pearson, 1975). Nilsson and Rosenberg (2001) describe four successional stages along a gradient of increasing organic enrichment, defined by community composition. The stages include: an oxidized habitat with a highly diverse community, a transitional community with an increase in the abundance of opportunist species, a community dominated by opportunistic species, and an azoic habitat void of any macrobenthic species. Pearson and Rosenberg (1978) hypothesized that this biological relationship exists in any marine area that is affected by organic enrichment.

The degree of OM enrichment from aquaculture depends on a multitude of factors including farming practices, bathymetric characteristics, oceanographic condition (i.e., current speed and direction; temperatures), habitat type and the assimilative capacity of the local ecosystem (Kalantzi & Ioannis, 2006). Spatially, the extent of impact can be limited to the area underneath the cages (Wildish & Pohle, 2005; Tomassetti et al., 2009) or extend to distances < 1 km (Holmer, 1991). A study by Pohle et al. (2001) conducted in Atlantic Canada shows regional changes to benthic macrofauna as a result of increased amounts of OM, up to 200 m away from cages.

Temporal studies conducted on OM enrichment from finfish aquaculture have monitored changes to the benthos throughout a production cycle with a continued supply of OM (Tomassetti et al., 2009) or have focused on benthic recovery once cessation of OM enrichment occurs (Brooks et al., 2004). Recovery of the benthos has been shown to take place in as little as few months to >5 years post aquaculture production (Brooks et

al., 2003; 2004). Hamoutene et al. (2013, 2014) indicated that sites in NL post fallow still had hypoxic sediments and displayed an apparent lack of change in sediment condition. The impacts of OM enrichment from aquaculture are variable (Brooks et al., 2003); this variation can be caused by site characteristics, sampling protocols and analysis of the data (Kalantzi & Ioannis, 2006).

Conventional methods to measure OM enrichment, sediment grabs and cores, have been mainly utilized for soft sediments (Hall-Spencer et al., 2006) and are often ineffective on predominantly hard substrates (Bungay, 2012; Hamoutene et al., 2013). In some regions such as the south coast of Newfoundland, the substrates beneath finfish aquaculture sites are mixed/predominantly rocky (Anderson et al. 2005, Bungay 2012, Hamoutene et al., 2013; Hamoutene, 2014). Out of the > 120 biological and geochemical variables that have been used to determine benthic changes due to aquaculture (Kalantzi & Karakassis, 2006), none have been developed for hard substrates. The evaluation of methods to document benthic impacts of aquaculture in NL has concluded that due to the hard and patchy nature of the south coast, video analysis is a preferred method. However, the use of video imaging as a monitoring tool in the NL region is in its infancy and an exploration of how benthic communities are affected by OM enrichment over temporal and spatial scales has not yet been completed.

The present study uses video imaging to document the natural temporal variation in subtidal benthic organisms in the Coast of Bays region of NL and compare it to that in sites affected by OM enrichment from finfish cages and in sites undergoing fallow. Further, relationships between benthic organism presence and abundance according to distance from finfish cages are examined at production and fallow sites.

In this chapter, the abundance and composition of visible epibenthic organisms will be compared and analyzed on temporal and spatial scales using two approaches: first considering all sites (two non-impacted sites, two production sites and two fallow sites); then considering sites grouped according to similar depth characteristics. The aim of this chapter is to determine spatial and temporal trends in epibenthic community change in relation to OM enrichment from aquaculture sites while accounting for natural seasonal changes and spatial patchiness.

3.2. Methods

Underwater surveys were conducted at six locations on the South Coast of Newfoundland: two production sites (P), two non-production sites (NP) and two fallow sites (F), as described in Chapter 2. An underwater video camera and environmental monitoring equipment were lowered to the seafloor to capture images and obtain data of the physical and biological attributes of the benthic environment. Images were obtained at 20 m intervals along three transects at each site (Chapter 2).

3.2.1 Grouping of organisms

The level of taxonomic resolution that can be achieved using video data varies according to the phylum considered (DFO, 2012). Biological data were categorized into larger taxonomic groups due to low abundances of single species. Taxa that could be individually counted were grouped into 5 categories: Echinoderms, Cnidaria, Annelids,

Porifera and Molluscs (Table 5). Species that were quantified by % cover were grouped into 4 categories: *Beggiatoa spp.*, OPC, coralline algae and macroalgae (Table 5). Species quantified by % cover were separated from abundance data in subsequent analyses. For example, *Beggiatoa spp.* cover was only recorded as % cover and not quantified as abundance. Stations that lacked, in both abundance and % cover, any species (not including indicator species) were termed "no visible species".

3.2.2 Data processing

Depth characteristics differed between the two sets of sites outlined in Chapter 2. Group 1 (NP1, P1, F1) had greater maximum and average depths than group 2 (NP2, P2, F2). Therefore, when graphically displaying sites (except when comparing production stages), these two groups were separated.

Sampling protocols differed in the 2011 and 2012 sampling seasons. In this study we will investigate changes temporally over the whole time period and seasonally comparing within one sampling year (i.e., 2011 or 2012). During the 2011 field season, only one sample (one image per video recording) was collected for each station (geographic location); however four images, considered as replicates, were collected during the 2012 field season and data from these four images were averaged. The number of stations sampled varied slightly at different sampling dates and sites due to sampling design, constraints of video cable length, movement of cages, and adverse weather conditions. To graphically represent abundance and % cover values, averages per station (2500 cm^2 as per quadrat size) were presented. Data presented according to distance from

cage are averages of stations along the three transects. All data calculations, statistical analyses, and graphical representations were done using R Statistical Software version 0.97.318 (R. Development Core Team, 2005).

Table 5. Species identified and corresponding taxonomic group classification

Species	**Taxonomic Group**
Pectinidae	Molluscs
Mytilus sp.	Molluscs
Polymastia sp.	Porifera
Halichondria sp.	Porifera
Melonchora sp.	Porifera
Serpula sp.	Annelids
Gersemia sp.	Cnidaria
Stomphia sp.	Cnidaria
Stronglocentotidae	Echinoderms
Asterias vulgaris	Echinoderms
Laminaria sp.	Macroalgae
Chondrus sp.	Macroalgae
Hildenbrandia sp.	Coralline algae
Lithothamnium sp.	Coralline algae
OPC (Family Dorvilleidae)	OPC
Beggiatoa spp.	*Beggiatoa spp.*

3.2.3 Statistical analysis

The complete dataset was analyzed to determine biological composition differences among sites, and to test whether the total abundance, abundance within each taxonomic group, and the percent cover of each non-enumerable taxon vary: 1) among production stages (comparing stations from non-production, production and fallow sites); 2) among production stages, comparing sets of sites having similar depth distributions (i.e. considering separately NP1, P1 and F1, and NP2, P2 and F2, see Chapter 2); 3) according to sampling date within each site; and 4) according to distance from cage within particular sites.

As the distribution of the data violated ANOVA assumptions (homoscedasticity), non-parametric Kruskal Wallis tests were used to test for differences between data sets. When analyzed, data sets revealed similar residual distributions. If significant, multiple comparisons tests were used with a p-value of 0.05 for statistical significance.

3.3. Results

3.3.1 Epibenthic composition by production stage

3.3.1.1 Composition and abundance of enumerable organisms

All taxon groups were represented in each of the three production stages (P, NP, F). However, total abundance and the abundances of each of the taxon groups differed with each production stage. The abundance of visible, enumerable organisms per station was highest at NP sites with an average of 6.35 ± 7.13 individuals per station. Porifera and

annelids were the predominant taxa at NP stations, where they represented on average 67% and 19% of observed individuals, respectively. The P sites had the lowest average abundance of enumerable organisms, at 1.35 ± 3.44 individuals per station. Porifera were also predominant at P sites, consisting of 46% of the average abundance per station. F sites had an average abundance of 1.80 ± 5.57 individuals per station, mainly consisting of molluscs which comprised 61% of the average abundance per station. Molluscs existed at all sites however the species comprising this taxon differed between stages: at NP and P the dominant species were pectinids, comprising 89% and 97% of all molluscs, respectively. Molluscs at F sites were mainly comprised of *Mytilus* sp. (as visualised through the presence of shells), accounting for 99% of the mollusc abundance. Notably, NP, P and F had similar average abundances of cnidarians per station (Figure 17).

The Kruskal Wallis test revealed significant differences in abundances for all taxonomic groups between the production stages (Table 6). Specifically, post-hoc tests revealed that the abundances of all categories of organisms, and the total abundance at the NP sites were greater than those at the P and F sites, except for molluscs. Pairwise comparisons between the abundance of organisms at P and F sites showed that only poriferans differed significantly, being more abundant at the P sites than the F sites.

3.3.1.2 Percent cover of non-enumerable taxa

Macroalgae and coralline algae were present in all the production stages; however *Beggiatoa spp.* and OPC were only present in the P and F stages. Non-enumerable taxa ranged in percent cover from absent at specific stations (0% cover) to complete coverage of a station (100% cover).

At the NP sites, 83.7% of the stations contained non-enumerable organisms which consisted of macroalgae and/or coralline algae; no OPC or *Beggiatoa spp.* were observed. At P1 sites, *Beggiatoa spp.* was present with an average % cover per station of 9.55 ± 20.10%. At stations where *Beggiatoa spp.* was present it covered on average 29.96 ± 25.65% of the grid area. Likewise, at the F sites *Beggiatoa spp.*, occurred at 46.9 % of the stations with an average percent cover of 27.62 ± 39.92 per station. At stations where *Beggiatoa spp.* was present at F sites it covered on average 59.11 ± 39.37% of the grid area. OPC was present at both the P and F stages, and where present, they had an average % cover per station of 23.47 ± 33.09% and 16.13 ± 23.69%, respectively (Figure 17b); 10.8 % of stations in P and 11.7% of stations in F contained OPC.

The Kruskal Wallis test revealed significant differences in *Beggiatoa spp.*, OPC, macroalgae and coralline algae cover between production groups. Post-hoc tests showed that the percent cover of *Beggiatoa spp.*, macroalgae and coralline algae was significantly (p-value < 0.01) different among all production stages (Table 6). *Beggiatoa spp.* % cover was greatest at the F sites and absent at the NP sites, while the percent cover of both macroalgae and coralline algae were greatest at the NP sites and lowest at the F sites. The percent cover of OPC was not significantly different between the F and P stages, but differed significantly between both those groups and the NP sites (Table 6).

3.3.1.3 Images with no visible organisms

The NP sites had the lowest percentage of stations with no visible organisms (2.2 % of all images). P and F sites had greater amounts of images with no visible organisms with 38.9 % and 64.4 % at P and F stages, respectively.

3.3.2 Epibenthic composition by production stage, comparing similar depths

3.3.2.1 Sites NP1, P1, F1

Sites NP1, P1 and F1 were previously determined to be comparable based on depths (Chapter 2). Among these sites, the average total abundance of enumerable organisms was highest in NP1 with 10.77 ± 7.95 individuals per station. When groups of enumerable organisms were considered separately, the highest average abundance within each group, with the exception of molluscs, was found at NP1 (Table 7). Porifera and annelids were the predominant taxa in NP1 and comprised 65% and 23% of the average total abundance per station, respectively. F1 had the lowest average abundance with 1.86 ± 6.58 organisms per station. Molluscs were the predominant taxon at F1, where they formed 74% of the average total abundance. P1 had an average total abundance per station of 3.29 ± 5.35 individuals with about half of the enumerable organisms consisting of poriferans (Figure 18).

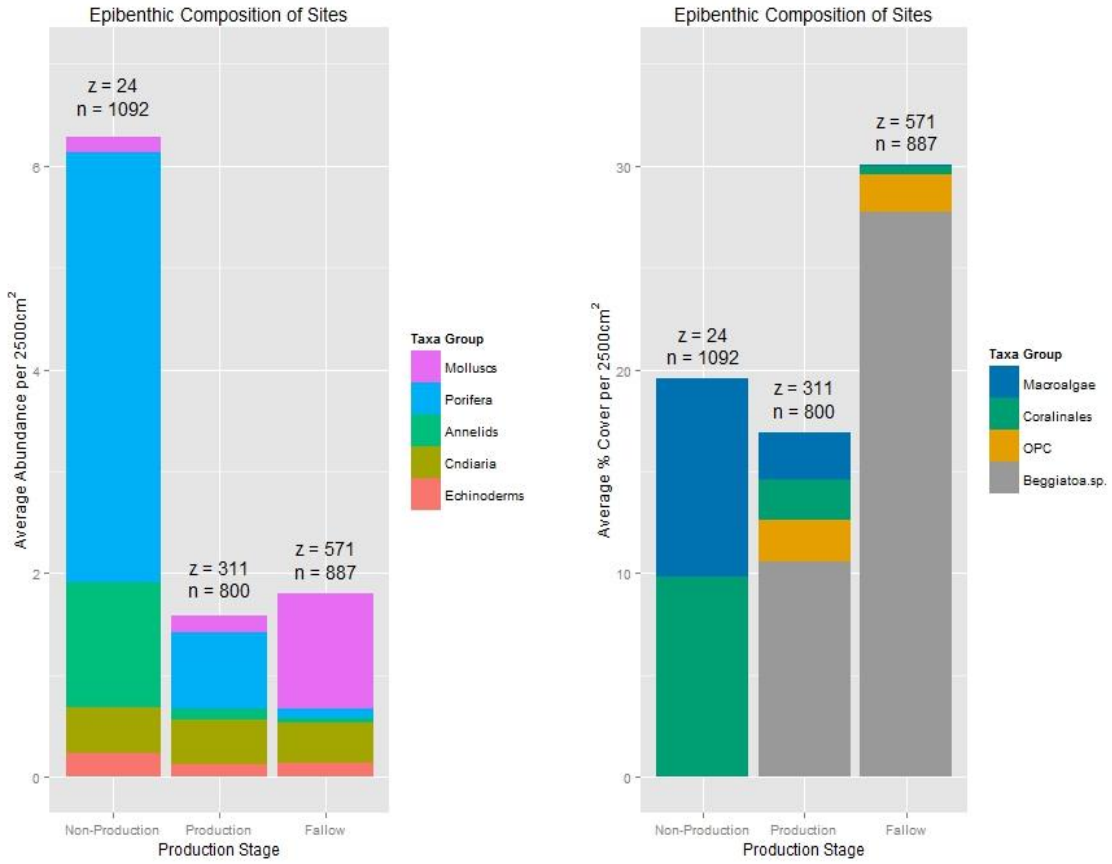

Figure 17. Average epibenthic abundance and % cover of observed taxonomic groups per station, with the latter grouped according to aquaculture production stage. Data from all sampling dates and study sites are considered. The number of image (n) examined for each production stage and the number of image with no visible organisms (z) is indicated above each bar.

Table 6. Results of Kruskal Wallis analyses comparing the total abundance, the abundances of organisms grouped by higher taxonomic rank, and the % cover of non-enumerable organisms (averages ± standard deviations) between sites grouped by production stage, with all dates considered. P-values are presented along with the results of multiple comparison tests, with letters denoting significant differences among groups. ***: $p < 0.001$.

	p - value	Non - Production	Production	Fallow
Total abundance	< 0.001 (***)	6.30 ± 7.10 (a)	1.35 ± 3.44 (b)	1.80 ± 5.57 (b)
Echinoderms	< 0.001 (***)	0.24 ± 0.71(a)	0.10 ± 0.52 (b)	0.14 ± 0.53 (b)
Cnidaria	< 0.001 (***)	0.44 ± 0.96 (a)	0.41 ± 1.62 (b)	0.40 ± 1.43 (b)
Annelids	< 0.001 (***)	1.22 ± 2.57 (a)	0.08 ± 0.55 (b)	0.04 ± 0.30 (b)
Porifera	< 0.001 (***)	4.21 ± 4.94 (a)	0.60 ± 2.00 (b)	0.10 ± 0.40 (c)
Molluscs	< 0.001 (***)	0.15 ± 0.78 (a)	0.12 ± 0.86 (a)	1.10 ± 5.30 (a)
Beggiatoa spp.	< 0.001 (***)	0.00 ± 0.00 (a)	9.50 ± 20.10 (b)	27.6 ± 39.9 (c)
OPC	< 0.001 (***)	0.00 ± 0.00 (a)	2.30 ± 12.30 (b)	1.80 ± 9.50 (b)
Macroalgae	< 0.001 (***)	9.72 ± 19.8 (a)	2.47 ± 9.13 (b)	0.06 ± 0.51 (c)
Coralline algae	< 0.001 (***)	9.81 ± 15.94 (a)	2.78 ± 8.10 (b)	0.36 ± 2.45 (c)

The Kruskal-Wallis test revealed significant differences in the abundance of all categories of organisms between NP1, P1 and F1 (Table 7). The total abundance and the

abundance of poriferans differed significantly between sites, with highest abundances at NP1 and lowest at F1. As for echinoderms, cnidarians and annelids, post-hoc tests showed that abundances were higher at NP1 than at P1 and F1, with no significant difference in abundance between the latter two sites. However, post hoc multiple comparison tests revealed no differences in the abundance of molluscs between sites.

The non-enumerable organisms at NP1 were solely comprised of coralline algae and macroalgae. At both P1 and F1, non-enumerable organisms were mainly comprised of *Beggiatoa spp.* and OPC. *Beggiatoa spp.* had an average % cover per station of 19.17 ± 25.67% and 29.03 ± 37.40% and OPC of 4.4 ± 16.75% and 4.23 ± 14.33% for P1 and F1, respectively. The Kruskal-Wallis test revealed significant differences in the abundance of all categories of organisms between sites NP1, P1 and F1 (Table 7). Macroalgae and coralline algae had a significantly greater % cover at N1 than at P1 and F1 (with coralline algae % cover also being significantly lower at F1 than P1). The percent cover of *Beggiatoa spp.* was significantly lower at NP1 than at F1 and P1, but that of OPC only differed significantly from F1.

The percentage of images from NP1, P1 and F1 with no visible species is 3.0%, 31.2% and 66.8%, respectively.

3.3.2.2 NP2, P2, F2

The average total abundance per station was highest in NP2 (2.47 ± 2.81), with 72% of enumerable organisms being poriferans (1.81 ± 2.32 organisms per station). P2 had the lowest average total abundance with only 0.48 ± 1.39 organisms per station. Echinoderms and annelids were not observed at this site. All sites had similar numbers of cnidarians

with average abundances per station of 0.21 ± 0.82, 0.26 ± 0.63, and 0.46 ± 1.32 for NP2, P2, F2, respectively. F2 had high numbers of molluscs (average abundance per station of 0.92 ± 4.37) comprising 51% of the total average abundance per station at this site (Figure 18).

Kruskal Wallis tests showed that abundances within all categories except Cnidaria differed significantly between sites (Table 8). Post-hoc analyses indicated that abundances of poriferans were greater at NP2 than at P2 and F2 and that echinoderm abundance was lower (nil) at P2 than at NP2 and F2.

Non-enumerable organisms at NP2 consisted of macroalgae and coralline algae only, with average % cover per station of 15.82 ± 23.64% and 12.69 ± 16.11%, respectively. At F2, non-enumerable organisms were mainly comprised of *Beggiatoa spp.* with an average % cover per station of 26.72 ± 41.80. *Beggiatoa spp.* was present in P2 but with only 4.74 ± 14.34% cover per station; coralline algae and OPC were also observed at this site (Figure 18). Kruskal Wallis analyses revealed significant differences in the percent cover of all non-enumerable organisms among sites NP2, P2 and F2. The percent cover of *Beggiatoa spp.* was smallest at P2 and greatest at F2, while that of coralline algae and macroalgae were greatest at NP2 and lowest at F2 (Table 8).

The percentages of images from NP2, P2 and F2 with no visible species are 1.5%, 43.8% and 62.9%, respectively.

Table 7. Results of Kruskal Wallis analyses comparing the total abundance, the abundances of organisms grouped by higher taxonomic rank, and the % cover of non-enumerable organisms (averages ± standard deviations) between sites NP1, P1 and F1 (all dates considered). P-values are presented along with the results of multiple comparison tests, with letters denoting significant differences among groups. ***: $p < 0.001$

	p - values	NP1	P1	F1
Total abundance	< 0.001 (***)	10.77 ± 7.95 (a)	3.29 ± 5.35 (b)	1.86 ± 6.58 (c)
Echinoderms	< 0.001 (***)	0.49 ± 0.99 (a)	0.31 ± 0.89 (b)	0.002 ± 0.02 (b)
Cnidaria	< 0.001 (***)	0.71 ± 1.06 (a)	0.73 ± 2.7 (b)	0.32 ± 1.57 (b)
Annelids	< 0.001 (***)	2.47 ± 3.32 (a)	0.26 ± 0.98 (b)	0.03 ± 0.24 (b)
Porifera	< 0.001 (***)	6.96 ± 5.67 (a)	1.70 ± 3.21 (b)	0.11 ± 0.4 (c)
Molluscs	< 0.001 (***)	0.02 ± 0.11 (a)	0.22 ± 0.63 (a)	1.4 ± 6.38 (a)
Beggiatoa spp.	< 0.001 (***)	0.00 ± 0.00 (a)	19.17 ± 25.66 (b)	26.72 ± 41.80 (b)
OPC	< 0.001 (***)	0.00 ± 0.00 (a)	4.23 ± 16.75 (a,b)	4.42 ± 14.33 (b)
Macroalgae	< 0.001 (***)	2.76 ± 10.74 (a)	0.63 ± 3.22 (b)	0.05 ± 0.08 (b)
Coralline algae	< 0.001 (***)	6.53 ± 15.14 (a)	3.45 ± 4.34 (b)	0.09 ± 0.67 (c)

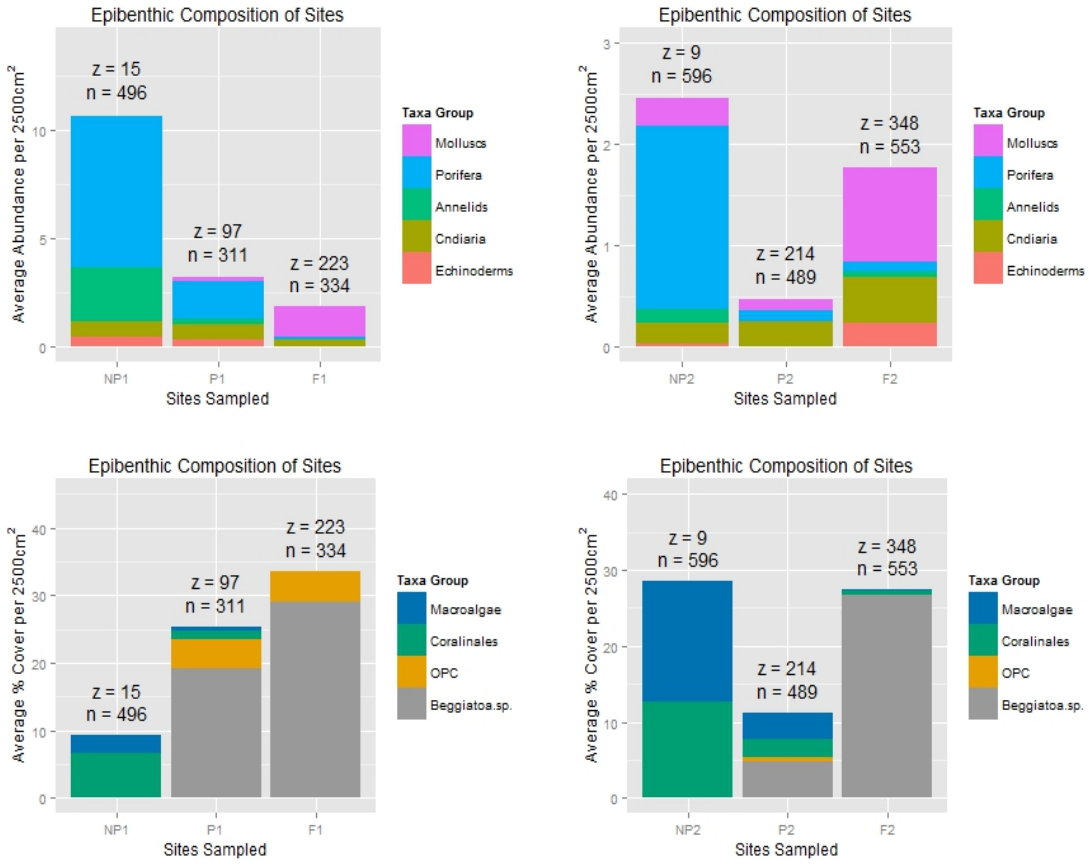

Figure 18. Average epibenthic abundance and % cover of observed taxonomic groups per station, with sites NP1, P1 and F1, and sites NP2, P2 and F2 considered separately. Data from all sampling dates are compiled. The number of images (n) examined for each production stage and the number of images with no visible organisms (z) are indicated above each bar.

Table 8. Results of Kruskal Wallis analyses comparing the total abundance, the abundances of organisms grouped by higher taxonomic rank, and the % cover of non-enumerable organisms (averages ± standard deviations) at sites NP2, P2 and F2 (all dates considered). P-values are presented along with the results of multiple comparison tests, with letters denoting significant differences among groups. ***: $p < 0.001$.

	p - values	NP2	P2	F2
Total abundance	< 0.001 (***)	2.47 ± 2.81 (a)	3.29 ± 1.25 (b)	1.76 ± 4.71 (c)
Echinoderms	< 0.001 (***)	0.02 ± 0.13 (a)	0.00 ± 0.00 (b)	0.24 ± 0.69 (a)
Cnidaria	0.343	0.21 ± 0.82	0.26 ± 0.63	0.46 ± 1.32
Annelids	< 0.001 (***)	0.14 ± 0.53 (a)	0.00 ± 0.00 (a)	0.05 ± 0.39 (a)
Porifera	< 0.001 (***)	1.81 ± 2.33 (a)	0.08 ± 3.21 (b)	0.10 ± 0.48 (b)
Molluscs	< 0.001 (***)	0.27 ± 1.04 (a)	0.08 ± 0.94 (a)	0.92 ± 4.37 (a)
Beggiatoa spp.	< 0.001 (***)	0.00 ± 0.00 (a)	4.73 ± 14.34 (b)	26.6 ± 41.7 (c)
OPC	< 0.001 (***)	0.00 ± 0.00 (a)	1.37 ± 9.54 (a)	0.03 ± 0.32 (a)
Macroalgae	< 0.001 (***)	15.80 ± 23.64 (a)	3.32 ± 10.73 (b)	0.06 ± 0.53 (c)
Coralline algae	< 0.001 (***)	12.69 ± 16.11 (a)	3.45 ± 9.27 (b)	0.55 ± 3.16 (c)

3.3.3 Temporal changes in epibenthic composition

3.3.3.1 Sites NP1, P1, F1

The total abundance of enumerable organisms, and the percent cover of macroalgae and coralline algae at NP1 remained relatively constant throughout time, with the highest abundance per station occurring in July 2011 (13.10 ± 9.57) and the lowest in

September 2011 (8.26 ± 7.27). Although the total abundance did not change significantly with time, the abundance of cnidaria, annelids and echinoderms differed significantly between time periods, as did the percent cover of macroalgae (Table 9).

Total abundances at P1 were lower at every date sampled than at NP1. The highest total average abundance per station was recorded in June 2012 with 4.60 ± 6.15 individuals per station and the lowest abundance occurred in August 2012 with an average abundance of 1.55 ± 2.50 individuals per station (Figure 19). The indicator species *Beggiatoa spp.* and OPC differed significantly through time. *Beggiatoa spp.* coverage was highest in August 2011, with an average % cover per station of 43.79 ± 34.47 and was lowest in Oct/Nov 2012 (7.58 ± 14.01%). OPC percent cover was highest in October 2011 (19.21 ± 37.97) and lowest (0.00 ± 0.00%) in August 2011 and in Oct/Nov 2012 (0.00 ± 0.00%).

At F1, the total abundance of enumerable organisms per station was lower at each date sampled compared to NP1. The highest total abundance per station at F1 was recorded in August 2012 (4.68 ± 9.43) and the lowest abundance in July 2011 (zero individuals). Molluscs differed significantly through time and were the most dominant taxon observed in August 2011, June 2012, and August 2012, comprising 84%, 68% and 77% of the average abundance per station, respectively. *Beggiatoa spp.* percent cover was highest in June 2012 (48.00 ± 43.68%) and lowest in July 2011 (17.6 ± 28.99%). OPC percent cover was highest in July 2011 (12.90 ± 23.27 %) and lowest (0.20 ± 0.54 %) in September 2011.

NP1 had no images classified as having no visible organisms in July 2011 and September 2011, however the highest percentage of images with no visible organisms occurred in June 2012 (5.3% of images). The percentage of images with no visible organisms at P1 was highest in October 2011 at 36.8% and was lowest (25.0%) in August 2011 and September 2011. F1 had high percentages of images with no visible organisms and in July 2011, 100% of the images had no visible organisms. However, at the last sampling period, only 50.8% of the images at F1 had no visible organisms.

Table 9. Results of Kruskal Wallis analyses comparing the total abundance, the abundances of organisms grouped by higher taxonomic rank, and the % cover of non-enumerable organisms between sites NP1, P1 and F1, according to sampling date. P-values are presented. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

	NP1	P1	F1
Total abundance	0.137	0.521	< 0.001 (***)
Echinoderms	0.031 (*)	0.639	0.437
Cnidaria	< 0.001 (***)	0.357	0.081
Annelids	0.019 (*)	0.006 (**)	0.493
Porifera	0.231	0.651	0.081
Molluscs	0.153	0.117	< 0.001 (***)
Beggiatoa spp.	NA	0.003 (**)	0.001 (**)
OPC	NA	0.003 (**)	< 0.001 (***)
Macroalgae	0.002 (**)	0.664	0.708
Coralline algae	0.808	< 0.001 (***)	0.004 (**)

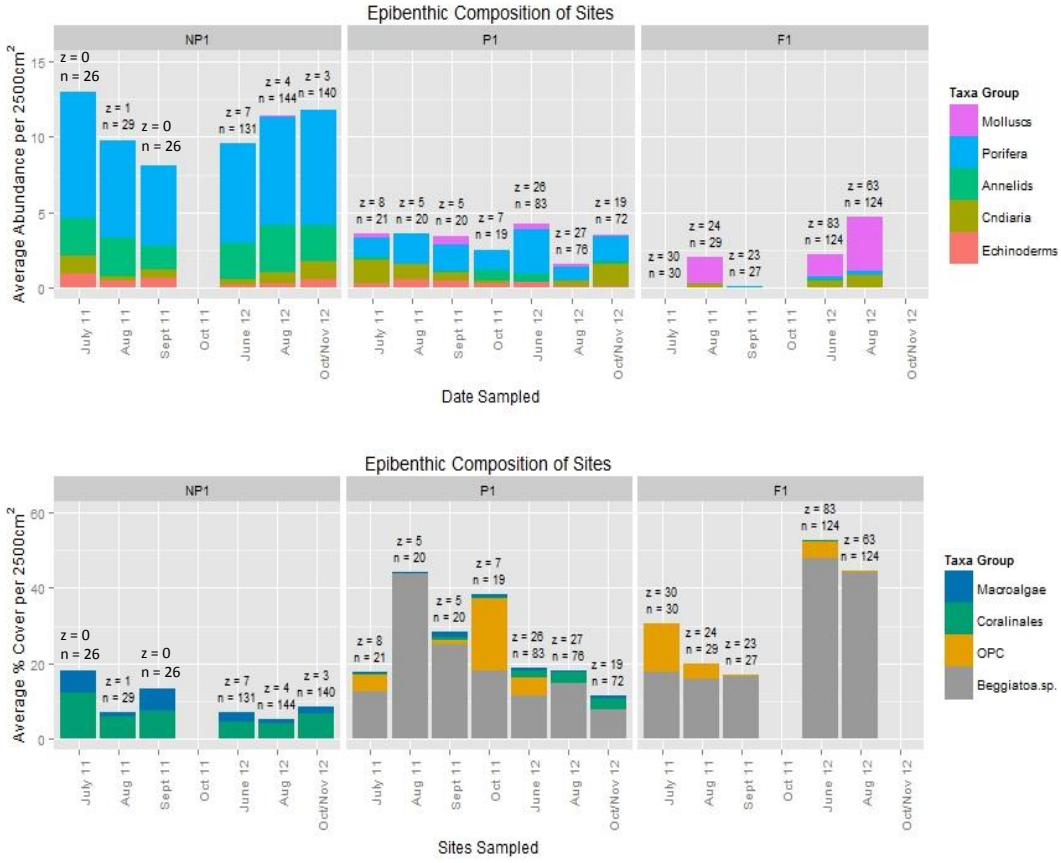

Figure 19. Average epibenthic abundance and % cover of observed taxonomic groups per station, at sites NP1, P1 and F1. The number of images (n) examined at each site and the number of images with no visible organisms (z) are indicated above each bar.

3.3.3.2 Sites NP2, P2, F2

At NP2, the total abundance of enumerable organisms and the percent cover of macroalgae and coralline algae varied significantly over time with the highest abundance occurring in Oct/Nov 2012 (4.60 ± 2.65) and the lowest abundance (1.00 ± 1.78) in October 2011. Annelids, porifera, molluscs and coralline algae displayed significant changes over time (Table 10).

At P2, the abundance and percent cover of organisms was lower at all dates than at NP2. Additionally, the total abundance, the abundance of cnidaria and porifera, and the percent cover of macroalgae and coralline algae differed significantly throughout time at P2 (Table 10). The percent *Beggiatoa spp.* cover at P2 varied with time and was the highest in August 2011 (9.29 ± 20.22) and lowest in October 2011 (0.80 ± 4.04).

At F2, molluscs appeared to increase in abundance during each year (i.e., from September 2011 to October 2011 and from June 2012 to Oct/Nov 2012; Figure 20). The % cover of *Beggiatoa spp.* varied with time, however no statistical difference was found (Table 10). At F2, OPC varied significantly with time but percent cover values were very low, ranging from 0 – 0.16 average % cover per station. Coralline algae showed a similar pattern, varying significantly with time, but with low average % cover per station (0.35 – 0.74 %).

At NP2, there were no images with no visible organisms in September 2011; the highest percentage of images with no visible organisms occurred in June 2012 (3.0% of images). At P1, the percentage of images with no visible organisms peaked at 55.5% in

October 2011 and was the lowest in August 2012 (35.9 %). F1 had high percentages of images with no visible organisms and in September 2011, 81% of the images had no visible organisms. However, the last sampling period had the lowest (56.2%) percentage of images with no visible organisms.

Table 10. Results of Kruskal Wallis analyses comparing the total abundance, the abundances of organisms grouped by higher taxonomic rank, and the % cover of non-enumerable organisms at sites NP2, P2 and F2, according to sampling date. P-values are presented. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

	NP2	P2	F2
Total abundance	< 0.001 (***)	0.031 (*)	< 0.001 (***)
Echinoderms	0.4028	NA	< 0.001 (***)
Cnidaria	0.1085	0.017 (*)	0.205
Annelids	0.006 (**)	NA	0.701
Porifera	< 0.001 (***)	0.050 (*)	0.650
Molluscs	< 0.001 (***)	0.120	< 0.001 (***)
Beggiatoa spp.	NA	0.046 (*)	0.651
OPC	NA	0.106	0.005 (***)
Macroalgae	0.172	0.040 (*)	0.198
Coralline algae	0.005 (**)	0.004 (**)	0.826

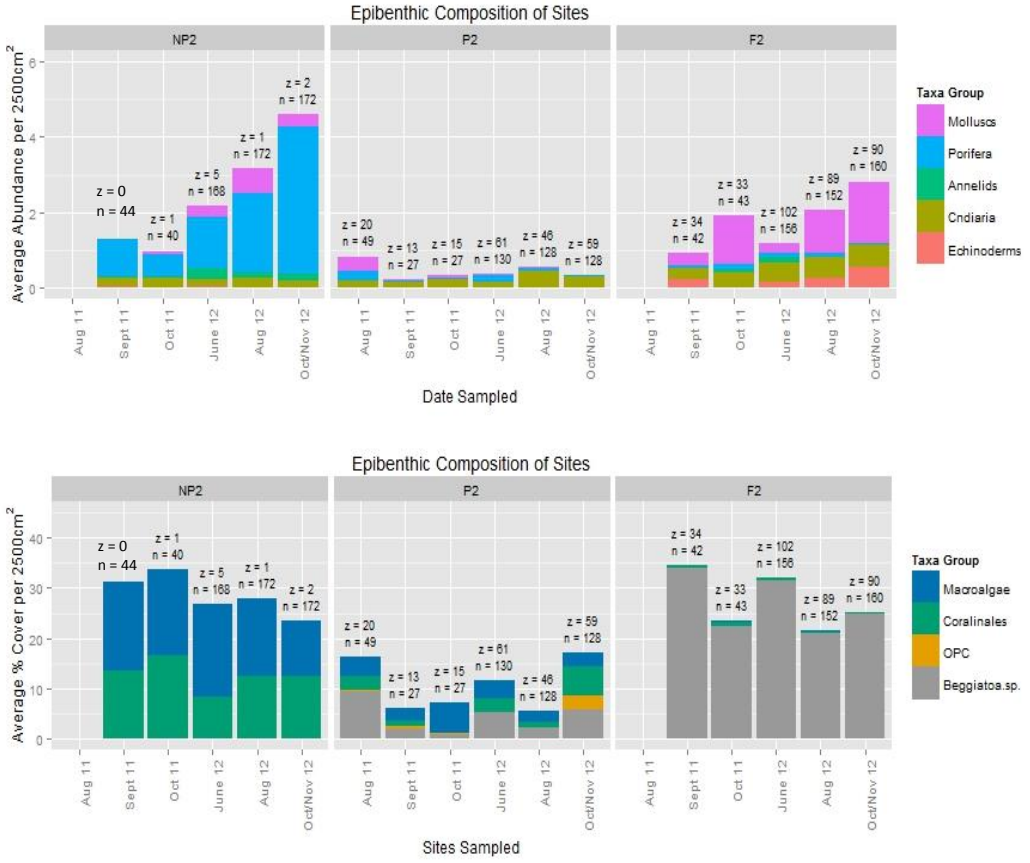

Figure 20. Average epibenthic abundance and % cover of observed taxonomic groups per station, at sites NP2, P2 and F2. The number of images (n) examined for each site and the number of images with no visible organisms (z) are indicated above each bar.

3.3.4 Epibenthic composition with distance to cage

3.3.4.1 P1 and F1

The abundance of enumerable organisms at P1 varied with distance to cage, with the highest abundances occurring at stations that were 100 and 120 m from the edge of the cage. The 100 m station had a total average abundance per station of 6.32 ± 9.08, most of which (51% of the total average abundance) consisted of cnidaria. The 120 m station had a total average abundance of 5.92 ± 8.41 and was predominately comprised of porifera which accounted for 63% of the total average abundance. Significant differences in the abundance of cnidaria and porifera with distance to cage were observed (Table 11). The percent cover of *Beggiatoa spp.* differed significantly according to distance to cage (Table 11). *Beggiatoa spp.* and OPC coverage tended to decrease with increasing distance from cage edge but differences were not significant (Figure 21, Table 11).

At F1, the total abundance of enumerable organisms and the abundance of cnidaria and molluscs differed significantly with distance to cage (Table 11). The average percent cover of *Beggiatoa spp.* differed significantly with distance to cage (Table 11), and tended to decrease with increasing distance from cage edge (Figure 21): the highest average % cover occurred at 20 m from cages (44.92 ± 43.30%) and the lowest occurred at 160 m from cages (7.63 ± 19.58%). The percent cover of OPC showed a similar trend but differences were not statistically significant (Figure 21, Table 11).

Spatially, the greatest amount of images with no visible organisms at P1 (39.3%) occurred at 140 m from cage edge, and the lowest (12.5% of images) occurred at 100 m.

At F1 the highest amount of images with no visible organisms occurred at 20 m (88.6%) and the lowest (23.3%) at 100 m.

3.3.4.2 P2

At P2, the total abundance per station and the abundance of cnidaria decreased significantly with distance from cages (Table 12). The percent cover of *Beggiatoa spp.* and OPC differed significantly with distance to cage (Table 12) and tended to decrease with increasing distance (Figure 22). The percent cover of macroalgae was highest at 160 m (10.95 ± 24.07%) while that of coralline algae was highest at 180 m (7.74 ± 9.90%); the percent cover of those two taxa varied significantly with distance to cage (Table 12).

The images with no visible organisms at P2 were most prevalent at 0 m (59.49% of the images) and the lowest at the furthest distance (180 m) from cages (11.5% of images).

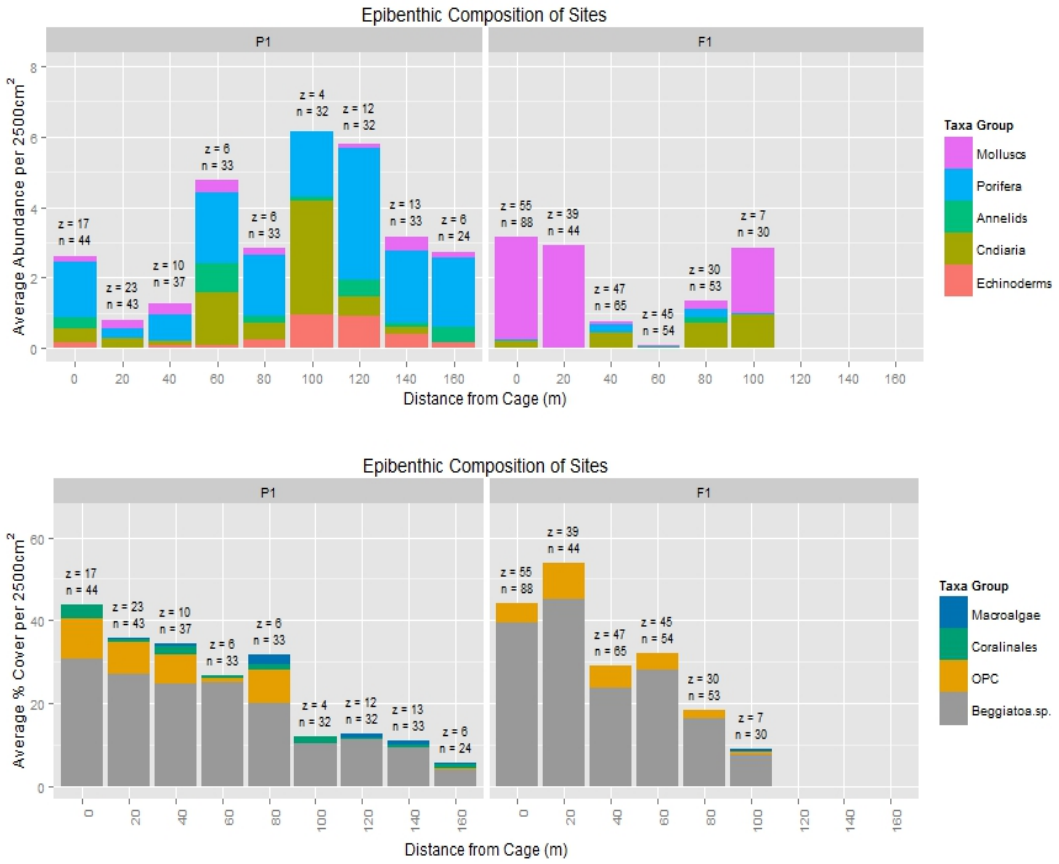

Figure 21. Average epibenthic abundance and % cover of observed taxonomic groups per station, for sites P1 and F1. Data from all dates are considered. The number of images (n) examined for each production stage and the number of images with no visible organisms (z) are indicated above each bar.

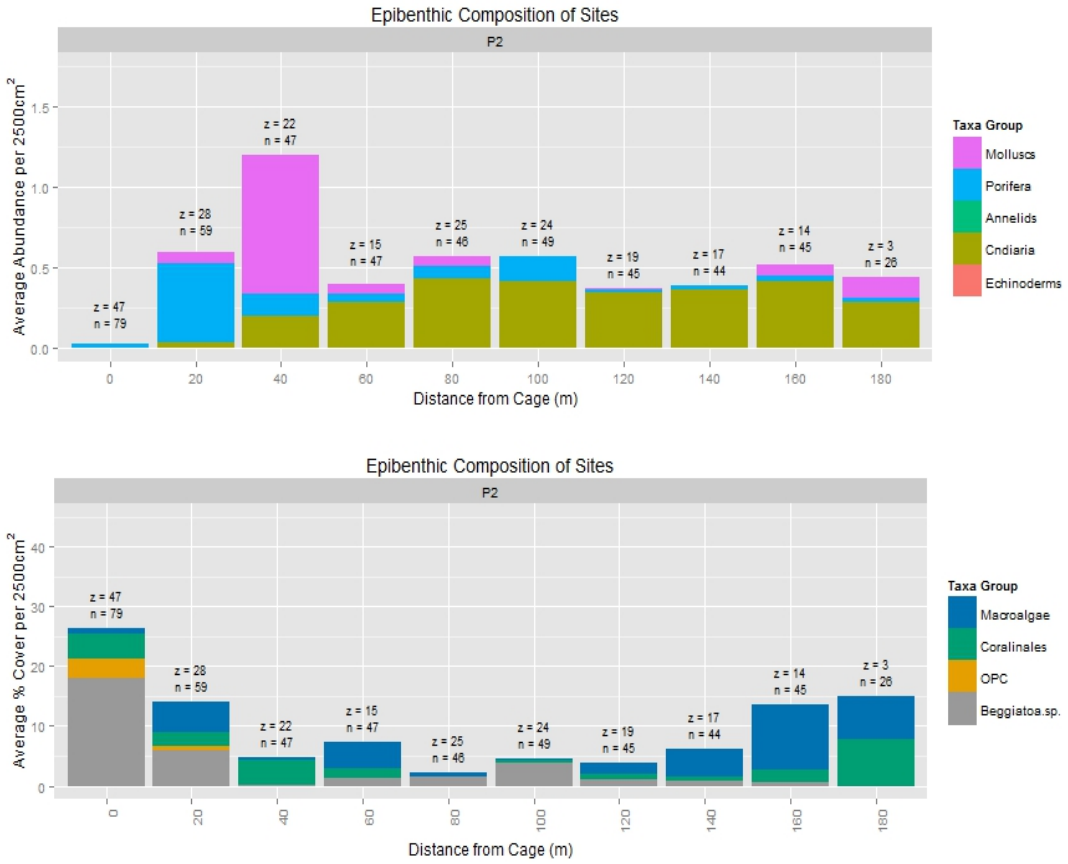

Figure 22. Average epibenthic abundance and % cover of observed taxonomic groups per station, at site P2 (all dates considered). The number of images (n) examined for each production stage and the number of images with no visible organisms (z) is indicated above each bar.

Table 11. Results of Kruskal Wallis analyses comparing the total abundance, the abundances of organisms grouped by higher taxonomic rank, and the % cover of non-enumerable organisms between sites P1 and F1 according to distance from cage edge (all dates considered). P-values are presented. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

	P1	F1
Total abundance	0.128	0.039 (*)
Echinoderms	0.022 (*)	0.365
Cnidaria	0.005 (**)	0.012 (*)
Annelids	0.355	0.362
Porifera	0.269	0.104
Molluscs	0.362	0.122 (*)
Beggiatoa spp.	0.003 (**)	0.005 (**)
OPC	0.198	0.765
Macroalgae	0.616	0.478
Coralline algae	0.543	0.118

Table 12. Results of Kruskal Wallis analyses comparing the total abundance, the abundances of organisms grouped by higher taxonomic rank, and the % cover of non-enumerable organisms between stations at various distances from cage edge within site P2 at all sampling periods. P-values are presented. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

	P2
Total abundance	0.026 (*)
Echinoderms	NA
Cnidaria	< 0.001 (***)
Annelids	NA
Porifera	0.971
Molluscs	0.472
Beggiatoa spp.	< 0.001 (***)
OPC	< 0.001 (***)
Macroalgae	< 0.001 (***)
Coralline algae	< 0.001 (***)

3.4. Discussion

The aim of this study was to investigate potential spatial and temporal changes in the composition of epibenthic assemblages resulting from organic enrichment and/or seasonal effects.

3.4.1 Differences between production stages

The present study shows that all biological variables, with the exception of the abundance of molluscs in the Kruskal Wallis multiple comparisons, differed significantly between the NP stage and both the P and F stages (all stations and sampling periods considered). Differences observed in the abundance of non-indicator species and in the presence of the indicator species among production stages are biologically significant because they indicate modifications to the benthic habitat (Gray & Elliot, 2009). Both the P and F sites had a significantly lower total abundance of enumerable organisms than the NP sites. OM loading can cause localized hypoxia and anoxia due to the consumption of oxygen from microorganisms (Hargrave et al., 2008). Areas with reduced oxygen concentrations are inhospitable to many species as they can negatively affect physiological responses and cause mortality (Gray et al., 2002). The low abundances and the high amounts of stations devoid of enumerable organisms at the F and P stages may be explained by hypoxia or anoxia at certain stations. Further, the P and F sites were colonized by indicator species (*Beggiatoa spp.* and OPC), which were not observed at the NP site. Hamoutene et al. (2013) showed that these indicators are valid bio-indicators of organic enrichment at aquaculture sites on the south coast of NL.

According to the Pearson-Rosenberg model (1978) of organic enrichment, at certain levels along enrichment gradient sediments become reduced and have higher abundance of organisms due to the high concentration of opportunistic species (Pearson & Rosenberg, 1978; Nilsson & Rosenberg, 2000). Although the results of the present

study appear to contradict this model of organic enrichment with decreased abundance in reduced sediments (Pearson & Rosenberg 1978) it should be noted that the abundance measurement did not include taxa calculated by % cover. In addition, we did not include infaunal species which could have altered abundance values when dealing with patchy substrates. Indicator species, assumed to be in high numbers in proximity to organic enrichment (Nilsson & Rosenberg 2000), were measured as % cover and were not enumerated; therefore, abundances at production and fallow sites would have been greater if indicator species had been quantified and incorporated into the abundance calculation. Although indicator species were not included into abundance calculations, there were other biologically significant differences between production stages. The abundance and percent coverage of macroalgae, coralline algae and porifera were significantly lower at the P and F sites. Photosynthetic organisms and sponges are sensitive to smothering by OM (Hall-Spencer et al., 2006) and the organic loading from the aquaculture sites most likely caused their decline at both the P and F sites. This analysis provided further evidence of OM smothering and the possible development of reduced conditions. After the cessation of organic loading it was expected that the abundance of all non-indicator species would increase at an F site; however, this was not apparent for algal species. The percentage cover of algal species was highest in the NP sites, followed by the P sites, with F sites showing the lowest percent cover. Hall-Spencer et al (2006) indicated that a two year fallow period was inadequate in allowing maerl beds to recover due to slow growth characteristics of that species. Similarly, the fallow period was insufficient to support the recolonization of algal species in our study.

3.4.2 Differences between sites

When groups of sites having similar depth characteristics were considered separately, changes in epibenthic composition were similar to those observed when comparing all sites. In both groups 1 and 2, comparisons of the three stages of production revealed significant differences in the total abundance of organisms, in the abundance of porifera, and in the percent coverage of macroalgae and coralline algae.

Not all enumerable species decreased in abundance at P and F sites: molluscs (predominantly mussels) were abundant at both F sites. The abundance of molluscs differed significantly among sites within both groups of sites 1 and 2; however, multiple comparison tests could not differentiate sites maybe due to the high standard deviation of the images. Mussels were observed in abundance on derelict gear (ropes, polar circles) at both F1 and F2 when sampling. The high abundance of molluscs at the F sites is most likely due to biofouling mussels falling off derelict gear at both of these sites. Biofouling is a common problem in finfish aquaculture industries globally and mussels are known to foul aquaculture gear in temperate regions (Bloecher et al., 2013).

It is clear that aquaculture production and the resulting organic enrichment have an impact on epibenthic community composition. These changes include a reduction in the overall abundance, algal species, sponges, and the presence of indicator species at both the P and F sites including an increase in molluscs in both the F sites. Many other studies have shown comparable results (e.g. Hall-Spencer et al., 2006). However, as aquaculture sites were here repeatedly examined, we have the opportunity to examine how aquaculture might affect epibenthic communities on a finer temporal scale, and

attempt to differentiate natural changes from those related to organic enrichment from aquaculture activities.

3.4.3 Changes on a temporal scale

Temporal variations in benthic composition were observed within every site in group 1 (NP1, P1, F1); however, no consistent, significant trend existed among all sites. OPC coverage was highest in October 2011 and was low or absent at other time periods, suggesting sensitivity to temperature, seasonal life history, or to organic loading. Life history traits of OPC are relatively unknown; further studies should investigate OPC environmental preferences and tolerance to aid in understanding their distribution on a temporal scale. Our data suggest a temperature tolerance of OPC as its presence was essentially limited to 1.8 – 4.7° C. Furthermore as temperature increased from June 2012 – Oct/Nov 2012 (Figure 3, Chapter 2) the % cover of OPC decreased in both F1 and P1 (Figure 19). However, this trend did not exist July 2011 – October 2011 and could be related to its biological relationship with *Beggiatoa spp.*, organic loading or temporal oceanographic conditions. Additionally, images with void of enumerable organisms decreased with time at F1 and could be signs of recovery at that site. This trend will be further analyzed in Chapter 4.

Interestingly, mollusc abundance differed significantly among time periods, with abundances being highest in August 2011 and 2012. As mentioned previously, biofouling of derelict gear by mussels was observed at both F sites. It is possible that they began

falling off derelict gear as their size increased after a period of optimal mussel growth in the summer and/or from the onset of fall storms (Boghen, 1995).

Temporal variations in benthic composition in group 2 (NP2, P2, F2) differed from those observed in group 1. The abundance of enumerable organisms differed significantly over time unlike in P1 and NP1. As sites in group 2 are shallower, the temperature varied over time (Figure 6, Chapter 1). Benthic species distribution can be affected by temperature (Bergen et al., 2001); seasonal changes in temperature could have caused significant changes to the abundances of enumerable organisms in this group of sites. Annelids at both NP1 and NP2 varied significantly over time suggesting seasonal or temporal influences. Annelids were shown to have strong seasonal patterns in other areas and peak in abundance in the summer (Stanwell-Smith et al., 1999).

Organic enrichment of P2 appeared to lead to rapid decreases in the abundance of enumerable species, with lower values observed after the first sampling period, and the possible development of reduced conditions that were unfavourable to certain taxa. In general, fishes are more sensitive to hypoxia than crustaceans and echinoderms, which in turn are more sensitive than annelids, whilst molluscs are the least sensitive (Gray et al., 2002). Although molluscs were observed at P2, there were no echinoderms or annelids at this site, suggesting a degree of hypoxia unsuitable to those taxa but perhaps tolerable for molluscs. Alternatively, although molluscs were observed on the seafloor they may not have been alive.

Interestingly, at F2, the total abundance of enumerable organisms as well as the abundance of echinoderms and molluscs differed significantly over time and were highest in the last sampling period (i.e., 14 months of fallow). The increased abundances and

appearance of echinoderms and annelids could be signs of recovery at F2 (Pereira et al., 2004). The echinoderms observed here likely use deposit feeding as a feeding strategy, and could have aided in both the ingestion of organics and bioturbation (Holmer et al., 2005) allowing sediments to recover. Although deposit feeders are associated with enriched environments (Pearson & Rosenberg, 1978), the high abundance and proportion of echinoderms could be an indication of recovery. Annelid abundance at F2 was not elevated at the last sampling period, although this could be explained by natural seasonal variation, as mentioned previously. F2 showed similar patterns of F1 in which images void of species decreased over a temporal scale. This trend will be further analyzed in Chapter 4.

It is clear that some of the epibenthic assemblage changes observed in this study are due to seasonal or temporal variation while others are related to either increasing OM loading or to the cessation of organic enrichment. Annelids and molluscs appeared to be affected by temperature or season (or by other stochastic events such as recruitment) as well as by organic enrichment. Macroalgal species and sponges reacted negatively to organic enrichment: at production and fallow sites, they appeared in much lower abundances compared to NP sites and showed no signs of recovery with time. However, echinoderms increased in abundance over time at F2, suggesting recovery; further temporal sampling is required to determine if this trend is consistent. Echinoderms are quite motile and can travel major distances to sites by chemical gradients of food

The percent coverage of both indicator species changed significant with time with no clear pattern, although some temporal changes in OPC could be related to temperature preferences (Chapter 2). In part, temporal or seasonal variability in fish feed input and

stock biomass (Linfoot et al., 1990) could explain those inconsistent changes on a temporal scale. In addition, analyzing changes in indicator species on a spatial scale could be useful, as Pearson and Rosenberg (1978) noted that organic enrichment affects benthic communities on both temporal and spatial scales concurrently.

3.4.4 Changes on a spatial scale

Wildish and Pohle (2005) investigated changes in benthic communities in New Brunswick, Canada, and stated that changes in benthic communities in proximity to aquaculture sites were mainly constrained to a defined cage footprint; however, Pohle et al. (2001) found regional impacts extending as far as 200 m even though currents were as low as < 5 cm/s. Our study revealed the presence of indicator species as far away as 160 m from cages, suggesting that organic enrichment extended at least to that distance. However, a decreasing % cover of indicator species was observed with increasing distance.

At P1 and F1, stations at 100 m from cage edge were affected by organic enrichment: *Beggiatoa spp.* was present at these stations, but in low amounts (7 – 10 % cover). The OM enrichment at a certain distance from cages (e.g, approximately 100 m away) could have led to greater abundances of cnidaria, as observed for certain taxa in other studies (Vetter & Dayton, 1999). Cnidaria varied significantly with distance at P1, F1 and F2. The abundance of cnidarians was higher at 100 m from cage edge than at non-impacted sites. Echinoderms showed a similar pattern with the highest abundances at P1 and F1 located at 100 m from cages, and higher than at site NP1. These patterns

correspond to the transition zone within generalized SAB diagrams as described by Pearson & Rosenberg (1978) but further analysis at multiple sites is recommended to determine if this trend is consistent.

Interestingly, at P2, the *Beggiatoa spp.* trend was not consistent through all distances. Furthermore, images void of enumerable species decreased with increasing distance. Both trends could be due to bathymetric properties of that area. Spatial maps could help determine epibenthic trends due to bathymetric characteristics and water current and circulation effect. The percent cover of macroalgae and coralline algae increased with increasing distance from cages and could be partly to do with the bathymetric characteristics of the area and decreasing organic enrichment.

Spatial patterns of organic enrichment were more predictable and were comparable to those described by the Pearson & Rosenberg model. Opportunistic/indicator species coverage decreased as distance from the source of organic enrichment increased, and abundances of echinoderms and cnidarians increased at a transitional zone but decreased at greater distances, where they closely resembled abundances at non-impacted zones. Echinoderms may benefit from intermediate organic enrichment as a food source and/or the abundance of molluscs could be attracted to certain sites near cages (Crawford et al., 2002 Mente et al., 2010). However, sponges and the algal species seemed sensitive to organic enrichment up to distances of 160 m from cages. Determining the spatial extent of OM settlement could provide more detail on enrichment effects and help correlate biotic changes with deposition.

3.5. Conclusions

Organic enrichment led to decreases in the abundance of sponges, macroalgae and coralline algae; however molluscs proliferated during the fallow period but were most likely due to biofouling rather than recolonization. These trends were consistent whether or not sites were grouped according to similar depth characteristics. On a temporal scale, the impact of organic enrichment from aquaculture production appeared immediate, effecting changes to epibenthic assemblages even within the first sampling period. Sponges, macroalgae and coralline algae were the most sensitive taxa to organic enrichment, and exhibited no sign of recovery during fallowing.

The presence of indicator species at P and F stages provided validation that organic enrichment had occurred. OPC and *Beggiatoa spp.* were present soon after aquaculture production began although OPC showed seasonal variability. *Beggiatoa spp.* displayed no clear pattern of change in percent coverage through time. It is unclear if the percent cover of indicator species could be used to quantitatively compare sites or used in regulatory thresholds. Our data did show that the percent cover of *Beggiatoa spp.* and OPC did decrease with distance. This trend of decreasing % cover of the indicator species is expected as the intensity of organic enrichment would decrease over distance due to dilution and limitations of its particle distribution. *Beggiatoa spp.* was expected to increase throughout a production cycle and decrease throughout the fallow cycle. However, although the % cover varied at different time periods a clear increase or decrease in percent cover was not observed.

Echinoderms increased in abundance during the fallow period at F2 and could indicate recovery. Increased abundances of echinoderms and cnidaria at 100 m could also be indicative of a transition zone to “natural” conditions. In conclusion, organic enrichment impacts epibenthic assemblages by both increasing and decreasing specific taxon groups. Furthermore, changes are likely related to habitat specific characteristics are bathymetric properties.

3.6 References

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Chapter 4. Spatial and temporal distribution of biological indicators of organic enrichment *(Beggiatoa spp.* and OPC*)* in relation to aquaculture cages and local bathymetry

4.1. Introduction

The sedimentation of organic matter (OM) is a complex process that depends on several factors: farming practices (e.g. feeding quantity and rate), fish conditions (e.g., age, size, biomass) and physical conditions (e.g. currents, winds, bathymetry) (Corner et al., 2006). Aquaculture sites are subject to dynamic conditions, with changes in farm management, seasonal oceanographic fluctuations and temporal variations in biological communities occurring (Perez et al., 2002; Cromey et al., 2002; Sanz-Lazaro et al., 2011) and ultimately altering the rate and spatial extent of organic deposition. Additionally, the intensity of organic enrichment can change drastically over relatively short distances (Cromey et al., 2002). This combination of factors can lead to complex spatial and temporal patterns of organic enrichment**.**

On the south coast of Newfoundland, two biological indicators, *Beggiatoa spp.* and Opportunistic Polychaete Complexes (OPC), were found to be associated with organic enrichment at finfish aquaculture sites (Bungay; 2012; Hamoutene et al., 2013). The presence and abundance of these organisms can be used to determine the spatial extent of organic enrichment originating from salmon aquaculture sites. For example, the impact of marine fish aquaculture on the seafloor has been modeled using Geographic Information Systems (GIS) to display the presence and abundance of indicator species

relative to the spatial extent of cages and the local bathymetry (Perez et al., 2002; Corner et al., 2006).

The objective of this chapter is to investigate the spatial and temporal distribution of biological indicators of organic enrichment (*Beggiatoa spp.* and OPC) in relation to aquaculture cages and local bathymetry. First, bathymetric maps of two study sites (a production and a fallow site) were generated, and the percent cover of both indicators at sampling stations were displayed on those maps, along with the presence (and, if appropriate, the percent cover) of other, non-indicator species. Sampling was performed at different times to capture temporal changes in the percent cover of those indicators of organic enrichment. Changes in the spatial distribution and abundance of indicator species at the production site can help to assess how changes occur in the benthic habitat along with a continuous source of organic loading. At the fallow site, changes in indicator and non-indicator species may reveal recovery after organic loading has stopped.

4.2. Methods

4.2.1 Sampling sites

Two sites were chosen for this analysis due to their comparable depth characteristics, P1 and F1 (Chapter 2). These sites were chosen specifically due to the number and distance of sampling of stations that extended from cage edge. Three transects were utilized to gain a better understanding of how differential waste dispersion

due to possible influences from current direction might affect organic distribution and corresponding changes in the epibenthic communities.

4.2.2 Mapping

Bathymetric maps were created using ArcGiS software. The coastline of Newfoundland was generated based on a shapefile produced from a National Topographic Service 1:50000 scale map. Depths and corresponding coordinates were obtained from multibeam sonar surveys and from the depth recorders that were used during video sampling (see Chapter 2). To create the bathymetric shapefile, data was interpolated across a surface using inverse distance weighted interpolation.

Biological data (see below) was plotted on maps based on coordinates obtained during video sampling (for procedure and equipment, see Chapter 2). Cage locations were extracted from environmental monitoring reports. However, over the sampling period, cages moved and/or were rearranged periodically. In order to standardize the results, stations located at cage edge were always referred to as “0 m”, with subsequent images obtained each 20 m from the first station, along transects (as described in Chapter 2).

4.2.3 Data processing

Based on video monitoring and macrobenthic community identification as described in chapter 2, during the 2011 field season, one sample was collected at each

station; four images were collected at each station during the 2012 field season, with resulting data representing averages for each station.

Data are presented as pie charts showing OPC and *Beggiatoa spp.* percentage cover determined from benthic image analysis (the area determination technique is described in Chapter 2). For each time and station, *Beggiatoa spp.* and OPC are represented by their respective percentage cover on a pie chart, and the remaining percentage is shown in green if other organisms were observed (i.e., non-indicator species, or NIS), or in grey it no other organisms were seen (i.e., no visible species, or NVS). For example, if *Beggiatoa spp.* and/or OPC were present at a station along with no visible species, then ”no visible species” was calculated as: 100 – (% cover *Beggiatoa spp.* + % cover OPC). If a station was devoid of any visible species it was classified as 100% “no visible species” (i.e., a grey circle). If a station only contained visible organisms other than the indicators, it was classified as 100% “non indicator species” (i.e., a green circle).

4.3. Results

4.3.1 Bathymetry of sites

4.3.1.1 P1

P1 is located over a steep depth gradient, with the majority of stations (81%) located over depths of 50 – 100 m. The increase in depth from 50 to 100 m occurs rapidly (i.e., over < 50 m in horizontal distance) and relatively evenly as distance from the shoreline

increases, in the area considered herein. The rectangular area occupied by cages has its greatest length parallel to the shoreline, and the cage location changed over the sampling period, being situated over deeper water at later sampling periods (July 2011 – June 2012) (Figures 23 – 29).

4.3.1.2 F1

The majority (89%) of stations at F1 are located over depths of 50 – 100 m. However, the seafloor at F1 appears to be relatively less steep than at P1 although it also displays increased depth as distance from shoreline increases. The approximately rectangular area that was previously occupied by cages has its longest side parallel to the shoreline (Figures 30 – 34).

4.3.2 Spatial benthic cover

4.3.2.1 P1

Throughout P1 and at all sampling periods, certain trends in the presence and percent coverage of benthic organisms were observed, along with apparent patchiness. In general, IOE (indicators of organic enrichment: *Beggiatoa spp.* and OPC) were observed in close proximity to cages, and NIS appeared to be more common at locations furthest away from the spatial extent of cages (Figures 23 – 29).

On the first sampling date (July 2011), all benthic cover groups (i.e., *Beggiatoa* sp, OPC and NIS) were observed on the three transects (Figure 23). Along the short transect perpendicular to the coastline, mainly NIS were observed, along with < 25% coverage of

Beggiatoa spp. On the transect to the east of cages, no IOE were seen, with stations either being bare or containing NIS. Along the transect to the west of cages, IOE were dominant, particularly between 50 and 100 m depth, and within 60 m of the cage edge. Some NIS and a bare station were also documented along this transect.

In August 2011, *Beggiatoa spp.* appeared along the eastern transect at up to 100 m distance from cages, and declining in % cover with distance from cage edge (Figure 24). Along the short perpendicular transect, the station closest to the cage was dominated by *Beggiatoa spp.*, and the next station was bare. *Beggiatoa spp.* were seen at all stations (including a shallow station) on the transect to the west of cages, but no OPC were visible at this time.

In September 2011, *Beggiatoa spp.* on the eastern transect decreased in percent cover with distance from cage edge (and were present at up to 100 m from cages); NIS were observed at > 40 m from cages (Figure 25). Only NIS were observed on the transect perpendicular to the shoreline. All stations on the western transect contained IOE, generally covering less than half of the station area. The following month, *Beggiatoa spp.* coverage on the eastern transect had slightly decreased, and the western transect showed only IOE at up to 100 m from cages, with *Beggiatoa spp.* recorded at up to 140 m from cages (Figure). OPC were predominant at 0, 20, 40 and 80 m from cages along the western transect.

In the second year, very few IOE were seen on the eastern transect, and the percent cover of IOE where observed was generally lower than in 2011. Along the transect

perpendicular to the coastline, only IOE (mainly *Beggiatoa spp.*) were observed in June 2012 (Figure 27), but NIS were predominant in August (Figure 28) - no observations were made along this transect in Oct/Nov 2012 (Figure 29). Stations on the western transect contained a decreasing percent cover of IOE (*Beggiatoa spp.* and OPC) with increasing distance from cages, to a maximum distance of 120 m from cages in June 2012, *Beggiatoa spp.* (up to 10.3 % cover) at up to 80 m from cages in August 2012, and a low coverage of *Beggiatoa spp.* at up to 120 m from cages in Oct/Nov 2012; NIS were present along this transect at all sampling dates in 2012 (but were rare in August 2012). Three bare stations were noted along the western transect in August 2012, and one in Oct/Nov 2012 (Figures 27 – 29).

Temporally, *Beggiatoa spp.* was observed at the greatest proportion of stations (85%) in August 2011 and at the lowest proportion of stations (42%) in August 2012 (Figure 35). OPC was most prevalent in June 2012, occurring in 36% of stations, and least prevalent in August 2011 and Oct/Nov 2012 in which it was absent. NIS were most prevalent in Oct/Nov 2012, occurring at 85% of the stations, while in October 2011 they were only present in 57% of the stations. Stations classified as NVS (or a combination of an IOE and no other visible organisms) were observed at all survey periods. Bare stations were located at various areas relative to the cages, on the three transects, and showed no clear temporal pattern.

4.3.2.2 F1

Patchiness in benthic organism presence and coverage was observed at site F1, throughout all sampling periods, with no obvious spatial gradients or difference between

transects. *Beggiatoa spp.*, OPC, and areas with no visible species were common within and in close proximity to the area previously occupied by cages. Furthermore, NIS appeared to be more common at stations that were furthest from the area covered by cages.

Over the first year, most stations were either bare or contained *Beggiatoa spp.* (Figures 30 – 32). In addition, OPC decreased in presence and percent cover from June to October, and NIS were only seen in August and September (Figures 31, 32). In 2012, there were fewer stations with NIS, but *Beggiatoa spp.* remained abundant. OPC were observed at two stations underneath the cage area in July, but were not seen in August (Figures 33, 34).

Beggiatoa spp. occurred at the highest proportion of stations (74%) in August 2012, and in the lowest proportion of stations (43%) in July 2011 at start of production. OPC were seen at 60% of stations at the first sampling period (July 2011), but were only seen at 10% of stations in the last sampling period (August 2012). NIS were not seen in July 2011 but increased in prevalence up to Oct/Nov 2012, where they were observed in 58% of the stations, throughout the sampling area (Figure 34). In July 2011, 90% of the stations were classified as having no visible species compared to 20% of stations in June and August 2012 (Figure 36).

Site – P1
Time – July 11

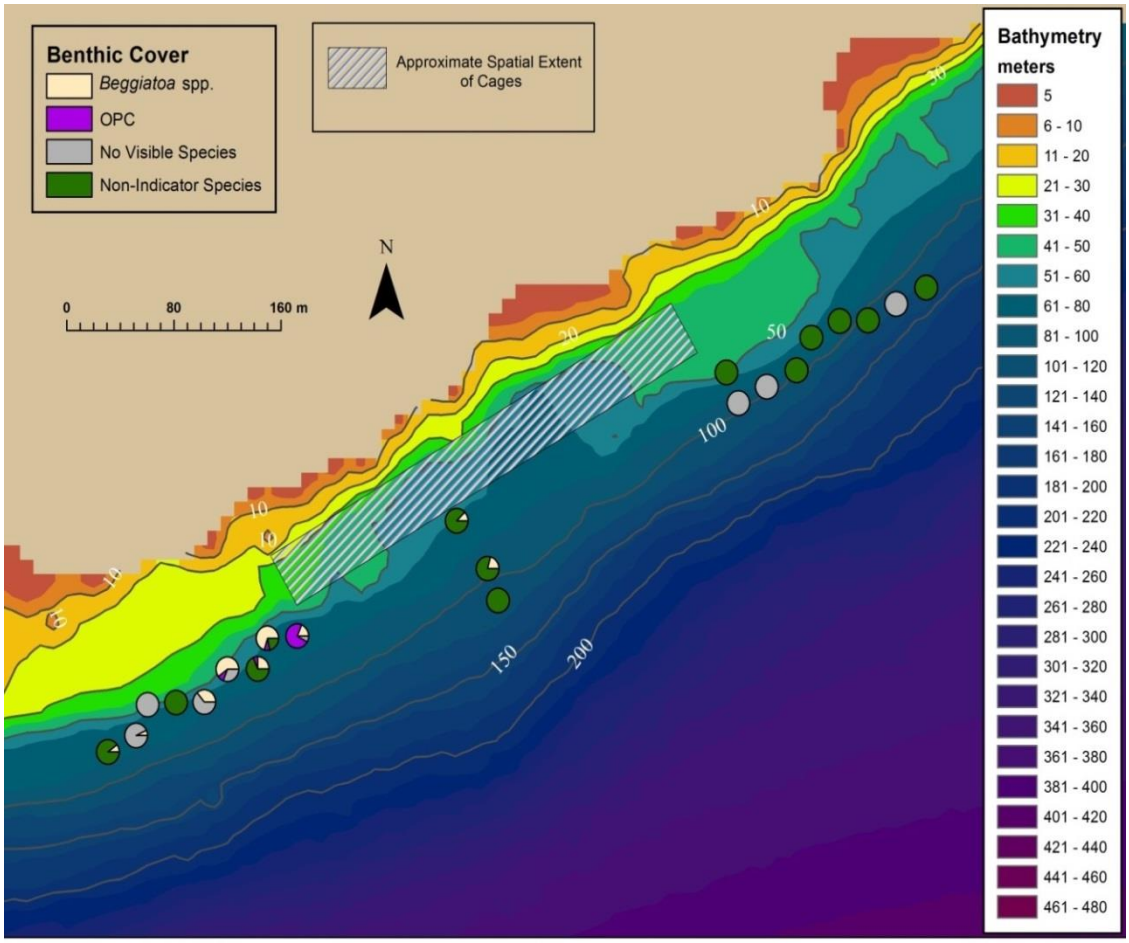

Figure 23. Bathymetric map of P1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in July 2011.

Site – P1
Time – Aug 11

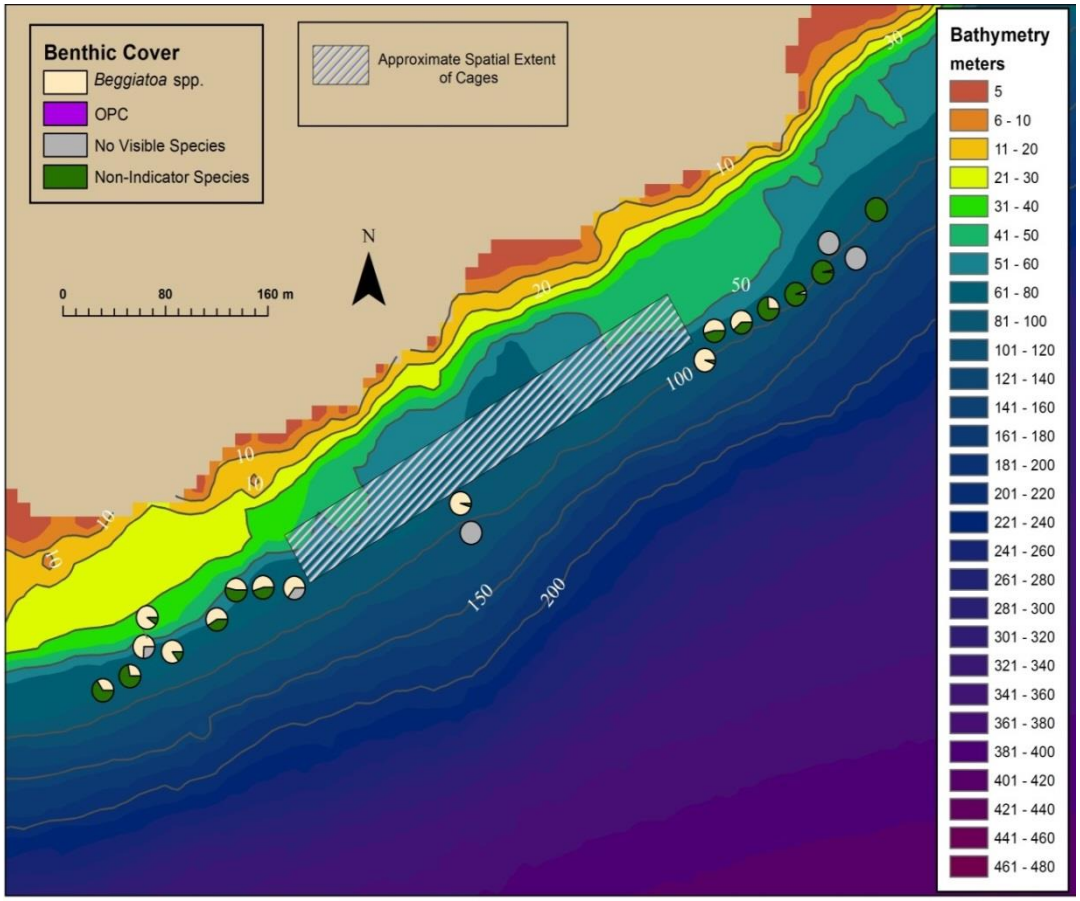

Figure 24. Bathymetric map of P1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in August 2011.

Site – P1
Time – Sept 11

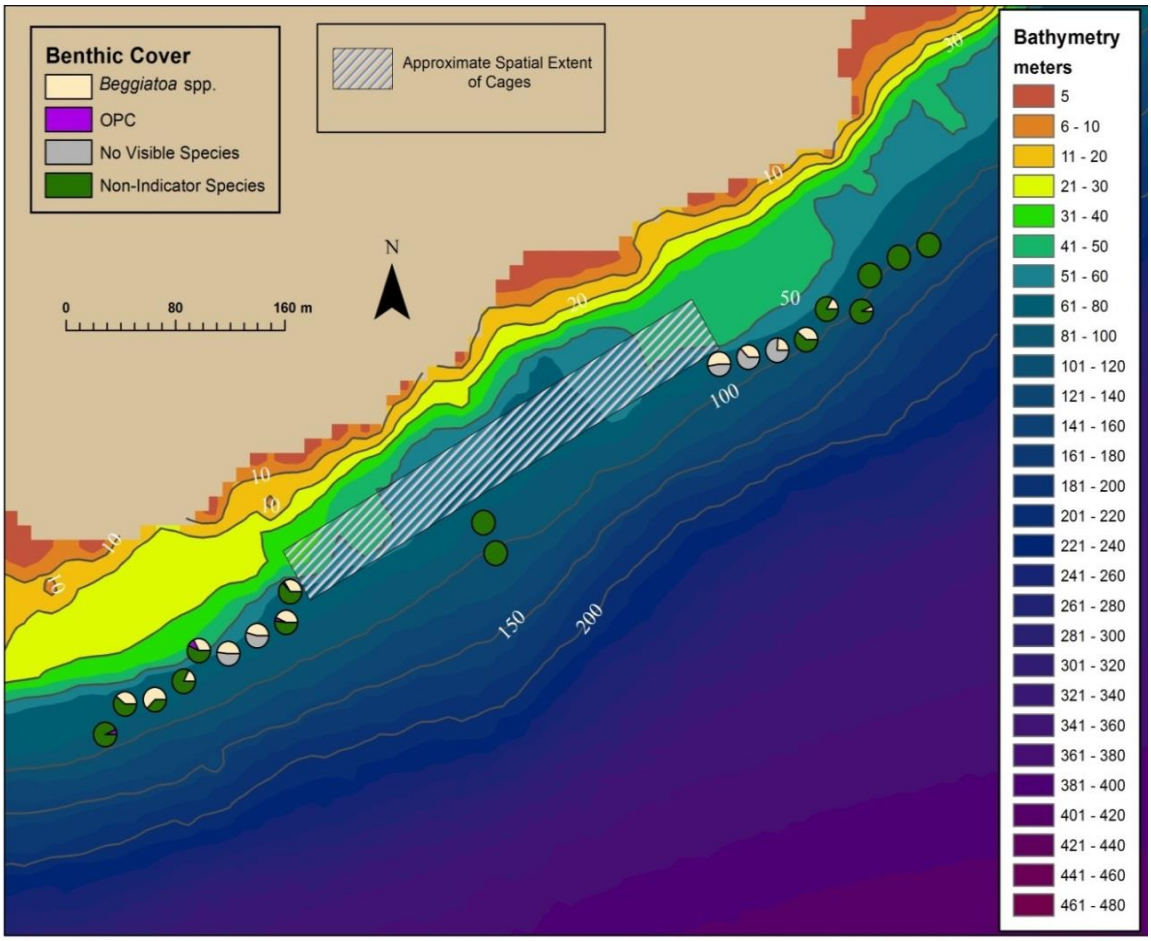

Figure 25. Bathymetric map of P1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in September 2011.

Site – P1
Time – Oct 11

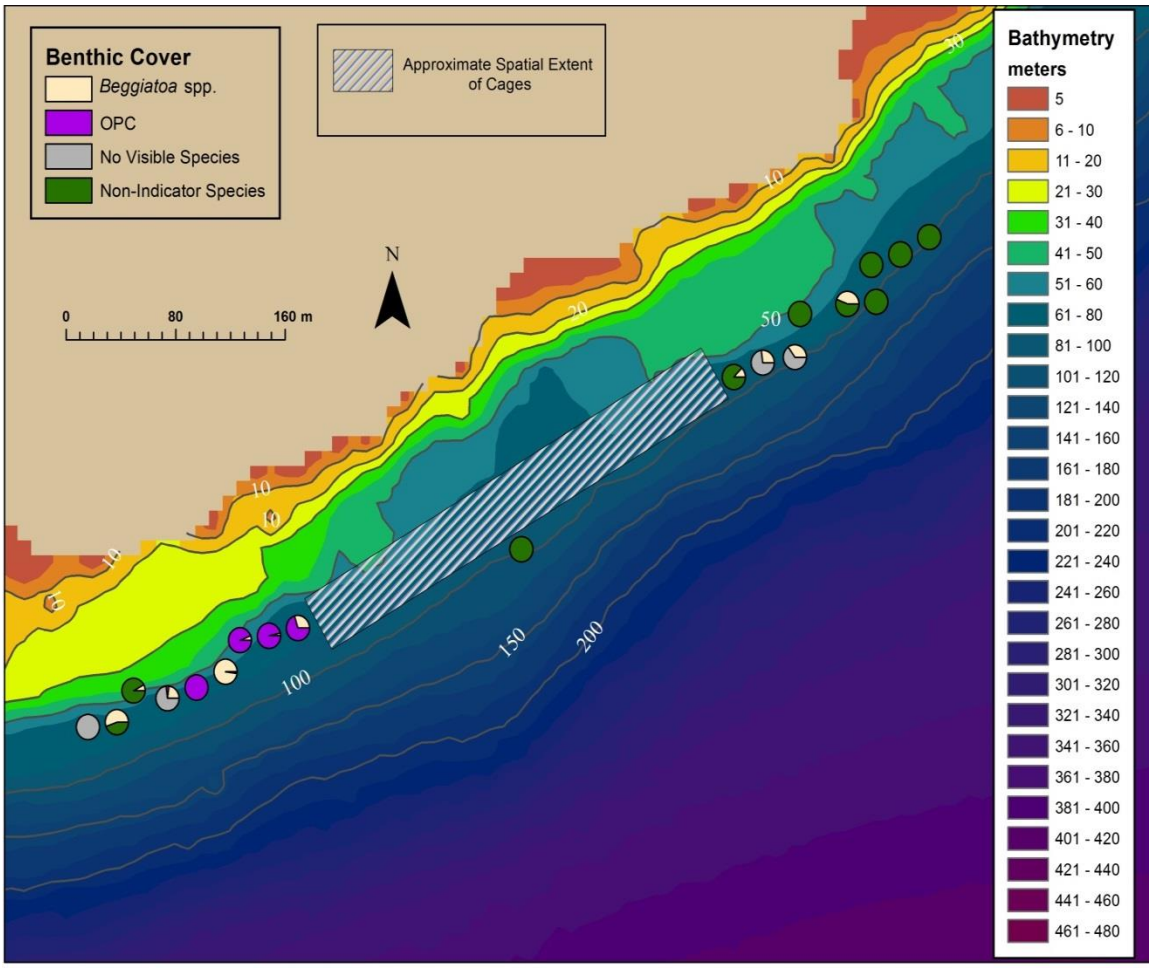

Figure 26: Bathymetric map of P1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in October 2011.

Site – P1
Time – June 12

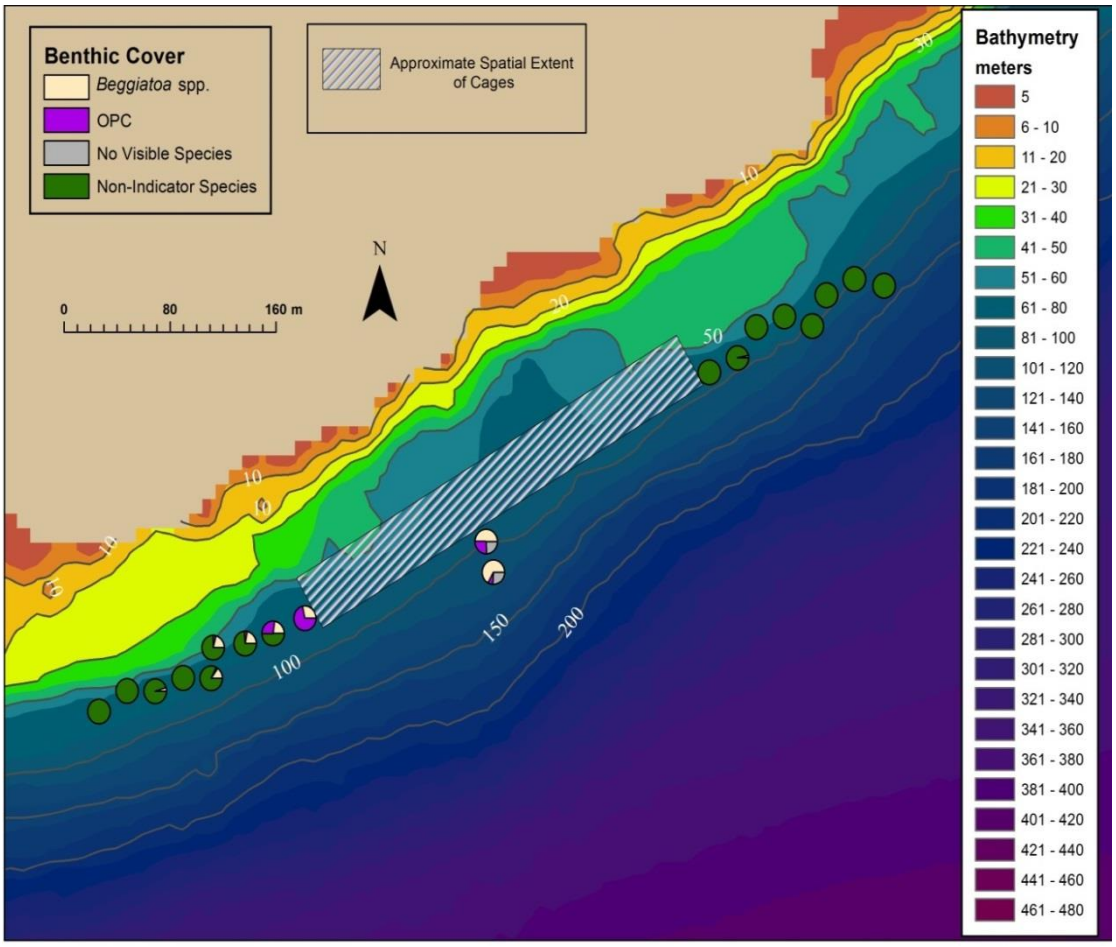

Figure 27. Bathymetric map of P1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in June 2012.

Site – P1
Time – Aug 12

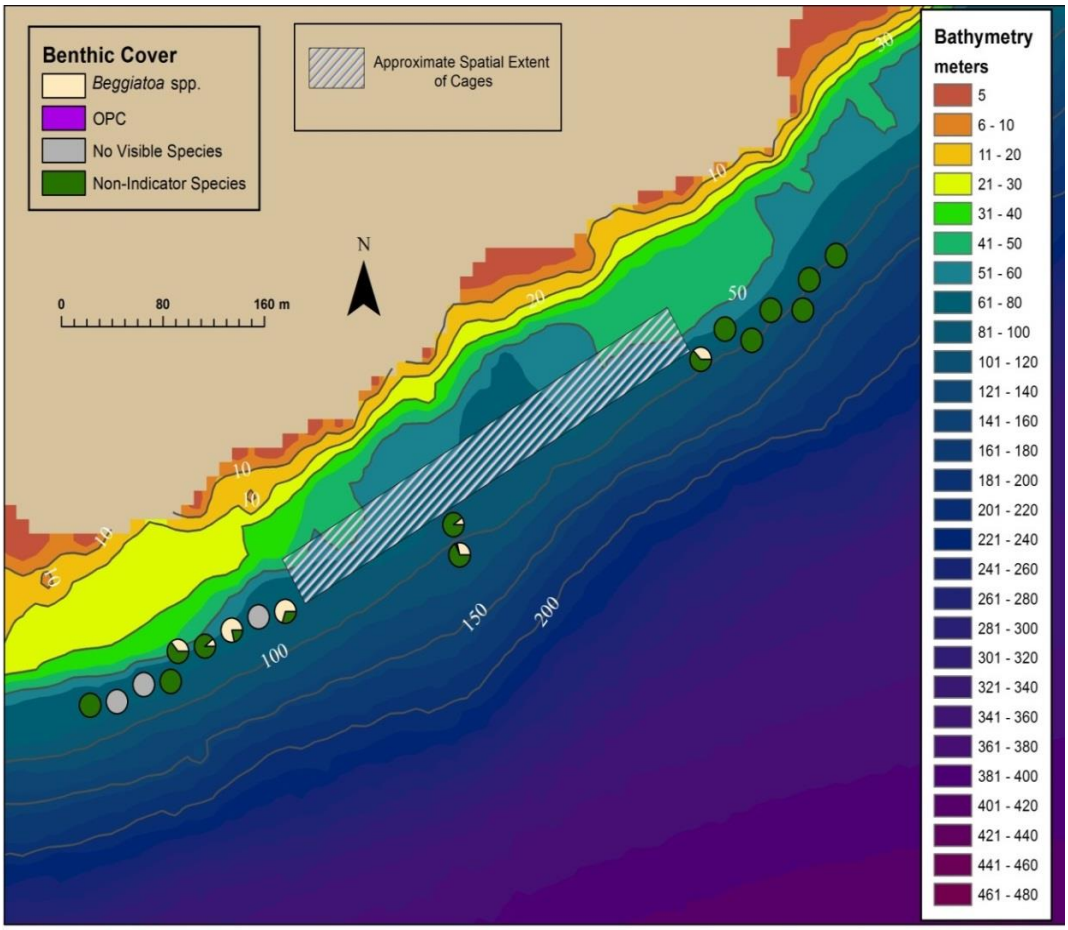

Figure 28. Bathymetric map of P1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in August 2012.

Site – P1
Time – Oct/Nov 12

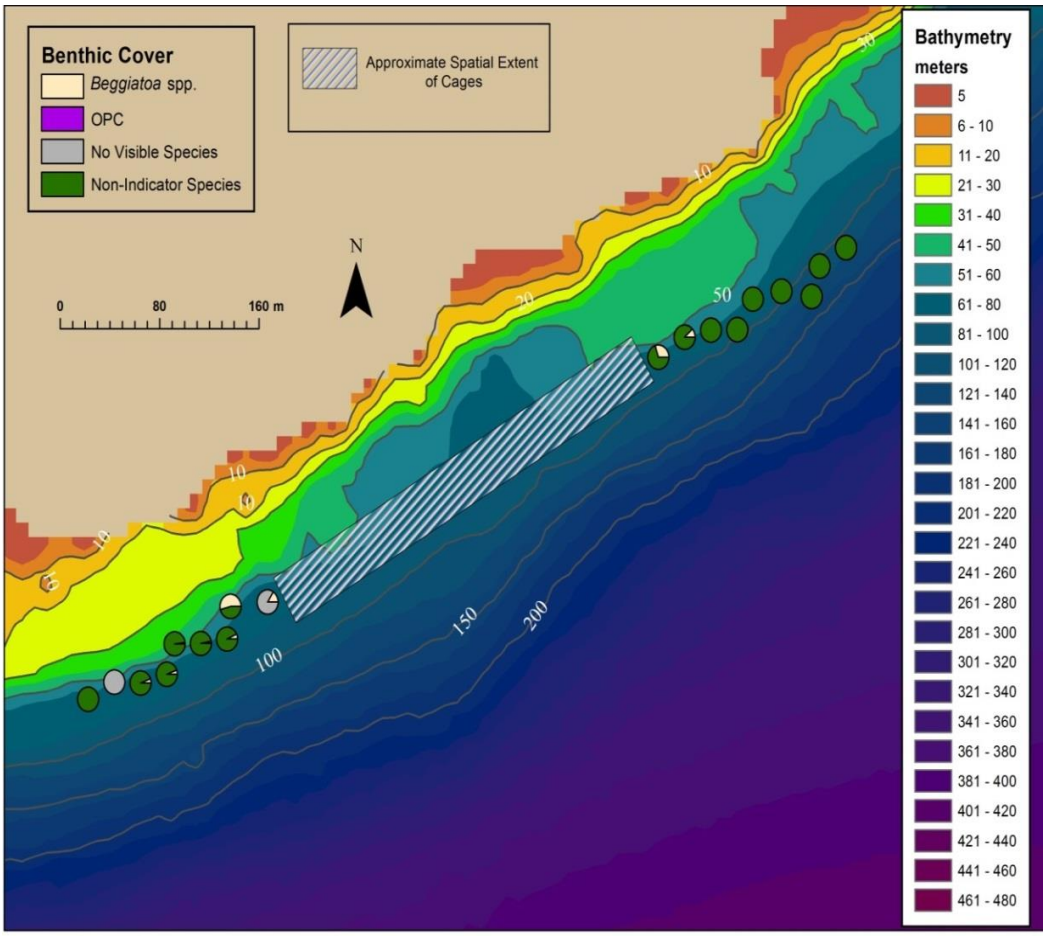

Figure 29. Bathymetric map of P1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in Oct/Nov 2012.

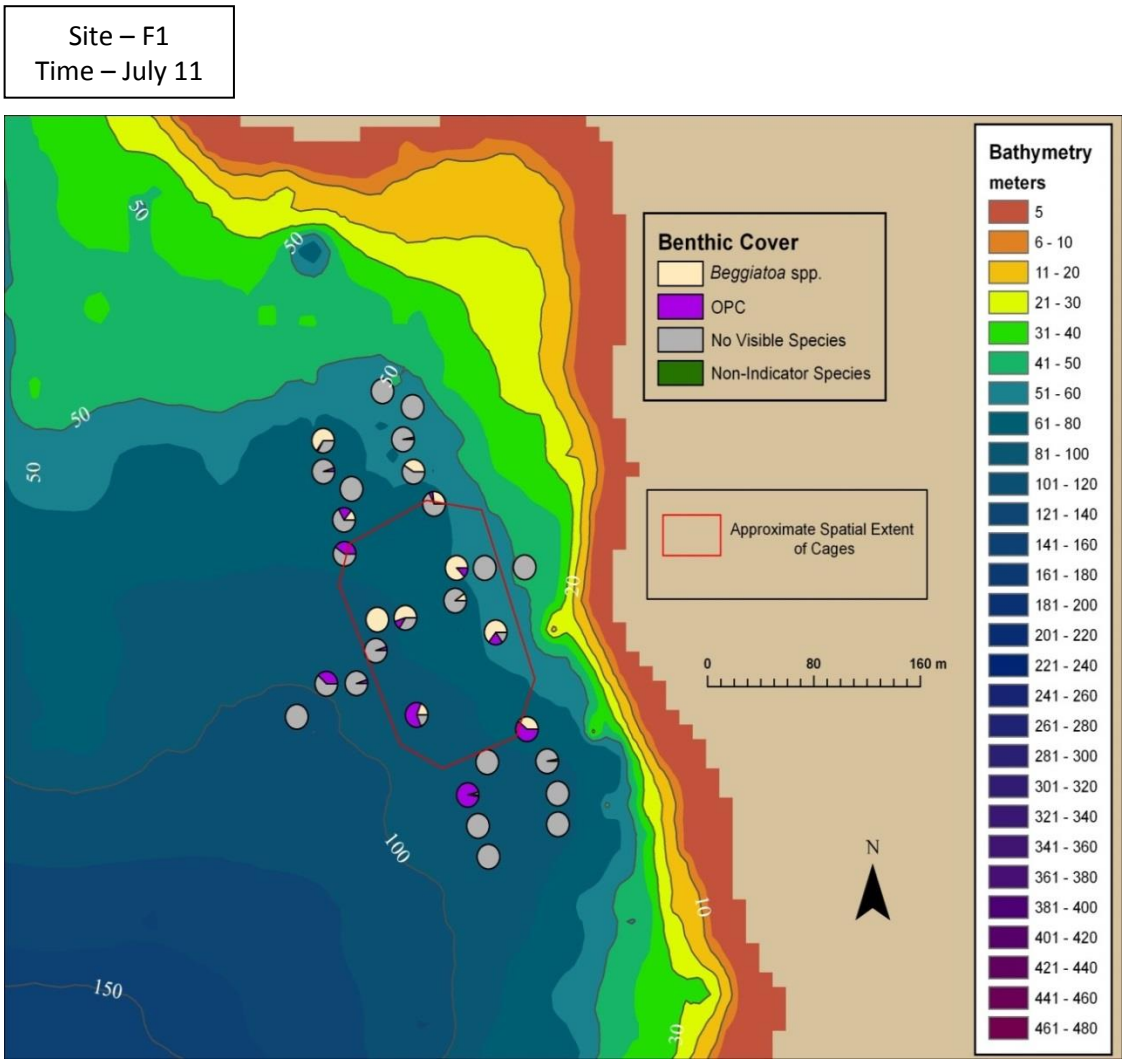

Figure 30. Bathymetric map of F1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in July 2011.

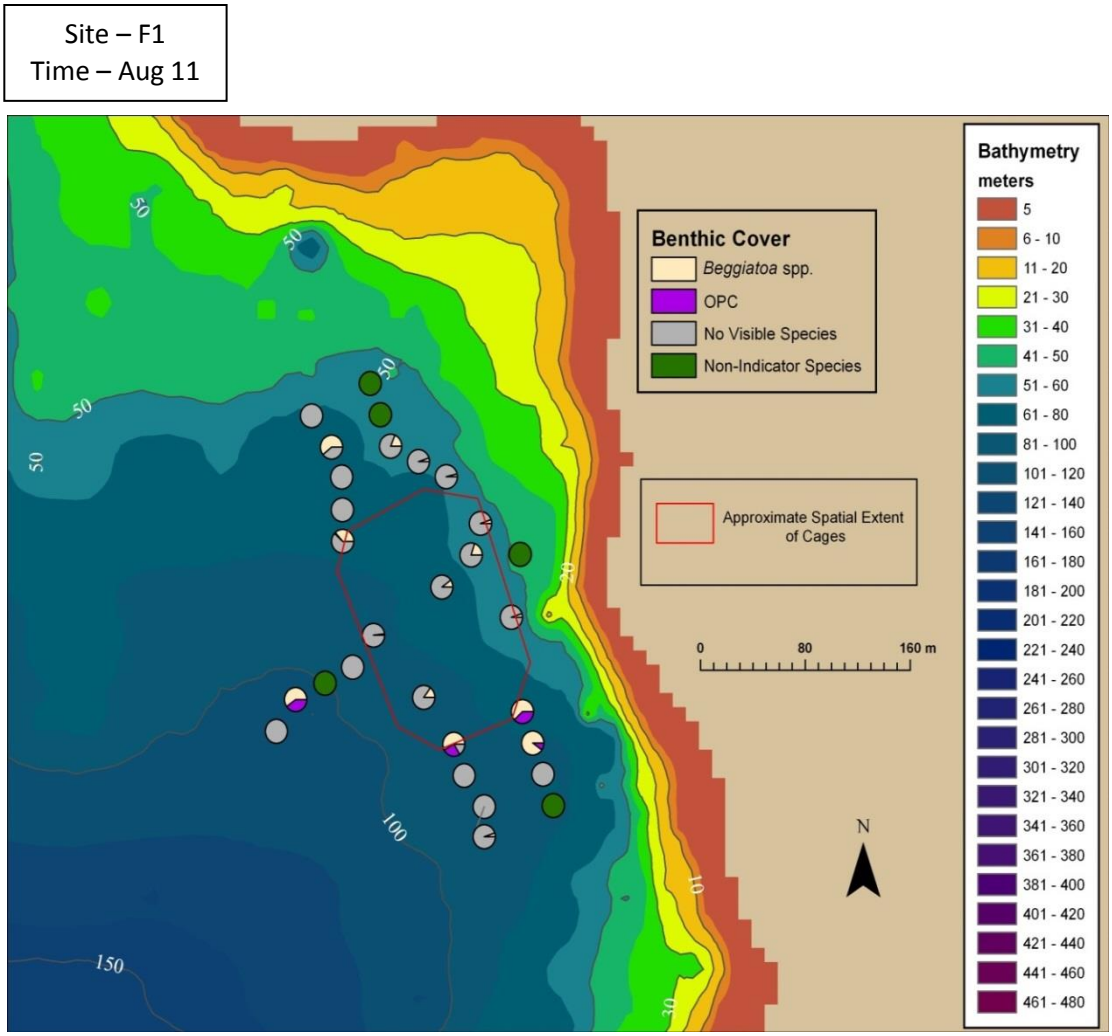

Figure 31. Bathymetric map of F1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in August 2011.

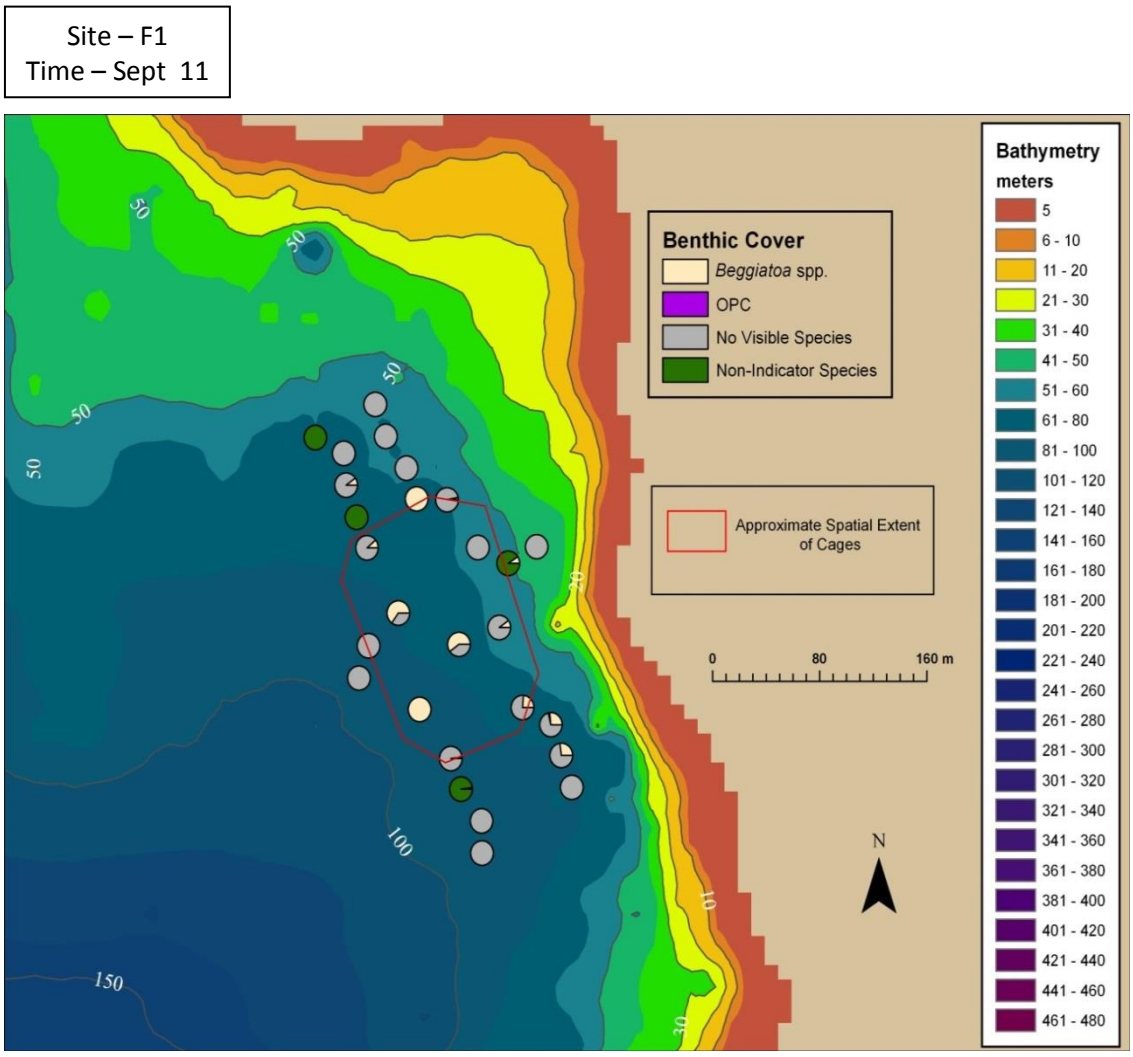

Figure 32. Bathymetric map of F1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in September 2011.

Site – P1
Time – June 12

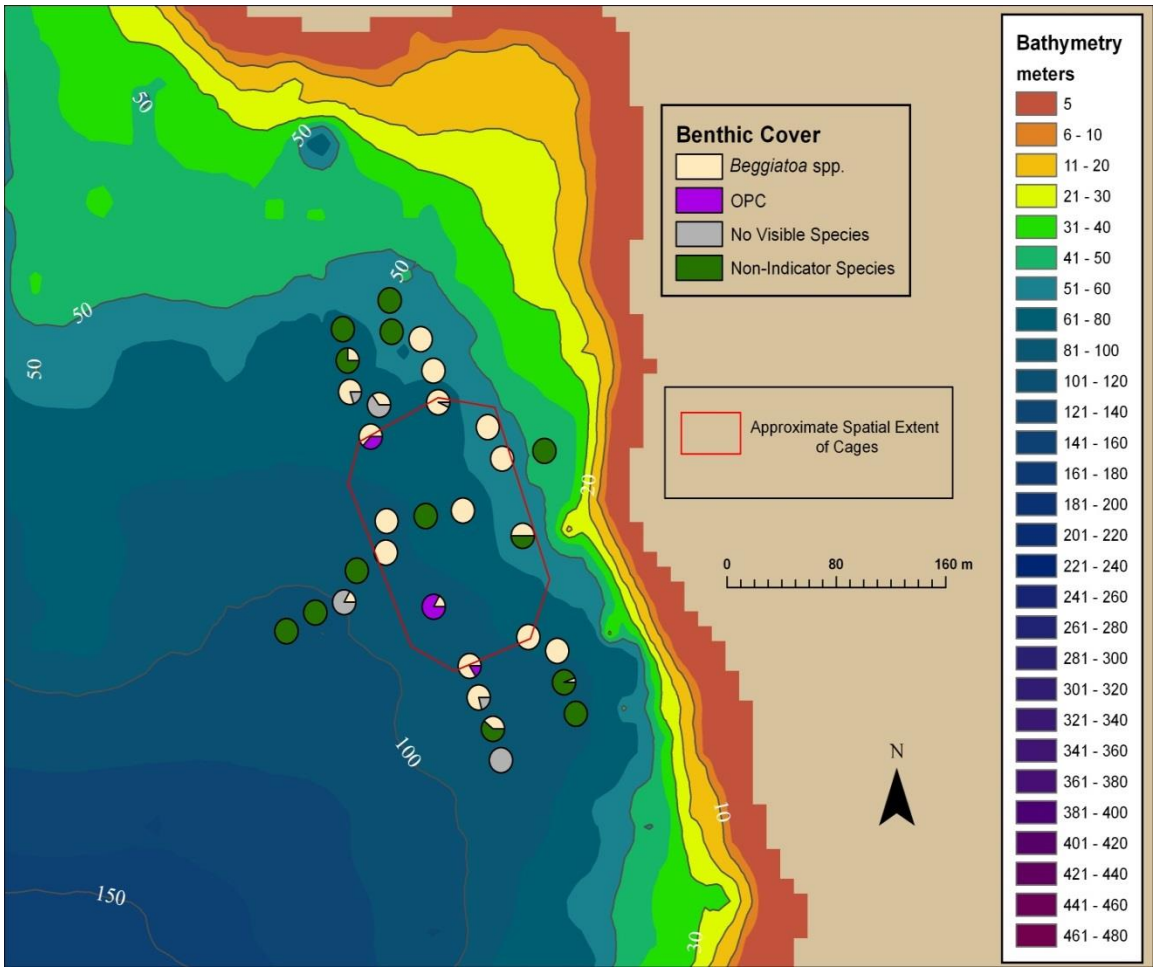

Figure 33: Bathymetric Map of F1 with Benthic Cover composition at stations sampled on the South Coast of Newfoundland on June 2012.

Site – F1
Time – Aug 12

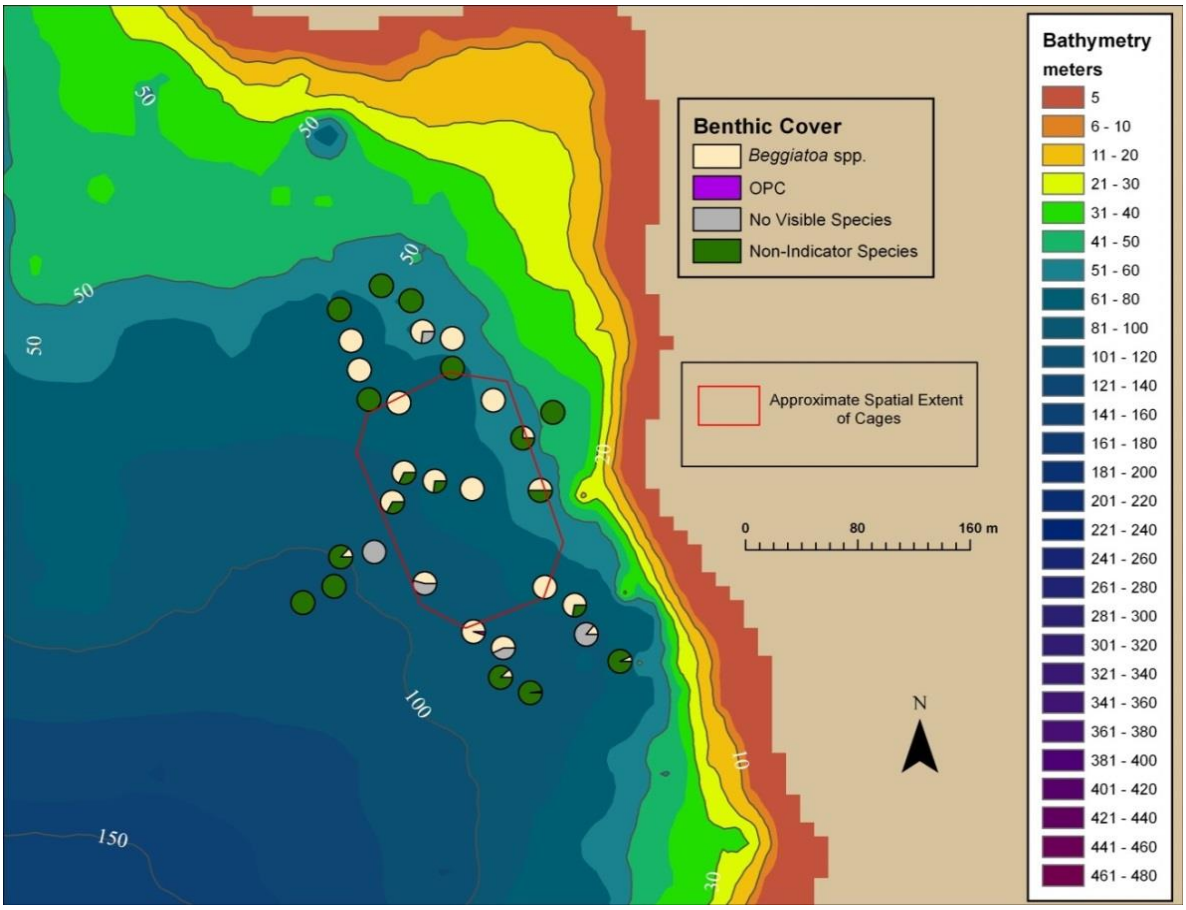

Figure 34. Bathymetric map of F1 with benthic cover composition at stations sampled on the South Coast of Newfoundland in August 2012.

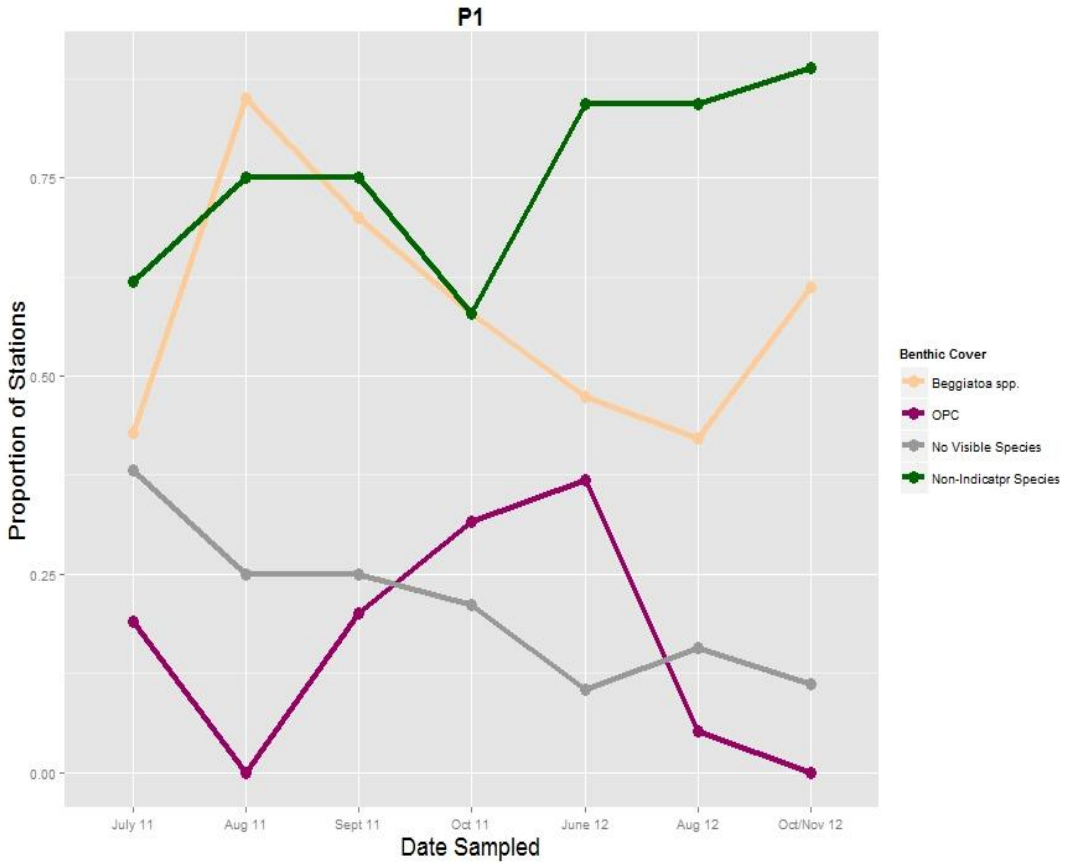

Figure 35. Proportion of stations at P1 where *Beggiatoa spp.*, OPC, other non-indicator species or no visible species were observed at each sampling date.

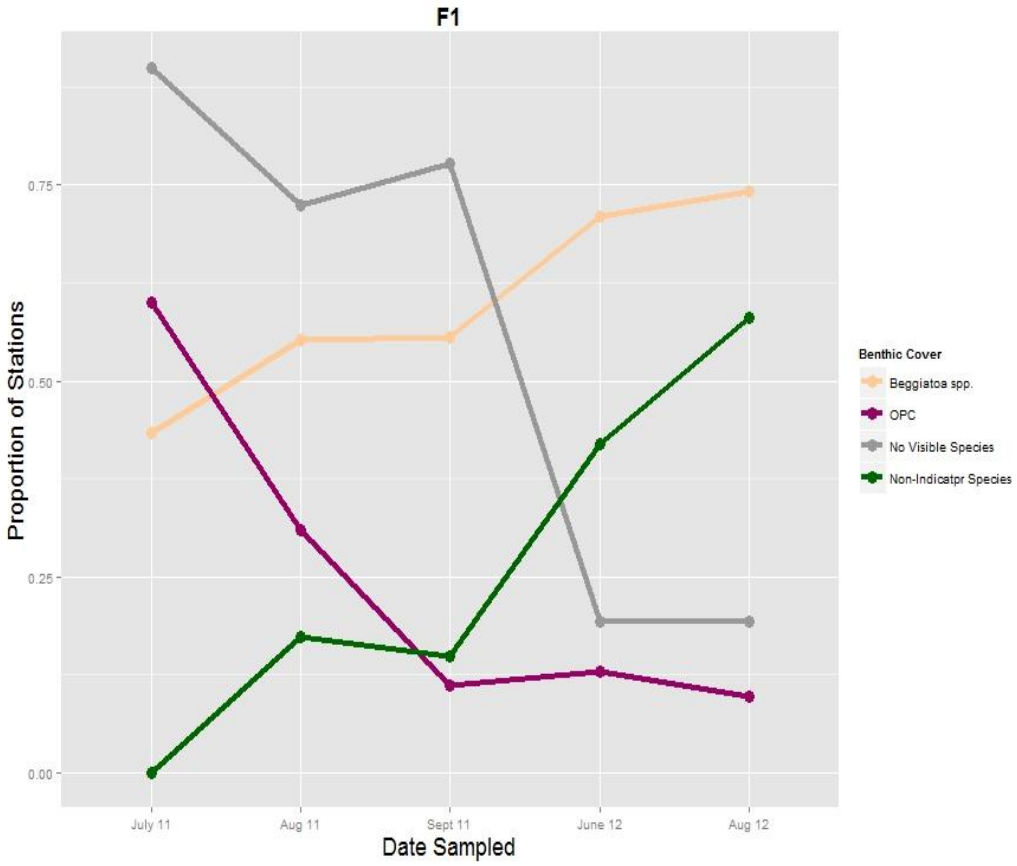

Figure 36. Proportion of stations at F1 where *Beggiatoa spp.*, OPC, other non-indicator species or no visible species were observed at each sampling date

4.4. Discussion

The aim of this chapter was to investigate spatial and temporal effects of organic enrichment due to aquaculture by comparing the distribution of *Beggiatoa spp.*, OPC and other benthic organisms within a production and a fallow site.

4.4.1 Spatial patterns

Spatial patterns were similar at both sites (P1 and F1) studied and across sampling periods. *Beggiatoa spp.*, OPC and, to some extent, patches without visible benthic organisms, were more often found in proximity to cage edge. Other benthic organisms were more prevalent as distance from cage edge increased. These findings complement the results found in Chapter 3: Figure 21 displays a lower abundance and higher percent cover of indicator species at stations close to cage edge. However, as the distance increases from cage edge an increase in benthic abundance and decrease in percent cover of indicator species is apparent. Similarly, other studies have shown that *Beggiatoa spp.* and opportunistic species are more abundant close to aquaculture sites (Hall-Spencer et al., 2006; Hargrave et al., 2008; Paxton & Davey, 2010), with other benthic species appearing as distance from the source of organic enrichment increases (Nilsson & Rosenberg 2000; Keeley et al., 2013).

There were noticeable differences in benthic cover among the transects. At P1, the western transect appeared to be affected more by organic enrichment, which could be due to currents influencing deposition (Sanz-Lazaro et al., 2011; Huang et al., 2012).

4.4.2 Temporal patterns

4.4.2.1 Site P1

The temporal patterns of presence and percent cover *Beggiatoa spp.* at P1 were complex, with no obvious trend through time. The highest proportion of stations with *Beggiatoa spp.* occurred in August 2011; the increase in the spatial cover of *Beggiatoa spp.* since July 2011 is likely due to the continuous loading of OM from aquaculture cages. Continuous loading of OM has been shown to be associated with an increase in the presence of *Beggiatoa spp.* in other studies (Keeley et al., 2013). However, the decrease in the number of stations containing *Beggiatoa spp.* from August 2011 – October 2011 was unexpected. A possible reason for the decrease of stations containing *Beggiatoa spp.* could due to increased concentrations of sulphide in the sediment which has been suggested as a limiting factor of the development of *Beggiatoa spp.* (Hamoutene, 2014). Furthermore, although the number of stations containing *Beggiatoa spp.* decreased from August 11 – October 11, the proportion of stations containing OPC increased. *Beggiatoa spp.* could have been present but the development of OPC could have covered bacterial mats not allowing us to detect them. In part, the decrease in the proportion of stations with *Beggiatoa spp.* could also be due to a combination of sampling accuracy and the bathymetry of P1. The bathymetric maps display a steep depth gradient perpendicular to the shoreline with rapid depth changes over relatively short distances. Differences in bathymetry can influence the deposition of OM (Perez et al. 2002) and in turn could affect the distribution of *Beggiatoa spp.*, leading to high spatial patchiness. Furthermore, the GPS sampling accuracy is within 10 m and the video surveys are unlikely to have

covered the exact same locations at different sampling dates, particularly given that cages moved between sampling dates. Therefore, it is possible that the local distribution and percent cover of *Beggiatoa spp.* may have remained constant from July 2011 – October 11, but this was not detected due to the sampling limitations explained above.

In June 2012, a decrease in the proportion of stations with *Beggiatoa spp.* was observed compared to the 2011 sampling dates and could denote a partial recovery in winter of 2011 when feeding rates are decreased (Karakassis et al., 1998). A decrease of *Beggiatoa spp.* from June 2012 to August 2012 was unexpected, however the number stations with no visible species did increase and could indicate the development of hypoxic or anoxic conditions over that time period, with conditions unsuitable for *Beggiatoa spp.* (Macleod et al., 2004; Hamoutene, 2014). The increase in proportion in stations with *Beggiatoa spp.* from June 2012 – Oct/Nov 2012 should be interpreted with caution as the % cover of *Beggiatoa spp.* was the lowest at all sampling periods during this period (Figure 19). Harvesting was initiated in Oct/Nov 2012, and as feeding decreased and/or ceased prior to harvesting, the intensity of organic loading was reduced between June 2012 – Oct/Nov 2012. The reduced loading could have allowed for partial recovery from anoxic to hypoxic conditions as stations with *Beggiatoa spp.* increased in proportion and stations with NVS were observed less frequently.

OPC presence and coverage varied temporally: these organisms were most abundant in June 2012 (the coldest sampling period) and were absent in Oct/Nov 2012 and August 2011 (the warmest sampling dates). The temporal pattern in OPC presence may indicate that these organisms are restricted to temperatures below 4°C (as suggested in Figure 12,

Chapter 2). Furthermore, *Beggiatoa spp.* and OPC often co-occur, and there appears to be a relationship between the two as OPC only appears at stations in P1 after the appearance of *Beggiatoa* sp (Figures 23 – 29). Little is known about the life history or feeding mode of polychaetes forming OPC, but they may feed on *Beggiatoa spp.*: species forming OPC in Newfoundland belong to the family Dorvilleidae (Murray et al., 2012), in which species from oxygen-minimum zones consume mat-forming microbes (Levin et al., 2013). At stations where only OPC were observed, *Beggiatoa spp.* may have been located beneath OPC where they were not visible on benthic images. Further, both *Beggiatoa spp.* and OPC may be present beneath the surface of sediments or flocculent matter, indicating that reported OPC and *Beggiatoa spp.* data may be underestimated (and that some sites with areas of NVS may in reality contain IOE).

The highest proportion of stations with NVS was observed in July 2011, with decreasing reports of this category thereafter. In these areas, the benthos may have been initially smothered by OM (Pearson, 1975) and later colonized by *Beggiatoa spp.* as organic loading increased (Brooks et al., 2003). Data must also be interpreted with caution as the sampling design changed from 2011 – 2012: only one sample was obtained during the 2011 field season, while four images were taken in 2012. As the probability of encountering an organism was greater in 2012 given the larger area examined overall per station, this likely led to fewer stations being reported as having NVS in the second year.

The lowest proportion of stations with NIS was reported in October 2011 and the highest proportion in October/November 2012. The highest proportion of stations with NIS in Oct/Nov 2012 was unexpected and could be due to the sampling design and/or

dispersion patterns of organics due to the highly variable current speeds/directions in this area (Sebastien Donnet, pers. comm). The occurrence of few organisms at the beginning of the first year may have been due to increased organic loading which can lead to habitats void of macrobenthic species (Nilsson & Rosenberg, 2000). The highest mean abundance per station was observed in June 2012 and the lowest in August 2012, however no statistical significant differences in abundances were observed (Figure 19). The change in sampling design between the two seasons likely led to a higher proportion of stations with reported NIS in 2012; this should be evaluated carefully as August 2012 had the lowest abundance of NIS out of all sampling periods.

4.4.2.2 Site F1

The proportion of stations with *Beggiatoa spp.* and NIS increased through time, while the proportion of stations with OPC and NVS showed the opposite trend. Macleod et al. (2006) observed that *Beggiatoa spp.* was not apparent immediately after the removal of cages. *Beggiatoa spp.* increased in area coverage and thickness during the first month of the fallow period and was visible up to 8 months thereafter (Macleod et al., 2006). Therefore it is probable that conditions at F1 were unfavourable for *Beggiatoa spp.* initially, but that microbial mats could form as sediments slowly became oxidized or sulphide fluxes became adequate for growth (Hamoutene, 2014). Alternatively, *Beggiatoa spp.* could be present in deeper sediments but not visible of surface sediments (Hamoutene, 2014). Such changes in sediment chemistry could explain the inverse relationship in the proportion of stations with *Beggiatoa spp.* and with NVS, as *Beggiatoa* mats progressively spread into areas that were previously bare. The proportion of stations

with OPC decreased rapidly over time as has been noted during fallow periods in other studies (Mazzola et al., 2000; Macleod et al., 2007), possibly due to temperature constraints in our study and relationships between OPC and *Beggiatoa spp.* at F1.

4.5. Conclusions

Organic loading on the benthos is a complex process that can be dependent on farming practices, oceanographic conditions, bathymetric characteristics, substrate type and the local biological capacity to assimilate organics. All parameters that affect deposition differ between sites and can cause variable spatial and temporal distributions of organic enrichment. Our study showed not only that *Beggiatoa spp.* and OPC are associated with organic enrichment as they predominately occur in close proximity to cages, but also that areas with no visible species could be another indicator of reduced conditions and should be evaluated when conducting video surveys. Furthermore, our study indicated that the temporal patterns of indicator species are complex. *Beggiatoa spp.* appearance seemed to display a lag time with respect to the beginning of organic enrichment. From one month into production, *Beggiatoa spp.* increased in presence and % cover throughout the duration of farming, and continued to increase in spatial coverage, albeit slowly, at the fallow site. OPC showed temporal variation that could be attributed to temperature preferences. Interestingly, OPC occurred in areas where *Beggiatoa spp.* was previously observed; however, the biological interactions between those IOE are unknown and further studies should investigate their relationship. This study highlights the importance of continued environmental monitoring to determine the

spatial and temporal impacts of organic enrichment originating from aquaculture sites. Maps provide a useful visual supplement for the regulators and industry to understand impact on a spatial scale. Furthermore it should also be noted that the fallow site aquaculture impact footprint remained unchanged in our study period of 2 years. Further investigation is needed to understand how long temporally organic enrichment from aquaculture affects an area.

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Chapter 5. General conclusions

5.1 Benthic changes and relationship to aquaculture production

As the NL aquaculture industry continues to grow, it is essential that it be managed in a manner that is environmentally sustainable (NLDFA 2014). Regulatory thresholds on the impacts of OM enrichment originating from aquaculture operations to the benthic environment must be developed and enforced. Therefore, knowledge must be gained in the south coast of NL concerning changes to the benthic habitat resulting from the increase of OM in sediments. Unlike other jurisdictions across Canada, where soft sediment sampling is used (BCMOE, 2002; NLDFA, 2010; NSDFA, 2011; DELG, 2012) to determine benthic changes, the south coast of NL consists of rocky and patchy substrates (Anderson et al., 2005; Bungay 2012; Hamoutene et al. 2013, Hamoutene 2014) where soft sediment sampling is ineffective and video surveys are the preferred method to investigate and document changes in epibenthic habitat. Through video surveys and complementary environmental monitoring equipment, this study characterized abiotic factors, identified the epibiota and documented changes on temporal and spatial scales.

As in other studies (Anderson et al., 2005; Hamoutene et al., 2013, Hamoutene, 2014; Bungay, 2012) we showed that the bottom composition consisted of rocky and patchy substrates. The substrate composition of these sites also changed on a temporal scale, possibly through the influence of sedimentation from OM (Karakassis, 1998, Klaoudatos et al., 2006) and inaccuracies in our sampling design. Although substrate type varied between sites, the study highlighted two sets of sites that had comparable depth

and temperature characteristics. These sets were initially separated to investigate if epibiota displayed abiotic preferences (Chapter 2). However, for the most part, organisms were unrestricted by abiotic factors although few had noticeable temperature and depth preferences (OPC, family Comasteridae, algal species). Biota did however show a relationship with the various aquaculture production stages with *Beggiatoa spp.*, OPC and genus *Strongylocentrotus* present at only production and fallow sites but absent from non-production sites. The video survey helped classify and identify the species that inhabit the south coast of NL but also helped identify species that are possibly attracted to OM enrichment. These findings are important for regulatory purposes and habitat protection because the video survey can readily identify sensitive habitats, rare/endangered species and or species of economic importance.

Both the production stage and the fallow stage differed significantly from the non-production stage in total abundance and abundances in all taxon groups except the molluscs (Chapter 3). Furthermore both bio-indicators were only present at sites that had or were currently experiencing aquaculture production. Certain groups (sponges, algal species) were more sensitive to OM enrichment and declined significantly in abundance or percent cover when compared to non-production sites. However other taxon groups such as the molluscs increased in abundance. Molluscs likely increased in abundance due to fall off from biofouled gear and net washing activities.

Temporally, OM enrichment appeared to impact epibenthic change immediately as changes were observed after one month of aquaculture production. A possible trend of epibenthic recovery was noticed in the fallow sites, with a slight increase in abundances

at the later sampling periods (June 2012 – August 2012 at fallow sites). However epibenthic composition at fallow sites were not comparable to the non-production sites and it is likely that return to original conditions did not occur. In addition, at least OPC and/or *Beggiatoa spp.* was present at all sampling periods throughout the fallow stage indicating reduced conditions. Future studies should analyze fallow sites for extended periods of time (> 2 years) to identify suitable time periods that would allow for benthic recovery.

Spatially, OM enrichment and its effects diminished with increasing distance from aquaculture cages as expected. Bio-indicator species and images with zero visible species were more prevalent close to cage edge but decreased with increasing distance. Interestingly, increased abundances were observed at intermediate distances and could be associated with certain species (i.e. sea urchins) using the OM as a food source. Further investigation of the intermediate effects of organic enrichment should be evaluated to gain a better understanding of the potential beneficial effect of aquaculture impact.

Bio-indicators and images with zero visible species were in close proximity to aquaculture operations regardless of the bathymetry (Chapter 4). It appeared that the bio-indicators and images with no visible species followed a transitional pattern. Stations close to cage edge and likely heavily impacted by OM were initially devoid of species, followed by colonization of *Beggiatoa spp.*, and then OPC. These stations might be initially covered by OM and conditions could be unsuitable for any visible epibenthic species. From the accumulation of OM, the resulting habitat likely attracts *Beggiatoa spp.* who then colonize these areas. OPC then seems to follows after *Beggiatoa spp.* Further

studies should focus on the direct relationship between *Beggiatoa spp.* and OPC to gain understanding on how these bio-indicators interact. Although these transitional patterns of bio-indicators and stations with no visible species were observed, further research should be done as it is likely that the patchy nature of the habitat, farming practices and our sampling protocol contributed to the complexity of these transitions. Permanently fixed time lapse camera focused on the sea bottom could be used to see if clear trends exist between these groups. Images with no visible species should be identified and evaluated in environmental monitoring programs.

In conclusion, the OM enrichment from aquaculture causes epibenthic changes in the south coast of NL. Transitions are complex and variable, likely dependent on farming practices, environmental factors, and assimilative capacity of the environment. The complexity and variability reinforce the idea that these sites should be continuously monitored pre and post aquaculture production. OM enrichment appears to impact these habitats immediately no return to baseline conditions in the sites evaluated in this study. Our data suggests that neither bio-indicator species showed clear changes in presence or percent cover over temporal scales, either at sites with continuous loading of OM or in recovery from OM. Images with zero visible species should be considered concurrently with bio-indicators to determine the extent of OM enrichment impact.

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