EFFECT OF THE ENVIRONMENT ON CONDITION AND QUALITY OF CULTURED BLUE MUSSELS (*MYTILUS EDULIS*) WITH REFERENCE TO CULTURE DEPTH AND POST-HARVEST PRACTICES

by

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ABSTRACT

The condition and quality of cultured blue mussels (*Mytilus edulis*) are affected by various environmental characteristics including temperature, salinity, food concentration, composition and year-to-year variability, waves, tides, and currents. Mussels are a keystone species in the ecosystem, affecting the surrounding environment through filtration, biodeposition and nutrient recycling.

This study evaluated the effects of culture depth and post-harvest handling on cultured blue mussels in Newfoundland, Canada. Depth was examined over two years; three shallow water (5 m depth) and three deep water sites (15 m depth) were compared for environmental characteristics, mussel physiological stress response, growth, and biochemical composition. The area examined presented complex hydrodynamic characteristics; deep water sites appeared to be located more often near or within the pycnocline than shallow water sites. Deep water sites presented lower temperatures than shallow sites from spring to fall. Physiological stress response varied seasonally, but was unaffected by culture depth. In Year 1 shallow and deep water mussels presented similar growth, while in Year 2 deep water mussels showed better final condition. Lipid and glycogen showed seasonal variation, but no significant differences between shallow and deep water were noted. Fatty acid profiles showed a higher content of omega-3s PUFA in deep water sites at the end of Year 2. Under extreme weather conditions, deep water appeared to provide a more stable environment for mussel growth than shallow water.

Harvested mussels were kept under ambient live-holding conditions for one month during the fall, winter, and spring seasons. They were compared to freshly harvested

mussels for condition, biochemical profile and palatability. A progressive loss of dry tissue weight and an increase in water content were shown over the holding period during the fall and spring seasons, when compared to field controls. The biochemical analysis suggested seasonal changes; differences in triacylglycerol content were found in the spring season when compared with controls. The palatability data indicated that the panellists were unable to determine a difference between mussels kept in holding and those freshly harvested from the site. This study presents new knowledge for mussel farming, especially in terms of environmental interactions and deep water culture.

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List of Abbreviation and Symbols

ADCP	Acoustic Doppler Current Profiler
ANOVA	Analysis of variance
ARA	Arachidonic acid
ATP5β	Adenosine triphosphate 5 β
ATP	Adenosine triphosphate
BCD	Bulley's Cove deep
BCS1	Bulley's Cove shallow 1
BCS2	Bulley's Cove shallow 2
BMP	Best management practices
$CaCO_3$	Calcium carbonate
cDNA	Complementary DNA
CTD	Conductivity, temperature, depth instrument
CV	Coefficient of variation
DFA	Department of Fisheries and Aquaculture
DHA (22:6ω3)	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
EA	Echo Amplitude
EDTA	Ethylenediaminetetraacetic acid
EF1	Elongation factor 1
EPA (20:5ω3)	Eicosapentaenoic acid
FAME	Fatty acids methyl esters
FARM	Farm Aquaculture Resource Management model
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GC	Gas chromatograph
GSH	Glutathione
GSTp	Glutathione S-transferase p
GTP	Guanosine triphosphate
H_2O_2	Hydrogen peroxide
HH3	Histone H3
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HSP	Heat shock protein
ICES	International Council for the Exploration of the Sea
IMTA	Integrated multi-trophic aquaculture
KOH	Potassium hydroxide
MID	Mouse Island deep
mRNA	Messenger RNA
MSX	Multi-nucleated unknown disease
NADP	Nicotinamide adenine dinucleotide phosphate
NAFC	Northwest Atlantic Fisheries Centre
NAIA	Newfoundland Aquaculture Industry Association

NCBI	National Centre for Biotechnology Information
NMID	Non-methylene interrupted dienoic fatty acids
$NH4^+$	Ammonium
NTC	Non-template controls
O_2^-	Superoxide
PAHs	Polycyclic aromatic hydrocarbon
PCA	Principal components analysis
PCB	Polychlorinated biphenyl
PCO	Principal coordinates analysis
PCR	Polymerase Chain Reaction
PL	Phospholipids
PUFA	Polyunsaturated fatty acids
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPOL2-3	DNA-directed RNA polymerase II polypeptide C
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SAD	South Arm deep
SAS	South Arm shallow
SD	Standard deviation
SE	Standard error
SOD	Superoxide dismutase
ST	Sterol
TAG	Triacylglycerols
TBE	Tris borate ethylenediaminetetraacetic acid
TLC	Thin layer chromatography
ω3	Omega-3 fatty acids
ω6DPA (22:5ω6)	ω6 docosapentaeonic acid

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CHAPTER 1

Introduction and Overview

1.1. Introduction

In an ecosystem, biotic and abiotic components of the environment are subject to complex interactions important in sustaining the system itself. An organism that survives and thrives in a given environment has succeeded in adapting to both the biotic and abiotic influences; any condition that is outside of the organism's tolerance is defined as a limiting factor. Organisms, therefore, survive in a zone delimited by lower and upper limits of such factors (Dame, 1996).

The blue mussel, *Mytilus edulis*, is a bivalve mollusc; its spatial distribution and physiology are affected by the environmental components described above. Temperature, salinity or a combination of the two are the main factor affecting blue mussel growth and health (Dame, 1996). However, other factors such as the food supply including phytoplankton composition and quantity, adequate water motion, tides and suspended sediment can affect mussel survival and health (Dame, 1996). These environmental factors need to be taken into consideration when developing mussel aquaculture. From an aquaculture science perspective, it is important to investigate the effect of different environmental conditions on mussel growth, health, and quality to find the most suitable environment for mussel production, while promoting environmental sustainability and seafood quality.

The origin of blue mussel aquaculture dates back to the 13th century in France. Starting in the 1970s, technological developments and new culture techniques were introduced (FAO, 2015). Currently blue mussels are cultured primarily in Europe and North America. However, other countries such as China have also started developing this type of aquaculture. In 2013, global *M. edulis* production reached 197,831 tonnes (FAO, 2015).

The first objective of the present project was to investigate, characterize and compare seasonal changes in the environmental conditions found in open deep water blue mussel (*Mytilus edulis*) culture sites and shallow coastal sites in Notre Dame Bay, Newfoundland. This comparison was needed in order to define differences between culture depths and thus characteristics affecting ecological carrying capacity and consequently environmental sustainability of the mussel industry. In order to achieve these objectives deep and shallow water culture sites were compared based on environmental characteristics during different seasons for two consecutive years. Moreover, the present study aimed to investigate the relationship between seasonal changes driven by the environment and the health and quality of the cultured mussels. This comparison was necessary to determine whether deep water sites have increased potential for aquaculture sustainability, helping to reduce and mitigate environmental effects of mussel culture. Showing that open deep water mussel culture in Notre Dame Bay is feasible could help alleviate the pressure from carrying capacity limits in coastal zones in the future. In order to achieve this goal, blue mussels cultured on long-lines in deep (headline located at 15 m depth) and shallow (headline located at 5 m depth) water

were compared for growth, condition, biochemical profile and physiological stress responses. Finally, the post-harvest practice of ambient live holding (maintenance of harvested live mussels in tanks with continuous seawater circulation) was examined to investigate potential seasonal changes in condition and palatability of mussels kept under standard ambient wet-holding conditions and to determine if held mussels differed seasonally from fresh mussels harvested directly from the farm. The information collected in this project should help the aquaculture industry decide whether the utilization of deep water sites in Newfoundland can be valuable for mussel culture. Similarly, investigating the effects of post-harvest handling on mussel physiology provides advice on the development of best practices for mussel growers. This research will increase knowledge of blue mussel (*Mytilus edulis*) physiology, metabolism and health under different environmental conditions, both on the farm and in the processing/holding facility.

1.2. Statement of Hypotheses

Hypothesis #1 (Chapter 3): deep (~15 m) and shallow (~5 m) culture sites differ with regard to environmental characteristics. Deep water sites present a more stable culture environment than shallow water sites.

Hypothesis #2 (Chapter 4): deep and shallow water cultured mussels differ in environmental stress responses. Deep water mussels present a lower immune and oxidative stress response than shallow water mussels; the expression of defensin, superoxide dismutase (SOD), heat shock protein (HSP) and glutathione S-transferase p (GSTp) are lower in deep water cultured mussels.

Hypothesis #3 (Chapter 5): deep and shallow water cultured mussels differ in growth, lipid classes and fatty acid profile, as well as glycogen content. Deep water mussels present better condition, and quality than shallow water mussels.

Hypothesis #4 (Chapter 6): mussels subjected to long-term, live holding post-harvest differ in quality from freshly harvested mussels. Mussels in long-term live holding conditions present decreased condition, and quality compared to freshly harvested mussels.

1.3. Literature review

For the purpose of the thesis, a review of the environmental conditions affecting the blue mussel, and more generally bivalve culture, will be presented. However, the relationship between environment and the mussel is by its nature bilateral and mutual. Therefore, I would also like to emphasize the importance of the effects of the mussel and the culture of other bivalves on the surrounding environment. Successful aquaculture comes from a delicate balance between production (economic gain) and environmental sustainability (acceptable effect of culture on the surrounding ecosystem), therefore the reciprocal mussel-environment interaction has to be investigated as a whole. For this reason a chapter of this thesis (Chapter 2) will be dedicated to the effects of mussels and bivalve aquaculture on the environment.

1.3.1. Blue mussel (*Mytilus edulis*)

Mytilus edulis, the common blue mussel, is a member of the *Mytilus edulis* species complex; the complex also includes *M. trossulus* and *M. galloprovincialis* and natural

hybridization can occur between the species of the complex (Comesaña *et al*., 1999). *M. edulis* is a benthic, semi-sessile suspension feeder (Bayne *et al*., 1976). It is a widely distributed bivalve species that can be found in boreal and temperate waters in the northern hemisphere, from the Arctic to California, on both Atlantic and Pacific coasts. It is also present in the Mediterranean and on the North African coast (Seed, 1976). The limiting factor for the distribution of *M. edulis* appears to be temperature, which can affect both survival and breeding (Seed, 1976). Blue mussels are found in the littoral and sublittoral zones, and occasionally in deeper zones. They are present in both brackish and marine environments and are therefore tolerant of a large range of salinities from 4-5‰ to fully marine conditions (Seed, 1976). Mussel growth is affected by a variety of factors, many of which are related to the surrounding environment. Other than the aforementioned temperature, additional important factor influencing mussel growth and condition include food intake and absorption, tidal exposure, waves, currents, salinity, and population density (Seed, 1976). Optimal conditions for shell and tissue growth in *M. edulis* include water temperatures between 10 and 20°C and a salinity around 25-30‰ (Bayne, 1976). However, mussels are relatively tolerant of adverse environmental conditions including oxygen-deficiency or even anoxia as well as prolonged exposure to air and sub-optimal temperature and salinity. In such cases they undergo fundamental biochemical changes until a new physiological balance, called acclimation, is reached (Bayne *et al*., 1976).

1.3.2. Blue mussel aquaculture in Newfoundland

The Newfoundland mussel aquaculture industry has been steadily growing over the past decade. Since 2000, production has increased from 1,051 to 4,354 tonnes in 2013

(although preliminary data suggest a decrease in production for 2014); the industry is currently investigating new market opportunities and initiatives including the establishment of organic status in 2013 (NAIA, 2011; DFA, 2015). In Newfoundland, the blue mussel, *Mytilus edulis* L., is farmed mostly on the northeast coast of the island, in Notre Dame Bay, although some farms also occur on the south coast, in Connaigre Bay. Mussels produced in this Province, as in the rest of Atlantic Canada, are grown by suspended longline culture. Market size is 50-75 mm and mussels may be harvested throughout the year, depending on meat quality, yield, and shelf life (Mallet & Myrand, 1995; Brown *et al*., 2000). In Newfoundland, mussel culture sites are typically situated in sheltered near shore areas, such as river mouths, estuaries, harbours and shallow bays.

Once harvested, fresh mussels are typically shipped to a variety of local and international markets. However, geography, extreme weather conditions, and mechanical failures may create delays in the harvest of fresh product or affect its timely shipping to market. In such situations, harvested fresh mussels may require stockpiling or extended holding in ambient or environmentally controlled, wet-storage facilities prior to processing.

1.3.3. Effect of the environment on cultured blue mussels

The growth and biochemical profile of blue mussels are greatly affected by the environment in which they exist. Various environmental characteristics have been shown to play a key role in mussel health and growth. Previous studies in Notre Dame Bay, Newfoundland, showed a significant relationship between mussel growth, temperature, and salinity, suggesting that those environmental characteristics highly influence mussel

growth and health (Khan *et al*., 2006; Alkanani *et al*., 2007). The concentration of phytoplankton in the water, its composition and year-to-year variability can also affect the biochemical characteristics of blue mussels seasonally (Okumus and Stirling, 1998; Orban *et al*., 2002; Ventrella *et al*., 2008; Pleissner *et al*., 2012; Irisarri *et al*., 2015). Moreover, storms, tidal cycles, and current speed can resuspend bottom material, increasing the concentration of seston but reducing the quality of the food available for bivalve filter-feeders (Cranford *et al*., 2011; Karayücel *et al*., 2013). Current speed and flow also affect phytoplankton transport, mussel clearance rate, and settling of organic and inorganic material on the cultured bivalves, while extreme waves can cause mortality and limit food intake (Fréchette *et al*., 1989; Buck, 2007). Often it is a complex interaction between all the aforementioned environmental parameters that influence growth and health of the animal. Bayne & Worrall (1980) showed that food quantity, quality, physiological availability, and temperature were all affecting *Mytilus edulis* somatic growth and fecundity, thus emphasizing the importance of evaluating environmental characteristics and how they alter mussel condition, and the final quality of the harvested product.

The evaluation of environmental characteristics surrounding an aquaculture farm can be carried out using a number of techniques. Multi-parameter sondes can be located at different depths in the water column and maintained in place for a period of time to monitor environmental conditions such as temperature, salinity, pH, dissolved oxygen and chlorophyll *a* (Burt *et al*., 2012). Conductivity, temperature and depth (CTD) profiles can be used to provide vertical sections of the water column's main physical parameters such as temperature and salinity (Ramón *et al*., 2007; Saurel *et al*., 2007; Parrish *et al*.,

2009). Acoustic Doppler Current Profilers (ADCPs) can be positioned on the seabed to measure current velocity (Saurel *et al*., 2007). Moreover, seawater can be collected at different depths and analyzed for chlorophyll *a*, particulate matter, and organic and inorganic nutrient content (Parrish, 1998; Ramón *et al*., 2007; Saurel *et al*., 2007). Finally, photopigment analysis can be used to measure phytoplankton composition (Li *et al*., 2015).

Cultured blue mussels and other bivalves are traditionally assessed for condition using a variety of methods and calculations (Lutz *et al*., 1980; Lucas & Beninger, 1985; Davenport & Cheng, 1987). Condition indices are used to quantify the quality of a product for the market and can also be used as an index of growth and health of the animals (Lucas & Beninger, 1985). One of the indices that can be used is the ratio of the dry weight to wet weight which measures the proportion of water contained in an organism; a high proportion of water often signifies poor physiological condition and it is present in situations such as prolonged starvation (Lucas & Beninger, 1985). Other calculations such as the shell to length relationship, length-weight relationship (allometry), volumetric condition indices and cooked meat yield have also been used to assess bivalve growth (Lutz *et al*., 1980; Lucas & Beninger, 1985; Davenport & Cheng, 1987; Hemachandra & Thippeswamy, 2008).

The biochemical composition of the blue mussel is an important indicator of condition and health and it changes as a result of interaction between temperature and the quality and quantity of the diet and the mussel reproductive cycle. The total level of lipid, the fatty acid composition, and glycogen content also vary depending on the diet and the temperature; lipids and glycogen storage occurs in the reproductive tissues; therefore,

biochemical levels also depend on gametogenesis (Orban *et al*., 2002; Ventrella *et al*., 2008; Martínez-Pita *et al*., 2012; Karayücel *et al*., 2013; Irisarri *et al*., 2015).

In recent years quantification of mRNA expression for genes involved in protection against environmental stressors have been studied in bivalves and more specifically in mussels (Banni *et al*., 2011; Li *et al*., 2009; Núñez-Acuña *et al*., 2012). Changes in water temperature may result in oxidative stress. The bivalve response to this type of stress produces an increase in the concentration and activity of certain enzymes such as glutathione S-transferases (GST), which play a key role in cellular detoxification; therefore, thermal stress and seasonal climate variation induce increased levels of GSTp gene expression in bivalves (Power & Sheehan, 1996; Kim *et al*., 2009). Heat shock protein (HSP) gene expression is elevated after acute thermal stress (high temperatures) and changes with local environmental conditions (Banni *et al*., 2011; Núñez-Acuña *et al*., 2012). It has also beennsuggested that HSP down-regulation can be caused by a low salinity environment, while GSTp activity appears independent of salinity changes (Lyons *et al*., 2003). Superoxide dismutase (SOD) activity shows a relationship with food availability and appears to be overexpressed in association with low chlorophyll *a* concentrations (Núñez-Acuña *et al*., 2012). Both salinity and temperature appear to influence the gene expression of Defensin, an antimicrobial peptide gene involved in the bivalve immune-response (Li *et al*., 2009).

Harvested mussel quality is in large part constituted by the sensory perception and the consumer acceptability of the final product; these aspects therefore need to be considered (Hyldig & Green-Petersen, 2004). Sensory evaluation is used to determine non-specific sensory difference between treatments and to describe and categorize different samples

(Carpenter *et al*., 2000). This type of test is a practical tool for evaluating quality and freshness of seafood during the complete production chain (Hyldig & Green-Petersen, 2004). It has been shown that a relationship exists between lipid constituents, content and their autoxidation state, and the sensory profile and aroma of aquaculture products (Morita *et al*., 2003; Frank *et al*., 2009).

One of the goals of blue mussel aquaculture is to identify the main environmental characteristics that affect growth, and quality in the specific location of the farm. It is a goal of aquaculture research to understand how the culture techniques can be adapted to take advantage of suitable environmental conditions and how the environment can be utilized in the most sustainable way. Mussel culture depth and location, and post-harvesting practices have the potential to influence the environmental parameters to which the bivalves are exposed. Therefore culture depth and post-harvesting processes need to be taken into consideration when discussing how the environment affects mussels culture.

1.3.3.1. Culture depth

The environmental characteristics of a mussel farm can vary with culture depth. It has been suggested that water temperature and salinity may be more stable in deeper water; therefore seasonally dependent environmental and biological stress that affect condition in cultured shellfish could be potentially reduced in deep water culture (Buck *et al*., 2005; Yu *et al*., 2010; Chavez-Villalba *et al*., 2010; Cheney *et al*., 2010). Sheltered nearshore areas, typically used for mussel culture, have more potential for being affected by land

runoff than deep open water sites, especially during times of significant precipitation, and therefore can be exposed to contaminants of land origin such as coliform bacteria (*E. coli*), industrial pollution, and fertilizers (Cheney *et al*., 2010). Also, recent studies indicated that deep water chlorophyll *a* maxima are possible due to thermal and saline stratification (Ogilvie *et al*., 2004). Similarly, natural upwelling events generated by topographic forcing near capes, promontories, shelves and other deep water structures can cause an additional influx of nutrients and particles into the water column at depth (Pitcher *et al*., 2010). The combined potential improvement in water quality and concentrated food sources suggest the possibility of enhanced culture conditions and decreased stress for the animals; therefore, offshore deep water culture has the potential to improve mussel growth and health.

In nearshore shallow water culture, aquaculture-related benthic deposition and impact have also become an important issue (Hartstein & Rowden, 2004; Fabi *et al*., 2009; Frechette, 2012). The sources of deposition are mainly from mussel drop-off, including shell debris, and from organic material associated with mussel faeces and pseudofaeces (Frechette, 2012; Hartstein & Rowden, 2004). Culture sites should present sufficient depth and adequate water circulation in order to disperse wastes and reduce bottom enrichment (Cheney *et al*., 2010). Recent studies indicate that benthic deposition in open-sea, deep water mussel culture sites has minimal effects on the benthic domain and that it is comparable to reference sites without aquaculture influence (Fabi *et al*., 2009; Frechette, 2012). These observations suggest that deep water mussel culture would have a lesser impact on the benthic environment; changes in culture technology and improvements in practices have the potential to increase the environmental sustainability

of aquaculture in Newfoundland and around the world. Increased culture depth therefore has the potential to improve carrying capacity limits and thus deep water mussel culture may contribute to increased sustainability (McKindsey *et al*., 2006; Cheney *et al*., 2010; Duarte *et al*., 2012). Moreover, it has been shown that the culture system itself can modify water flow; however, in a more dynamic system such as deeper and more open waters the flow modification is reduced (Cheney *et al*., 2010). The concerns about nearshore shallow water sites, the potential improvement in mussel health and condition, and the reduced environmental impact of farms in deep water sites have stimulated increased interest in developing offshore deep water bivalve culture (Cheney *et al*., 2010). Preliminary data from sites in Asia, the United States, New Zealand and Europe suggest that overall condition is improved in deep water sites for a variety of shellfish species (Langan & Horton, 2003; Buck, 2007; Yu *et al*., 2010). A more stable environment can increase growth by lowering mortality, reducing turbidity and fouling, and improving water exchange (Cheney *et al*., 2010). However, little is understood about how the deep water environment specifically affects health and condition in blue mussels (*Mytilus edulis*) compared to the traditional coastal shallow water environment and how these benefits may be quantified. It is of fundamental importance to understand these relationships in order to make decisions on the feasibility of deep water sites for mussel culture, especially when considering the considerable effort and expense involved in their development (Cheney *et al*., 2010). The rationale for the experiments presented in Chapters 3, 4 and 5 emerged from the intent to investigate how the deep water environment affects blue mussels as compared to coastal shallow water culture.

1.3.3.2. Post-harvesting practice of live holding

When cultured mussels reach the market size of 50-75 mm they are harvested, depending on meat quality, and market demand and price (Mallet & Myrand, 1995; Brown *et al*., 2000). Harvest is carried out on a boat equipped with a boom and a hydraulic winch; the harvested mussels are transferred in 100-1000 L plastic insulated containers and transported to a processing plant, where they are declumped, graded, washed and debyssed (Mallet & Myrand, 1995). Following this automated process, the bivalves are visually inspected and broken shells, undersize and dead individuals are manually removed. The remaining mussels are packed and sent to the market in insulated refrigerated trucks (Mallet & Myrand, 1995).

When geography, weather conditions, and mechanical failures create delays in the harvest of fresh product or affect the shipping of product to market, harvested fresh mussels may require extended holding in ambient or environmentally controlled, wet-storage facilities prior to processing. This common practice in the aquaculture industry can greatly affect the environment to which the mussels are subject and consequently their health. Condition, physiological stress responses, and biochemical characteristics of mussels subject to wet holding can be modified by temperature, mussel density, food availability, and reproductive condition (Seed, 1976; Karayücel & Karayücel, 2000; Harding *et al*., 2004; Wyatt *et al*, 2013; Wyatt *et al*., 2014). Depending on season, the storage of mussels over extended periods up to three months under ambient conditions has been found to adversely affect overall condition index and lead to mortality (Wyatt *et al*, 2013). Additionally, mussels held for the same period showed a significant increase in stress responses compared to field controls (Wyatt *et al*., 2013; Wyatt *et al*., 2014). Handå

et al. (2013) showed that mussels held under starved conditions are able to reduce their oxygen consumption and metabolic rate, in order to maintain weight. However, physiological compensation may not be possible when starvation is combined with other environmental stressors, such as low oxygen and elevated ammonia concentrations. During extended holding, mussel density is a factor that can play an important role. High densities have been correlated negatively with mussel growth and health, leading to increased mortality in extreme cases, due to competition for food resources and space (Alluno-Bruscia *et al*., 2001; Lauzon-Guay *et al*., 2005; Cubillo *et al*., 2012). To date no studies have investigated variability in the biochemical composition of mussel tissue and final product quality following extended holding in processing facilities under ambient conditions. From this lack of information emerged the rationale for the experiment presented in Chapter 6 where the results of mussels exposed to ambient live holding are reported.

1.4. References

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CO-AUTHORSHIP STATEMENT

The initial idea for the project was proposed by Dr. Murray. The design of the study and the specific objectives of each research paper were discussed and finalized by myself with the help of Dr. Murray, and then discussed with the supervisory committee.

I prepared and took part in all the field campaigns; I participated in deployment and recovery of the instruments for environmental monitoring and collected the mussel samples. I conducted the laboratory analyses for condition, lipids, fatty acids, glycogen and physiological stress responses; I prepared and carried out the palatability testing. I completed the data analysis and prepared this thesis manuscript.

Chapter 2 is a review generated from the comprehensive examination and written by myself, following suggestions from the comprehensive examination committee.

Dr. Murray is co-author on Chapters 3, 4, 5 and 6. He provided expertise and guidance for the experimental design. He reviewed all the chapters in this thesis.

Dr. Parrish is co-author on Chapters 3, 5 and 6. He provided expertise and guidance for the biochemical analyses and the environmental sampling. He reviewed all the chapters in this thesis.

Chapter 3 is co-authored by Sebastien Donnet (Fisheries and Oceans Canada). He provided expertise and guidance for oceanographic data collection, analysis and interpretation.

Chapter 4 is co-authored by Larina Carroll (Fisheries and Oceans Canada) and Dr. Helen Gurney-Smith. They provided expertise and guidance for the physiological stress genes analysis and for the RT-qPCR protocol.

Chapter 6 is co-authored by Cyr Couturier. He provided expertise for mussel handling and condition analysis.

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Chapter 6: Gallardi, D., Hobbs, K., Mills, T., Couturier, C., Parrish, C.C., and Murray, H.M. (2014). Effects of extended ambient live holding on cultured blue mussels (*Mytilus edulis* L.) with reference to condition index, lipid profile, glycogen content and organoleptic testing. Aquaculture 430, 149-158.

CHAPTER 2

Effects of bivalve aquaculture on the marine environment and their possible mitigation: a review

2.1 Introduction

Global bivalve aquaculture production has been increasing constantly over the past 20 years. The main bivalves cultured in the world are oysters, clams, scallops, and mussels (World Health Organization, 2010). Bivalve aquaculture is considered to have a lower environmental impact compared to finfish culture, since it requires minimal addition to the environment. For the main species cultured the food is supplied by the environment itself and the wastes return nutrients and minerals to the ecosystem (Cranford *et al*., 2003). However, bivalves have the ability to maintain, modify and create entire habitats due to their effects on suspended particles and their shell formations (Dame, 1996; Cranford *et al*., 2003). The majority of research on interaction between bivalve culture and the environment has concentrated on mussels and oysters, probably because the production of other bivalves such as clams and scallops is greater in Asia, where aquaculture and its effects have been a culturally accepted part of the coastal environment for centuries (McKindsey *et al*., 2011).

This chapter presents a review of the effects of bivalve aquaculture on the environment, particularly in estuarine and coastal zones, the current prevention and

mitigation strategies, and highlights how bivalve culture can positively interact with the environment.

Bivalves are suspension feeders that perform their functions in a range of habitats, in particular estuaries, lagoons and coastal oceanic systems. They gain nourishment by filtering suspended particles such as phytoplankton and detritus from the water column (Dame, 1996). It has been calculated that an adult oyster can filter on average 15 to 55 $L \cdot day^{-1}$ of seawater (Powell *et al*., 1992; Rice, 2008). Bivalve by-products are dissolved ammonium and biodeposits comprised of feces and pseudofeces. Bivalves sequester nitrogen in the form of protein in meat and shell and stabilize phytoplankton growth dynamics through moderation of ammonia cycling in the water column. They are therefore considered "keystone" species which exert "top-down" control of phytoplankton by grazing but also "bottom-up" control through biodeposition and promotion of nutrient removal (Dame, 1996; Newell *et al*., 2002; Newell, 2004; Rice, 2008; National Research Council, 2010). Bivalves such as oysters and mussels respond to increased levels of phytoplankton and detritus in the water column with increased filtration capacity and production of pseudofeces (Tenore & Dunstan, 1873). In contrast bivalves such as clams adjust their clearance rates rather than increasing production of pseudofeces (Malouf & Bricelj, 1989; McKindsey *et al*., 2006a; National Research Council, 2010). These processes affect the food web, biogeochemical cycling, and the physical and chemical environment, potentially modifying habitats and ecological functioning (Dame, 1996; National Research Council, 2010).

Bivalve aquaculture therefore has the ability to affect the environment in both negative and positive ways, with a variety of near and far field cascading effects on

different parts of the ecosystem, including influencing primary and secondary productivity and community structure. Culture structures and operations can alter water flow, sediment composition and sedimentation rate, and disturb the benthic flora and other marine organisms (McKindsey *et al*., 2007; Dumbauld *et al*., 2009; National Research Council, 2010; McKindsey *et al*., 2011).

Estuaries are often a preferred site for bivalve culture and therefore are the environments examined by the majority of the literature. The bivalves that can exert the highest influence on the environment are oysters and mussels, since they maintain high clearance rates and reject large numbers of particles as pseudofeces (Cranford *et al*., 2003; Newell, 2004). The direct comparison of impacts between Manila clam (*Tapes philippinarum*) and mussel (*Mytilus galloprovincialis*) culture located in the same body of water has in fact shown a greater impact for mussel culture (Nizzoli *et al*., 2006).

There is more than one way a system can react to bivalve culture:

- Bivalves can redirect energy away from the zooplankton, replacing its ecological role and grazing on phytoplankton, leading to less energy passing up to the higher pelagic trophic level (Horsted *et al*., 1988).
- Bivalves can direct energy away from other benthic filter-feeders, decreasing their population and opening an ecological niche for benthic deposit feeders (Gibbs, 2007).
- Finally, bivalves can redirect energy from the bacteria in the microbial loop by recycling particulate nitrogen to inorganic nitrogen and reducing nitrogen fixation,

due to higher free ammonium presence, resulting in a noticeable increase in rates of primary productivity and less detritus (Gibbs, 2007).

It is however likely that these three processes can occur concurrently (Gibbs, 2007). Based on Gavine and McKinnon's (2002) hazard assessment for oysters (Pacific *Crassostrea gigas*, Sydney and rock *Saccostrea spp.* and pearl *Pinctada spp*.) and blue mussel (*Mytilus edulis*) culture in Australia, the higher risk of impact was associated with the deterioration of sediment quality, the alteration of sediment physical structure and the impact on seagrass beds.

The effects of bivalve aquaculture on the environment have been classified in different ways throughout the literature. For the purpose of this manuscript they will be divided in four main effects: on the water column, on the sediment, effects of bivalve introduction and effects on other marine species (including marine mammals and birds; Appendix A- Table A1).

2.2 Ecological effects of bivalve aquaculture

2.2.1 Effects on water column and nutrients

Bivalves affect the water column through particle filtration, and through modification of the nutrient cycle, with direct excretion and microbially mediated remineralisation of their organic deposits in sediments (McKindsey *et al*., 2006a; National Research Council, 2010). Large bivalve assemblages have the ability to modify phytoplankton populations and blooms. They can regulate the abundance of phytoplankton in shallow seas and reduce phytoplankton bloom intensity. Reduced turbidity due to bivalve grazing can

increase light, a limiting factor for the growth of other species, such as macroalgae (Kaspar *et al*., 1985; Cranford *et al*., 2003; Newell 2004; Cranford *et al*., 2006; McKindsey *et al*., 2006a). Grazing can reinforce seasonal successional cycles in phytoplankton composition. Picoplankton (0.2-2 μm) can be favored by warmer waters and by changes in relative abundance of organic and inorganic nitrogen and it is also generally retained less efficiently on the gills of bivalves, while nanoplankton (2-20 μm) is preferentially removed by grazing. Therefore, during warmer seasons, with the contributing role of bivalves, picoplankton becomes relatively more abundant than larger species (Newell 2004; Anderson *et al*., 2006; Fabi *et al*., 2009).

The effects of bivalves on nutrient cycling include marked changes in the nitrogen distribution (Rodhouse & Roden, 1987), especially contribution of nitrogen in the form of ammonium (NH_4^+), removal of particulate phosphorus and nitrogen through biodeposition and recycling of silicate through transfer from water column to the sediment. The ammonium excreted by bivalves is immediately available for primary production; therefore bivalves have a positive effect on primary production by increasing the nitrogen turnover in the water column. Bivalves such as mussels may also concentrate certain metals like copper in their pseudofeces (Kaspar *et al*., 1985; Dame, 1996; Cranford *et al*., 2003). The extent to which the overall nutrient budget and primary production are affected by bivalves is related to their abundance, location, system flushing rate and residence time (Rodhouse & Roden, 1987). Therefore, a population of cultured bivalves has the potential to modify the nutrient cycle in coastal ecosystems in that carbon and nitrogen ingested as phytoplankton are converted into other forms and concentrated near the culture area; moreover, competition between bivalves and other

grazing species such as zooplankton arises (Rodhouse & Roden, 1987). It has been postulated that changes in relative concentration of silica, nitrogen, and phosphorus could facilitate growth of harmful phytoplankton classes. For instance, promotion of algal blooms of *Pseudo-nitzschia* in relation to eutrophication has been demonstrated (Parsons *et al*., 2002a), though a direct link between these blooms and bivalve culture sites is still speculation. In the end bivalve aquaculture is a net remover of nutrients from the ecosystem through harvesting of the product (Kaspar *et al*., 1985; Dame, 1996; Gavine & McKinnon, 2002; Cranford *et al*., 2003; Anderson *et al*., 2006; National Research Council, 2010).

2.2.2 Effects on sediments and benthic habitat

Bivalve filter-feeders effectively remove natural suspended matter with a diameter between 1 to 7 µm, depending on species, and return large fecal pellets of ~500-3000 µm. These pellets rapidly settle to the seabed, particularly when slow or poor water flushing and exchange conditions exist. This particle repacking diverts primary production and energy from planktonic to benthic food webs (Dame, 1996; Cranford *et al*., 2003). Much of the research regarding bivalve aquaculture and bottom sediments focuses on the effects of increased organic load to the sediments from biodeposition, habitat modification associated with culture gear and consequent changes in local fauna (McKindsey *et al*., 2006a; National Research Council, 2010).

In the sediment, the rate of accumulation or dispersion of biodeposits and the severity of impacts created by bivalves depend on water depth and prevailing currents close to the seafloor. In the literature, a variety of observations on cultured bivalve biodeposition have been reported, including little or negligible impact, low sedimentation rates and absence of major changes in benthic infauna (Chamberlain *et al*., 2001; Crawford *et al*., 2003; da Costa & Nalesso, 2006). In other cases the benthic community showed strong long-term effects. For example, it has been shown that adult mussels (*Mytilus edulis*) are able to increase natural sedimentation rates by an average factor of 26 (Cranford *et al*., 2003). Kaspar et al. (1985) noted consistently higher nitrogen pools in sediments under a mussel farm, suggesting accumulation of inorganic nitrogen. Again, under a blue mussel farm, Dahlback and Gunnarsson (1981) found a sedimentation rate (3 g $C \cdot m^{-2} \cdot d^{-1}$) three time higher than at the reference site. When the organic biodeposition reaches high levels, its decomposition can increase oxygen demand and generate an anaerobic environment that promotes ammonification, sulfate reduction and silicate flux increase (Dahlback & Gunnarsson, 1981; Dame, 1996; Cranford *et al*., 2003; Newell, 2004; Anderson *et al*., 2006; McKindsey *et al*., 2006a; Callier *et al*., 2009). Benthic responses to organic enrichment include an increase in bacterial abundance, meiofauna community and biomass, and reduction in macrobenthic infaunal abundance and diversity (Kaspar *et al*., 1985; Chamberlain *et al*., 2001; Cranford *et al*., 2003; Callier *et al*., 2009). Bivalves have the capability to transform a diverse benthic community dominated by suspension feeders (bivalves, crustaceans and some polychaetes) into one dominated by smaller opportunistic deposit feeders, such as polychaetes, scavengers, carnivores and hydrogen sulphide-

tolerant species (Kaspar *et al*., 1985; Hartstein & Rowden, 2004; Callier *et al*., 2009; Dumbauld *et al*., 2009; Fabi *et al*., 2009; Forrest *et al*., 2009; Fréchette, 2012).

Despite the variety of impact severity, there is a consensus on the fact that aquaculture activities in intertidal, high-energy, well flushed areas generally results in dispersal of the organic biodeposits with a lesser impact on sediments. In contrast, culture in sub-tidal, quiescent, low-energy areas can potentially produce a large accumulation of biodeposits and therefore have a greater localized impact on the benthos (Chamberlain *et al*., 2001; Gavine & McKinnon, 2002; Hartstein & Rowden, 2004; Newell, 2004; da Costa & Nalesso, 2006; Fabi *et al*., 2009; National Research Council, 2010; Fréchette, 2012). In addition to the physical and hydrodynamic characteristics of the site, the scale of the operation and the culture technique used will determine the impact of bivalve culture on sediments and benthic populations. For instance, activities such as mussel harvesting by bottom dredging practiced in Germany, Netherlands, Irish Sea and Maine results in greater impacts on benthic habitats than off-bottom culture (National Research Council, 2010).

It is important to mention the processes related to bivalve shell formation, which capture carbon in the form of calcium carbonate. After natural mortality of the bivalve, carbon can be sequestrated in the surface sediment, where it provides local buffering against ocean acidification, through dissolution of the shells. Moreover, there is a positive feedback process between bivalves and carbonate addition to the sediment: bivalve carbonate producers, especially reef builders, provide a critical sedimentary constituent promoting the long-term survival of their own species (National Research Council, 2010). However, with current aquaculture practices the source of carbonate is extracted from the

marine system and discarded on land. This practice coupled with ocean acidification can accelerate carbonate loss in estuarine and coastal systems and diminish the positive feedback and the provision of habitat that support recruitment, growth and survival of the bivalves themselves (National Research Council, 2010). Bivalve shells also have the potential to change seabed topography and hydrodynamic conditions and therefore provide novel habitats which would normally not occur in a given environment (Anderson *et al*., 2006; McKindsey *et al*., 2006a; Forrest *et al*., 2009; Fréchette, 2012). It has been indicated that a mussel farm situated in a sheltered site can add up to 10 $cm \cdot year^{-1}$ of biodeposit largely constituted by shells, resulting in changes to the seabed up to 20 m from the farm boundaries (Hartstein & Rowden, 2004; Fréchette, 2012). The accumulation of this material can provide sites of attachment for large epibiota, such as tunicates, sponges and calcareous polychaetes (Kaspar *et al*., 1985). Oysters are an important bivalve to consider when discussing shell deposition since oyster reefs are known to provide a habitat that can support a diversity of taxa. When oyster culture is located on soft-sediment habitats, dominated by flat sand or mud, its impact could result in fundamental long-term shifts in benthic community composition (Dame, 1996; McKindsey *et al*., 2006a; Forrest *et al*., 2009). It has been observed that both oyster and mussel introduction in soft-sediment areas generates an increase in diversity and abundance of infauna and epifauna. Therefore, the establishment of a bivalve culture operation has the ability to shift soft-sediment to hard-bottom, where communities are generally more diverse, have greater biomass and are more productive (McKindsey *et al*., 2007; McKindsey *et al*., 2011). In addition, aquaculture structures such as bags, anchors,

and ropes can both change the hydrodynamic processes of an area, redirecting water flow, and alter the benthic habitat (McKindsey, 2010; National Research Council, 2010).

The effects on the sediments caused by bottom clam aquaculture are not as extensively studied as those of oysters and mussels. However, this type of aquaculture deserves mention, since it involves a number of practices which largely modify the physical environment, clearing intertidal and beach zones of rocks, wood debris and competing species (unwanted species of clams, mussels and barnacles). Also predator species such as snails and starfish are removed and gravel may be added to encourage growth and stabilize the sediments. The culture is then covered with anti-predator netting, which can facilitate the growth of other species otherwise not suitable for coastal areas and can also trap fish. The removal of rocks and debris has the opposite effect of netting on hard-bottom species, since their main natural attachment sites are removed (McKindsey, 2010).

2.2.3 Effects on native pelagic and benthic species

Cultured bivalves affect planktonic and benthic food webs by modifying, repacking and increasing the sedimentation rate of fine suspended particles, thus potentially altering the availability of food resources to other species. The physical structure of the farm and the fouling organisms that concentrate on bivalves and culture structures create an attraction for a variety of species. Crabs, other crustaceans, shellfish and demersal fish seem to benefit from culture activities as a result of increased food availability under bivalve

suspended culture. In some cases it has been shown that the diet of crabs in the vicinity of farms switched from algae to mussels as the main component (Gavine & McKinnon, 2002; Cranford *et al*., 2003; McKindsey *et al*., 2006a; McKindsey *et al*., 2011). Lobsters (*Homarus americanus*) appear to be attracted by both the presence of anchor blocks and other structural components of mussel farms, used as refuge, and by the increased food supply constituted by the bivalves themselves and by other species attracted by the farm (D'Amours *et al*., 2008; McKindsey *et al*., 2011). It has been observed that kelp (*Laminaria longicrursis*) grows abundantly on cultured blue mussel (*Mytilus edulis*) lines, with new tissue growth both in summer and winter, suggesting that this alga is taking advantage of nutrient release from the bivalves as well as increased light availability near the surface (McKindsey *et al*., 2006a). Bivalve aquaculture can displace or disturb seagrass with culture structures and operations, the farm can also provide an unnatural hard substrate, cause physical modification of flow and sediments, and shading from light, which affects growth and survival of both macroalgae and seagrass. Moreover, bottom-cultured oysters can affect seagrass also by severing the plants with the sharp tips of their shells (Gavine & McKinnon, 2002; Forrest *et al*., 2009; National Research Council, 2010). Carnivorous fish can be attracted in areas with bivalve farms by an increase in benthic herbivorous fauna; this increase is due to a rise in microphytobenthos, an important food source for the benthic herbivorous fauna, which is in turn caused by increased water clarity from bivalve filtration (Newell, 2004).

In contrast, zooplankton and larval fish that depend on suspended seston as food can compete with bivalves for food (Rodhouse & Roden, 1987). Also, bivalves, e.g. *Mercenaria* and *Mytilus* species, have the ability to significantly reduce the abundance of

microzooplankton and mesozooplankton through filtering while oysters may have the capacity to filter and remove larvae of some invertebrate species. However, the importance of this last impact under natural conditions is still unknown (Cranford *et al*., 2003; McKindsey *et al*., 2006a; Fabi *et al*., 2009; National Research Council, 2010). Lastly it is significant to note that certain aquaculture practices, such as collection of wild bivalve seed, can have a potential negative impact on native stocks of the cultured species, if collection is done on large proportions of the stocks (Gavine & McKinnon, 2002; McKindsey *et al*., 2007).

2.2.4 Introduction of non-native species

To diversify the number of species used in culture operations, introduction of non-native species has been largely employed in aquaculture. The most renowned examples are the introduction of Pacific oyster (*Crassostrea gigas*) on the Pacific and Atlantic coast of North America, in Europe, Australia and New Zealand, and the introduction of Mediterranean mussels (*Mytilus galloprovincialis*) in South Africa (McKindsey *et al*., 2007). Non-native bivalve species often exhibit faster growth rates, better resilience to diseases, physiological stress and higher reproductive effort than equivalent native species. Therefore, they can become a superior competitor for resources, with the risk of naturalizing and establishing self-sustaining populations and dominating endemic species, they can also inbreed with native species and induce genetic changes in the natural populations (McKindsey *et al*., 2007; National Research Council, 2010). They can influence biodiversity, local community composition, and the performance of the whole

ecosystem and they are more likely to have negative far-field effects compared to cultured endemic bivalve species. However, there appears to be a lack of knowledge on how non-native oysters and other non-native bivalves impact community and ecosystem level structure and function (McKindsey *et al*., 2007; National Research Council, 2010).

2.2.4.1 Diseases and pest introduction

Numerous diseases have been transferred via movement of infected bivalve stocks. In many cases the fact that the translocated bivalves harbored a disease agent was unknown due to lack of basic knowledge of the disease or inadequate testing and monitoring before the transfer. This has been the case for different oyster diseases. For example, *Haplosporidium nelsoni*, the causative agent of MSX disease, is a parasite that infects Pacific oysters causing little disease and mortality whereas it greatly affects eastern oysters. Its transfer via movement of Pacific oysters has been the cause of a major decline in eastern oyster population in Chesapeake and Delaware Bays (Andrews, 1988; National Research Council, 2010). Norcardiosis, caused by the bacterium *Nocardia crassostreae*, is thought to have originated in Japan and then spread to North America with Pacific oyster transfers (Forrest *et al*., 2009). Another renowned and documented case is the introduction of the parasite *Bonamia ostreae* from the United States to Europe. European flat oysters (*Ostrea edulis*) transferred from California to France and Spain appear to be the cause of a devastating crash of the flat oyster population in Europe. In this case,

however, the presence of the parasite and the high mortality it causes were known and the seed transferred were erroneously declared disease-free (Elston & Ford, 2011).

The intentional introduction of non-native bivalves has often resulted in the associated unintentional transfer of non-native organisms that "hitchhiked" with the introduced species. Non-native species can hitchhike within the bivalve, on the bivalve, in water or equipment, in the sediment contained in empty shells or even with other hitchhiking organisms (McKindsey *et al*., 2007). In San Francisco Bay it has been estimated that 20% of the non-native species resulted from the shipment of eastern (*Crassostrea virginica*) and Pacific oyster (*Crassostrea gigas*). Many of these species are now important predators and competitors of the resident fauna and flora, as well as pests. In the North Sea it has been estimated that 40% of non-indigenous species hitchhiked with oyster culture, and 43 exotic macroalgae species were introduced in Southern France (Verlaque, 2000). This type of introduction contributed historically, at least as much as international shipping to the spread of exotic species (McKindsey *et al*., 2007; Forrest *et al*., 2009; National Research Council, 2010; McKindsey *et al*., 2011). Biofouling organisms have often been introduced with cultured bivalves, especially oysters and mussels. These bivalves are highly vulnerable to biofouling due to their aggregated shells and culture structures thus providing substrate for the settlement of fouling organisms. Pests associated with their transfers include macroalgae (*Codium fragile* spp. *tomentosoides* and *Undaria pinnatifida*), tunicates (*Ciona intestinalis* and *Styela clava*) and gastropods (common slipper shell, *Crepidula fornicata*). In some cases, non-native biofoulers have proliferated, reducing local biodiversity and changing population and community structure in coastal systems. In addition, bivalves may be treated with antifouling agents

in order to eliminate or reduce fouling. These treatments are almost universally done in situ. Antifouling agents such as hypochlorite and acetic acid may therefore be added to the environment, and the physical removal of fouling may cause the invasive species to spread or deposit on the bottom (McKindsey *et al*., 2007; Forrest *et al*., 2009; National Research Council, 2010).

A particular case of introduction, with repercussion on public health, is the possible transfer of harmful phytoplankton species. It has been proven that the harmful dinoflagellate *Alexandrium* spp. can be transported in the digestive tract and therefore introduced into new environments by mussel, oyster, clam and scallop, especially at the more robust cysts stages but also at vegetative cell stage. The viability of the algal cells appears to be significantly reduced at 48 hours post-filtration; therefore, a depuration period of 48 h pre-introduction could minimize the risk of transfer of harmful algae (Bricelj & Shumway, 1998; Hégaret *et al*., 2008; reviewed by McKindsey *et al*., 2007; McKindsey *et al*., 2011).

It is important to point out that the introduction of non-native bivalve species for aquaculture purposes is now highly regulated by national and international laws greatly reducing the probability of the introduction of diseases and pests. A code of practice for the introduction of non-native species developed by the International Council for the Exploration of the Sea (ICES) has been adopted by many countries and includes preventive measures such as quarantine, extensive disease testing, and hatchery breeding so that only first-generation offspring can be released in open water (National Research Council, 2010; Hedgecock, 2011).

2.2.5 Effects on marine mammals and seabirds

Bivalve aquaculture operations have the potential to impact marine mammals by causing entanglement from farm structures and litter, changes to prey abundance, and reducing habitats through noise and farm structures. These impacts have only been identified as potential and not yet demonstrated directly for bivalve culture, with the exception of a case of entanglement in mussel spat collectors of two Bryde's whales in New Zealand (McKindsey *et al*., 2006a; Forrest *et al*., 2009; National Research Council, 2010).

Bivalve culture can affect seabirds due to alteration of food sources, displacement of habitat and noise disturbance (Forrest *et al*., 2009). When non-native oyster culture is introduced in soft-sediment areas, an increase in abundance of birds may occur, and sea ducks appear to be strongly attracted by bivalve aquaculture operations (Žydelis *et al*., 2009). During mechanical harvesting of clams an increase in the feeding activities of gulls and waders is observed. In these cases aquaculture created a new habitat for associated fauna (McKindsey *et al*., 2007; Žydelis *et al*., 2009; McKindsey, 2010). However, diving ducks are considered one of the most important predators of cultured bivalves, particularly mussels, and cause considerable damage to farmers (McKindsey *et al*., 2006a). They generally prey preferentially on small mussels, however they cause damage to collectors and commercial mussel rope culture (Varennes *et al*., 2013). It has been calculated that eider ducks can remove up to 2.6 Kg of mussels in a day and the total loss for the farm can be anywhere from 30 up to 75% of the production (McKindsey *et al*., 2006a; Varennes *et al*., 2013). Many methods have been employed to deter bird predation, including acoustic deterrents, nets, and gunfire (McKindsey *et al*., 2006a). The

debate on the most sustainable deterrent is still open. Exclusion nets are currently being studied in order to determine the correct mesh and twine size for different duck species, as shown by Varennes et al. (2013).

2.3 Evaluation, control, and prevention of bivalve aquaculture effects

There are different environmental indicators that have been used to evaluate the interaction between bivalves and the surrounding environment. For effective prevention and mitigation of bivalve aquaculture effects, the indicators used need to encompass water quality effects, benthic effects, changes in biodiversity, habitat transformation, and carrying capacity (Hargreaves, 2011; summary in Appendix A- Table A2). There are indicators that help to understand the movement of water and nutrients in the area of interest such as the ones described by Gibbs (2007):

- Clearance efficiency: the ratio between the number of days that the water takes to clear an inlet and the number of days it would take for the bivalve to process all the water in the inlet (CE = Rt/Ct; CE= days/days).
- Filtration pressure: the ratio between the total carbon extracted by bivalves in the water column and total carbon fixed by autotrophs (FP = Bf/Pp).
- Regulation ratio: the phytoplankton turnover rate over the ratio of the daily volume of water cleared by bivalves to the total volume of water (RR = Tc/Tp).
- Depletion footprint: it can be measured by fluorometry and conductivity-temperature-depth (CTD) instruments (Gibbs, 2007).

Moreover, nutrient concentration, dissolved oxygen, bacterial abundance, phytoplankton biomass and population size structure can be measured. Other indicators are used to measure the health of sediments and benthic habitat: redox potential, sediment oxygen concentration, similarity indices, biodiversity metrics, and presence/abundance of indicator species. Finally, there are socio-economic indicators that evaluate the impact and the interest around bivalve culture. For example, social acceptability of the culture, supply availability and livelihood security for the local communities (Cranford *et al*., 2006; Cranford *et al*., 2012). The information obtained from these indicators can be used independently to give a snapshot of the status of the system and the impact of a farm; however, it should be used to feed a carrying capacity model or a risk-type matrix as part of a larger management plan (Gibbs, 2007).

Many efforts have been concentrated on developing complex, numerical, hydrodynamic carrying capacity models, which take into consideration currents, nutrient, plankton and zooplankton (Gibbs, 2007). However, most of the modeling has focused on production carrying capacity (optimized level of production of the target species), with few modeling efforts on ecological carrying capacity which considers the whole ecosystem. Given the complexity of carrying capacity models and evaluation, they require expertise in many areas and therefore these models are always the result of collaboration between experts in different fields (McKindsey *et al*., 2006b). Modeling is one of the few tools capable of assessing aquaculture sustainability while also considering the cumulative effects of human activities and resident and invasive species. It is therefore the most complete tool to assess and prevent aquaculture impacts on the environment (Cranford *et al*., 2012). A good example of modeling for bivalve aquaculture

is the complex Farm Aquaculture Resource Management (FARM) model developed by European researchers (Ferreira *et al*., 2009), which includes information about production and environmental impact for bivalve farms. It combines hydrodynamics, biogeochemistry, population dynamics, and economics into a management tool that became a strong decision-support tool for both growers and regulators. This model has been tested for a variety of systems around Europe, from open coast to estuaries, and on a wide range of cultured bivalves such as the Pacific oyster, blue and Mediterranean mussel, and Manila clam. The FARM model also shows the indicators of positive impact provided by bivalve culture in helping to reduce eutrophication in the coastal zones (Ferreira *et al*., 2009). Also, in Canada, a bio-physical ecosystem model assessing the environmental effects, particularly regarding nutrient cycling, of bivalve aquaculture in coastal waters has been developed and validated. This model includes both benthic and pelagic components and the cycling of limiting nutrients (Dowd, 2005).

Best management practices (BMP) and performance standards have been used as means of prevention of unacceptable environmental interactions and they are often developed by the industry group itself. Regulatory and certification standards can be developed by the public authority and by the buyers. The goal of these standards is a more sustainable, effective and acceptable aquaculture operation. However, to reduce or limit environmental impacts of bivalve culture, standards should be developed and implemented at the ecosystem level (National Research Council, 2010; Hargreaves, 2011).

All the means of prevention and impact assessment presented above can be gathered into an ecosystem-based management (EBM) strategy for bivalve culture. Ecosystem-

based management is defined as the comprehensive integrated management of human activities implemented to identify and take action on influences that are critical for the ecosystem. It is a tool to achieve sustainable uses of the ecosystem, maintaining its integrity and encompassing interaction between ecological, social, and economic systems (Cranford *et al*., 2012). In brief, a bivalve culture, ecosystem-based management strategy requires models to assess carrying capacity, policies for hazard identification, risk assessment and management, environmental monitoring programs, impact assessment and communication. It should incorporate the best available scientific knowledge, address phytoplankton interactions, impact on the seabed and interactions between farms, consider cost versus benefits, the potential ecological services provided by cultured bivalves, social issues and economic impact (Cranford *et al*., 2006; Cranford *et al*., 2012).

2.4 Positive effects of bivalve culture

The functions of water clarification and biodeposition that characterize filter-feeding bivalves are valuable providers of ecological services to shallow water ecosystems. Bivalves help buffer estuaries and coastal ocean waters against excessive phytoplankton blooms in response to anthropogenic loading of nitrogen, counteracting the symptoms of eutrophication; they also remove inorganic sediments from suspension, counteracting coastal water turbidity. The biodeposition created by mussels and oysters, through the creation of sediment anoxic microzones where denitrifying bacteria are promoted, induce denitrification, which also helps to counteract eutrophication by returning nitrogen into

the atmosphere as inert nitrogen gas (Officer et al., 1982; Dame, 1996; Newell, 2004; Newell & Koch, 2004; National Research Council, 2010). Moreover, the enhancement of water clarity due to filtration allows deeper light penetration and therefore can increase the growth of seagrasses that are important nursery habitat for many fish, crustaceans and molluscs; bivalves are therefore capable of enhancing estuarine nursery habitats (Newell & Koch, 2004; Cerco & Noel, 2007; National Research Council, 2010). These natural functions of bivalves can be employed in aquaculture not only to mitigate the environmental effects of the culture, but also to create added value and services for the surrounding environment.

2.4.1 Restoration

Natural shellfish populations around the world are in decline due to over exploitation from fisheries, decline in estuarine condition and in smaller part to introduction of diseases (Brumbaugh *et al*., 2006; Fulford *et al*., 2007). As the natural populations decline the important ecosystem services that bivalves provide also drop off and both the water column and the benthic habitat can be affected. It has therefore been suggested that bivalve restoration should be a component of restoring historical baseline conditions and functioning of estuaries or some other pre-determined, acceptable condition. The restoration of oysters in the Chesapeake Bay is the most famous example of a bivalve restoration effort (Ulanowicz & Tuttle, 1992; Newell 2004; Cerco & Noel, 2007; Fulford *et al*., 2007; National Research Council, 2010; Burkholder & Shumway, 2011). Bivalve

aquaculture can be considered as an estuarine and coastal ecosystem restoration tool, it could serve to mitigate water quality issues, such as excess chlorophyll *a* and turbidity and even level of contaminants. Although bivalve culture does not provide the same structure created by wild aggregated bivalves, the culture gear itself can provide a structural habitat.

It has been suggested that farmers should receive a compensation for mitigation based on the level of improvement achieved, in addition to selling their product, hence enhancing locally grown seafood production. However, for bivalve aquaculture to work as a restoration tool and give net benefits to the environment, regular removal and responsible disposal of non-native fouling needs to be undertaken. Moreover, human activities have to be closely controlled to avoid disturbance of any valued species, including birds and marine mammals (National Research Council, 2010; Burkholder & Shumway, 2011).

2.4.2 Re-eutrophication

In Sweden, blue mussels (*Mytilus edulis*) have been used for a study on "agro-aqua recycling". Cultured mussels were used to reduce the effects of eutrophication created by excess nutrients discharged in coastal waters from farm land runoff and rural living. They were then harvested and re-used as seafood or in agricultural operations as feedstuff and fertilizer (Lindahl, 2011). The use of harvested mussels as a substitute for fishmeal in poultry feed was investigated and was successful (Jönsson & Elwinger, 2009; Lindahl, 2011). Moreover, the remainder of mussels and shells proved to be a valuable land

fertilizer, especially interesting for organic farmers who cannot use commercial fertilizers. This model of re-eutrophication, nutrient trading and mussel farming resulted therefore in a successful solution for society, environment, and industry (Edebo *et al.*, 2000; Lindahl, 2011). The study site has been evaluated for 1.5 years after the beginning of the culture operation, and in all cases presented a net removal of nitrogen from the system (Carlsson *et al.*, 2012). Research in this field is currently ongoing in other European countries, such as Denmark and the Netherlands.

2.4.3 Integrated multi-trophic aquaculture (IMTA)

Mussels, oysters and sea scallops have been involved in studies of polyculture systems (Parsons *et al.*, 2002b; Navarrete-Mier *et al.*, 2010; Chopin *et al.*, 2012). Generally, polyculture or integrated multi-trophic aquaculture (IMTA) combines artificially fed aquaculture such as finfish or shrimp with extractive aquaculture. The latter utilizes filter-feeding organisms, the bivalves, to remove excess organic nutrients and seaweeds to remove the excess inorganic nutrients, in order to reduce the environmental impact of fed aquaculture. The bivalves perform as biological filters and environmental cleaners. This type of culture is based on the principle that the solution to pollution is not dilution but extraction and conversion (Navarrete-Mier *et al.*, 2010; Chopin *et al.*, 2012). A possible further gain can be the fact that it has been experimentally demonstrated that blue mussels (*Mytilus edulis*) ingest sea-lice (*Lepeophtheirus salmonis*) at the copepodid stage and therefore could be a valuable help in controlling sea-lice infestation in farmed salmon

(Molloy *et al*., 2011). However, Navarrete-Mier et al. (2010) showed that excess organic matter produced by a finfish farm in open-water systems was not used by bivalves; the authors conclude therefore that polyculture may not be relevant for diminishing the environmental impact of finfish farms located in highly hydrodynamic areas. It is the opinion of the author that the effects of bivalves in a polyculture system have to be carefully studied with regard to the hydrodynamics of the area for an extended period of time, as the amount of fish waste in the diet of IMTA bivalves varies with season (Mazzola & Sarà, 2001). Critical limitations of the effectiveness of mussels in removing excess organic nutrients with current IMTA practices are presented by Cranford et al. (2013).

2.4.4 Remediation

Gifford et al. (2004) suggested the use of pearl oysters as bio-remediators in polluted environments. The interest in using pearl oysters, such as *Pinctada imbricata* and *Pinctada margaritifera*, for bioremediation is highly attractive since the market value for these species is in the pearl and not in the meat. The authors suggest the possibility of culturing different pearl oyster species in environments polluted by heavy metals, such as lead, copper, zinc and iron. Organopollutants, such as PCBs and petroleum hydrocarbons, have been also shown to accumulate in bivalve meat. Finally, pearl oysters can be used as natural filters to clean waters of bacteria, viruses and protozoa from human and animal waste (Gifford *et al*., 2004). Pearl oysters have been shown to accumulate significant

amounts of pollutants in both meat and shells and therefore they are good candidates for remediation of polluted waters (Gifford *et al*., 2005). However, in order to make bivalve remediation a viable alternative, the disposal of harvested contaminated oysters needs to be assessed and addressed. Moreover, tolerance limits of the pearl oyster to pollutants needs to be investigated, together with their effect on pearl quality (Gifford *et al*., 2005).

In Australia, rock oyster (*Dendostrea folium*) and Sydney cockle (*Anadara trapezia*) have been experimentally used to remediate water in ponds previously used for prawn culture. These ponds were rich in suspended silts and sediment loads; the bivalve that showed the highest survival was the Sydney cockle (Palmer & Rutherford, 2011).

Another possible form of remediation is the use of bivalves to control phytoplankton biomass and prevent harmful algal blooms; in mesocosm experiments northern quahog (*Mercenaria mercenaria*) was able to control the ability of *Aureococcus anophagefferens* to dominate the phytoplankton and to create brown tides that negatively affect other economically important bivalves (Cerrato *et al*., 2004).

2.5 Conclusions

The success of bivalve aquaculture is highly dependent on water quality and a healthy ecosystem. There is no doubt that efforts are underway to pursue sustainable culture that protects and maintains the supporting environment by operating within the ecological carrying capacity. The author agrees, however, with Hargreaves (2011) when he states that sustainability is not an endpoint, but rather a trajectory of constant improvement. Bivalve culture needs to keep evolving, modifying and striving to couple benefits for the

farmers with benefits for the environment. “Constant improvement” also encompasses identifying what research can do for bivalve aquaculture. Polyculture, bivalve restoration and use to counteract human eutrophication are directions worth exploring, and ecosystem-based management should be developed and implemented in areas with bivalve culture operations. To do this, more knowledge on the direct effects of bivalve culture on the water column and nutrients is needed. To have the most accurate picture of culture impacts Nizzoli et al. (2006) suggested the need to monitor nutrients sequentially both in suspended culture and the benthic environment. Constant improvement requires extensive interdisciplinary collaboration because the interaction between bivalve culture and the environment is complex and encompasses many disciplines such as biology, ecology, chemistry, oceanography, and social sciences.

Ultimately, as stated by McKindsey et al. (2011), there is a need to evaluate which of the effects of bivalve aquaculture on the environment are important and which ones are not, and what should be the goal of management. Often negative and positive effects of bivalve aquaculture are closely related and shown together and often the same effect can be considered both negative and positive, depending on the situation. On balance, whether or not bivalve culture has a negative or positive effect depends on the social and economic values that are used to weight the different components (McKindsey *et al.*, 2011). These values are not a privilege of the industry or the scientists, and have to be chosen in collaboration with the whole of society.

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CHAPTER 3

Environmental conditions in shallow and deep water blue mussel culture sites in Notre Dame Bay, Newfoundland, Canada

3.1. Introduction

The growth of bivalves can be affected by many factors, the most important being water temperature, food quantity and quality, and seasonal cycles. Other factors such as salinity, oxygen consumption, currents and wave action can also exert an effect on bivalve physiology (Seed, 1976; Penney *et al*., 2001). Therefore, knowledge of environmental conditions, such as water column characteristics and vertical structure, is fundamental to understanding overall bivalve growth and physiology.

In shelf seas, the vertical structure of the water column results from a competition between buoyancy inputs due to surface heating and freshwater fluxes (runoff and precipitation), and the mixing/stirring effects of tides and winds (Simpson & Sharples, 2012). When heating increases, positive buoyancy increases (i.e., surface water density decreases) and tends to stabilize the water column; the water can then stratify (Parrish *et al.*, 2005). In colder months, however, when heat is lost from the sea-surface, buoyancy contributes to stirring due to increasing surface density which, in turn, results in surface water moving deeper, mixing the water column on its way down. In addition to atmospheric heating, surface buoyancy can be increased by freshwater inputs (runoff,

precipitation, sea-ice melting) which will tend to enhance the seasonal stratification. Therefore, mid-latitude shelf seas are generally stratified during the summer and well mixed during the winter months. Simplistically, seasonal stratification will separate the water column into two layers: one surface layer heated, potentially freshened, and stirred by the atmosphere and a bottom layer stirred by tides, separated by a high gradient region (Simpson & Sharples, 2012). Together, heating and freshwater buoyancy inputs influence and define the water vertical density structure: heating creates a region of high vertical temperature gradient, or *thermocline*, freshening creates a region of high vertical salinity gradient, or *halocline* and together heating and freshening result in a region of high vertical density gradient, the *pycnocline* (Sharples *et al*., 2001; Parrish *et al*., 2005).

Phytoplankton need light and macronutrients such as nitrogen, phosphorus, and silica to thrive (Sharples *et al*., 2001; Valiela, 2010). The largest fluxes of nitrate and phosphate come from the deep ocean. However, in coastal waters nitrogen and phosphate are also supplied by rivers as a result of runoff from fertilised land and wastewaters. Nitrogen can be supplied also from the atmosphere and bacteria in the water column can remineralize organic material to supply nutrients. Silicate, however, is supplied by the weathering of rocks on land, by volcanism and hydrothermal vent activity; silicate can therefore be a limiting nutrient for the growth of algal groups such as diatoms (Valiela, 2010; Simpson & Sharples, 2012). Other factors such as temperature appear to have a smaller but clear effect on phytoplankton seasonal cycles and growth. Phytoplankton photosynthetic activity decreases significantly at temperatures below 1°C and colder water seems to also affect bacterial activity. Nevertheless, temperature is thought to have more important

consequences when treated as a covariate with other factors, such as photosynthetic pigments and carbon concentration (Valiela, 2010).

Seasonal water stratification affects primary production and the development of blooms, because the changes in the physical environment control the availability of light and nutrients to the phytoplankton (Valiela, 2010; Simpson & Sharples, 2012). During the earlier stages of pycnocline formation, as stratification develops, phytoplankton are split into two communities; the cells in the surface water become trapped in the new surface mixed layer and will receive enough light to initiate a bloom, while the cells in the bottom mixed layer will not receive sufficient light and their number will start to decline (Simpson & Sharples, 2012). During the spring bloom the majority of phytoplankton are constituted by large cells, such as diatoms, which are more efficient at transferring energy through the food chain due to their size and are thus major contributors to production in coastal waters (Parrish *et al*., 1995; Parrish, 1998; Trottet *et al*., 2008). Diatom dominance during the spring bloom can be due to different reasons; they are suited for turbulent waters and are therefore present in the pre-bloom water column, where they can take advantage of the developing stratification; they have a high growth rate in the environment where nutrients are not limited and are armored with silicate and therefore are subject to lower grazing (Safi & Gibbs, 2003; Simpson & Sharples, 2012). The spring bloom is sustained until depletion of nutrients in the surface layer becomes a limiting factor (Parrish *et al*., 2005). However, a rapid and temporary remixing of water, caused for example by a strong burst of wind, can resupply nutrients; subsequent heating and/or freshening restabilizes the water column and can trigger another bloom. Spring blooms can easily be subject to inter-annual variability, particularly in timing, since different

weather conditions lead to differences in air-sea exchanges of heat and in wind stirring (Parrish *et al*., 2005; Parrish *et al*., 2009; Simpson & Sharples, 2012).

At the pycnocline, another important physical force is responsible for mixing, internal waves. These are mainly generated by stratified flow over steep topography and shelf edges. Internal waves are extremely important in driving vertical mixing and in maintaining biogeochemical fluxes in an interior region that is far from both the surface and the bottom (e.g., through the seasonal pycnocline) where most of the input of mechanical energy (wind and tide stirring, respectively) into the water column occurs (Simpson & Sharples, 2012).

The shelf edges are often regions with high biological production, since different physical processes are responsible for the transport of nutrients and the increase of primary productivity. Wind force at the surface can create upwelling, exporting surface shelf water and importing nutrient rich deep sea water; often during upwelling events, the phytoplankton population is dominated by diatoms. Topographical changes in the slope can create eddies and meanders and consequently increase water and nutrient transfer. Internal tide, internal mixing, and vertical turbulent mixing can affect the pycnocline and therefore the nutrient distribution (Archambault *et al*., 1999; Penney *et al*., 2001; Simpson & Sharples, 2012).

To take advantage of the physical characteristics at the pycnocline, and its possible enhanced chlorophyll *a* concentration, bivalve deep water aquaculture is being developed in different parts of the world (Ogilvie *et al*., 2004; McKindsey *et al*., 2006; Cheney *et al*., 2010; Duarte *et al*., 2012). A Newfoundland-based company has already started to adapt existing technology for the utilization of deep water mussel sites in Notre Dame

Bay. Preliminary oceanographic data suggested that the region between 10 and 20 m depth is the most productive for mussel growth; the same region should also provide the most stable environment with a high level of phytoplankton and lower fluctuation in temperature and salinity (Deep Water Mussel Aquaculture Development (Phase II) Final Report, 2009).

The objective of this chapter is to present the characteristics of the physical environment of South Arm, an inlet of New Bay, Notre Dame Bay, where mussel aquaculture is present. This chapter aims also to compare the environmental conditions at different depths (5 m and 15 m) to determine the most suitable location in the water column for mussel culture.

3.2. Materials and Methods

3.2.1. Study site and experimental set-up

The study area was located in one of the numerous inlets of the North Coast of Newfoundland (Appendix B- Figure B2). South Arm is an inlet about 15 km long and 1-2 km wide. It is the southernmost branch of a larger embayment (New Bay) connected to the ocean (Notre Dame Bay) by an opening of about 4 km width and 440 m depth. The depth progressively diminishes from the mouth of New Bay towards the heads of the branches and, in South Arm, decreases from more than 200 m in the main channel to less than 40 m in the inner basin where most of the mussel sites studied were located. The

inner basin is separated from the main channel by a relatively long and gentle sill of less than 30 m limiting depth. The channel width at the sill location is less than 1 km.

The study was conducted between September 2012 and September 2014. Starting in September 2012 instruments were positioned in the water at three blue mussel culture sites: South Arm, Bulley's Cove and Mouse Island (Appendix B- Figure B1). South Arm included a shallow water site (SAS), with headline at 5 m depth (49° 20.131'N; 55° 19.680'W), and a deep water site (SAD), with headline at 15 m depth (49° 20.603'N; 55° 19.959'W). Bulley's Cove included two shallow water sites (5 m; Bulley's Cove shallow 1: 49° 20.959'N; 55° 20.847'W, BCS1; Bulley's Cove shallow 2: 49° 20.892'N; 55° 20.654'W, BCS2), and one deep water site (15 m; 49° 20.754'N; 55° 20.460'W, BCD). Mouse Island included only one deep water site (15 m; 49° 22.933'N; 55° 18.039'W, MID), due to the impossibility of having a shallow water site in this area because of the presence of pack ice in the spring season. During the second year of the experiment between December 2013 and May 2014 it was not possible to proceed with sampling and instrument retrieval, due to severe winter conditions and presence of ice that prevented access to the sampling sites; many instruments failed to record data due to battery depletion during these months, and therefore data gaps are present for this period.

3.2.2. Moored sondes

Multiparameter water quality instruments (YSI sondes 6600 V2, YSI; Yellow Springs Instrument, 2012) moored at the three sites (six stations) were used to continuously monitor water temperature (°C), salinity, dissolved oxygen (DO; mg L^{-1}) and chlorophyll

a (μg L^{-1}). A summary of the technical specifications of the sensors is presented in Table 3.1; temperature was expected to be measured with an accuracy of 0.15°C, salinity to ± 0.3 in seawater of 30 and DO to ± 1%. In practice, and based on a number of cross-quality checks, salinity was recorded to accuracy no better than 0.5 and numerous measurement legs suffered from calibration offsets that could only be partially corrected using the CTD (Conductivity, Temperature and Depth) profile data (Section 3.2.3). Thus, salinity time-series were excluded from the analysis.

The concentration of chlorophyll *a* is used to estimate phytoplankton abundance and phytoplankton biomass (Cullen, 1982). Photosynthetic organisms such as phytoplankton re-emit part of the energy absorbed as light; the measurement of this emitted light is the basis of the use of chlorophyll fluorescence as an indicator of phytoplankton biomass (Simpson & Sharples, 2012). However, fluorescence varies as a function of phytoplankton species composition and nutritional state, and as a function of light absorption (Cullen, 1982; Trottet *et al*., 2008). The chlorophyll *a* data obtained with the sondes were compared to discrete water samples analysed in the laboratory in order to verify the accuracy of the in situ measurements.

Data were collected monthly and processed to obtain a concatenation with consistent date/time stamp and variables output. 'Out-of-water' data (any data with depth < 1 m) and large chlorophyll *a* spikes (any data > 50 μg·L^{-1}) were removed; a low-pass filter was applied on all the variables (i.e., Depth, Temperature, Chlorophyll, DO) based on running-averages with a 24 hours window (i.e., averaged 24 hours data together and then moved along the time-series).

Table 3.1: YSI 6600 V2 sonde specifications (Yellow Springs Instrument, 2012).

	Depth (m)	**Temperature (^{o}C)**	**Salinity**	**Dissolved Oxygen [4] (%)**	**Chlorophyll ($\mu g \cdot L^{-1}$)**
RANGE	0 – 60	-5 – +50	0 – 70	0 – 500	0 – 400
ACCURACY	+/- 0.12	+/- 0.15	+/- 1%[1] or 0.1[2]	+/- 1%[5] or 1[6]	N/A
RESOLUTION	0.001	0.01	0.001 to 0.1[3]	0.1	0.1

[1] Of reading; e.g., 0.35 in waters of 35 in salinity. [2] Whichever greater (i.e., 0.1 for waters of 10 in salinity, above that is +/- 1%). [3] Range dependent. [4] ROX Optical. [5] Of reading. [6] Whichever is greater.

3.2.3. CTD

Starting on January 2013, monthly water profiles were collected at the six sampling sites. Data were collected using a CTD CastAway instrument (YSI, Sontek; SonTek/Yellow Springs Instrument, 2012). A summary of the technical specifications of the instrument is presented in Table 3.2.

Data were extracted from the instrument after each field trip and processed as per manufacturer recommended procedures. Salinity was derived from the temperature, conductivity and pressure data using standard UNESCO algorithms (Fofonoff & Millard, 1983). Each processed profile was checked individually for downcast/upcast consistency and erroneous data or spikes were filtered out prior to graphic presentation as a final product.

Table 3.2: CastAway CTD specifications (Sontek/YSI, 2012)

	Depth (m)	**Temperature (^{o}C)**	**Salinity**
RANGE	0 – 100	(-5) – (+45)	0 – 42
ACCURACY	+/- 0.25[1]	+/- 0.05	+/- 0.1
RESOLUTION	0.01	0.01	0.01

[1] Full scale range; e.q. to 0.25 dbar or ~0.25 m

3.2.4. ADCP

Water current velocity profiles were recorded using a Teledyne Rowe and Deines Instruments (T-RDI) Acoustic Doppler Current Profilers (ADCPs; Teledyne RD Instruments, 2011) during different seasons. The ADCPs were moored at about 2.0 m above the sea bottom; profiling the water column in 1 m water cells from about 5 m above sea bottom up to about 5 m below the sea-surface. For each of the water cells, current magnitude and direction were recorded at 30-60 min intervals for 2.5 minutes (depending on instrument setup; Table 3.3). Current speed standard deviation, a measure of uncertainty, was estimated to be equal to 0.96 $cm \cdot s^{-1}$ by the manufacturer's software at setup time. The length of each data record ranged from about 119 to 183 days (Table 3.3). Water temperature was also measured by the ADCPs at their mooring depth (i.e., about 2.0 m above sea bottom).

Table 3.3: ADCP deployments sampling details.

Deployment name (Figures 3.9-3.16)	Site name	Site depth (m)	Sampling Interval (min)	Deployment date	Recovery date	Record duration (days)
MF001	MID	57	40	19-Dec-2012	14-May-2013	146.0
MF002	SAD	30	30	16-May-2013	13-Nov-2013	183.3
MF003	SAD	38	60	13-Nov-2013	23-Apr-2014	182.4
MF004	MID	60	30	20-Aug-2014	16-Dec-2014	119.2

3.3. Results

3.3.1. Moored sondes

The comparison between chlorophyll *a* measured with the moored instruments and the laboratory analysis of water samples showed a discrepancy in absolute values, with the values obtained with the sondes larger than the discrete water samples (1-5 fold; Appendix D-Table D1). A complete calibration of the sondes with the discrete sample results was not possible, due to the low concentration of chlorophyll *a* at each time the water samples were taken. Therefore, the chlorophyll *a* results that follow will be discussed only qualitatively and not quantitatively.

3.3.1.1. Year 1

Water temperature at the beginning of Year 1 gradually decreased from October to late January (-1.5°C, the lowest for the year) and was maintained for the months of February and March (Figure 3.1a). During this period the temperature appeared to be similar between all the sites and between shallow and deep water. However, during late spring, separation between the shallow and deep water site temperature due to the formation of the seasonal thermocline is clear (Figure 3.1a). From late April until September the shallow water sites presented a water temperature warmer than the deep water sites; this difference in temperature reached up to a 5-10°C difference, occasionally even during the same day. From late April, the water temperature increased gradually from 0°C to a peak of 18°C in shallow water at the beginning of August; the peak for deep water sites was

approximately 15°C during the same period. It is also interesting to note that during the warmer months, when the thermocline was present, both shallow and deep water sites presented large variations in temperature (several degrees) consisting of events of one or more days duration (Figure 3.1a). Those events were, at times, periodic in nature, with a 3 days period, for example, in early to mid-May. The yearly standard deviation of the temperature for the shallow sites was larger (5.7-5.8°C) than the one for the deep water sites (4.5-4.8°C) indicating larger seasonality in shallow water (Appendix C- Table C1).

Dissolved oxygen saturation (100%) was relatively constant and similar between shallow and deep water sites from October until February (Figure 3.1b). From mid-February, an increase in oxygen saturation, more marked in the shallow water sites, can be observed, coinciding with the chlorophyll peaks due to the spring bloom (Figure 3.1b,c). After the spring event, the oxygen saturation remained high, approximately 110%, for the rest of the summer, but descended again in the fall (early September; statistics in Appendix C- Table C2).

Chlorophyll *a* levels for Year 1 were similar between sites and depth and they were constant from September until mid-February, when a steep increase (spring bloom) was observed (Figure 3.1c). Peaks of chlorophyll *a* occurred at the beginning of March, mid-March and beginning of May (Figure 3.1c). It is interesting to note that the first peak was present in all the sites but Mouse Island deep, while the second and third peaks were only found in South Arm deep and Bulley's Cove deep. It seems, therefore, that two of the three deep water sites presented a higher and longer lasting spring bloom effect, compared to the shallow water sites and also compared to Mouse Island deep. Mouse Island showed a distinct pattern from all the other sites; in this site, the chlorophyll *a*

increase during the spring bloom appeared to be lower but gradual and more sustained than in the other sites from mid-February until May (Figure 3.1c; statistics in Appendix C- Table C3).

3.3.1.2. Year 2

In Year 2, a similar pattern to the water temperature observed in Year 1 was observed during the fall and winter months. However, the gradual decrease in temperature was disturbed from mid-December to mid-January (Figure 3.2a); also, a temperature of 0°C was reached at mid-December, while in Year 1 the water did not reach 0°C until mid-January. In Newfoundland, the winter of 2013-2014 was unusually cold (DFO, 2015); heavy snowstorms and ice formation began in mid-December, around the same period when the water temperature shows the disturbance. From February until May 2014, only two sondes were continuously recording (Figure 3.2a-c); due to the formation of ice in the bay, it was not possible to retrieve the sondes monthly from December to May. Therefore, the majority of the sonde batteries failed before they could be retrieved and the data downloaded. However, from the two sondes recovered a constant water temperature was observed all winter long. Formation of the spring/summer thermocline was observed starting from mid-May, three weeks later than in Year 1 (Figure 3.2a). As in Year 1, a separation between shallow and deep water sites was observed during late spring and summer; however, the increase in temperature occurred in two distinct phases: a moderate increase from May to July and a large/steep one from mid-July to mid-August (~10°C and 15°C increase in the span of one month at deep and shallow sites, respectively). As in

Year 1, significant variations in temperature (on the order of a few degrees), at times periodic (e.g., August), was seen during this stratified season (Figure 3.2a). The yearly standard deviation of the temperature for the shallow sites was larger (5.0-5.4°C) than the one for the deep water sites (4.2-5.1°C) however, this difference was less than in Year 1 (Appendix C- Table C1).

Dissolved oxygen (DO) saturation during the winter of Year 2 appears to be lower than in Year 1 (between 90 to 95%) in general. As in Year 1 an increase in saturation coincided with the chlorophyll *a* increase during the spring bloom; DO saturation remains high and stable for the rest of the summer, decreasing only at the beginning of September (Figure 3.2b; statistics in Appendix C- Table C2).

Chlorophyll *a* levels were relatively low and similar among sites and depths until the beginning of February, when a peak was observed at Bulley's Cove shallow 2 (Figure 3.2c); this peak was possibly due to a small bloom happening under the ice that was still covering the bay. The spring bloom, however, started in April and lasted one month; a delay in the spring bloom was obvious compared to Year 1 (Figures 3.1c, 3.2c); also, the magnitude of the bloom appeared much smaller compared to Year 1, and presented only one peak, around mid-April (statistics in Appendix C- Table C3).

Offshore chlorophyll *a* measurements (Saint Anthony Basin) for the years 2012-2014 are reported in Appendix D (Pepin *et al*., 2015). A comparison with the data obtained in this study shows similarities in seasonal trends; however, the spring bloom peak appears earlier in the studied sites than offshore in both years of the study (Appendix D- Figure D1 a,b). A comparison of the phytoplankton seasonal cycle characteristics based on combined satellite chlorophyll *a* data measured off-shore (Zhai *et al.*, 2011; Pepin *et al.*,

2015) for the years 2013 and 2014 is presented in Appendix D (Figure D2). The comparison shows that the magnitude, duration, amplitude and concentration of the chlorophyll *a* bloom were all lower in 2014 (Pepin *et al.*, 2015), which supports the results obtained from the moored instruments during this study (Appendix D- Figure D2).

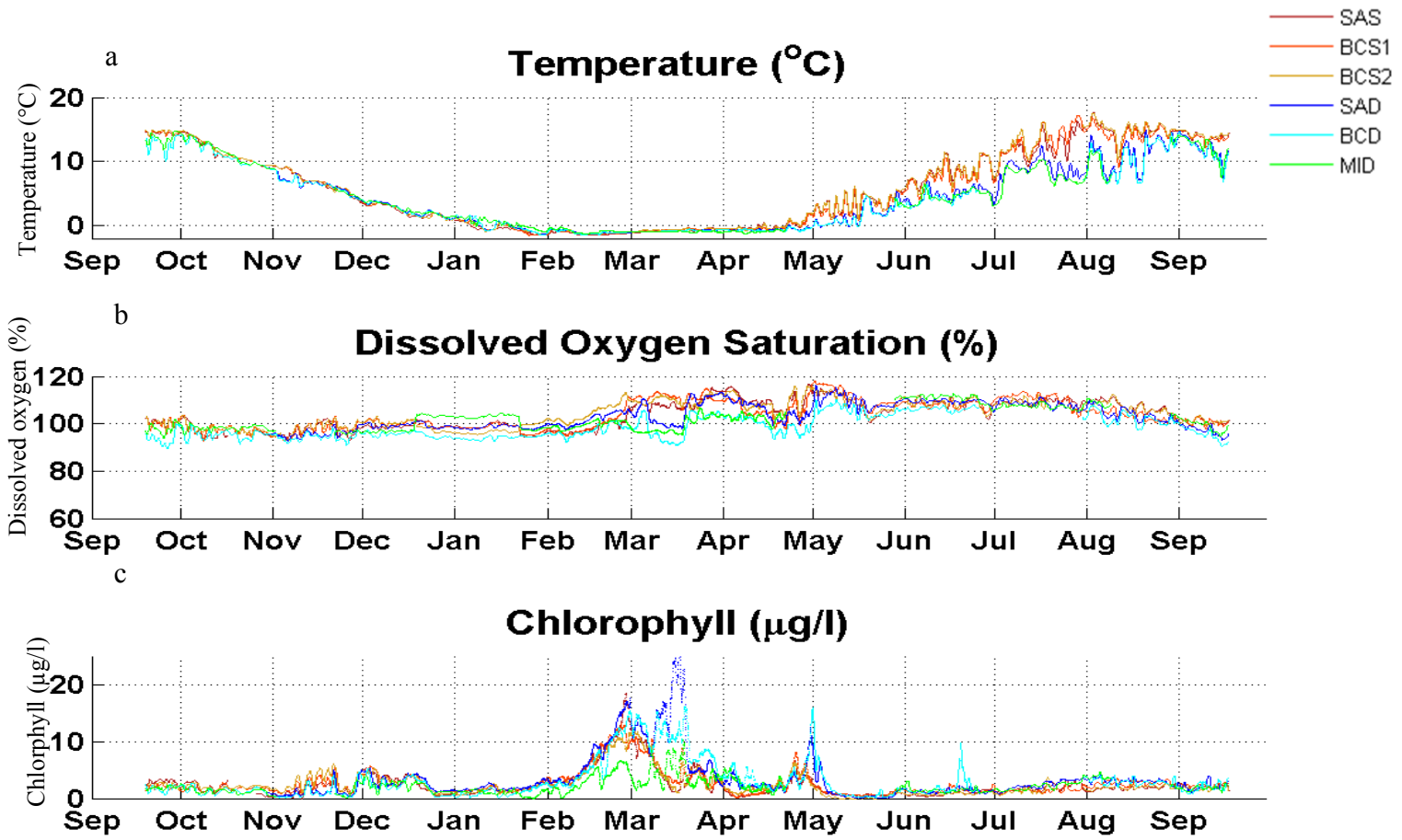

Figure 3.1: Moored sonde results-Year 1 for (a) temperature (°C), (b) dissolved oxygen (%) and (c) chlorophyll (µg/l). SAS= South Arm Shallow; BCS1= Bulley's Cove Shallow 1; BCS2= Bulley's Cove Shallow 2; SAD= South Arm Deep; BCD= Bulley's Cove Deep; MID= Mouse Island Deep.

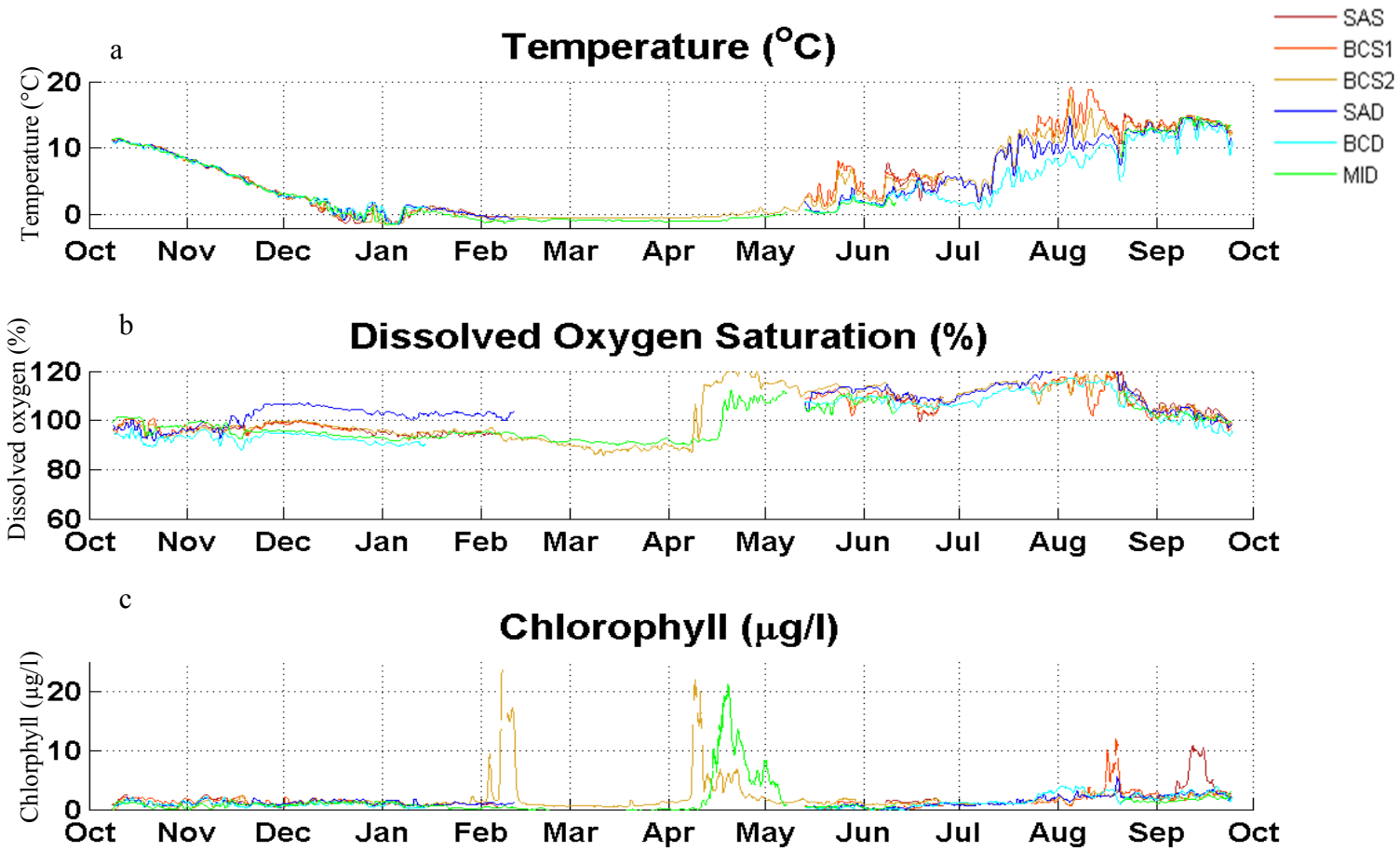

Figure 3.2: Moored sonde results-Year 2 for (a) temperature (°C), (b) dissolved oxygen (%) and (c) chlorophyll (µg/l). SAS= South Arm Shallow; BCS1= Bulley's Cove Shallow 1; BCS2= Bulley's Cove Shallow 2; SAD= South Arm Deep; BCD= Bulley's Cove Deep; MID= Mouse Island Deep.

3.3.2. CTD

3.3.2.1. Year 1

Monthly water profiles were started in January 2013. The profiles reveal an increase in water stratification starting in April (Figures 3.3-3.5). The temperature results show a strong seasonal cycle with values ranging from -1°C to 0°C in winter (January-February) to more than 15°C from late June to early October in the surface layer. The lowest temperatures were measured in late February (around -0.9°C) and the warmest in late July (up to 18°C near the surface). While the water column temperature appears homogeneous during fall and winter months (November, January and February), and very stratified in summer (approximately 8°C range from near surface to the 12 m depth), the extent of the thermocline could not be resolved fully due to the limitations of the profiles with depth. Overall, the shallow sites show notably warmer temperatures that were maintained for a longer period of time than the deeper sites. The thermocline buildup occurred between April and June, and while the timeline of the thermocline break-down cannot be resolved precisely from this data set alone, it might occur quite rapidly. The bottom of the thermocline was roughly located at the 10 m depth in April for South Arm Shallow, South Arm Deep and Bulley's Cove Deep (Figure 3.3a-b,e), while it was around the 5 m depth for Bulley's Cove Shallow 1 and 2 (Figure 3.3 c-d). In later spring-summer, the thermocline stabilizes at approximately 5-10 m for all the sites (Figure 3.3).

The salinity fields also present a seasonal cycle with saltier conditions in winter (January-February) and fresher conditions in summer to early fall (July to August). Fresher conditions seem to be also present in spring (late April and May). The maximum

salinity values (31.6-31.7) were measured in late February at the Bulley's Cove Shallow 2 site while the minimum (around 28.5-29) was found in late August, at the South Arm Shallow site. The salinity field is the most homogenous in winter (January-February) and presents the strongest stratification in late April and late July (about 2 units range within the upper 10 m of the water column). The base of the halocline was located at 5 m depth in April and remained at a similar depth during the spring-summer seasons (Figure 3.4).

Comparing Figure 3.4 and 3.5, similarities in profiles can be noted; the density of the water appears mostly dominated by the salinity in the system analysed here. Thus, as for the halocline, the pycnocline appears to form in the first 5 m of the water column.

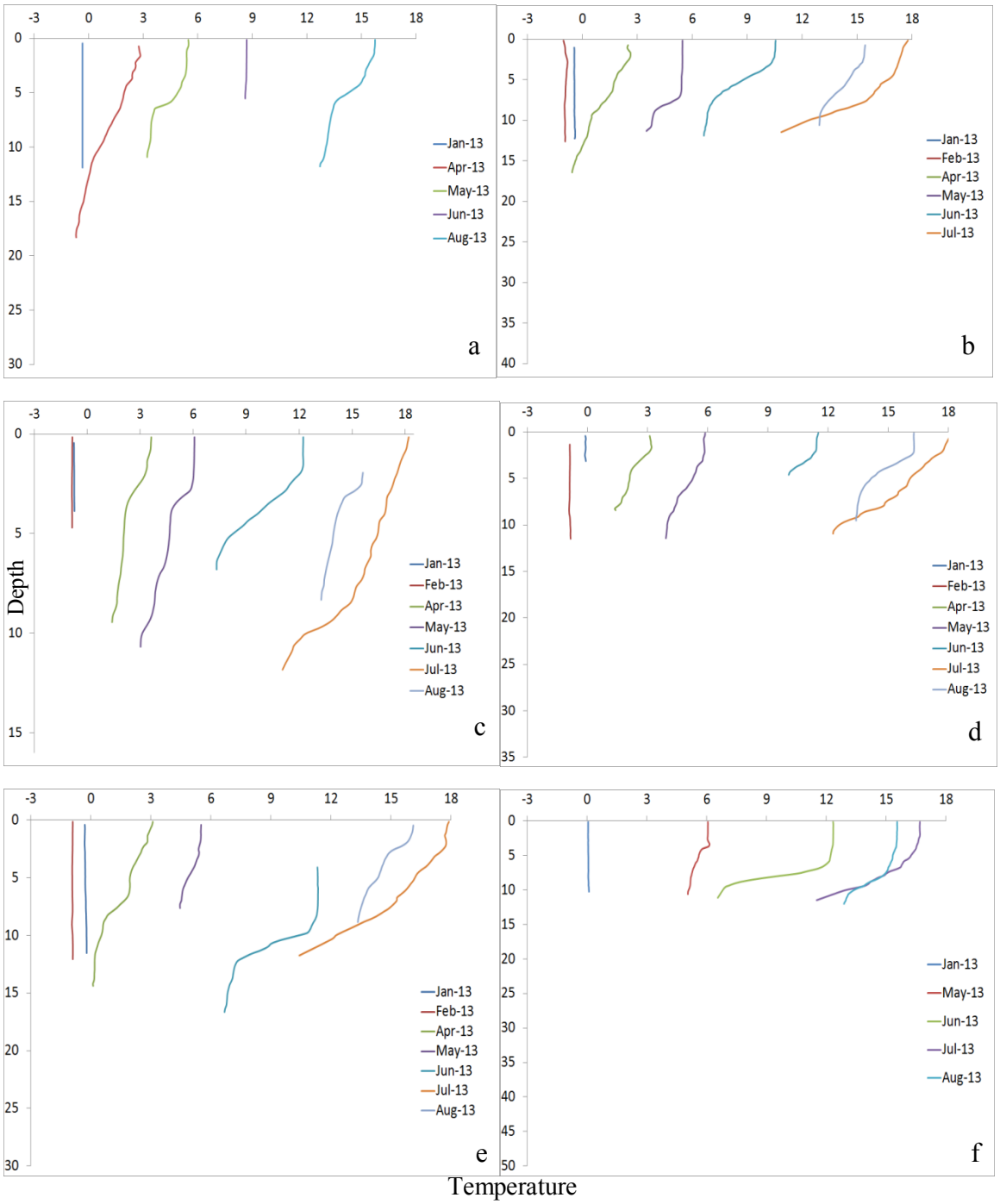

Figure 3.3: Year 1 temperature (°C) results of monthly CTD casts for (a) South Arm Shallow, (b) South Arm Deep, (c) Bulley's Cove Shallow 1, (d) Bulley's Cove Shallow 2, (e) Bulley's Cove Deep and (f) Mouse Island Deep; x-axis represents temperature (°C), y-axis represents depth (m) of the site.

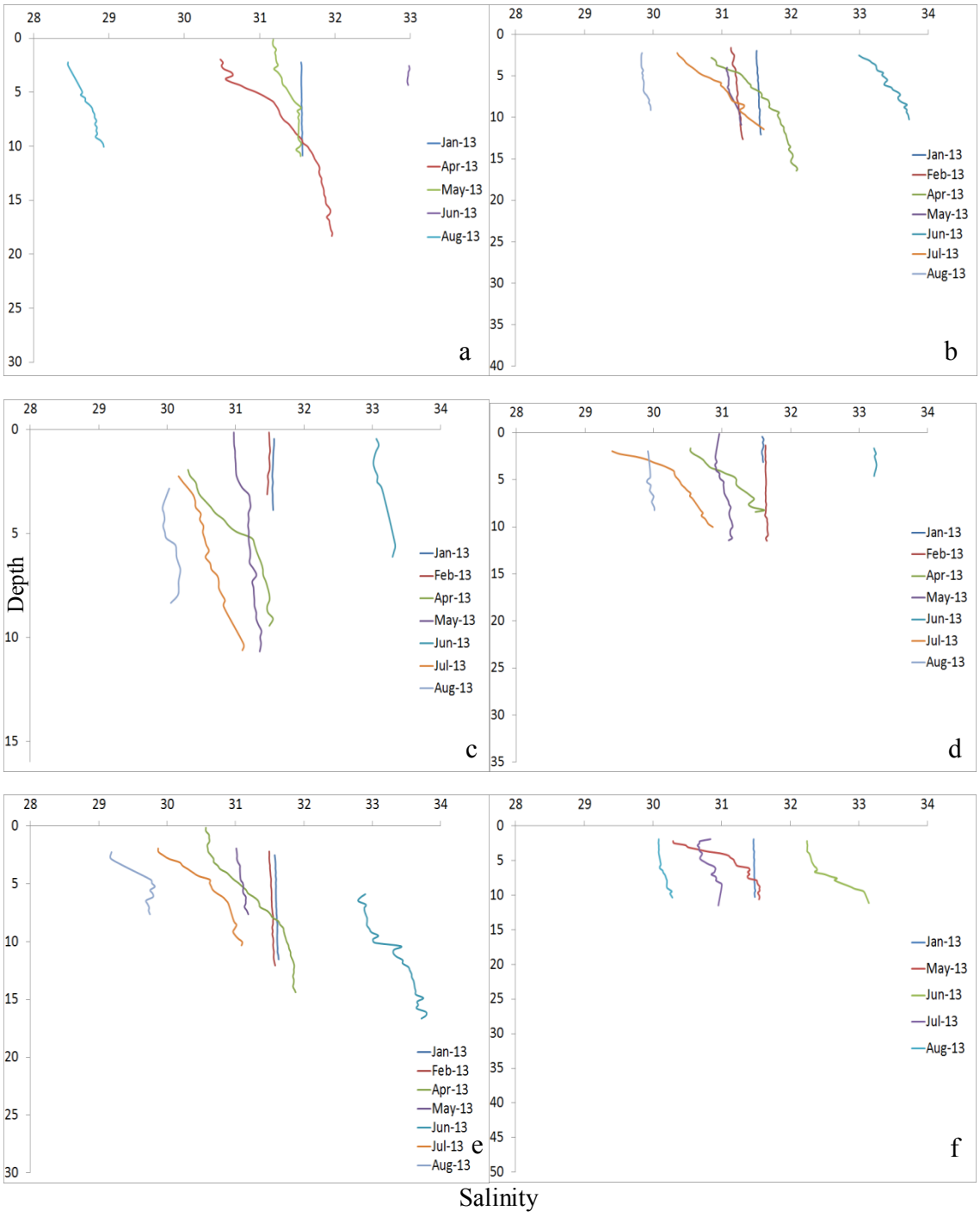

Figure 3.4: Year 1 salinity results of monthly CTD casts for (a) South Arm Shallow, (b) South Arm Deep, (c) Bulley's Cove Shallow 1, (d) Bulley's Cove Shallow 2, (e) Bulley's Cove Deep and (f) Mouse Island Deep; x-axis represents salinity, y-axis represents depth (m) of the site.

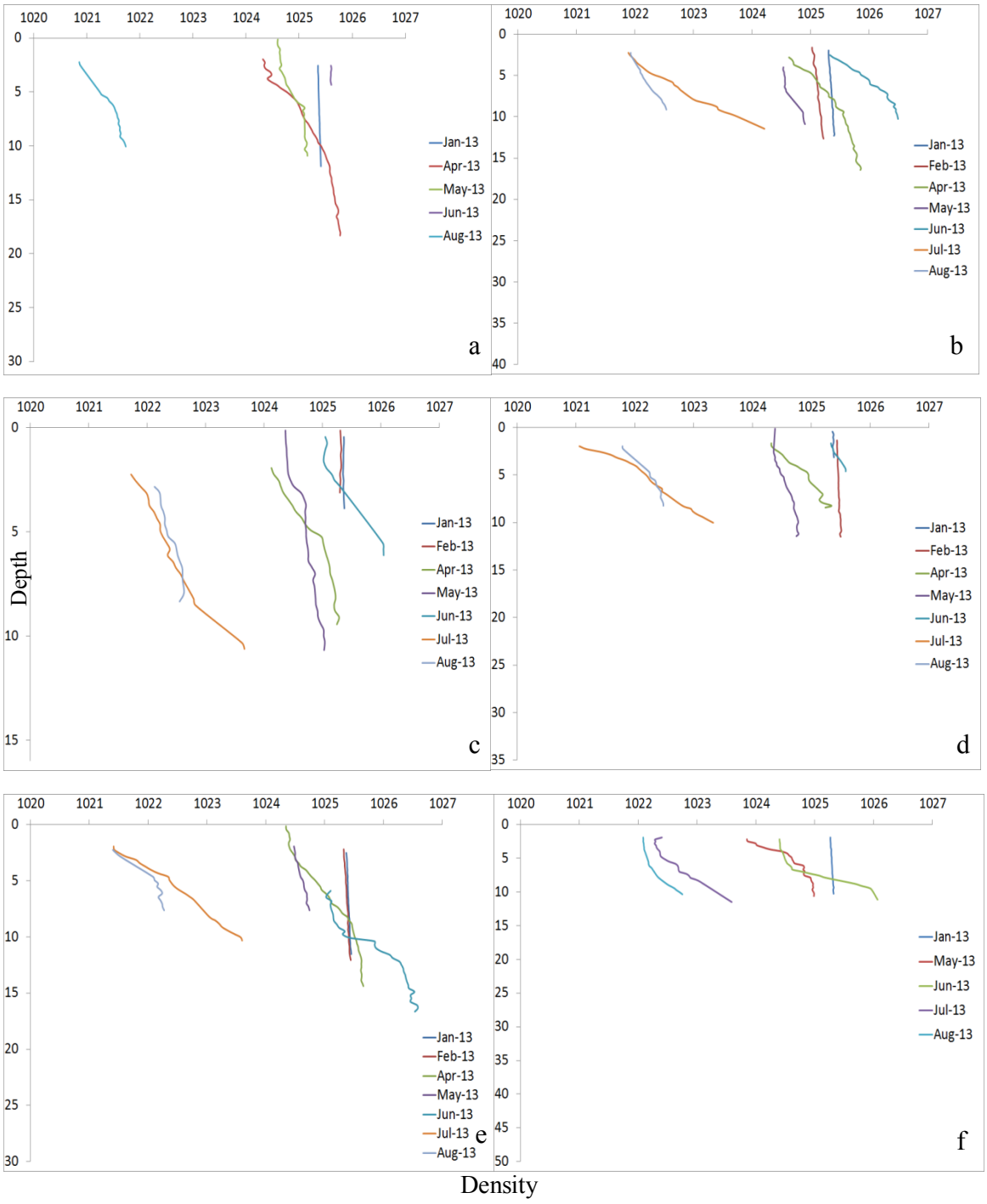

Figure 3.5: Year 1 density (kg m^{-3}) results of monthly CTD casts for (a) South Arm Shallow, (b) South Arm Deep, (c) Bulley's Cove Shallow 1, (d) Bulley's Cove Shallow 2, (e) Bulley's Cove Deep and (f) Mouse Island Deep; x-axis represents density (kg m^{-3}), y-axis represents depth (m) of the site.

3.3.2.2. Year 2

No CTD profiles were taken from December 2013 to May 2014 due to ice conditions in the bay. The temperature profiles of May 2014 were similar to those of April 2013, showing a delay of a month in the warming of the water between Year 1 and Year 2 (Figure 3.6). In June 2014 the profiles showed sub-surface temperature minima which are typical of high latitude (polar and sub-polar) regions. This profile was the result of a winter mixed layer that is "capped" with warmer water later in the season (June). Below that cold layer, a warmer layer was present; this likely resulted from advection (horizontal transport) of waters from a warmer location (Talley *et al*., 2011), which cannot, however, be identified in this case.

The water column was almost evenly stratified during the summer from the near-surface to a few meters above the bottom; a thick (~20 m depth) surface mixed layer appeared in September (Figure 3.6). In June 2014 low salinity (22-23) was observed at all sites (Figure 3.7). The halocline presented a complex profile; a two-step profile was observed in May, while evenly stratified water in the first 10 m was present in summer (June- August), probably resulting from offshore advection. In September a thick mixed-layer was observable; finally, a shallower mixed layer was present in October and November (Figure 3.7). As in Year 1, the water density profiles in Year 2 followed very closely the salinity profiles (Figure 3.7-3.8). Often during the course of the year the deep water mussel sites were located at the pycnocline. The density was at the highest point during the month of May at all the sites (Figure 3.8).

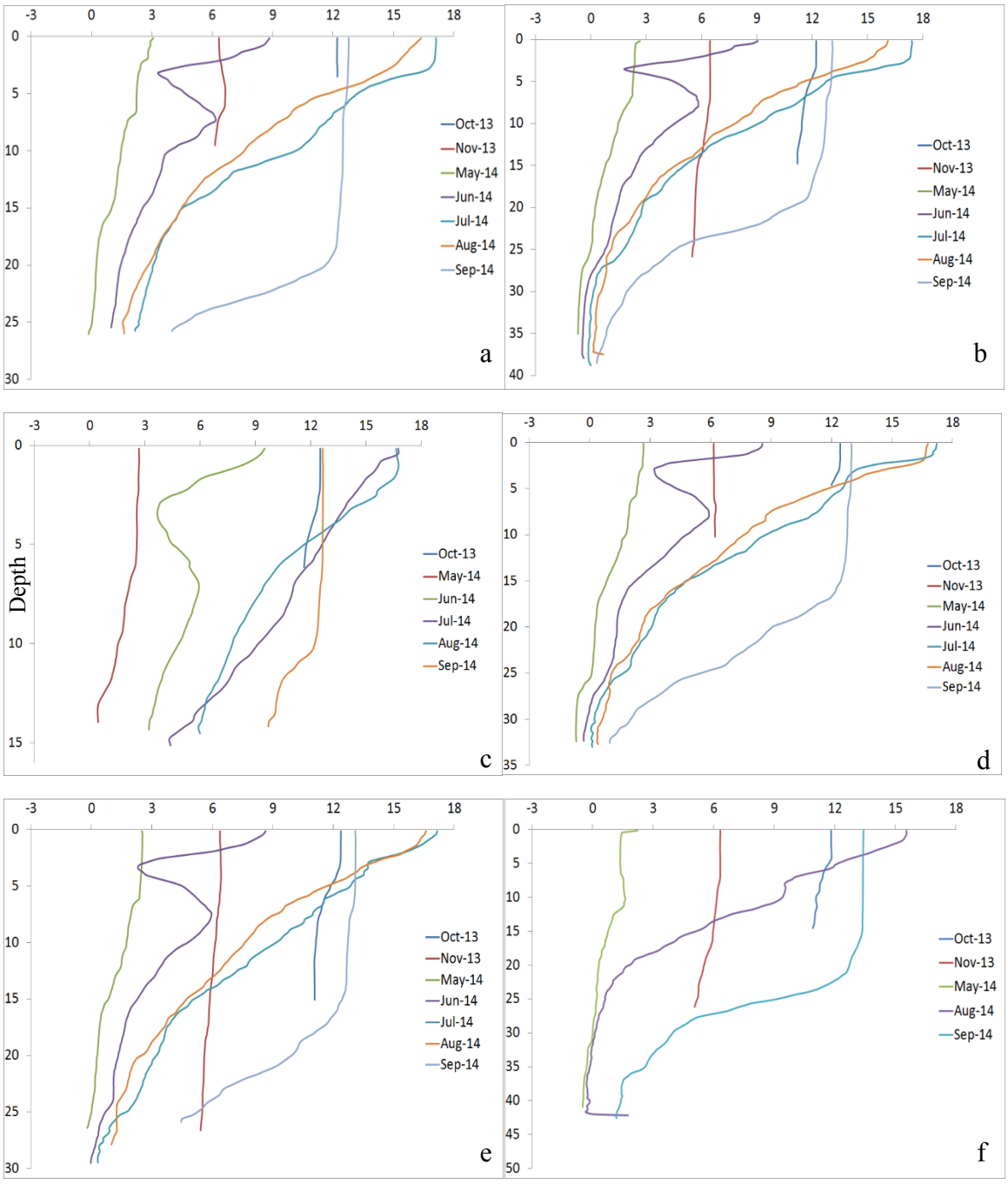

Figure 3.6: Year 2 temperature (°C) results of monthly CTD casts for (a) South Arm Shallow, (b) South Arm Deep, (c) Bulley's Cove Shallow 1, (d) Bulley's Cove Shallow 2, (e) Bulley's Cove Deep and (f) Mouse Island Deep; x-axis represents temperature (°C), y-axis represents depth (m) of the site.

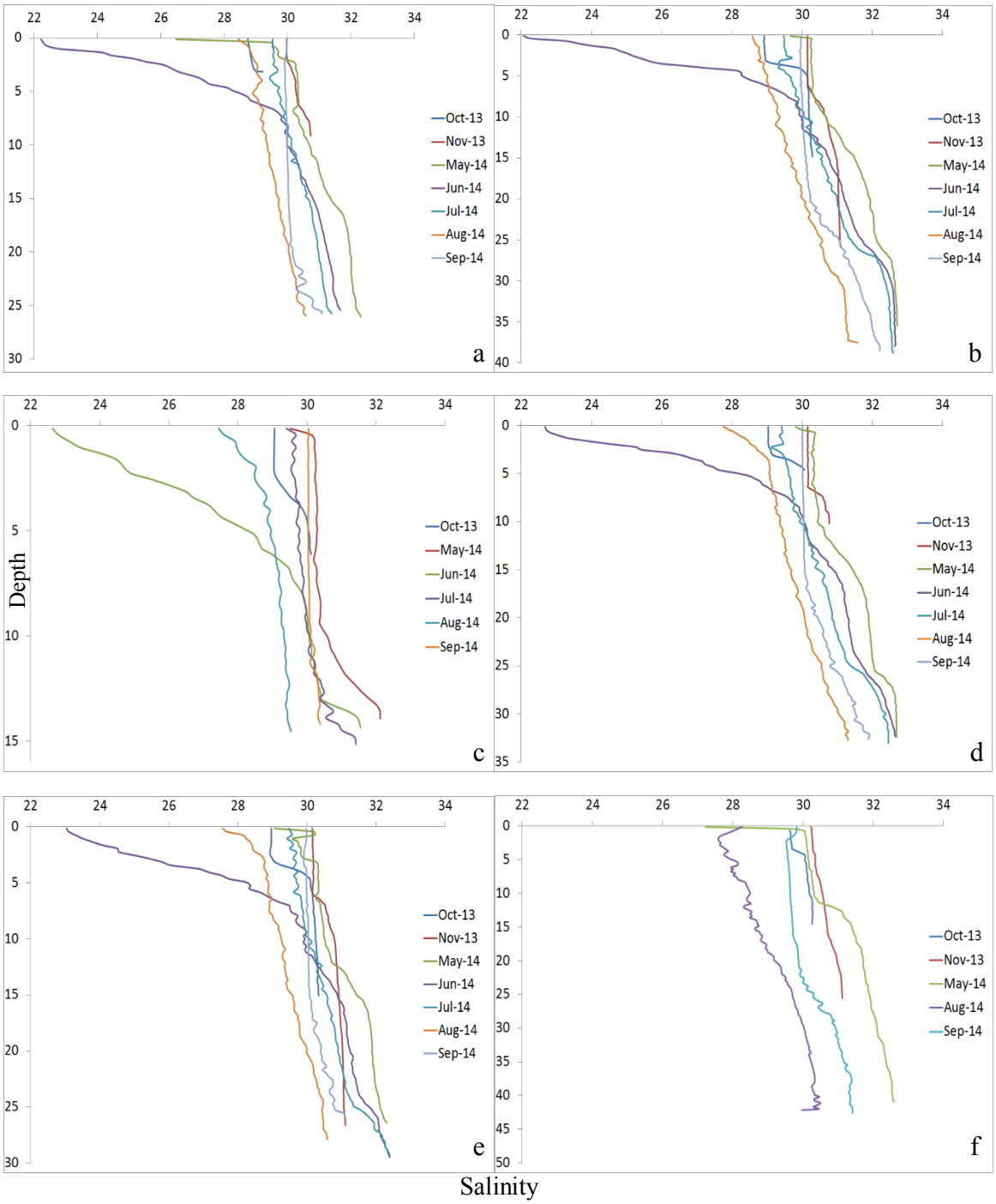

Figure 3.7: Year 2 salinity results of monthly CTD casts for (a) South Arm Shallow, (b) South Arm Deep, (c) Bulley's Cove Shallow 1, (d) Bulley's Cove Shallow 2, (e) Bulley's Cove Deep and (f) Mouse Island Deep; x-axis represents salinity, y-axis represents depth (m) of the site.

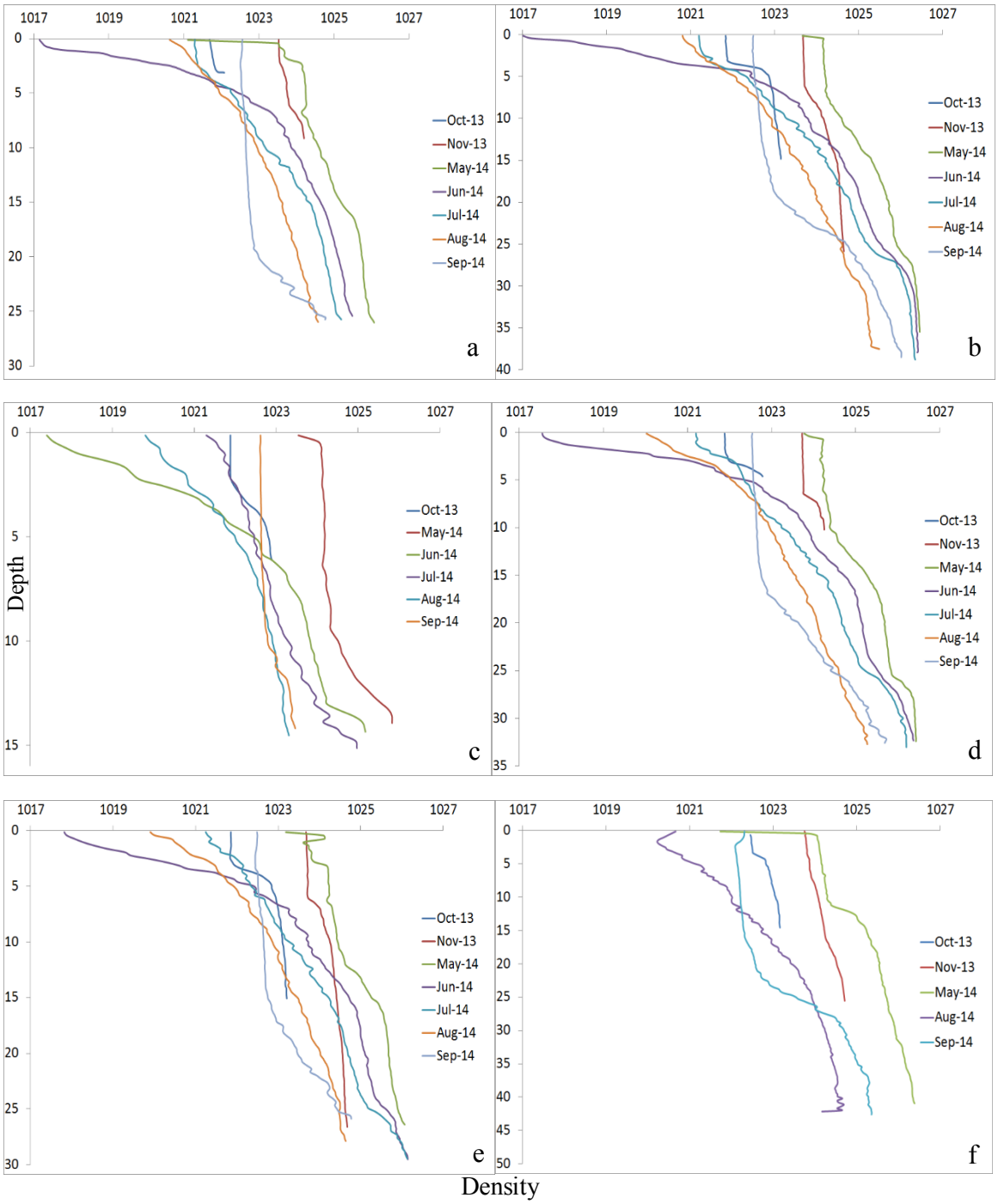

Figure 3.8: Year 2 density (kg m^{-3}) results of monthly CTD casts for (a) South Arm Shallow, (b) South Arm Deep, (c) Bulley's Cove Shallow 1, (d) Bulley's Cove Shallow 2, (e) Bulley's Cove Deep and (f) Mouse Island Deep; x-axis represents density (kg m^{-3}), y-axis represents depth (m) of the site.

3.3.3. ADCP

3.3.3.1. Mouse Island

The ADCP results showed sea-level variation (depth) dominated by semi-diurnal tides (Figure 3.9 a-c), with a tidal range in the order of 1 m. During the period between December 2012 and May 2013, near-bottom temperatures gradually decreased from 2°C to -1°C to a minimum of about –1.4°C at the end of April (Figure 3.9b). During the same period temperature showed some small periodic variations and some sharp drops and rises in the order of 0.5°C in a few hours or days (Figure 3.9b). During the period between August and December 2014, near-bottom temperatures gradually increased from <0°C at the end August to 3°C in early December, probably due to vertical mixing (Figure 3.9d). Measurements also showed noticeable (~2-5°C), periodic (few hours to a few weeks) variations from October to mid-November. Although of a much smaller scale, oscillations were also visible from the end of August to end of September (Figure 3.9d).

Between December 2012 and May 2013, the tidal contribution to currents appeared weak and only visible at times when currents were small and strong events seemed to be related to periods of significant winds (Figure 3.10a; more detailed figures in Appendix E- Figure E1). Layering processes (i.e., varying current direction within the water column at a given time) often seemed to take place during those events (Figure 3.10b). The current direction was represented by red and blue stripes; if only the tide effect was observable, straight and regular (twice a day) stripes, from surface to bottom, would be seen; while layering was shown as blue over red stripes or *vice versa* (Figure 3.10b). Interference in the ADCP record was observed between February and April (represented

by a white color band in Figure 3.10 a-c), caused by the presence of the mussel or mooring lines. The data within the obstruction were excluded from the analysis.

Diurnal zooplankton migration was visible in January and March 2013, as previously reported in Newfoundland by Record and de Young (2006; Figure 3.10c; more detailed figures in Appendix E- Figure E1). During August-December 2014, the current speeds appeared generally low (0.1 $m \cdot s^{-1}$ or less) with sporadic stronger events (0.2 $m \cdot s^{-1}$ or more; Figure 3.10d). These events appeared, however, more frequent in the top part of the water column (0-30 m depth) than during the period between December 2012 and May 2013. During the same period (August-December 2014) current directions showed some low and high frequency north-south flows (Figure 3.10e). High-frequency observations appeared to be related to the tides (semi-diurnal signal) and most visible in the lower part of the water column (about 30 m) from the end of August to early October 2014 (Figure 3.10e). The low-frequency observations appeared to occur over periods of a few days and dominated the whole water column from early October to the end of the record (Figure 3.10e). It should also be noted that those lower frequency events (or flow/circulation) did not affect the whole water column all at once; rather, the direction of the flow seemed to change progressively in time with depth from north to south or *vice versa*, indicating an effect of internal friction in the water column (e.g. 16-25 October 2014; more detailed figures in Appendix E- Figure E1). In the back-scatter data it was possible to observe a clear diurnal migration of zooplankton which occurs at varying depth, rising and lowering over time (Figure 3.10f). This migration was particularly visible from the beginning of the record (end of August) to the end of September. The vertical migration appeared to

be, in some instances, not only diurnal but also periodic with a period of the order of about a week (Figure 3.10f; more detailed figures in Appendix E- Figure E1).

Current speeds were small, about 0.025 - 0.05 $m \cdot s^{-1}$ (2.5 - 5 $cm \cdot s^{-1}$) on average (Figure 3.11 a-b); from December 2012 to May 2013 the flow most often was directed northward in the top 12.5 m and southward below (Figure 3.11a); while between August and December 2014 the most frequent direction is to the south over the whole water column, except near the bottom (below 50 m), where it veers towards the north-east, likely due to the effect of the bathymetry (Figure 3.11b). From August to December 2014, the residual currents were stronger than during the previous deployment, particularly between 10-30 m depth (Figure 3.11b).

For the period between December 2012 and May 2013, the strongest currents were of the order of 0.2-0.3 $m \cdot s^{-1}$ range except between 20-30 m where speeds slightly higher than 0.5 m/s were reached (Figure 3.11a). Similarly, from August to December 2014 strong currents in the order of 0.5 $m \cdot s^{-1}$ were observed between 20-30 m (Figure 3.11b). The strongest currents were generally towards the south except in near-surface and near-bottom parts of the water column where they were generally directed toward the northern quadrant (Figure 3.11b). Therefore, current direction appeared to change seasonally and with depth; between December 2012 and May 2013, at 5-6 m depth the currents were oriented north-south (along-channel) with prevalence towards the north, and therefore the outflow was toward the mouth of the inlet (Figure 3.12a); while at 15-16 m the prevalence was towards the south, with inflow toward the head of the inlet (Figure 3.12b). Between August and December 2014, at 5-6 m depth the currents were still oriented north-south (along-channel); however, with prevalence towards the south and with more

frequent stronger currents (Figure 3.12c). At 15-16 m depth, the currents were still oriented north-south, with a large/significant, prevalence towards the south (Figure 3.12d), with a larger proportion of currents higher than 0.1 $m \cdot s^{-1}$ (Figure 3.12d).

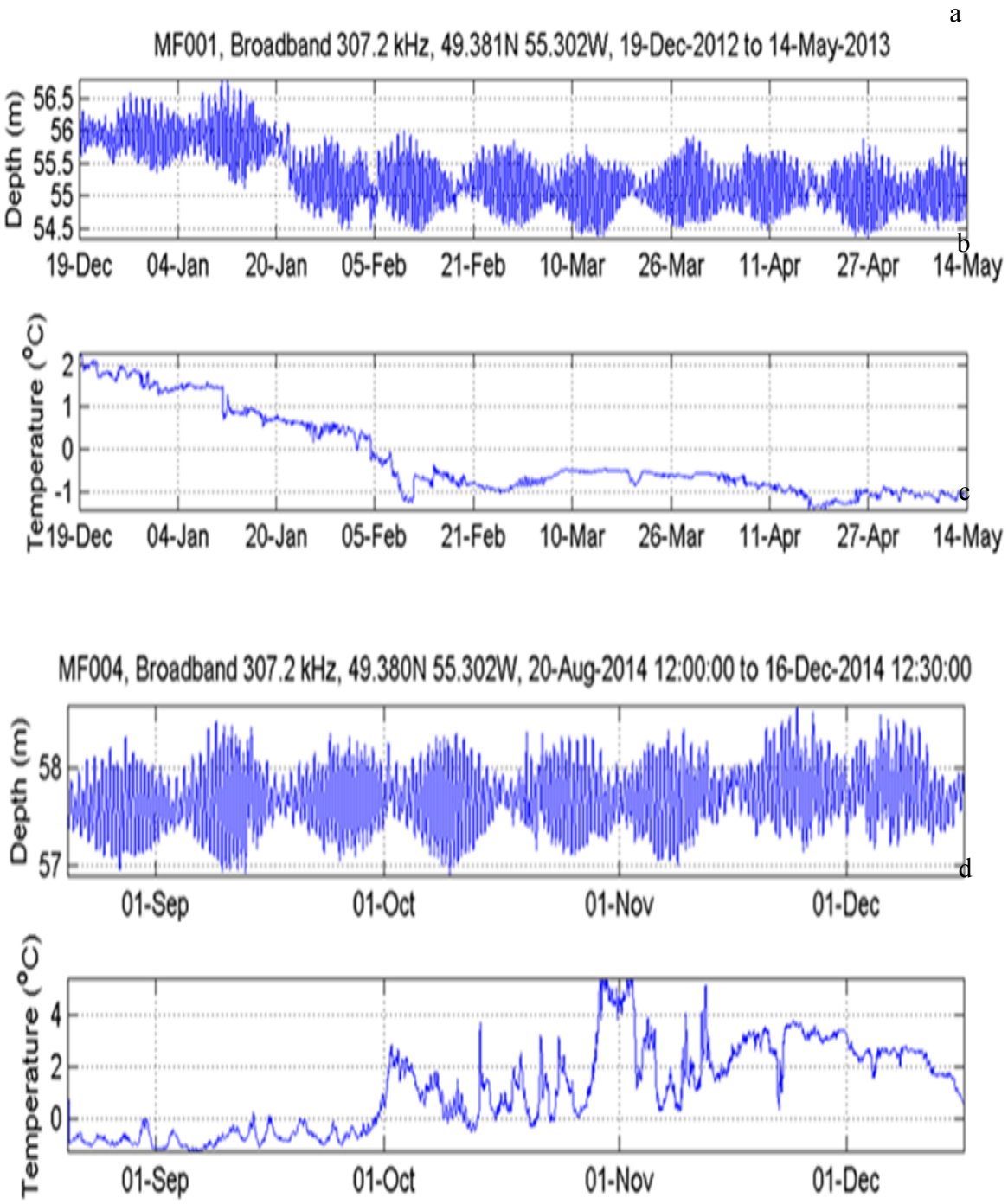

Figure 3.9: Mouse Island ADCP results for (a) depth (m) and (b) water temperature at instrument depth (~2 m from bottom; °C) during the periods of December 2012 to May 2013; results for (c) depth (m) and (d) water temperature (°C) during the period of August to December 2014. Drop in depth in (a) is due to unplanned movement of the instrument.

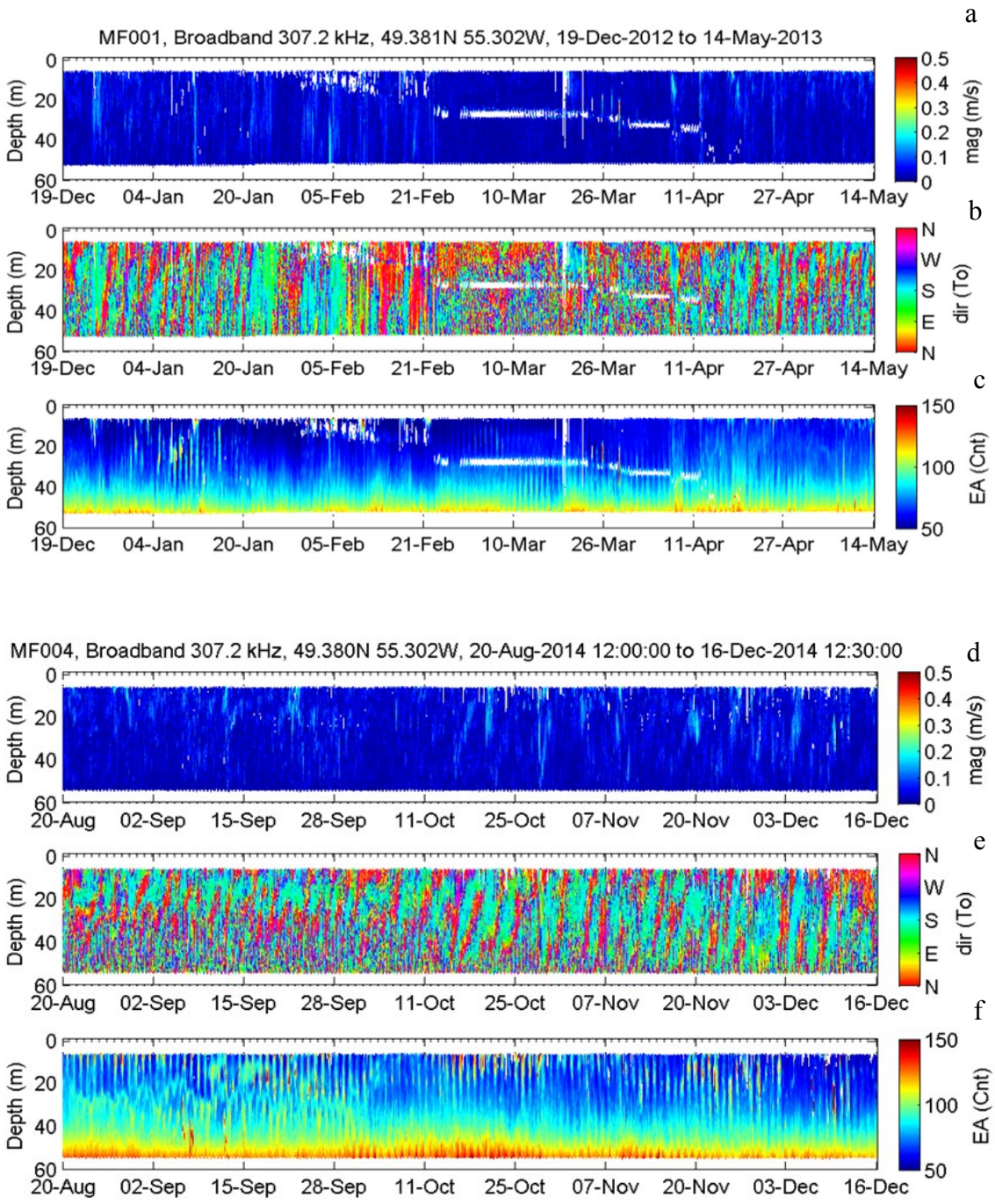

Figure 3.10: Mouse Island ADCP results for (a) current speed (mag, m s^{-1}), (b) direction and (c) back-scatter strength (EA = Echo Amplitude intensity, cnt = units based on voltage) for the period of December 2012 to May 2013; results for (d) current speed (mag, m s^{-1}), (e) direction and (f) back scatter strength for the period of August to December 2014. White color bands on the plot correspond to removed values after quality control of the ADCP data.

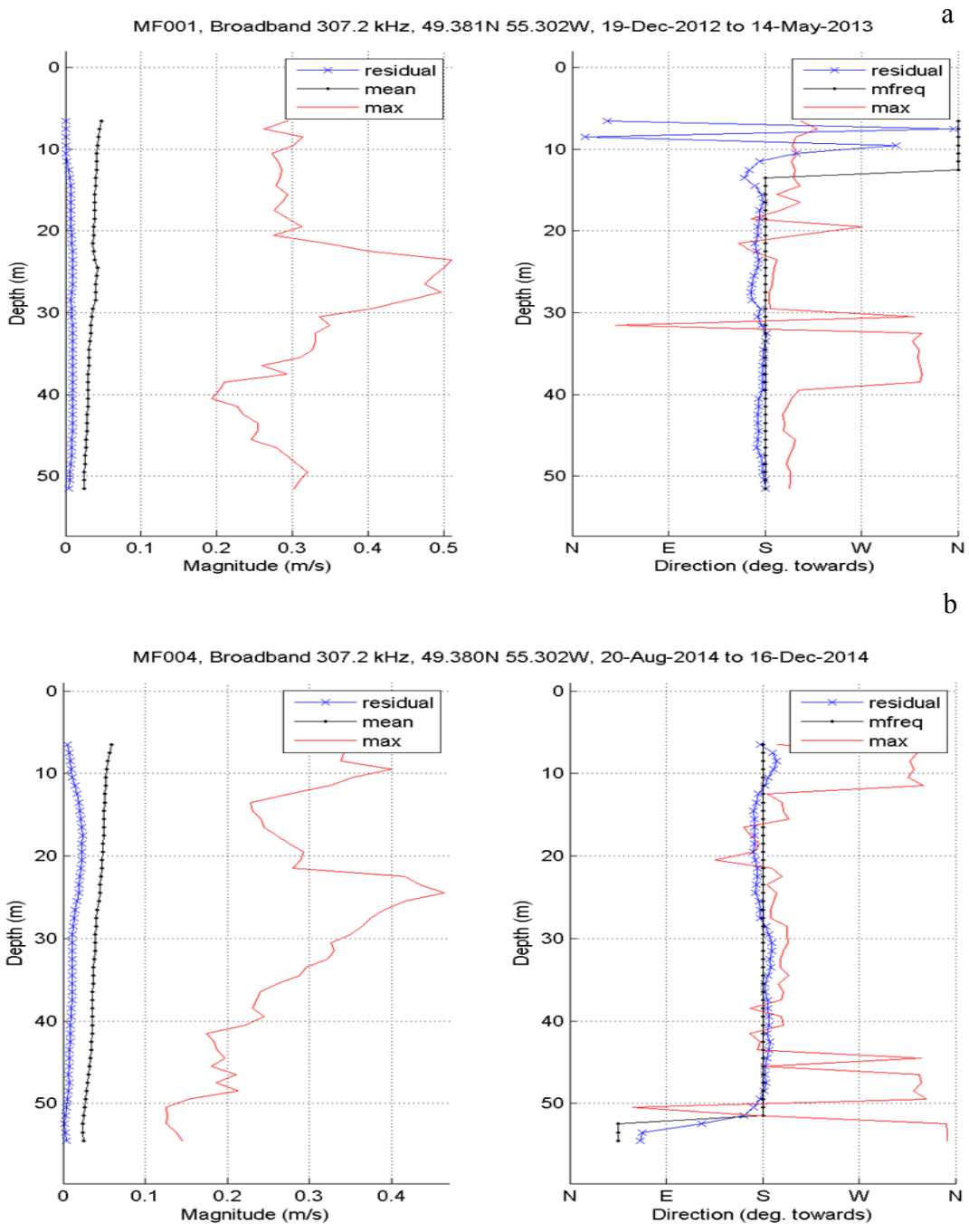

Figure 3.11: Mouse Island ADCP results for current magnitude ($m \cdot s^{-1}$) and direction for the periods of (a) December 2012 to May 2013 and (b) August to December 2014. Residual = vector averaged current (i.e. mean flow); mean = averaged current magnitude/speed; mfreq = most frequent current direction (based on 8 sectors); max = maximum current magnitude/speed observed and its associated direction.

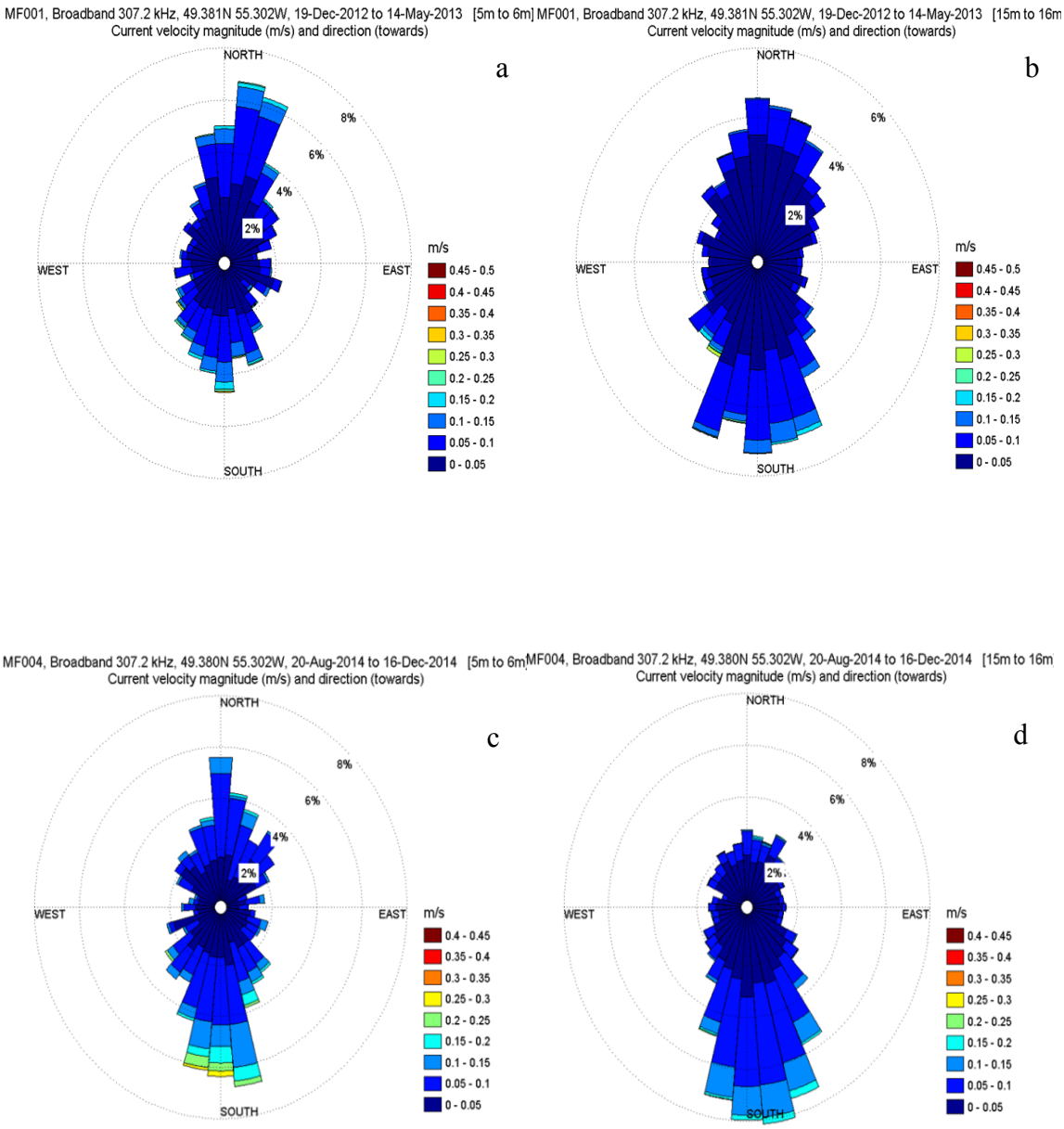

Figure 3.12: Mouse Island ADCP results for current speed ($m \cdot s^{-1}$) and direction at (a) 5-6 m depth and (b) 15-16 m depth for the period of December 2012 to May 2013 and (c) 5-6 m depth and (d) 15-16 m depth for the period of August to December 2014.

3.3.3.2. South Arm

As at the Mouse Island site, sea-level variation at the South Arm site was largely dominated by semi-diurnal tides with a tidal range around 1 m (Figure 3.13 a,c). Between May and November 2013 the near-bottom temperature gradually increased from <0°C in mid-May to around 6°C in mid-November (Figure 3.13b). The temperature showed some high-frequency variations all along the record that appeared to be related to the tides (i.e., semi-diurnal signal; Figure 3.13b). Moreover, some warming and cooling events having a more lasting effect were also visible (e.g., July, mid-October and early November; Figure 3.13b); these events may be related to water exchange between the South Arm inner basin and the main South Arm channel (cold water flowing above the sill). Between November 2013 and April 2014 the near-bottom temperature gradually decreased from around 5°C in mid-November to <0°C in early April, rising up again slightly towards the end of April (Figure 3.13d); however, during this period there was no large variation. Again, as in the previous period, strong cooling events (~1-2°C) are visible and may be related to water exchange between the South Arm Deep basin and the main South Arm channel (Figure 3.13d).

Current speeds appeared generally low (0.1 $m \cdot s^{-1}$ or less) with few strong events (0.2 $m \cdot s^{-1}$ or more; Figure 3.14 a,d). Currents were relatively stronger between mid-November and the end of December 2013, while they appeared very calm from February to April 2014 (Figure 3.14d).

From May 2013 to the end of January 2014 current directions showed a complex pattern of periodic north quadrant–south quadrant and layered flows (Figure 3.14 b,e;

more detailed figures in Appendix E- Figure E2). From February to April 2014 current directions were very erratic with some indication of northward flow in the upper half of the water column and southward flow in the lower half (Figure 3.14e; more detailed figures in Appendix E- Figure E2). Some diurnal migration patterns were also visible, particularly from the end of August (Figure 3.14c). During May and June 2013 important surface back-scatter was noted (Figure 3.14c). A remarkable diurnal pattern was visible in the November to April back-scatter record (Figure 3.14f; more detailed figures in Appendix E- Figure E2) with scatters staying at a specific depth; the depth appeared to be constant during February 2014 (Figure 3.14f).

The mean current speed between May and November 2013 was ≤ 0.05 $m \cdot s^{-1}$; generally decreasing with depth but slightly increasing between the 15-20 m depths (Figure 3.15a); the most frequent direction was toward the south to south-west near the surface. The maximum speed observed was around 0.2-0.25 $m \cdot s^{-1}$ throughout the whole water column (Figure 3.15a) with a general direction towards the south or east at some depth (e.g., between 5-10 m; Figure 3.15a). In the same period, residual speed was around 1 $cm \cdot s^{-1}$ or less, directed towards the south (Figure 3.15a).

Between November 2013 and April 2014, the mean speed was around 0.025 $m \cdot s^{-1}$, decreasing with depth, and most frequently flowing toward the northeast near the surface (down to about 15 m) and toward the southwest below (Figure 3.15b). The maximum speeds observed were around 0.2 $m \cdot s^{-1}$ throughout the whole water column with a peak/acceleration near the surface to ~0.3 $m \cdot s^{-1}$ (Figure 3.15b); direction was generally towards the north quadrant from 5 to 7 m, and directed to the southwest below this depth. During the same period very low residual speed (<1 $cm \cdot s^{-1}$) was observed, directed

towards the north near-surface and progressively veering towards the east, south, and southwest with depth (Figure 3.15b).

Current direction at the 5-6 m depth appeared to be oriented southwest-northeast, roughly oriented with the bathymetry (Figure 3.16 a,c); the prevalence was towards the southwest between May and November 2013 (net flow towards the head of the bay) and towards the northeast between November 2013 and April 2014 (net flow towards the mouth of the bay; Figure 3.16 a,c). At 15-16 m depth the current had a broader directional distribution than at 5-6 m depth during the period between May and November 2013; the general orientation was still south-southwest-north-northeast, with the stronger currents generally towards the south (Figure 3.16b). Between November 2013 and April 2014 the current had a south-southwest-northeast general orientation and it was slower than in the upper 5 m (Figure 3.16d). A clear seasonality pattern was observed, with southwest current direction prevailing between May and November, while northeast direction prevailed between November and April.

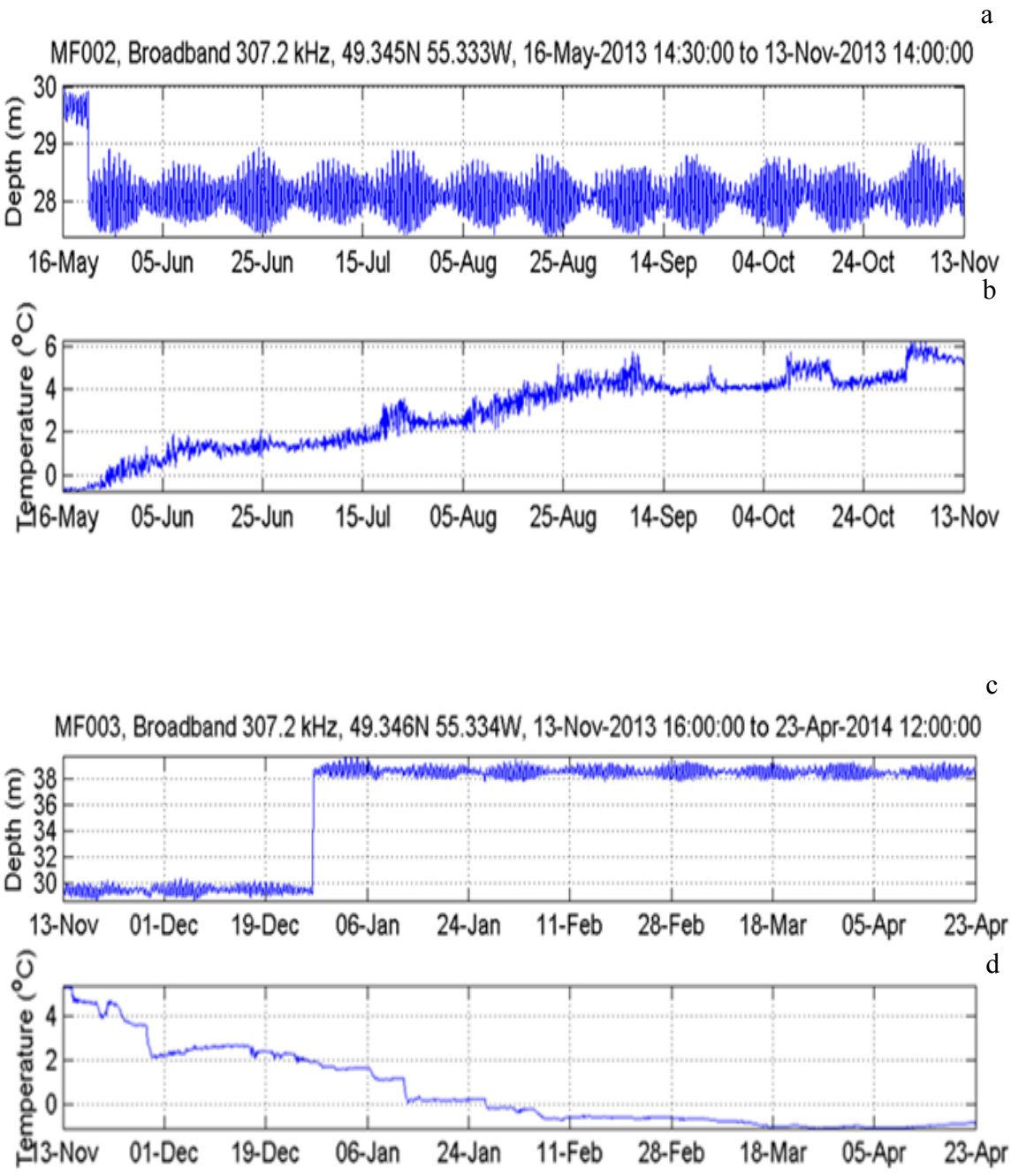

Figure 3.13: South Arm ADCP results for (a) depth (m) and (b) water temperature at instrument depth (~2 m from bottom; °C) during the periods of May to November 2013; results for (c) depth (m) and (d) water temperature (°C) during the period of November 2013 to April 2014. Drop in depth in (c) is due to unplanned movement of the instrument.

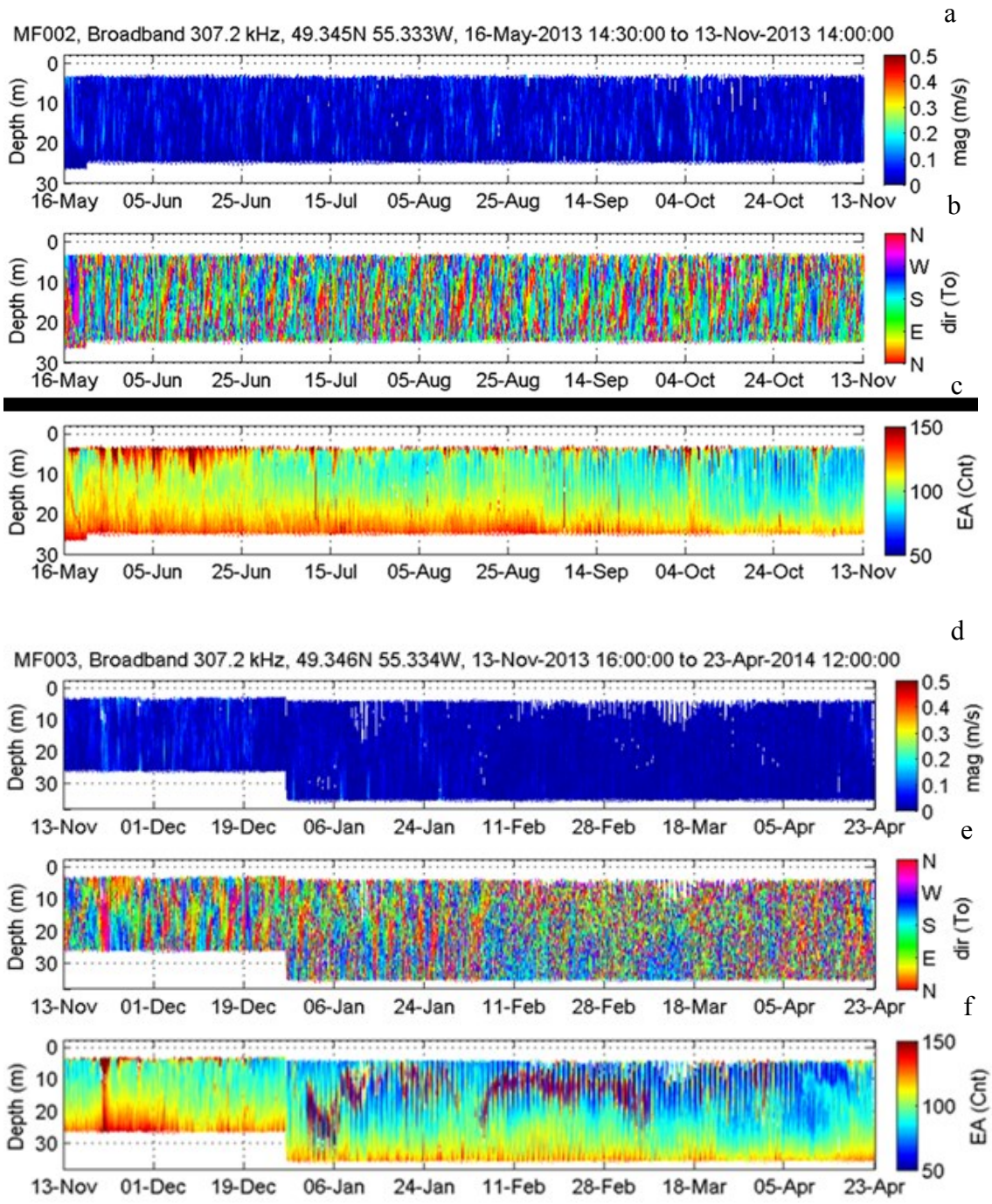

Figure 3.14: South Arm ADCP results for (a) current speed (mag, m s^{-1}), (b) direction and (c) back-scatter strength (EA = Echo Amplitude intensity, cnt = units based on voltage) for the period of May to November 2013; results for (d) current speed (m s^{-1}), (e) direction and (f) back scatter strength for the period of November 2013 to April 2014. White color bands on the plot correspond to removed values after quality control of the ADCP data.

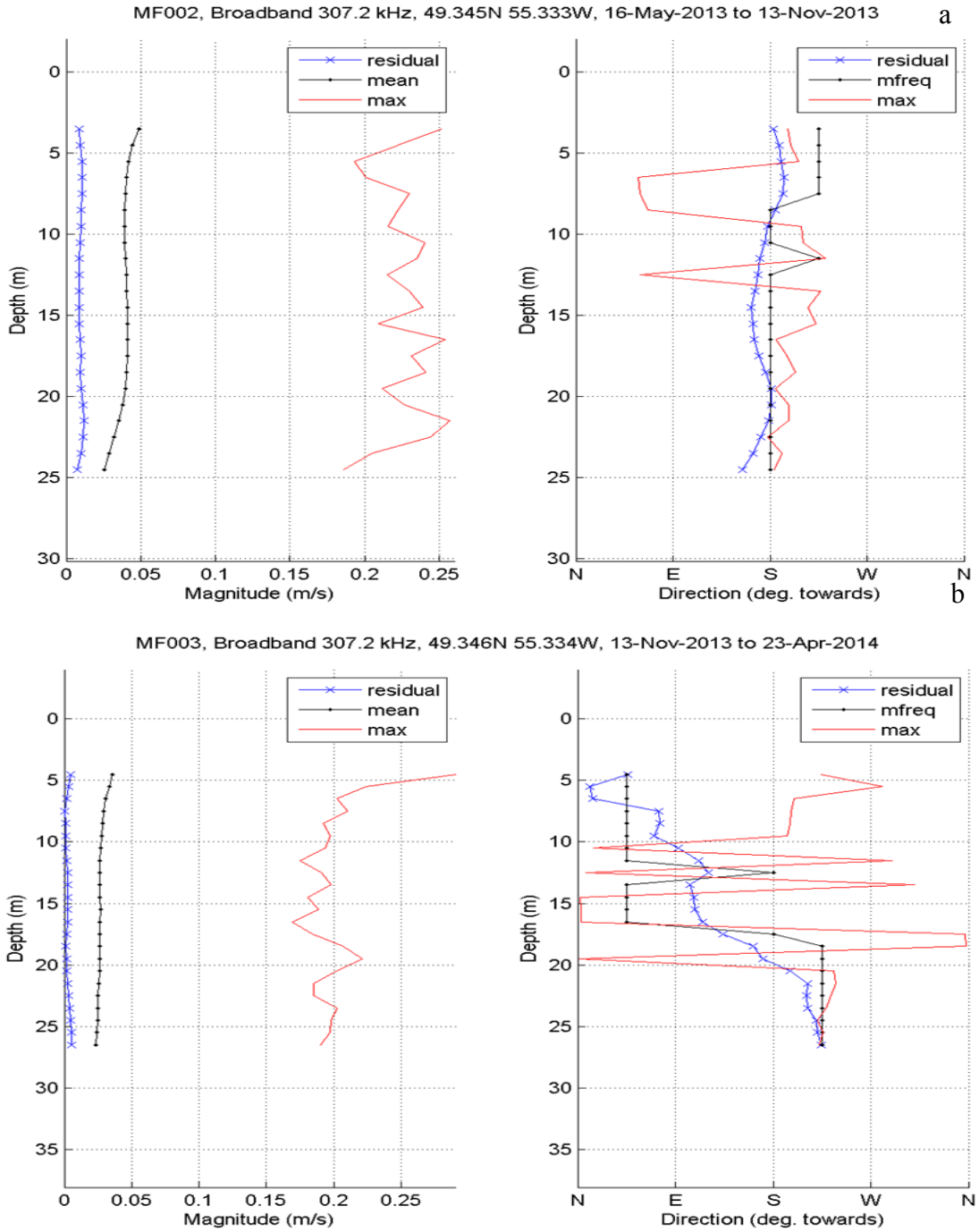

Figure 3.15: South Arm ADCP results for current magnitude (m s^{-1}) and direction for the periods of (a) May to November 2013 and (b) November 2013 to April 2014. Residual = vector averaged currents (i.e. mean flow); mean = averaged current magnitude/speed; mfreq = most frequent current direction (based on 8 sectors); max = maximum current magnitude/speed observed and its associated direction.

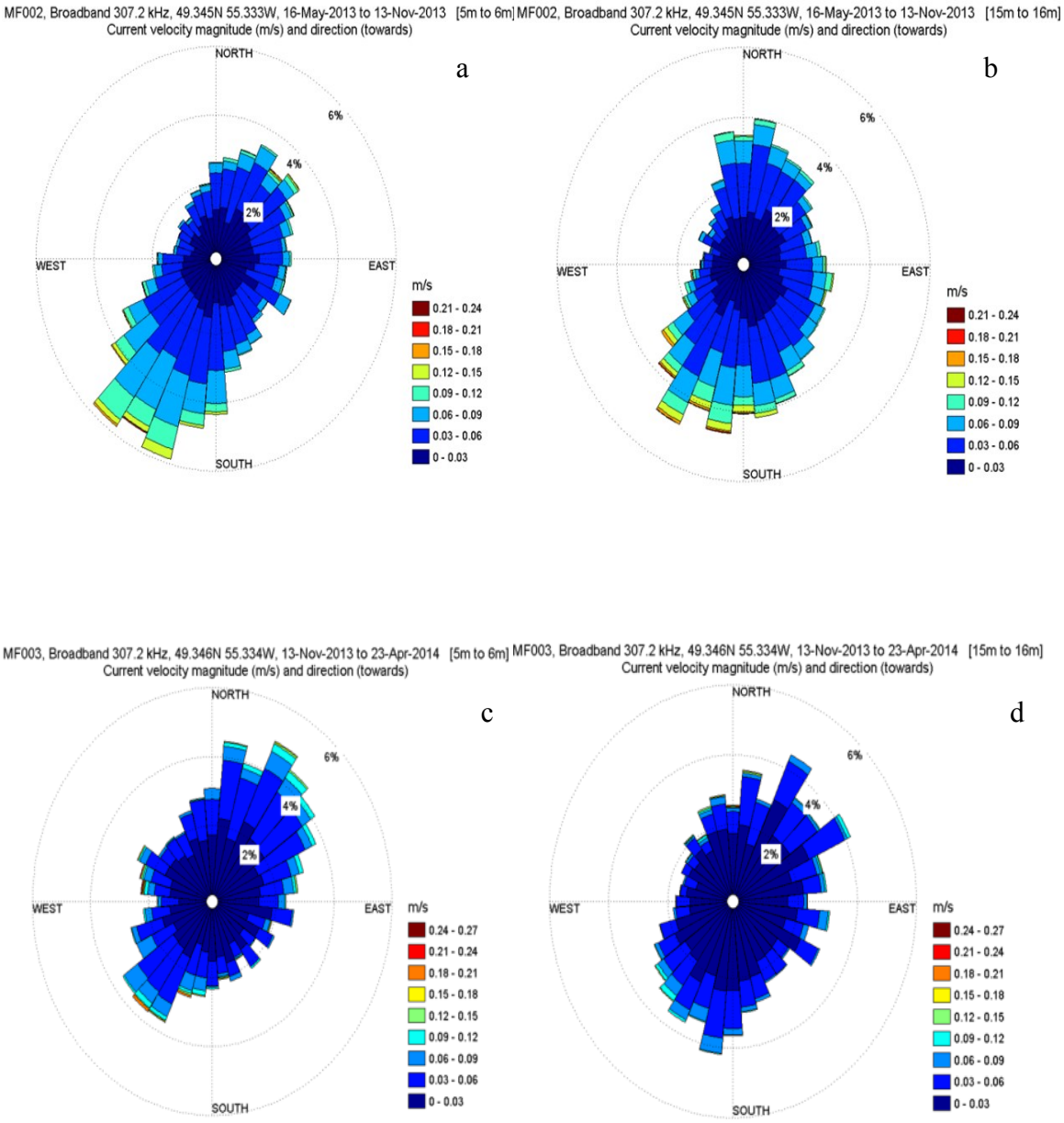

Figure 3.16: South Arm ADCP results for current speed ($m \cdot s^{-1}$) and direction at (a) 5-6 m depth and (b) 15-16 m depth for the period of May to November 2013 and (c) 5-6 m depth and (d) 15-16 m depth for the period of November 2013 to April 2014.

3.4. Discussion

Hydrography and physical processes mediate the delivery and availability of nutrients in the ocean (Archambault *et al*., 1999; Valiela, 2010; Nielsen & Vismann, 2014); therefore, their knowledge is fundamental in evaluating *Mytilus edulis* culture sites.

The area examined in this study presents complex hydrodynamic characteristics; it cannot be considered an estuary, since no main stream or river is present close to the area examined and no constant estuarine circulation was observed (surface layer outflow and bottom layer inflow; Dyer, 1997). Currents appear to be seasonal, with outflow in December-May and inflow in August-December at the surface (0-15 m) and slight inflow in December-May but largely inflow in August-December at subsurface for Mouse Island. South Arm presented inflow in May-November for the entire water column but outflow near the surface (0-15 m) and inflow near the bottom (below 15 m) in November-April. Currents are generally weak and the tide is small; therefore, none of these can be considered as the main driving forces. The results obtained with the moored instruments and the profiles show evidence of a seasonally stratified environment, dominated by changes of salinity (i.e., freshwater fluxes). The large change of salinity observed during the study, particularly in June 2014, could be associated with local ice melt, local runoff or influence external to the bay (Loder *et al*., 1998). Given the limited river input in the area (Appendix B-Figure B2), ice melt and offshore influence are thought to be dominant. The ADCP results suggest complex seasonal layered flows, with sporadic strong events which are probably related to atmospheric forcing, such as storms or strong wind events. The vertical profiles also indicate that wind mixing, particularly in fall when storms are

likely to be more frequent in the region, have a significant influence on water stratification and on the thermocline and pycnocline breakdown.

The cultured mussel sites examined in this study present differences in environmental characteristics: most of the sites are located in a basin, whereas Mouse Island is located in a channel, close to a land slope; its depth is greater and its surrounding bathymetry may have an effect on the hydrodynamics and on nutrient resuspension (Archambault *et al*., 1999; Simpson & Sharples, 2012). The topography could also possibly have an effect on nutrient distribution in South Arm; this site is located close to a sill, where internal waves may be generated, creating internal mixing and nutrient resuspension. However, this hypothesis could not be confirmed with the results obtained. Also, shallow and deep water sites appear to differ in other environmental conditions, at least for part of the year. During the late spring and summer seasons, the shallow water sites consistently present a higher water temperature compared to the deep water. The highest temperature reached during the year is significantly higher in shallow water (18-20°C) than in deep water (15°C). Another interesting difference between shallow and deep water is the presence, in Year 1, of a higher number of spring bloom peaks in the deep water sites, showing a longer-lasting bloom at depth. Mouse Island is an exception; this particular site presents a smaller, yet sustained spring bloom from mid-February to mid-April. Depth can affect chlorophyll *a* concentration; changes in the distribution of nutrients and chlorophyll *a* may be generated by the flow regime, often responsible for short term variability associated with the tidal cycle, internal waves and pycnocline depth (Gibbs *et al*., 1992). Although phytoplankton biomass appears to be the most important factor affecting mussel feeding behaviour, other factors, such as high concentration of silt/suspended sediment,

need to be taken into account, due to their effect on mussel feeding in estuaries and exposed coastal areas (Penney *et al*., 2001; Riisgård *et al*., 2011). The spring bloom appears to start in Mouse Island before it does offshore, by a few weeks to one month. The nutrients necessary for the initiation of the bloom do not likely come from rivers, due to the scarce presence of freshwater runoff in the area and absence of major rivers flowing into the bay. It is, therefore, possible to suggest that the water stratification starting earlier near the coast than offshore (subject to more mixing from the wind) creates the right conditions for a bloom before they can occur offshore (Simpson & Sharples, 2012).

The two years examined in this study presented different environmental conditions, due in particular to the exceptional winter of 2013-2014 (DFO, 2015). In Year 2, a delay of both the thermocline and halocline formation was observed, while the increase in the water temperature in the summer was steeper and quicker than in Year 1, with these characteristics being more accentuated in the shallow water sites. In Year 2 an obvious delay of the spring bloom is also clear; the bloom appears smaller and shorter compared to Year 1, consistent with the offshore chlorophyll data (Zhai *et al*., 2011; Pepin *et al*., 2015).

Current velocity can have a direct physical effect on suspension feeders; mussels have a lower and upper tolerance limit to current velocity. The lower limit is associated with no or very weak currents, due to refiltration and water that eventually becomes nutrient depleted; the higher limit is reached when clearance performance of mussels is affected or, in extreme cases, when mussels are dislodged (Widdows *et al*., 2002; Nielsen & Vismann, 2014). The upper current velocity tolerance limit for *M. edulis* has been shown to be around 0.6 m s^{-1}, with a decrease in clearance starting at currents higher than 0.2 m

s^{-1} (Wildish & Miyares, 1990; Nielsen & Vismann, 2014). In the area studied, currents appear to be weak and almost never reach the high threshold that might impair mussel feeding. Therefore, in both shallow and deep water, the feeding conditions appear ideal for *M. edulis*. However, even if mean current speed is similar between Mouse Island and South Arm, the strongest currents measured are slightly higher in Mouse Island. Also, at depth (15-16 m), in summer and fall, Mouse Island shows a more consistent current direction compared to South Arm. While this observation may not be enough to suggest a more stable environment for the mussels at Mouse Island, it could be worth considering this aspect on the potential for the dispersal of mussel biodeposits, which constitute a concern in mussel aquaculture. In fact, lower biodeposition is one of the reasons why interest in deep water mussel culture is developing (Hartstein & Rowden, 2004; Fabi *et al*., 2009; Frechette, 2012).

Finally, an interesting consideration comes from the possible presence of large zooplankton in the area of study. The zooplankton migration pattern noticed with the ADCP measurements can suggest changes in stratification of the water column and variation in the depth of the food supply (phytoplankton) in a relatively short period of weeks (Record & de Young, 2006). The pattern visible between November 2013 and April 2014 suggests a high number of zooplankton grazing below the ice. This is an important observation, since zooplankton may be a competitor of mussels for food, and their grazing pressure can vary between locations (Archambault *et al*., 1999).

Other environmental characteristics that could not be evaluated in this study may be important to fully understand the hydrodynamics of the area and their effect on shallow and deep water mussel sites. Shoreline configuration may have an important role, since it

can greatly affect both currents and phytoplankton concentration, and therefore mussel feeding; shoreline configuration can alter local hydrodynamic structure and consequently modify phytoplankton dispersion (Archambault *et al*., 1999). Turbulent mixing created by bottom roughness can control eddy diffusion and therefore influence the transport of suspended food (Fréchette *et al*., 1989; Penney *et al*., 2001). As mentioned previously, internal waves (vertical oscillation of thermocline and pycnocline depths) and complex layered currents could all have had an effect on the local hydrodynamics; however, more data collection and analysis would be necessary to fully resolve the magnitude of each of those water characteristics.

The retention period of phytoplankton should also to be taken into consideration; Archambault et al. (1999) found that larger embayments presented a longer retention period. Due to the complexity of the area studied, current velocity and phytoplankton concentration may not be a good predictor of mussel growth when taken into consideration singly. In order to estimate the food availability for mussels, and therefore the preferred location for culture, the flux of organic particles (a function of both currents and phytoplankton concentration) needs to be calculated (Newell, 1990; Archambault *et al*., 1999). Other mechanisms of nutrient transfer between the deep and surface layers, which cannot be resolved with the data currently available, may play a key role in food availability for mussels in the area.

3.5. Conclusions

Due to the complexity of the area examined, and the substantial differences between the two years of study, many questions about the region's environmental characteristics and hydrodynamics remain unanswered. The possible presence of upwelling/downwelling events and internal waves affecting nutrient distribution and resuspension is indicated but it could neither be excluded, nor confirmed. More data would be necessary.

However, some general conclusions can be outlined. Clearly, deep and shallow water mussel sites are different with respect to some environmental conditions, such as temperature and chlorophyll *a* concentration. The deep water sites appear often located near or within the pycnocline depth, where the chlorophyll *a* maximum layer should be found. Also, deep water sites, in Year 2, present a more stable environment for temperature and salinity; shallow water sites are subject to a more rapid and larger increases in temperature in summer and a higher freshwater influence in spring likely due to ice melting. Therefore, can be suggested that deep water sites indeed differ from shallow water and that they can constitute, at least for part of the year, a more stable environment for blue mussel (*Mytilus edulis*) culture.

3.6. References

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CHAPTER 4

Physiological stress response in gill and haemocytes from blue mussels (*Mytilus edulis* L.) cultured at different depths, including selection and validation of reference targets for RT-qPCR studies

4.1. Introduction

The blue mussel, *Mytilus edulis*, is commercially important cultured species in Canada. In 2013, Canadian farmed mussel production was 29,100 tonnes and valued at over $49.5 million (DFO, 2015). The majority of blue mussel producers are in the Atlantic Provinces, where mussels are grown by suspended longline culture (Mallet & Myrand, 1995; Canadian Aquaculture Industry Alliance, 2012). Over the past decade, in Newfoundland, the mussel aquaculture industry has been steadily growing. Since 2000, production has increased from 1,051 to 4,397 tonnes in 2012 and the industry is currently investigating new market opportunities and initiatives (NAIA, 2011; DFA, 2013).

In *Mytilus edulis* the gill is the major feeding organ, the first to come in contact with the external environment, and therefore is likely to be a major site for physiological and oxidative stress resulting from seasonal variation due to environmental changes and geochemical cycles (Bayne *et al*., 1976; Power & Sheehan, 1996; Chapter 3). The gills have also been shown to exhibit high levels of antioxidant enzymes in response to pollution (Manduzio *et al*., 2004). Haemocytes are cellular components of bivalve

haemolymph and they mediate immunity; they are also involved in digestion, nutrient transport and excretion (Bayne *et al*., 1976; Mitta *et al*., 2000). Haemocytes are therefore associated with various forms of antimicrobial activity and environmental stress responses (Mitta *et al*., 1999; Mayrand *et al*., 2005). In mussels, changes in genes expression due to an oxidative stress response are correlated with temperature, salinity, chlorophyll *a* levels, and freshwater influx (Lyons *et al*., 2003; Nuñez-Acuña *et al*., 2012). Moreover, the response profile and intensity of oxidative stress gene expression appears to be related to the mussel origin (Li *et al*., 2010). Environmental conditions such as temperature, nutrient availability, chlorophyll *a* and freshwater influx vary at different depths (Chapter 3) and consequently gene expression in gill tissue and haemocytes related to physiological stress may change between mussels cultured at different depths.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) provides a sensitive and reliable method for quantifying the expression of target genes (Bustin *et al*., 2005; Pfaffl, 2006). However, in gene expression analysis many variables need to be controlled: the amount of starting material, enzymatic efficiencies, paralogue specificity and differences between overall tissue transcriptional activity (Vandesompele *et al*., 2002). Additionally, target genes must be 'normalized' by establishing a stable expression baseline for comparative gene analysis using reference targets. Generally, these should be stable (constitutively expressed) and not vary in the tissue under experimental treatment or other investigation. The success of normalization depends upon the selection of appropriate reference targets and these genes need to be validated in the tissue of interest for stability before being used, as levels of expression vary considerably (Vandesompele *et al*., 2002). Therefore, it is vitally important to establish a highly robust protocol for the

identification of suitable normalization genes, to enable comparisons to target genes of interest.

Different studies have presented reference targets for various tissues from *Mytilus* spp. (Cellura *et al*., 2006; Cubero-Leon *et al*., 2012; Rola *et al*., 2012). However, while it has frequently been highlighted in many publications that no single gene can be reliably used as universal reference, many studies continue to use single reference targets and, as stated by Bustin *et al*. (2005), often those genes are not properly validated. Additionally, many publications do not properly describe the criteria and methodology used to select and validate reference targets. The inclusion of these data would greatly improve the reliability of the subsequent results and would provide increased confidence in the values from the genes of interest.

It is vital for the mussel aquaculture industry to determine optimal culturing conditions, paying specific attention to depth, and location. The present study aims to determine if depth, or location affect mussel performance (stress response), and therefore productivity, in a given season. It also aims to select and validate suitable reference targets that can be used for RT-qPCR analysis of oxidative stress and defense-relevant genes. The objective of this chapter is to evaluate if deep water cultured mussels present a lower oxidative and immune stress response than shallow water cultured ones.

4.2. Materials and Methods

4.2.1. Sample collection

Blue mussels (*Mytilus edulis*) were collected from a traditional longline culture set up at three locations (South Arm, Bulley's Cove and Mouse Island) in Notre Dame Bay, Newfoundland and Labrador, Canada. Linear mesh bags (socks) containing mussels were placed at two different depths (5 m and 15 m), at a total of six sampling sites (map in Appendix B). Adult mussels were collected every four months for two experiments of one year length each; Year 1 ran from September 2012 until September 2013, while Year 2 ran from October 2013 until September 2014.

At each sampling period 5 socks were collected from each of the 6 sampling sites; socks were collected from beginning, middle, and end of the line, each sock was then separated in 5 parts and the mussels pooled together, to obtain a randomized sample. Fifteen mussels from each of the 6 sampling sites were collected, dissected and gill tissue removed. The individual tissues were placed each in a 2 mL Eppendorf® tube either containing 1 mL of RNAlater (Ambion, Austin, TX) or directly flash-frozen on dry ice. Haemolymph from 25 mussels was also collected in five pools of five mussels each, to obtain a total of 2 mL of haemolymph/pool. The initial size of mussels did not allow individual sampling, due to the inadequate supply of haemolymph, therefore 5 mussels were pooled and this protocol was continued throughout the experiment. The haemolymph was extracted from the posterior abductor muscle, as described by Wyatt et al. (2013). Samples were transported to the Northwest Atlantic Fisheries Centre (NAFC),

St. John's, Newfoundland and Labrador, where they were stored at -80°C until further treatment (1-3 months).

4.2.2. RNA extraction and cDNA synthesis

Total RNA from 15 individual mussels taken from each site was extracted from 18-20 mg of gill tissue and then homogenized in 600 µL of lysis buffer containing β-mercaptoethanol, according to the manufacturer recommendations for the RNeasy Mini Kit (Qiagen, Mississauga, ON, Canada). Total RNA was extracted from resuspended haemocyte pellets as described by Wyatt et al. (2013), i.e. it was eluted in 50 µl of UltraPure water (Invitrogen, Carlsbad, CA). Subsequently, a maximum of ten individual gill samples and three pools of haemocytes per site were selected for RT-qPCR expression analyses based on RNA quality and quantity, as determined by spectrophotometry using the Nanodrop 2000 spectrophotometer (260/280 ratio ≥ 2.0; Thermo Fisher Scientific Inc., Wilmington, DE) and gel electrophoresis (single clear band on 1% agarose in TBE-Tris Borate EDTA: i.e., 100 mL of 1x TBE + 1.0 g of agarose + 6 µL of Ethidium Bromide, at 100 V for 30 min against a 1kb DNA ladder) (TrackIt, Invitrogen, Carlsbad, CA). When quality and quantity were not achievable for ten (or three) samples, the highest number of samples possible was used for the analysis.

Subsequently, cDNA was synthesized from 1 µg of purified total RNA using the QuantiTect Reverse Transcription Kit (Qiagen, Mississauga, ON, Canada) and incubated according to the manufacturer's suggested protocol. Reactions were performed in a 20 µl volume containing 1 µL of total RNA, 4 µL of 5x RT reaction mix, 1 µL of reverse

transcriptase enzyme mix, and 1 μL of RT primer mix (oligo-dT and random primers mix).

4.2.3. Selection of candidate reference targets and primer design

Primer sequences for candidate reference targets (Table 4.1) were obtained from two sources: 1) prior literature on blue mussel gene expression; and 2) an ongoing functional genomics study and database (Gurney-Smith *et al*., 2013; http://www.mytome.ca).

All primer sets tested were paralogue specific, as determined by BLAST query (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome) and were synthesized by Integrated DNA Technologies (IDT, Coralville, IA). Quality testing of primer sets was conducted using the 7500 Fast QPCR system (Applied Biosystems 7500 Fast 2.0) with SYBR green chemistry (Applied Biosystems) and a 3:1 five point serial dilution curve of pooled cDNA (comprised of six individuals, one per sampling site, with each individual contributing 1 μg of RNA to the pool). Primer sets that passed quality testing conformed to the following five criteria: 1) uniform spacing between the five amplification curves in the dilution series; 2) an R^2 value for the standard curve between 0.95 and 1.00, indicating a linear relationship between the serial dilutions; 3) amplification efficiency between 80 and 110%; 4) low variance (<0.5 cycles) among technical triplicates; 5) a single peak in the melt curve, indicating the absence of primer dimers and secondary (non-specific) products (Pfaffl, 2001). Based on these criteria, nine candidate reference targets were selected for further analysis: elongation factor 1 (EF1), alpha tubulin, ubiquitin c, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), histone

H3 (HH3), 18S ribosomal subunit, hypoxanthine-guanine phosphoribosyltransferase (HPRT), DNA-directed RNA polymerase II polypeptide C (RPOL2-3), and adenosine triphosphate 5 β (ATP5β) (Table 4.1). Oligonucleotide primers for these candidates were accessed through the MytOME database and NCBI GenBank depositions (Gurney-Smith *et al*., 2013; http://www.mytome.ca).

Table 4.1: Information on the 19 candidate reference targets and primers used in this study.

Transcript name (species and accession number)	Forward primer Reverse primer (5'-3')	Quality testing result	Primer source	Functional annotation[1]	GO Identifier[2]	Homolog species and accession number[3]	E-value/ %ID
Actin (*Mytilus galloprovincialis* AF157491)	AGGACTTGTAACCACC CACCGATCCAGAGTAT	Failed (multiple Tm peaks)	Anantharaman & Craft 2012	MF: nucleotide binding, ATP binding	GO:0005524	*Mytilus sp.* EF140761.1	0.0 (94%)
Actin (*Mytilus galloprovincialis* AJ625116)	TGTAACAAACTGGGACGATA AGCATGAGGAAGGGCATAAC	Failed (multiple Tm peaks)	Wang *et al.* 2013	N/F	N/F	*Mytilus galloprovincialis* AF157491	0.0 (98%)
Alpha Tubulin (*Mytilus edulis* KJ784485)	AATCGTCGACTTGGTCTTGG AGACGTTCCATAAGGAGTGAGG	Passed[4]	Gurney-Smith *et al.* 2013	BP: microtubule-based process, metabolic process; MF: nucleotide binding, GTPase activity, structural constituent of cytoskeleton, GTP binding; CC: microtubule	GO:0007017 GO:0008152 GO:0000166 GO:0003924 GO:0005200 GO:0005525 GO:0005874	*Mytilus galloprovincialis* KJ792080.1	0.0 (88%)

ATP5B (*Mytilus galloprovincialis* KJ792081)	TGCTCCAGCTACAACATTCG CAGGGTAAATACCCAACTCAGC	Passed[4]	Gurney-Smith *et al.* 2013	BP: transport, ion transport, metabolic process, ATP hydrolysis coupled proton transport, proton transport; MF: ATP binding, hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances; CC: proton-transporting two-sector ATPase complex, catalytic domain	GO:0006810 GO:0006811 GO:0008152 GO:0015991 GO:0015992 GO:0005524 GO:0016787 GO:0016820 GO:0033178	*Mytilus edulis* AY580269.1	0.0 (99%)
Cyclophilin A (*Mytilus edulis* KJ808670, KJ808671)	TGAGCATGGCTAATTCAGGTC GCCAAACACAACATGAGCAC	Passed	Gurney-Smith *et al.* 2013	BP: protein peptidyl-prolyl isomerization, protein folding; MF: peptidyl-prolyl cis-trans isomerase activity, isomerase activity	GO:0000413 GO:0006457 GO:0003755 GO:0016853	*Mus musculus* NM_008908.4	6e-18 (73%)
EF1 (*Mytilus galloprovincialis* AB162021)	CCAGAAGGAAGTCAGCAGTTAC TGTTGTCTCCGTGCCATCC	Passed[4]	Gurney-Smith *et al.* 2013	BP: translational elongation; MF: nucleotide binding, translation elongation factor activity, GTPase activity, GTP binding; CC: cytoplasm	GO:0006414 GO:0000166 GO:0003746 GO:0003924 GO:0005525 GO:0005737	*Mytilus edulis* AY580270.1	0.0 (99%)
EF1A (*Mytilus edulis* AF063420.1)	TGGTGAATTTGAAGCTGGTATCT CAATCATCTGTTTGACACCAAGA	Passed	Rola *et al.* 2012	BP: translational elongation; MF: nucleotide binding, translation elongation factor activity, GTPase activity, GTP binding; CC: cytoplasm	GO:0006414 GO:0000166 GO:0003746 GO:0003924 GO:0005525 GO:0005737	*Mytilus galloprovincialis* AB162021.0	0.0 (97%)

G6PDH (*Crassostrea gigas* AM076951, *Mytilus galloprovincialis* AJ516599)	CCACCTACTACAAATGCTGAGG ATCTACTGGCTGGATGCTCTTC	Passed	Gurney-Smith *et al.* 2013	BP: carbohydrate metabolic process, glucose metabolic process, oxidation-reduction process; MF: glucose-6-phosphate dehydrogenase activity, oxidoreductase activity, NADP binding	GO:0005975 GO:0006006 GO:0055114 GO:0004345 GO:0016491 GO:0050661	*Crassostrea gigas* NM_001305342.1	0.0 (100%), 2e-66 (76%)
GAPDH (*Mytilus galloprovincialis* KJ808668, *Mytilus edulis* KJ808669)	TCATCCCATCCTCAACAGG ATCTGGAACTGGTACCCTGAAG	Passed[4]	Gurney-Smith *et al.* 2013	BP: glucose metabolic process, glycolytic process, oxidation-reduction process; MF: glyceraldehyde-3-phosphate dehydrogenase (NAD+) (phosphorylating) activity, oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor, NADP binding, NAD binding	GO:0006006 GO:0006096 GO:0055114 GO:0004365 GO:0016491 GO:0016620 GO:0050661 GO:0051287	*Mytilus edulis* KJ808669.1 *Mytilus galloprovincialis* KJ808668.1	2e-74 (97%) 6e-75 (97%)
Helicase (*Mytilus galloprovincialis* DQ158075)	GCACTCATCAGAAGAAGGTGGC GCTCTCACTTGTGAAGGGTGAC	Failed (multiple Tm peaks)	Cubero-Leon *et al.* 2012	BP: mRNA splicing, via spliceosome; MF: helicase activity; CC: spliceosomal complex	GO:0000398 GO:0004386 GO:0005681	N/A	N/A
Histone H3 (*Mytilus edulis* KM042110, KM042119)	GCCATTTCAGCGATTAGTGAG TATGCTTCACTGGCTTCCTG	Passed[4]	Gurney-Smith *et al.* 2013	N/F	N/F	*Mytilus edulis* KM042112.1	0.0 (99%), 0.0 (100%)

HPRT (*Mytilus galloprovincialis* KJ817207)	TTCCAGCAGGTCTTGTAGCC GCTCCTCCTGAAAATCTTGC	Passed[4]	Gurney-Smith *et al.* 2013	N/F	N/F	*Crassostrea gigas* AB122067.1	7e-54 (86%)
Ribosomal subunit 18S (*Mytilus edulis* AY527062.1)	CGCGTTTATTAGATCAAAACCAG AAGTTGATAGGGCAGACATTTGA	Passed	Rola *et al.* 2012	N/F	N/F	*Mytilus trossulus* L33453.1	0.0 (99%)
Ribosomal subunit 18S (*Mytilus galloprovincialis* L33452)	TCGATGGTACGTGATATGCC CGTTTCTCATGCTCCCTCTC	Passed[4]	Gurney-Smith *et al.* 2013	N/F	N/F	*Mytilus trossulus* L33455.1	0.0 (99%)
Ribosomal subunit 28S (*Mytilus edulis* Z29550)	AGCCACTGCTTGCAGTTCTC ACTCGCGCACATGTTAGACTC	Failed (multiple Tm peaks)	Ciocan *et al.* 2011	N/F	N/F	*Mytilus galloprovincialis* AB103129.1	0.0 (99%)
Ribosomal subunit 28S (*Mytilus galloprovincialis* AB103129.1)	AAGCGGAGGAAAAGAAACTAAC TTTACCTCTAAGCGGTTTCAC	Failed (multiple Tm peaks)	Cellura *et al.* 2006	N/F	N/F	*Septifer virgatus* AB103126.1	0.0 (95%)
RPOL2-3 (*Mytilus edulis* KJ808672)	AAGTGGAATCCAACCTGTGG GGCCATTCTTCTGGTTTTGG	Passed[4]	Gurney-Smith *et al.* 2013	BP: transcription, DNA-templated; MF: DNA binding, DNA-directed RNA polymerase activity, protein dimerization activity	GO:0006351 GO:0003677 GO:0003899 GO:0046983	N/A	N/A

Tubulin (*Mytilus edulis* DQ174100)	TTGCAACCATCAAGACCAAG TGCAGACGGCTCTCTGT	Failed (amplification efficiency <80%)	Cubero-Leon *et al.* 2012	BP: microtubule-based process, metabolic process, protein polymerization; MF: GTPase activity, structural constituent of cytoskeleton, GTP binding; CC: microtubule, protein complex	GO:0007017 GO:0008152 GO:0051258 GO:0003924 GO:0005200 GO:0005525 GO:0005874 GO:0043234	*Mytilus galloprovincialis* HM537081.1	0.0 (93%)
Ubiquitin C (*Mytilus edulis* KJ808673)	GTTTTATCCCTGGCATCAGC AATGGGTTGGGGAGGTAAAG	Passed[4]	Gurney-Smith *et al.* 2013	BP: metabolic process; MF: hypoxanthine phosphoribosyltr-ansferase activity, transferase activity, transferring glycosyl groups, guanine phosphoribosyltr-ansferase activity	GO:0008152 GO:0004422 GO:0016740 GO:0016757 GO:0052657	N/A	N/A

[1]Functional annotation associated with the transcripts used in primer design for each target. Gene ontology (GO) categories: biological process (BP), molecular function (MF), and cellular component (CC). N/F: no functional annotation found for this transcript.
[2]GO identifiers are listed in the same relative order as the functional annotations (GO terms) with which they are associated.
[3]The best BLASTn hit, defined as having the lowest E-value (≤1e-10) and an associated gene name (i.e., not "hypothetical" or "predicted"), is shown. BLAST reports were collected on April 16th, 2015, and reflect the state of the GenBank nucleotide (nt) database as of that date. N/A: not applicable.
[4]Indicates primer sets used in GeNorm analysis for stability.

4.2.4. Reverse transcription quantitative PCR (RT-qPCR)

The nine candidate reference targets were assessed for stability across at least one third of the individuals for each sampling site (i.e., four individual gill samples from each of the six sampling sites). Each sample-reference target combination was run in triplicate (Pfaffl *et al*., 2002) in 96-well reaction plates using the 7500 Fast Real-Time PCR System with SYBR green chemistry. Non-template controls (NTC, blank) were included in each run. The RT-qPCR reaction volume was 13 µL and contained 2 µL of 10X dilute cDNA (diluted in nuclease-free water to a final volume of 200 µL), representing 10 ng of input from an individual, 50 nM of the forward and reverse primers of a set, and 1X Power SYBR Green Master Mix (Applied Biosystems). The RT-qPCR reactions were carried out using the following incubation conditions: one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min, and a final melting curve program consisting of a temperature increase from 60°C to 95°C at a ramp rate of 1%.

Four genes of interest involved in oxidative stress and immune-response in blue mussels (*Mytilus edulis*) were chosen to be investigated in the gill and haemocyte samples; defensin, heat-shock protein 70 (HSP-70), glutathione S-transferases p (GSTp) and superoxide dismutase (SOD) (Table 4.2). The genes of interest were chosen based on suitability of primer sets and physiological function. Each time point (initial, 4 months, 8 months and 12 months) of each year of study was treated as a single RT-qPCR experiment. The 10 gill samples and three haemocyte pools for each site and depth were run in triplicate in 96-well reaction plates using the 7500 Fast Real-Time PCR System with SYBR green chemistry. Non-template controls (NTC) and linker (i.e., pool of

individual gill samples from each of the sampling sites, allowing linking between plates) were included in each run; reference targets were chosen for each experiment using GeNorm (Vandesompele *et al*., 2002). The RT-qPCR reactions were carried out as described above.

Table 4.2: Gene-specific primers used in qPCR analysis.

Gene of Interest **(Accession number)**	**Metabolic Pathway**	**Forward primer** **Reverse primer** **(5'-3')**	**Primer Source**
Defensin (JN935272.1)	Host defense	TTAGTGCGTCTGCCGGTTAG GCATCAGCAACTTCAACAGC	In house design[1]
Heat-shock protein 70- HSP70 (AF172607.1)	Chaperone	GGGTGGTGGAACTTTTGATC CTCTTTGCCCTTTCACAAGC	In house design[1]
Glutathione S transferase p- GSTp (AY557404.1)	Conjugation of GSH and xenobiotics	ATACTGGCATATCTTGGCAGAAA ACGTAAGCCCCTCTGATATCTTC	Rola *et al*., 2012
Superoxide Dismutase- SOD (AJ581746.1)	O_2^- degradation	GTCCACATTTTCTCGCAGTTTAC ACCATCTCCTTTCAAGACACAAA	Rola *et al*., 2012

[1]Novel primer sets designed using Primer3 (Koressaar & Remm, 2007; Untergrasser *et al*., 2012; http://bioinfo.ut.ee/primer3/) for blue mussel transcripts available in public databases.

4.2.5. Analysis of gene expression stability for candidate reference targets

Two different methodologies for identifying the most stable reference gene(s) from among nine candidates were compared (using cDNA from the same third of individuals

for each gene): 1) candidate reference targets were run individually in a RT-qPCR experiment and were determined to be stably transcribed if transcript expression fell within 0.5 cycles for all samples tested; and 2) GeNorm (Vandesompele *et al*., 2002; qBASE+, Biogazelle) analysis was performed on the individual RT-qPCR experiments (from 1) to analyze multiple candidate reference targets simultaneously, and to determine the most stable gene or combination of genes that were suitable for use as reference targets. Conditions and procedures of RT-qPCR were as previously described.

GeNorm (Vandesompele *et al*., 2002), a Visual Basic Application (VBA) for Microsoft Excel, is based on the principle that the expression ratio of two stable reference targets is constant across samples. The pairwise variation of each reference target gene with all other such genes (the geometric mean) is calculated as the standard deviation of the logarithmic transformed expression ratios. A measure of control-gene stability (M-value) is determined as the average pairwise variation of a particular reference gene with all other candidate genes. Genes with the lowest M-value have the most stable expression; therefore the gene with the highest M-value is excluded from the analysis. The program recalculates new M-values for the remaining genes until the most stable combination of reference targets are found (Vandesompele *et al*., 2002). For each target, the coefficient of variation (CV) value is used to determine the gene-specific variation and validate the M-value. For the current study, individual gill tissues were considered heterologous tissue samples for the following reasons: 1) samples were obtained from a known hybrid zone (Bates & Innes, 1995; Penney & Hart, 1999; Murray *et al*., 2010); 2) tissues were not validated by histology, because gills are easily identified; and 3) the *Mytilus edulis*

genome has not yet been sequenced. Therefore, the default values for M and CV were changed to 1.0 and 0.5, respectively (Vandesompele *et al*., 2002).

4.2.6. Statistical analysis

Plate results were analyzed for gene expression in QBase+ (GeNorm; Vandesompele *et al*., 2002). Expression results were then analyzed with Sigmaplot (12.0 and successive versions) statistical and graphical software (Systat software). Data were tested for normality (Shapiro-Wilk test) and homogeneity of the variance and means ± SE were calculated. One-way analysis of variance and the appropriate post-hoc tests were conducted (Tukey's test). When the assumptions of homogeneity of the variance and normality were not met an ANOVA on ranks was conducted. Significance was set at $\alpha = 0.05$. ANOVA tables are presented in Appendix G (Table G1-G11).

4.3. Results

4.3.1. Selection and validation of suitable reference targets

Results for selection of reference targets for one qPCR experiment (Year 2 - initial time point) are presented. The same methodology was used for the reference targets selection in each experiment, resulting in the use of different combinations of reference targets. The specific reference targets used in each experiment are highlighted in Section 4.3.2.

4.3.1.1. Primer specificity and amplification efficiency

Primer pairs were validated in terms of efficiency and specificity. Not all primer sets passed validation; among the failed sets were primers that had been used in the published literature (Table 4.1). Amplification efficiency for the nine candidate reference targets used in the GeNorm test ranged from 81.0-94.2% (alpha tubulin and GAPDH, being the lowest and highest scoring primers, respectively; Table 4.3). Specificity of the amplification products was confirmed in the existing sequence database using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for each primer pair, and was confirmed by the presence of a single peak in the melt curve.

Table 4.3: Amplification efficiency for the 9 candidate reference targets (see text) used in GeNorm analysis.

Target[1]	**Amplification efficiency (%)**	**M-value**	**CV-value**	**Vn/Vn +1**	**ΔC_t**
Histone H3	88.9	1.077	0.428	---	4.9
GAPDH	94.2	1.093	0.651	---	5.1
EF1	83.7	0.933	0.366	0.172	4.9
ATP5B	81	0.983	0.397	0.146	4.7
Alpha Tubulin	82.4	0.916	0.328	0.147	4.5
RPOL2-3	83.4	0.973	0.330	0.136	3.0
Ubiquitin C	83.5	1.024	0.510	0.107	2.7
18S	84.4	1.250	0.587	0.129	4.7
HPRT	89.9	2.014	1.389	0.215	7.4

[1]Targets are listed by stability, as determined by GeNorm analysis, ranked from highest stability to lowest.

4.3.1.2. Expression profile of the candidate reference targets

The average threshold PCR cycle (Ct) values of the 9 candidate reference targets ranged from 10.4 (18S) to 31.8 (HPRT), across the 24 individual gill samples tested. Ubiquitin C was the most stably expressed, with ΔCt range of 2.7 cycles. Hypoxanthine guanine phosphoribosyl transferase (HPRT) was the least stably expressed, with a ΔCt range of 7.4 cycles. None of the candidates had a ΔCt range of <0.5 (Table 4.3). Therefore, GeNorm analysis was conducted to identify a suite of genes suitable for use as reference targets.

4.3.1.3. Expression stability of the candidate reference targets

Expression data for each candidate reference target was analyzed in QBase+ using the GeNorm algorithm to determine stability and the optimal number of targets required for accurate normalization in future RT-qPCR experiments using gill samples in a parallel study. Candidate reference targets were ranked based on their average expression stability (M value), coefficient of variability (CV value), and pairwise variability (Vn/Vn+1) (Table 4.3); GeNorm suggested that cut off values were <1.0, <0.5 and <0.15, respectively. In this case, GeNorm recommends that histone H3 (M=0.568; CV=0.428), GAPDH (M=0.575; CV=0.651) and EF1 (M=0.579; CV=0.366) be used together for the adequate normalization of targets of interest, due to the stability of expression, low variation and pairwise variability <0.15 (V3/V4=0.146) (Table 4.3; Fig. 4.1 a,b).

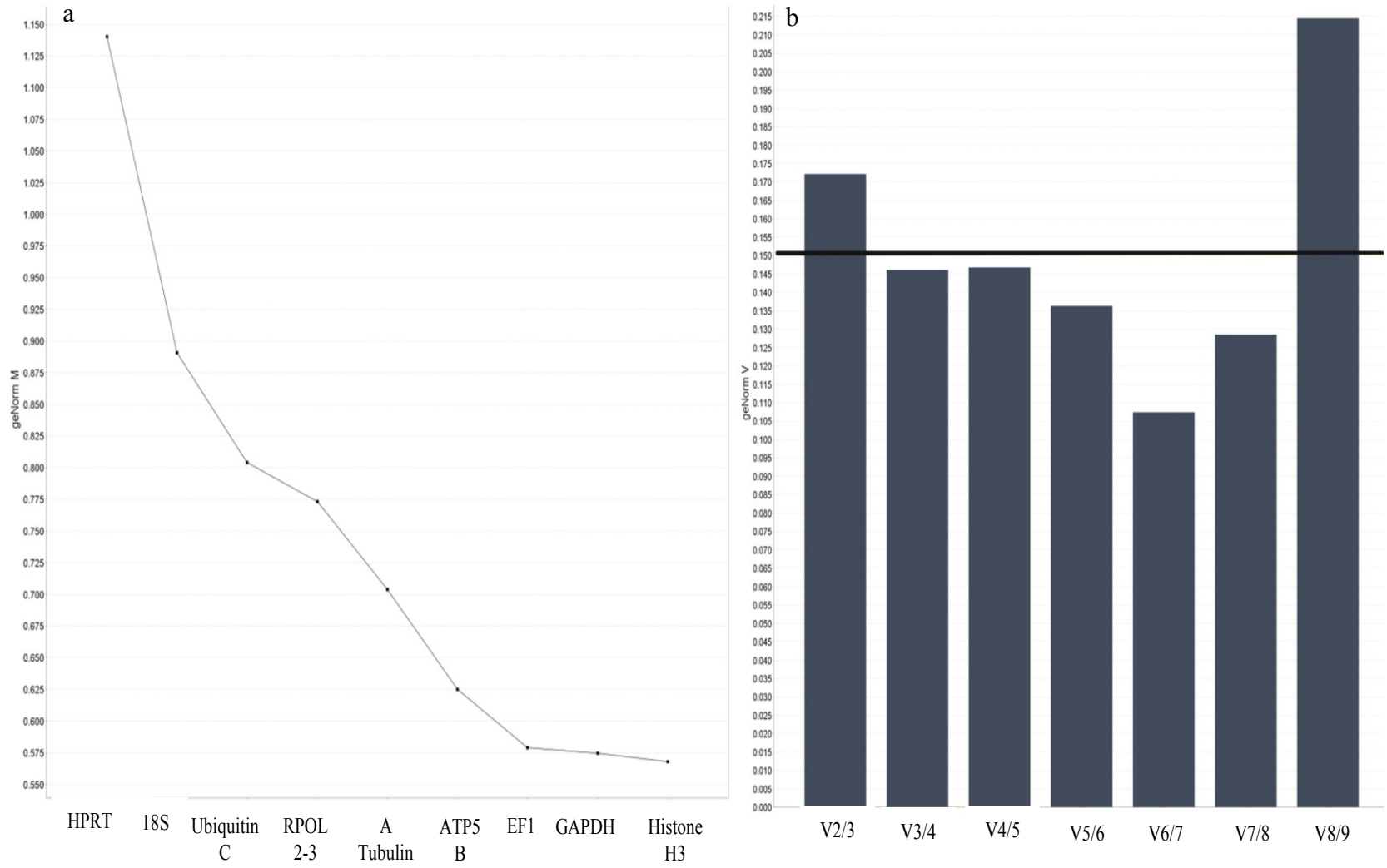

Figure 4.1: GeNorm results for candidate reference targets. (a) Average expression stability values (M: 0.550-1.150) of candidate reference targets by GeNorm and (b) determination of the optimal number (pairwise variability [Vn/Vn+1]) of the nine candidate reference targets by GeNorm. Black horizontal line represents cut off value of 0.15 for pairwise variability.

4.3.2. Physiological, stress-related gene expression

4.3.2.1. Year 1

4.3.2.1.1. Expression of immune and oxidative stress genes in mussel gill

ATP5B and EF1 A (Table 4.1) were used as gene expression reference targets for gill tissue samples at the initial time point. Interestingly three of the four stress genes analysed showed significant differences between sites; at the initial time point defensin expression was higher in Bulley's Cove deep compared to Bulley's Cove shallow 1 and Bulley's Cove shallow 2 ($p= 0.005$) (Figure 4.2a). Heat-shock protein presented higher expression in Bulley's Cove shallow 1 compared to Bulley's Cove shallow 2 and Mouse Island deep ($p=0.008$) (Figure 4.2b). Superoxide dismutase had a higher expression level in Bulley's Cove shallow 1 than in Mouse Island deep ($p=0.002$) (Figure 4.2d).

ATP5B and GAPDH (Table 4.1) were used as gene expression reference targets for gill tissue at the 4-month time point. Significant differences in gene expression were found between sites; glutathione S-transferase p showed a higher expression at Bulley's Cove shallow 1 than at Bulley's Cove shallow 2 ($p=0.019$) (Figure 4.3c). Superoxide dismutase had a higher relative expression level in Bulley's Cove deep compared to South Arm shallow ($p<0.001$) and Bulley's Cove shallow 1 ($p=0.002$) (Figure 4.3d). Also, South Arm deep presented a higher expression level than South Arm shallow ($p=0.027$) (Figure 4.3d).

ATP5B and histone (Table 4.1) were used as gene expression reference targets for gill tissue at the 8-month time point. Significant differences between sites at this particular

time point were observed only for the heat-shock protein; South Arm shallow presented a higher expression level of heat-shock protein than Bulley's Cove shallow 1 and Mouse Island deep ($p=0.009$) (Figure 4.4b).

ATP5B and GAPDH (Table 4.1) were used as gene expression reference targets for gill tissue at the 12-month time point. At this time point no significant differences were observed for physiological stress related genes among sites (Figure 4.5). Glutathione S-transferases p presented very low or no expression in all the samples analysed, therefore statistical analysis would not have been relevant and this gene was excluded from the analysis at this time point (not present in Figure 4.5).

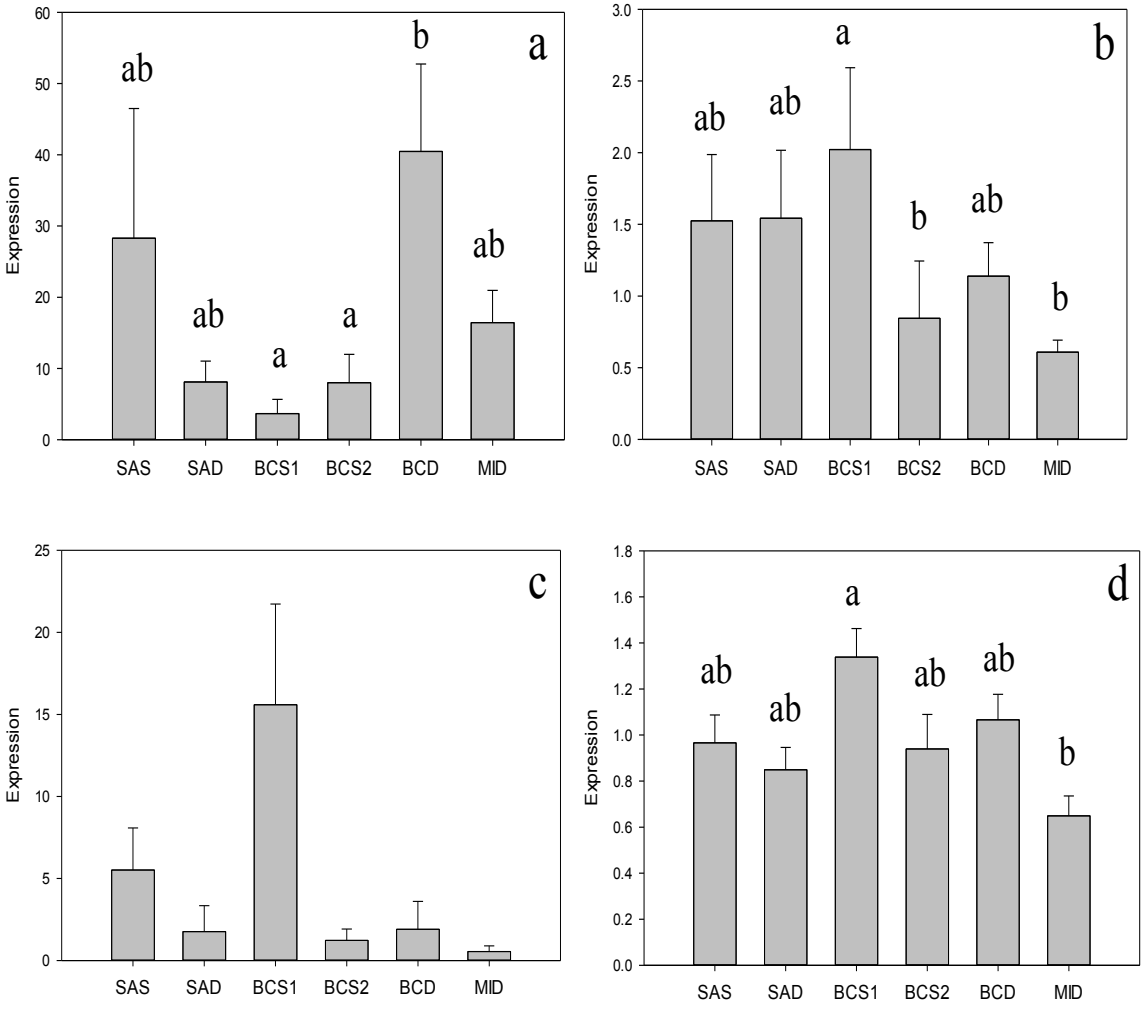

Figure 4.2: Immune and oxidative stress gene expression results for gill tissue Year 1-initial time for (a) defensin, (b) heat-shock protein 70, (c) glutathione S-transferases p, and (d) superoxide dismutase (n=8). Results are mean (± SE). Letters represent statistical significance (sites represented by different letters differ for stress response). SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep.

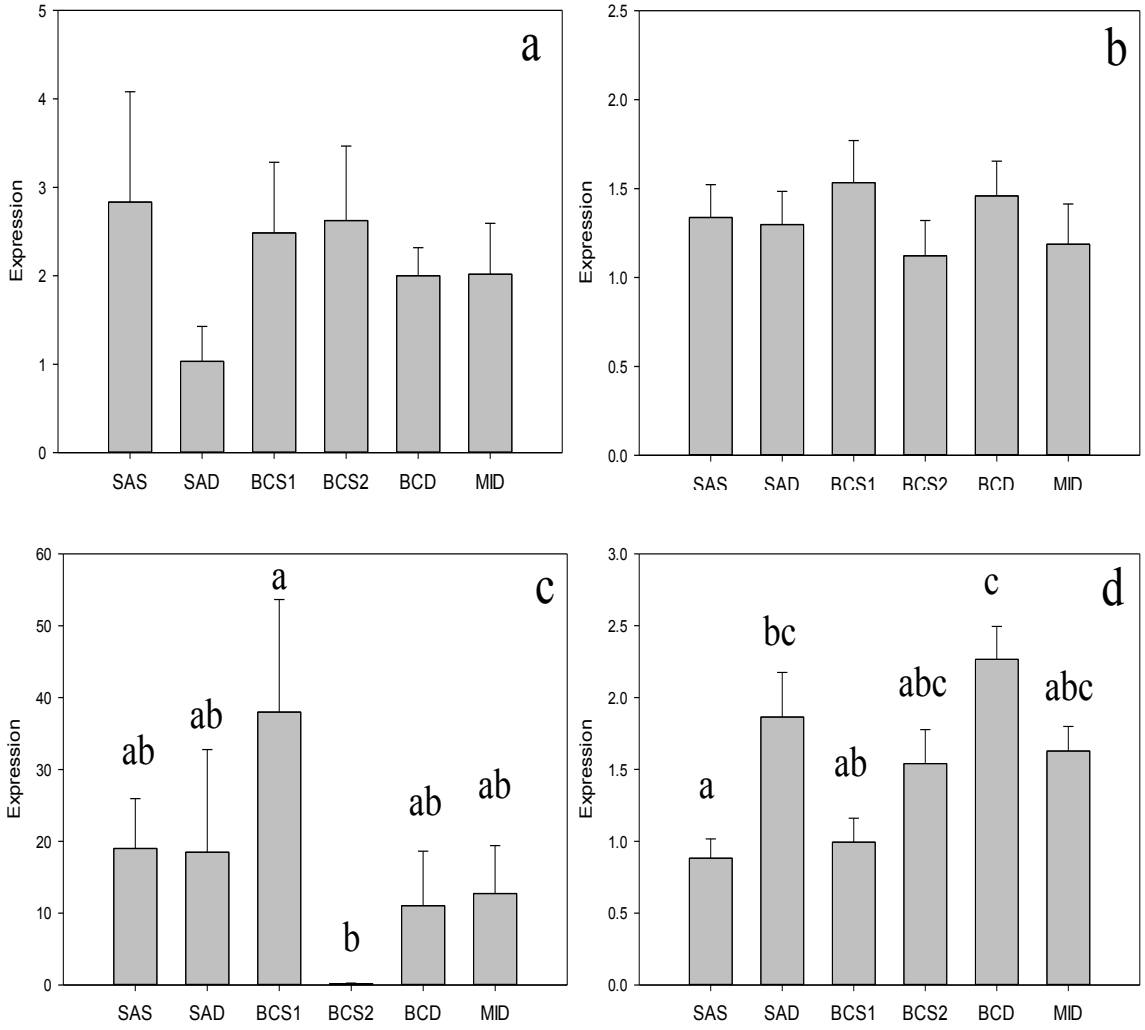

Figure 4.3: Immune and oxidative stress gene expression results for gill tissue Year 1-4 months for (a) defensin, (b) heat-shock protein 70, (c) glutathione S-transferases p and (d) superoxide dismutase (n=9-10). Results are mean (± SE). Letters represent statistical significance (sites represented by different letters differ for stress response). SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep.

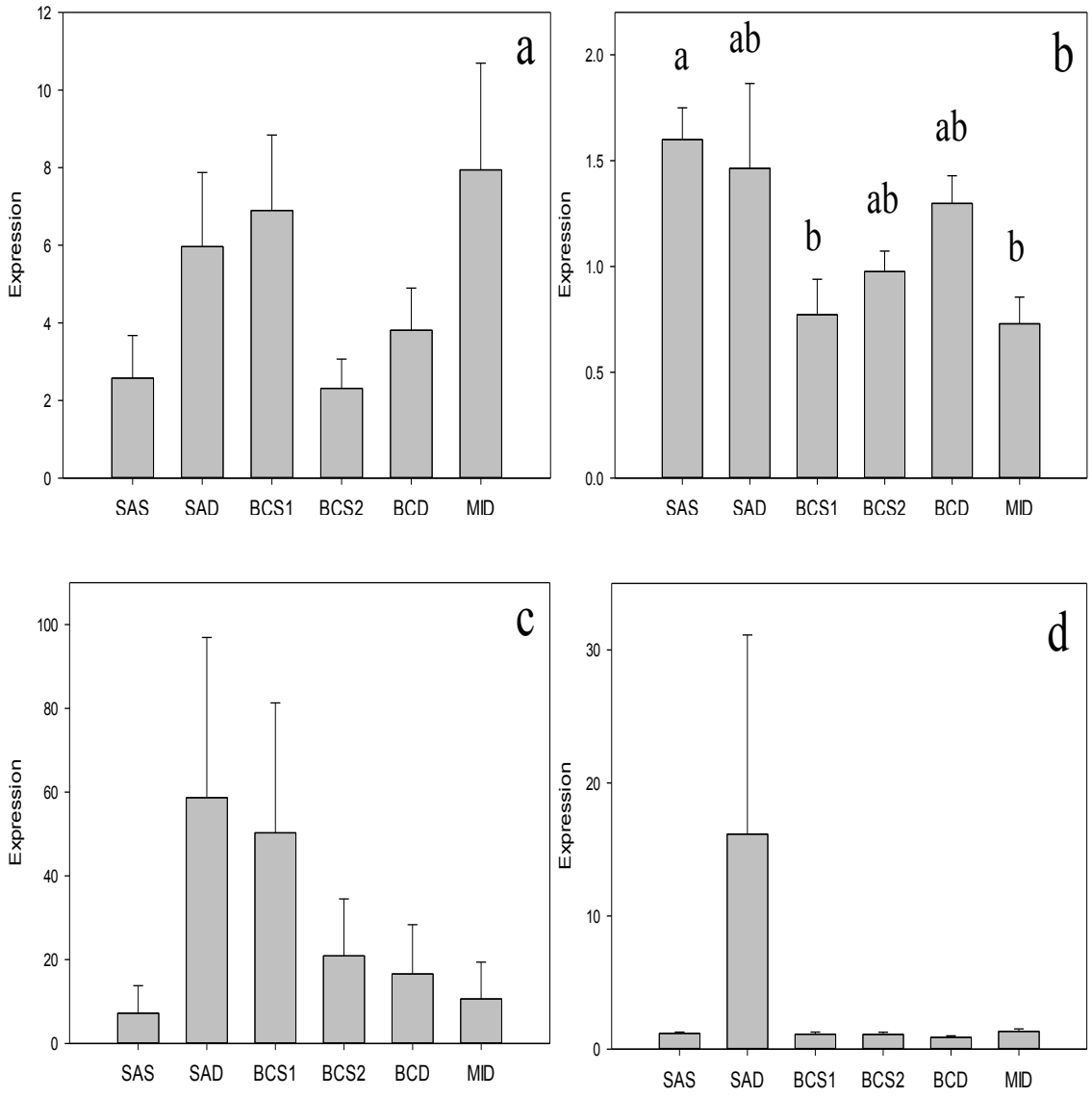

Figure 4.4: Immune and oxidative stress gene expression results for gill tissue Year 1-8 months for (a) defensin, (b) heat-shock protein 70, (c) glutathione S-transferases p and (d) superoxide dismutase (n=7-10). Results are mean (± SE). Letters represent statistical significance (sites represented by different letters differ for stress response). SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep.

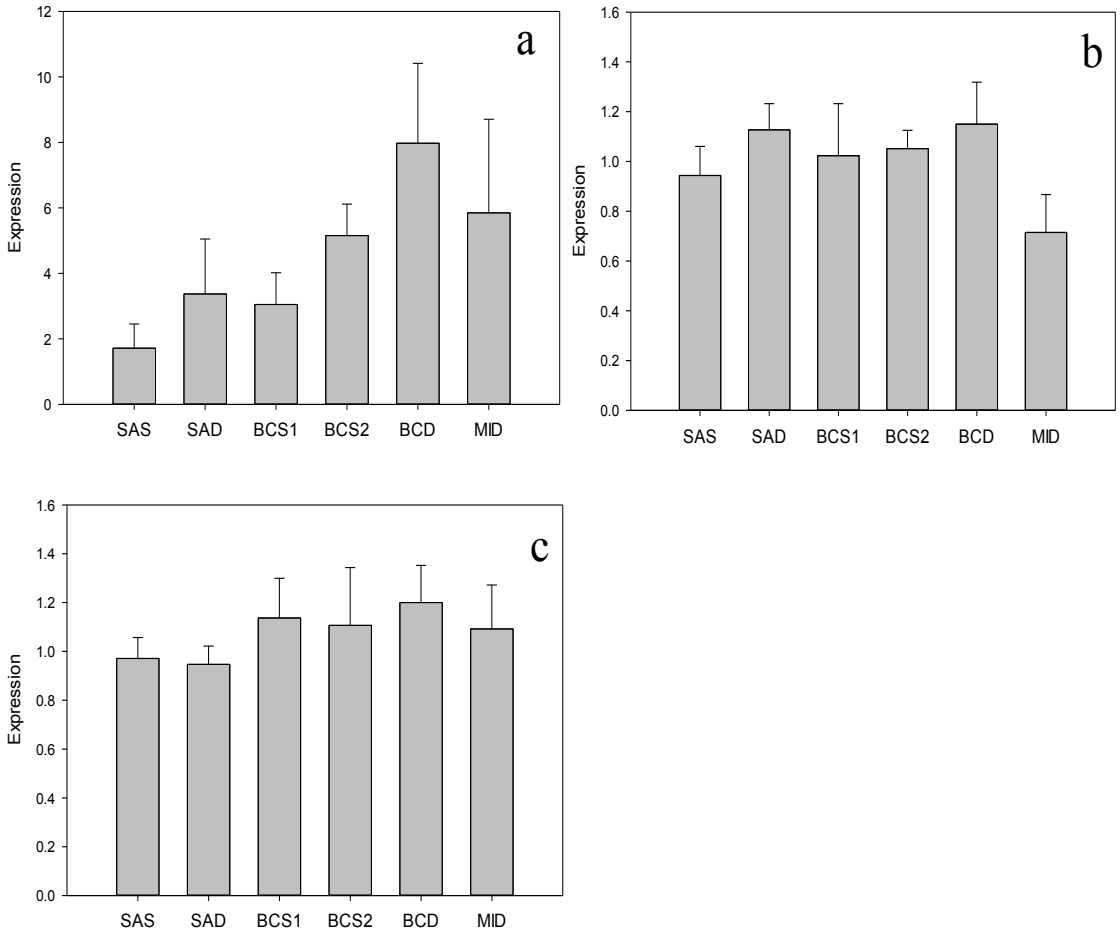

Figure 4.5: Immune and oxidative stress gene expression results for gill tissue Year 1-12 months for (a) defensin, (b) heat-shock protein 70, (c) superoxide dismutase (n=9-10). Glutathione S-transferases p was excluded from the analysis due to low/not shown expression. Results are mean (± SE). SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep. No significant differences were detected.

4.3.2.1.2. Expression of immune and oxidative stress gene in mussel haemocytes

EF1 and EF1 A (Table 4.1) were used as reference target genes in haemocyte tissue at the initial time point. EF1 A and Histone (Table 4.1) were used as reference targets for haemocyte tissue at the 4-month time point. ATP5B and GAPDH (Table 4.1) were used as reference targets for haemocytes at the 8-month time point. EF1 A and GAPDH (Table 4.1) were used as reference targets for haemocyte tissue at the 12-month time point. At all sample points in Year 1, expression of the four physiological stress genes did not differ among sites or between shallow and deep water in haemocytes (Figure 4.6-4.9). Glutathione S-transferases p presented very low or no expression levels in all samples analysed at the 12-month time point; statistical analysis would not have been relevant and this gene was consequently excluded from the analysis (not present in Figure 4.9).

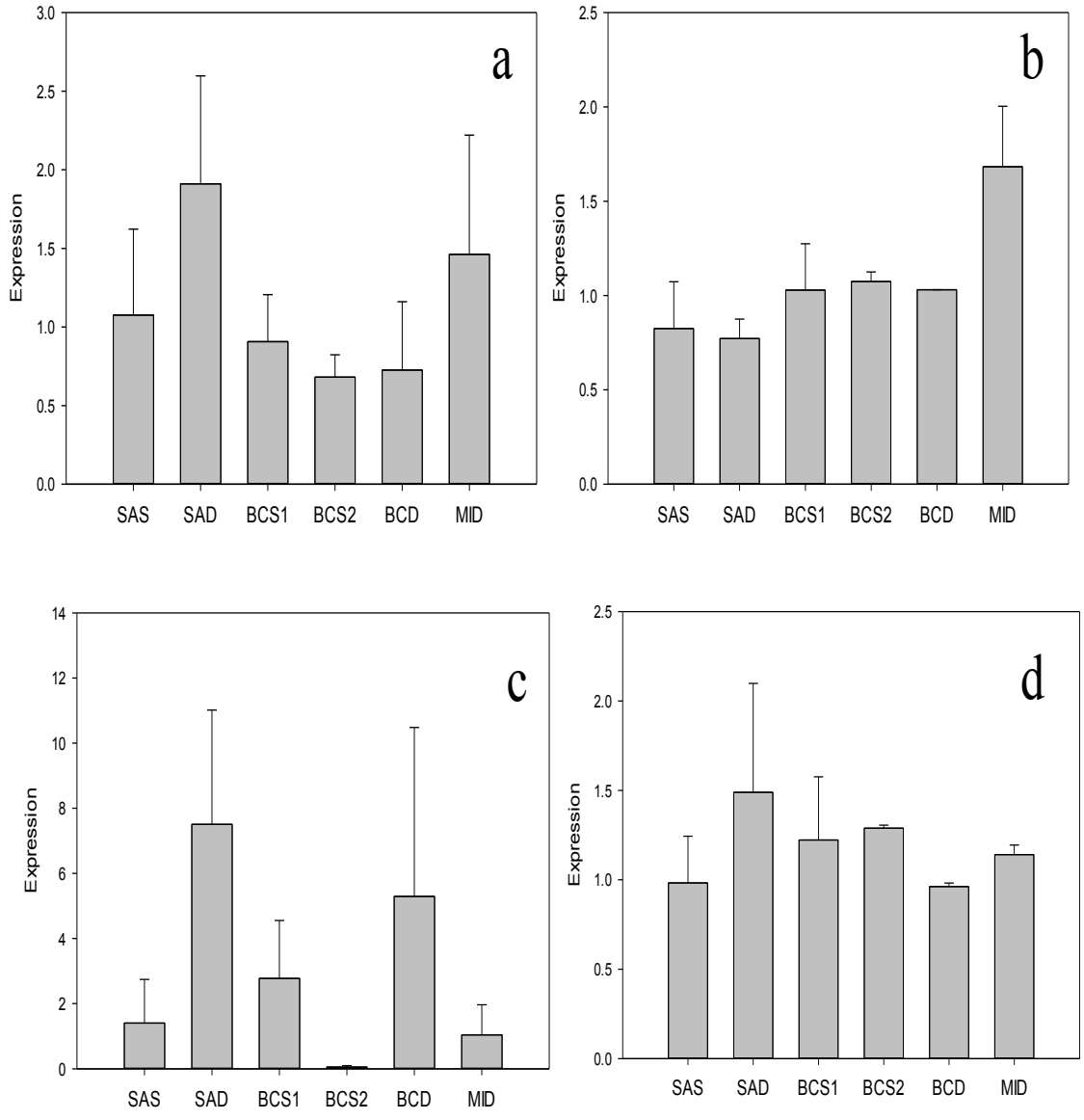

Figure 4.6: Immune and oxidative stress gene expression results for haemocyte tissue Year 1-Initial time for (a) defensin, (b) heat-shock protein 70, (c) glutathione S-transferases p and (d) superoxide dismutase (n=3). Results are mean (± SE). SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep. No significant differences were detected.

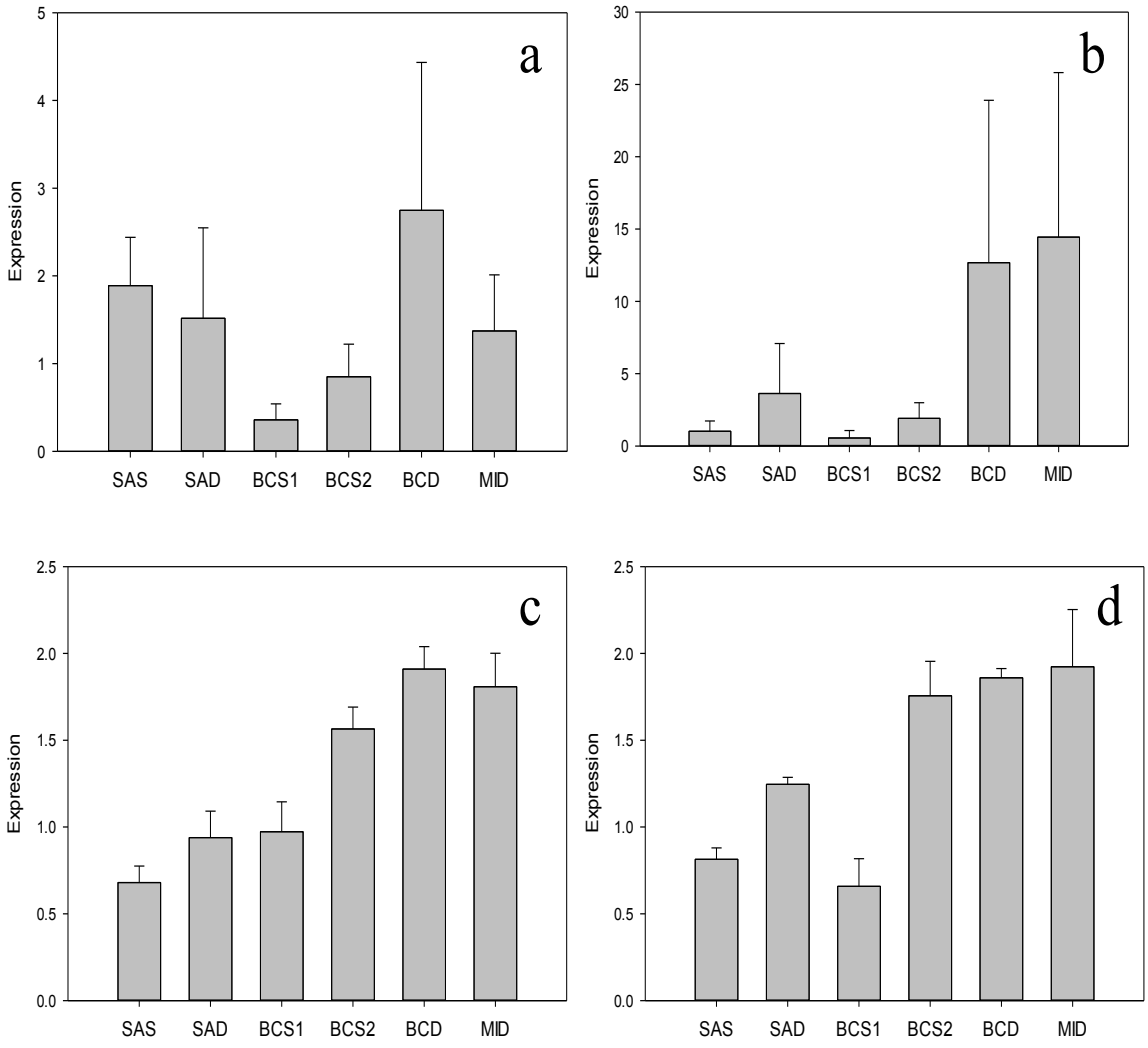

Figure 4.7: Immune and oxidative stress gene expression results for haemocyte tissue Year 1-4 months for (a) defensin, (b) heat-shock protein 70, (c) glutathione S-transferases p and (d) superoxide dismutase (n=2-3). Results are mean (± SE). SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep. No significant differences were detected.

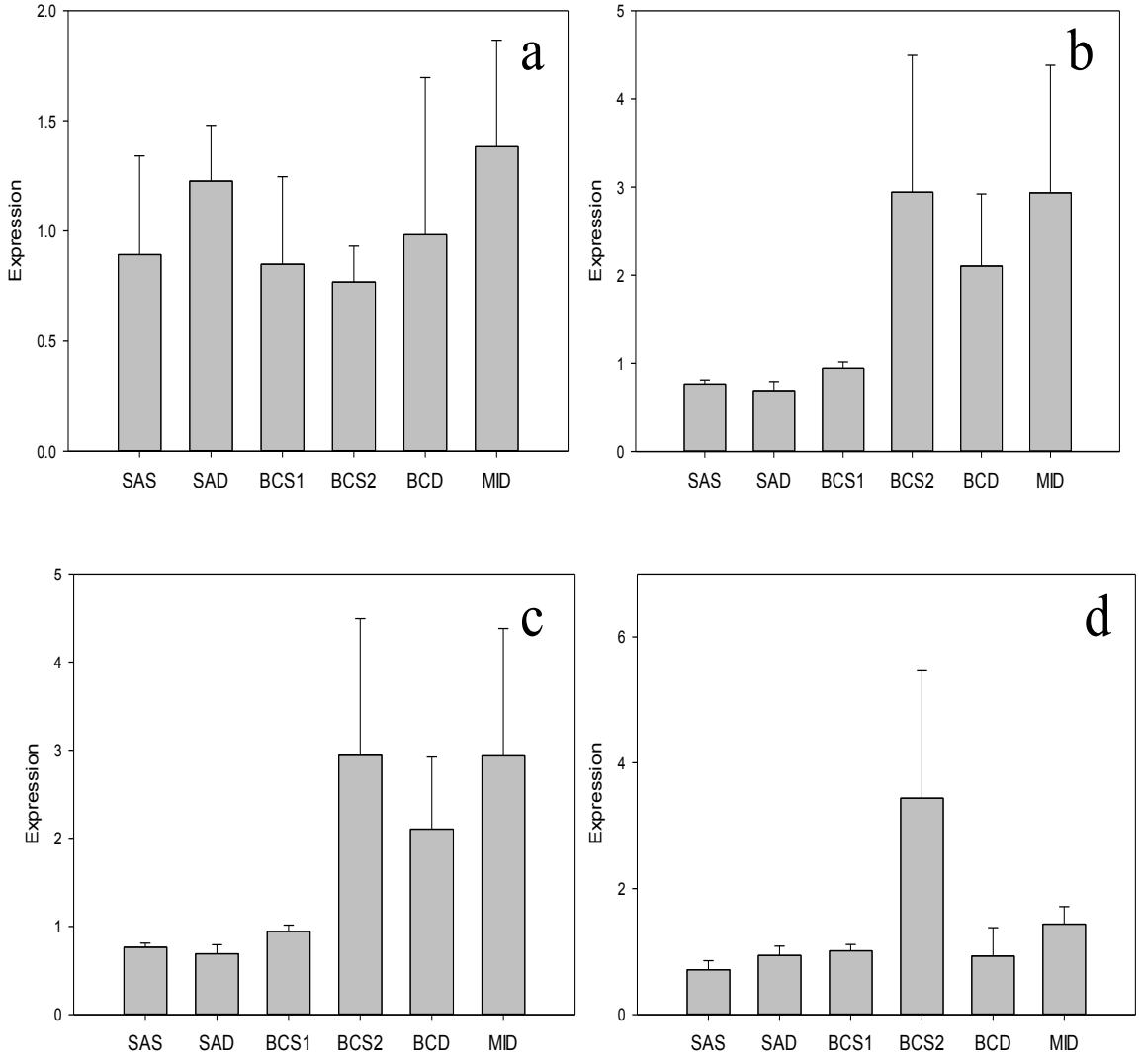

Figure 4.8: Immune and oxidative stress gene expression results for haemocyte tissue Year 1-8 months for (a) defensin, (b) heat-shock protein 70, (c) glutathione S-transferases p and (d) superoxide dismutase (n=2-3). Results are mean (± SE). SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep. No significant differences were detected.

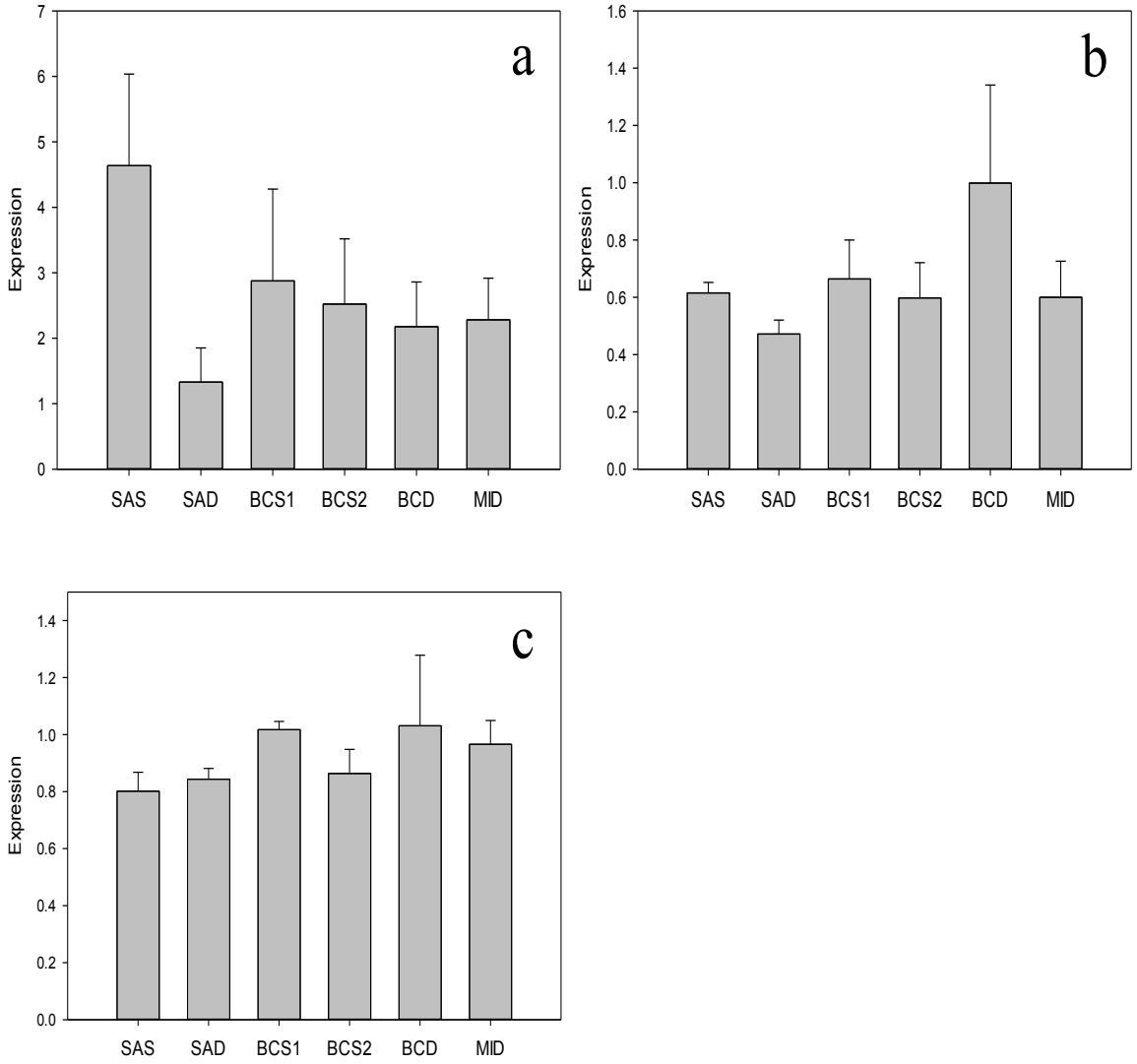

Figure 4.9: Immune and oxidative stress gene expression results for haemocyte tissue Year 1-12 months for (a) defensin, (b) heat-shock protein 70, (c) superoxide dismutase (n=2-3). Glutathione S-transferases p was excluded from the analysis, due to low/no detectable expression. Results are mean (± SE). SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep. No significant differences were detected.

4.3.2.2. Year 2

In Year 2 only gill tissue samples were analyzed, due to the poor quality of haemolymph and haemocytes collected, leading to a low number of available good quality samples for RT-qPCR, thus not allowing adequate sample size for statistical analysis. In Newfoundland winter 2014 was colder than usual; ice formation in the bay did not allow sampling in January 2014 (4-month time point), therefore tissues were not collected and results for 4-months samples are missing.

4.3.2.2.1. Expression of immune and oxidative stress genes in mussel gill

GAPDH, EF1 and histone (Table 4.1) were used as reference target genes for gill tissue expression at the initial time point. No differences were found among sites or between shallow and deep water for physiological stress expression at this time point (Figure 4.10).

ATP5B, EF1 A and GAPDH (Table 4.1) were used as reference target genes for gill tissue expression at the 8-month time point. Heat-shock protein showed lower expression in Bulley's Cove deep compared to South Arm shallow, Bulley's Cove shallow 1 and South Arm deep ($p<0.001$) (Figure 4.11b). Bulley's Cove shallow 2 showed lower heat-shock protein expression than South Arm shallow and South Arm deep ($p<0.001$) (Figure 4.11b).

ATP5B, Histone and GAPDH (Table 4.1) were used as reference target genes for gill tissue expression at the 12-month time point. Also at this time significant differences among sites were observed for heat-shock protein expression. Bulley's Cove shallow 1 showed lower expression levels compared to South Arm shallow and South Arm deep ($p<0.001$), while Bulley's Cove deep showed lower expression levels than South Arm deep ($p<0.001$) (Figure 4.12b).

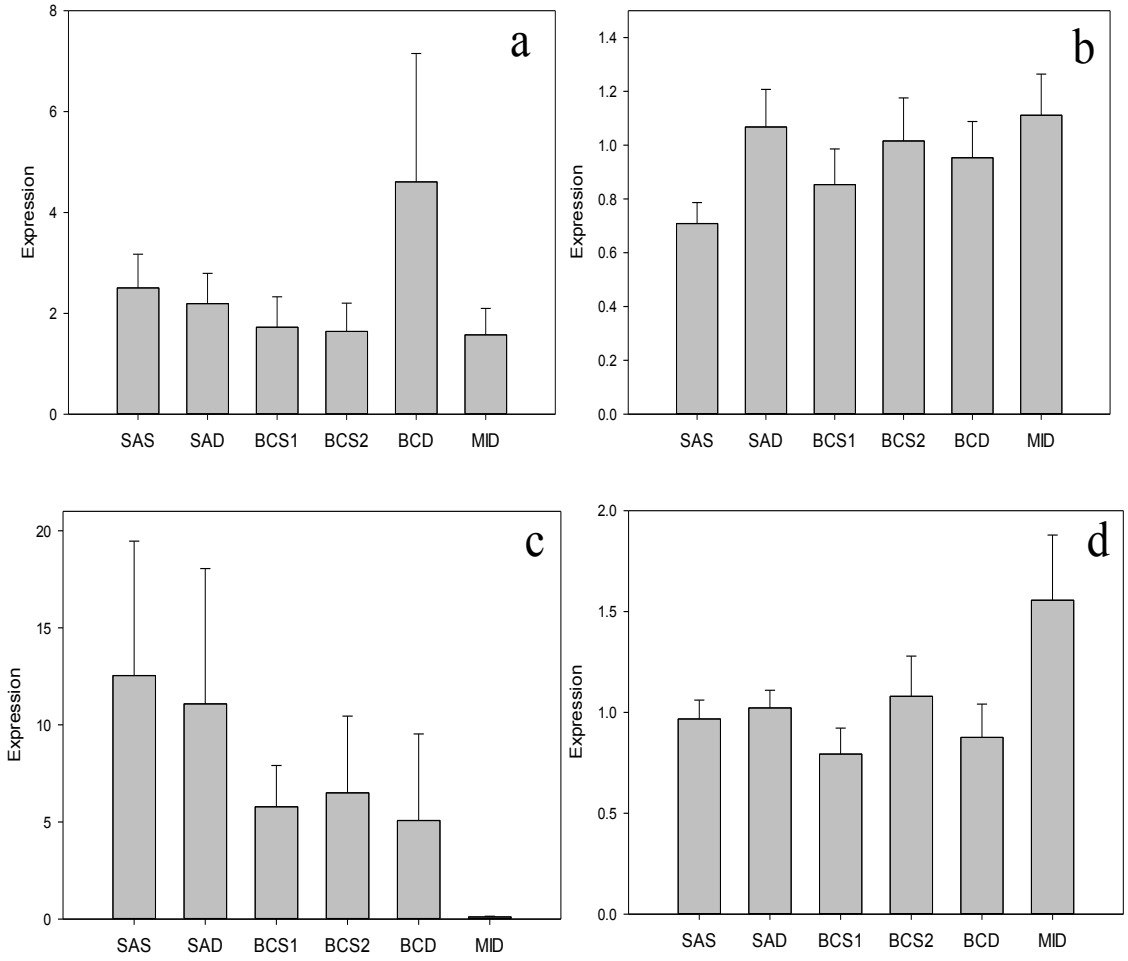

Figure 4.10: Immune and oxidative stress gene expression results for gill tissue Year 2-initial time for (a) defensin, (b) heat-shock protein 70, (c) glutathione S-transferases p and (d) superoxide dismutase (n=3-10). Results are mean (± SE). Letters represent statistical significance. SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep. No significant differences were detected.

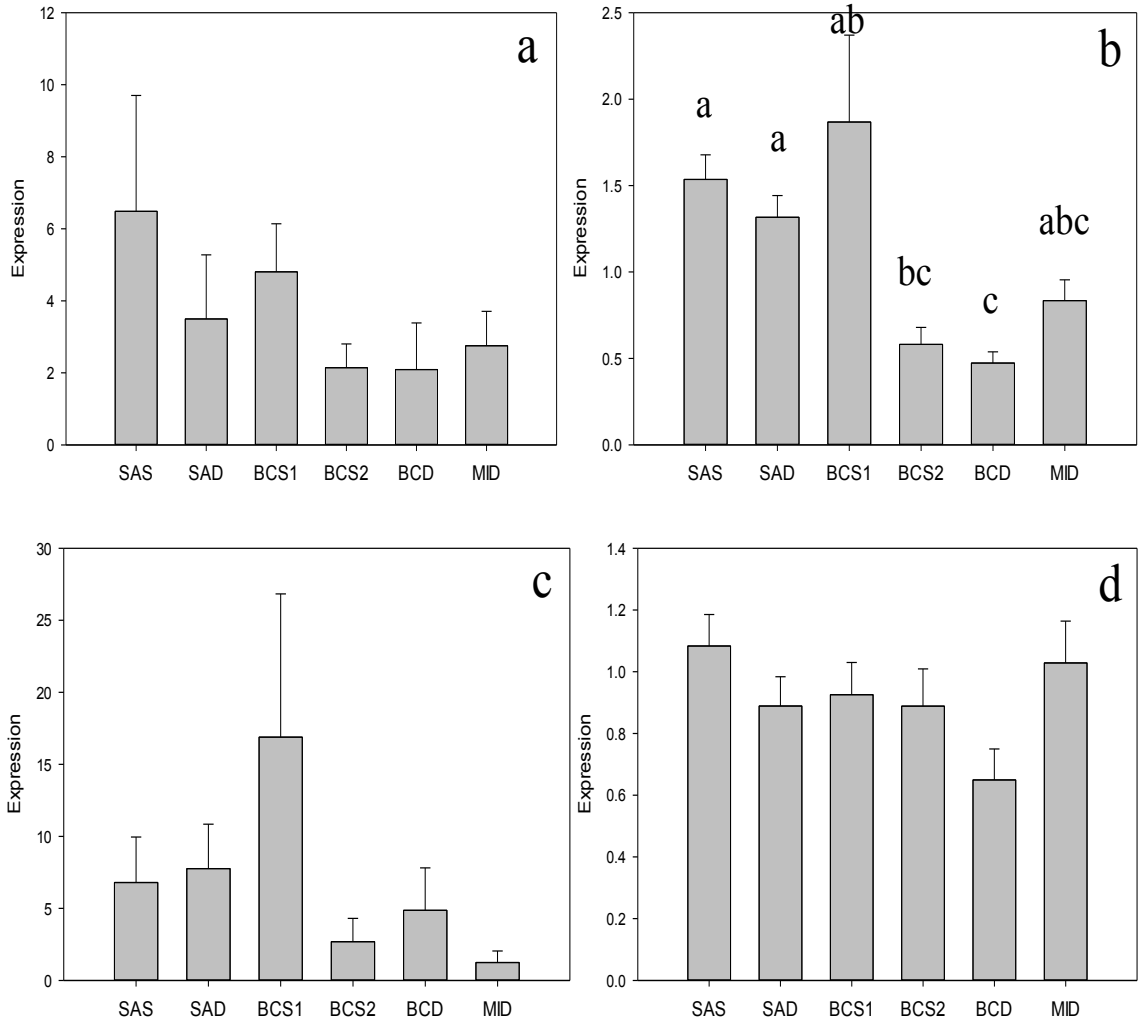

Figure 4.11: Immune and oxidative stress gene expression results for gill tissue Year 2-8 months for (a) defensin, (b) heat-shock protein 70, (c) glutathione S-transferases p and (d) superoxide dismutase (n=4-10). Results are mean (± SE). Letters represent statistical significance (sites represented by different letters differ for stress response). SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep.

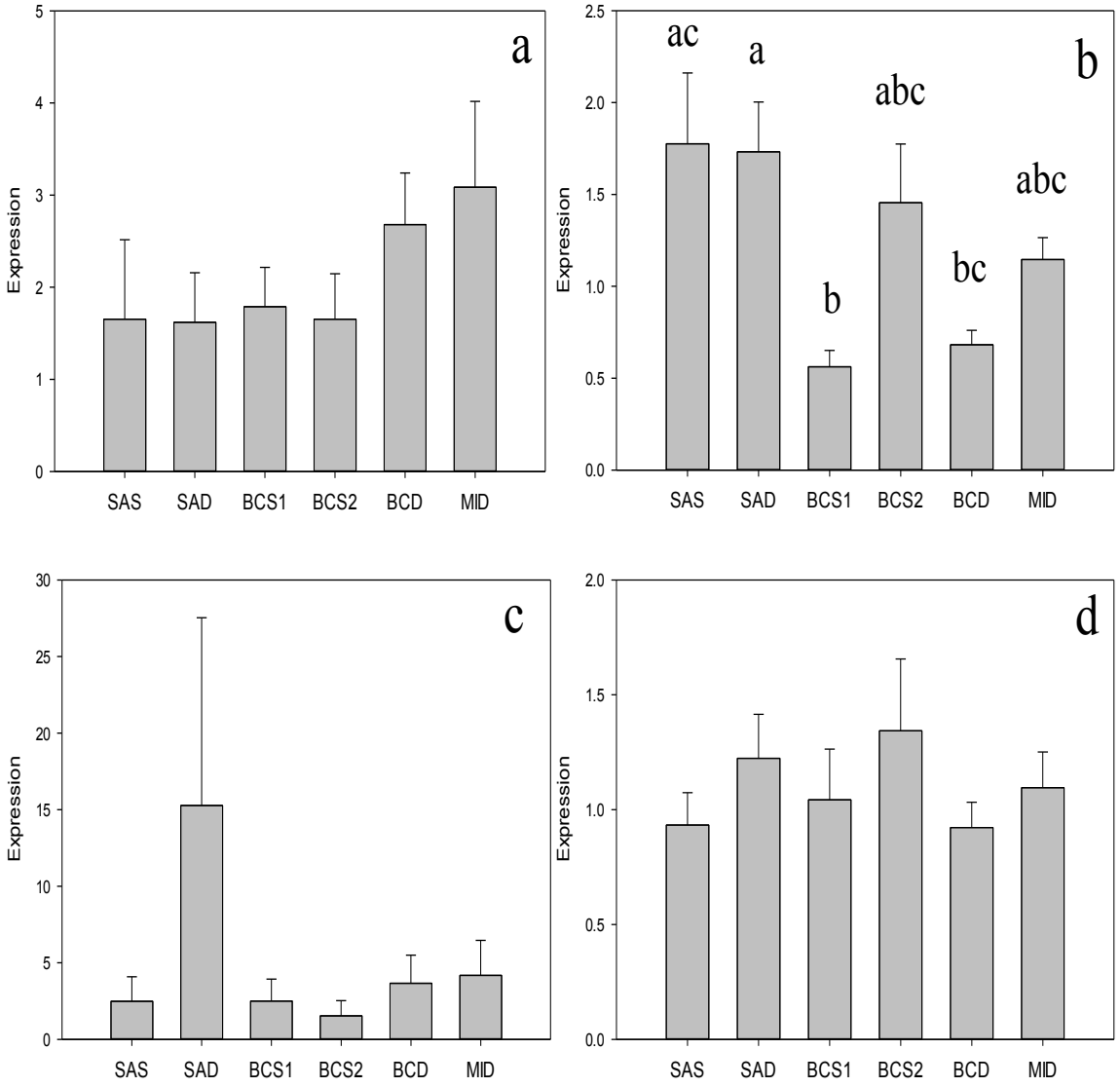

Figure 4.12: Immune and oxidative stress gene expression results for gill tissue Year 2-12 months for (a) defensin, (b) heat-shock protein 70, (c) glutathione S-transferases p and (d) superoxide dismutase (n= 9-10). Results are mean (± SE). Letters represent statistical significance (sites represented by different letters differ for stress response). SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep.

4.4. Discussion

Gene expression analysis is an important tool in many biological fields, providing physiological information on cellular function in response to environmental conditions. Typically, this involves using RT-qPCR methods for either small-scale studies or as validations of larger scale studies (RNA-Seq and microarray). In order to obtain reliable results, accurate normalization using reference genes is necessary (Vandesompele *et al*., 2002). Reference genes need to demonstrate stable expression in the tissue investigated and under the experimental conditions examined. However, reference gene stability can vary substantially under different experimental conditions and treatments, thus affecting the reliable quantification of genes of interest (Vandesompele *et al*., 2002). Single gene normalization has been effective in many studies, particularly those involving established model organisms or artificially selected cohorts (i.e., Meyer *et al*., 2013; Caspillo *et al*., 2014; Feng *et al*., 2014). However, this common practice can lead to flawed normalization, especially outside model systems, as genes may prove to be unstable and differentially expressed (Vandesompele *et al*., 2002). Therefore it is clear that evaluation studies to identify valid reference genes are critical for any such gene expression study, and must be performed for the particular species, tissue and experimental conditions evaluated.

In this study, it was impossible to use one single housekeeping gene as a normalizer. A candidate reference target is determined to be stably transcribed if transcript expression fell within 0.5 cycles for all samples tested; no candidate was expressed within 0.5 cycles for a third of the samples tested in the present study. In fact the minimum number of

cycles for transcript expression was within three. Consequently, the use of GeNorm (Vandesompele *et al*., 2002; qBASE+, Biogazelle) was implemented in order to find the optimal number of reference targets for *Mytilus edulis* gill tissue sampled from mussels cultured at different depths. This approach was necessary to obtain accurate quantification and quality assessment of the genes of interest and to generate biologically relevant results (Bustin *et al*., 2005).

For this study, 19 primers sets were initially selected to be tested as reference targets; six of these did not pass quality testing due to the presence of multiple peaks or low amplification efficiency. Among the primer sets that did not pass quality testing were two Ribosomal subunit 28S (*Mytilus galloprovincialis* and *Mytilus edulis*; Table 4.1); 28S is a commonly used normalizer in *Mytilus* studies and it has also been used as single housekeeping gene (Cellura *et al*., 2006; Li *et al*., 2008). From the thirteen primer sets that passed quality testing, four pairs were excluded from further analysis due to functional similarity with other transcripts (Table 4.1). The remaining nine primer sets were used for a GeNorm experiment (Table 4.3).

The optimal number of reference targets used for normalization suggested by GeNorm for the Year 2- initial time point experiment used as an example was three, which were highly stable and robust for further gene expression analysis of these tissues and conditions. A higher number of targets could have been used, as shown in Fig. 4.1b, but was not necessary for this study. Allocation of resources to include the identification of suitable reference genes will need to be taken into consideration for any reverse transcription qPCR study, to provide robust and informative results. The most stable reference targets for *Mytilus edulis* gill tissue from the Year 2- initial time point were

elongation factor 1 (EF1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and histone H3 (HH3). The elongation-factor 1 complex mediates the enzymatic delivery of aminoacyl tRNAs to the ribosome; EF1 is a protein involved in translational elongation. The latter presents stable expression in *M. edulis* at different stages of gametogenesis (Cubero-Leon *et al*., 2012), in the ovary of soft-shell clam *Mya arenaria* (Araya *et al*., 2008), in flat oysters *Ostrea edulis* (Morga *et al*., 2010), and king scallops, *Pecten maximus* (Mauriz *et al*., 2012), challenged with bacterial infections.

The enzyme GAPDH takes part in the glycolysis process and can initiate apoptosis (Tarze *et al*., 2007). This enzyme has been found to be a suitable reference target in *Ostrea edulis* challenged with bacterial infection (Morga *et al*., 2010), in testis of the giant scallop *Pecten maximus* (Mauriz *et al*., 2012), in larvae of Yesso scallop *Patinopecten yessoensis* (Feng *et al*., 2013) and in different tissues of oyster *Crassostrea gigas* (Dheilly *et al*., 2011). However, in other studies GAPDH was unsuitable as a reference target, probably due to its multiple functions (Lacroix *et al*., 2014).

The family of histone proteins is a constituent of eukaryotic chromatin and it has been divided into linker histones (H1) and core histones (H2A, H2B, H3 and H4); it is the most conserved of the eukaryotic proteins (Miller *et al*., 1993; Bouilly *et al*., 2010). HH3 has been suggested as reference target for *Patinopecten yessoensis* larvae (Feng *et al*., 2013).

Organisms and cells require a defence against reactive oxygen species (ROS), which are produced by aerobic metabolism. In order to inactivate ROS, antioxidants are produced; however, when ROS are present in greater numbers than antioxidants and such production exceeds their degradation by antioxidant systems, a state of physiological imbalance, called oxidative stress, is generated, potentially leading to cellular damage

(Sies, 1993; Lesser, 2006; Rola *et al*., 2012). The damage created by ROS may affect biological molecules, such as DNA, lipids, protein and carbohydrates in different ways; ROS can react with cell membrane lipids and, through peroxidation, affect enzyme activity and ATP production. They can also attack proteins and modify amino acids; additionally, they can inflict deletions and mutations in DNA (Sies, 1993; Lesser, 2006; Rola *et al*., 2012). Bivalve molluscs produce ROS in response to xenobiotics, changes in temperature and salinity, and during the cell-mediated immune response to pathogens. Bivalves are, however, generally resistant to normoxic-hypoxic-anoxic cycles, producing less ROS than other species in similar conditions and thus avoiding oxidative stress (Lesser, 2006; Li *et al*., 2009; Li *et al*., 2010).

Defensin, a small antimicrobial peptide involved in host defense, is positively influenced by temperature and salinity and has a high variability in expression at the individual level, but no significant inter-population differences (Li *et al*., 2009; Li *et al*., 2010; Núñez-Acuña *et al*., 2012). In this study defensin differed among sites only once. This happened at the beginning of the first year of the experiment, and could suggest that the environmental conditions present when the seed were collected might have indicated an immune significant host response. Moreover, the high variability at individual mussel level could also have contributed to the different expression of defensin (Li *et al*., 2009; Li *et al*., 2010; Núñez-Acuña *et al*., 2012).

Heat-shock proteins (HSP) are molecular chaperones that characterize heat shock response; they are involved in the mitigation of several stressors, such as temperature, hypoxia, pH, salinity and metal pollution; they also act as a signal to promote immune response under stress conditions such as bacterial infection (Lyons *et al*., 2003; Cellura *et*

al., 2006; Dutton & Hofmann, 2009; Rola *et al*., 2012). Heat-shock protein 70 (HSP 70), in particular, is a reliable indicator of stress in response to acute and long-term environmental variability (Dutton & Hofmann, 2009). Its response varies as a function of seasonal acclimatization and vertical zonation (Dutton & Hofmann, 2009; Núñez-Acuña *et al*., 2012); however, the patterns of HSP variation are complex and include not only latitudinal gradients but also large-scale abiotic influences, such as tidal cycles, wind speed, air temperature and precipitation (Dutton & Hofmann, 2009). Also, in *Mytilus* spp., HSP 70 is up-regulated at lower temperatures in mussels originating from higher latitude and therefore acclimated to colder temperatures (Dutton & Hofmann, 2009; Li *et al*., 2010). Moreover, in low salinity, HSP 70 appears down-regulated in *Mytilus edulis* (Lyons *et al*., 2003). In this study, HSP 70 is the gene of interest that presented the highest variability among sites, possibly reflecting the wide range of environmental parameters and conditions affecting its expression. It is interesting to note that HSP 70 varied in the fall and spring, when the environmental conditions were most different between sites, due to the presence of the thermocline, phytoplankton blooms and possibly due to the amount of precipitation. It did not, however, vary during winter, when the environment of shallow and deep water was similar. It is also interesting to point out that most of the time the lowest expression of HSP 70 was found in Bulley's Cove deep and Mouse Island deep. Results of this study suggest that HSP 70 could be a good and sensitive gene of interest to investigate environmental stress response in gills of *Mytilus edulis*. However, the large number of environmental parameters correlated to this protein expression make it difficult to differentiate what exact environmental characteristics affected it.

Glutathione-S-transferases (GSTs) are a group of enzymes that support oxidative stress response, inactivating reactive oxygen species (ROS). Their role is one of prevention, protecting against the formation of ROS which could generate other cascade products leading to damage. Essentially, GSTs connect the attacking reactive oxygen species to a less harmful product (Sies, 1993; Manduzio *et al*., 2004; Rola *et al*., 2012). In *Mytilus edulis* they can be used as an indicator of chemical pollution in the marine environment (Manduzio *et al*., 2004). The expression of GSTp in this study only once, during winter, presented differences between the sites examined, and showed high individual mussel variability, often making it impossible to detect significant differences between sites, due to a very high standard deviation between samples. Moreover, many mussels presented very low or non-existent GSTp expression. Low GST activity in bivalves has been noted in previous studies (Lee, 1988). Based on these results, it is suggested that GSTp is a less suitable gene of interest in investigating environmental stress response, especially in complex experimental field studies such as this one.

Superoxide dismutase (SOD) is an antioxidant enzyme responsible for dismutase O_2^- to H_2O_2; its role is to intercept a damaging species and deactivate it in order to avoid further activity, i.e., forming a nonradical end product from a radical compound (Sies, 1993; Manduzio *et al*., 2004; Lesser, 2006; Rola *et al*., 2012). Its mRNA levels increase in response to mechanical, chemical and biological stress, such as heat shock, metal and polycyclic aromatic hydrocarbon (PAH) exposure, as well as low chlorophyll *a* levels (Solé *et al*., 1994; Núñez-Acuña *et al*., 2012; Rola *et al*., 2012). This enzyme has been suggested to have a central role in the antioxidant defence in *Mytilus* (Solé *et al*., 1994). In the present study SOD varied between sites only in Year 1 (at the beginning and during

the winter). During winter, two of the three deep water sites showed higher expression of SOD than their respective shallow water sites; neither temperature nor chlorophyll *a* levels can explain this difference. It is possible however, that different environmental mechanical forcing could have acted on shallow and deep water sites at this time.

During the first year of study, unexpectedly at the initial time point, differences were found between sites for physiological stress response in gill tissue; three of the four genes of interest showed different expression. This was not expected since the mussels came from the same seed collectors and were graded and placed in the field a maximum of two weeks before the start of the experiment. The amount of time the mussels were exposed to shallow or deep water culture appears to be too short to result in physiological stress. However, it is possible that the mussels were still subjected to a period of acclimation, when initially sampled; this could explain the differences in expression for defensin, HSP70 and SOD. Interestingly, for both heat-shock protein and superoxide-dismutase the highest expression level is shown in Bulley's Cove shallow 1 and the lowest in Mouse Island deep. Also, in both years, mussels from Mouse Island deep showed expression of the genes of interest to be either similar to all the other sites or lower, but never higher. Furthermore, at the end of Year 1 no differences were found for oxidative stress expression in gill tissue, possibly indicating that the effects observed previously were not chronic and long lasting. No effect of culture condition on physiological stress gene expression was found in haemocytes.

Some seasonal variability was observed in gene expression, as highlighted in previous studies (Power & Sheehan, 1996; Manduzio *et al*., 2004; Li *et al*., 2009; Li *et al*., 2010). During fall sampling times (Year 1-initial time point and Year 2- 12 month time point)

differences among sites for defensin, HSP 70 and SOD expression were present. However, the other two fall sampling times, 12 months in Year 1 and initial time point in Year 2, did not show any significant differences in physiological stress. It is important to point out that the mussels for these two sampling points were collected within 3 weeks of each other. In both years, during spring sampling the sites exhibited different expression levels for HSP 70, while in the winter of Year 1 SOD and GSTp differed among sites; it has been reported in other studies that a higher GST activity in gills during the winter is possible, when the other antioxidant levels are low. During winter, significant biochemical changes occur in mussel gills, possibly affecting this tissue-specific antioxidant defence mechanism (Power & Sheehan, 1996; Manduzio *et al*., 2004).

4.5. Conclusion

The present study highlights the need for a thorough and robust method for selecting and validating reference targets for RT-qPCR normalization. Primer sets for previously used reference targets must be tested for each new investigation, as stability may not be conferred across or between experiments, under different experimental conditions, and for samples of different origins. Additionally, their presence in the published literature does not guarantee primer quality and specificity; thus it is necessary to always perform primer quality control, regardless of a candidate reference source. In this study, previously used reference targets may not have been suitable, due to the origin of the *Mytilus edulis* population used. Moreover, in Newfoundland, cultured mussel seed are collected from the

wild and therefore the local population presents higher variability in comparison to hatchery-produced blue mussels. The results of this experiment also support the use of GeNorm (Vandesompele *et al*., 2002; qBASE+, Biogazelle) analysis when selecting appropriate reference targets. This software may expedite selection, and also provides a series of genes that will be more suitable as stable reference targets. The use of a single reference gene increases the susceptibility to improper normalization and, therefore, to questionable conclusions. It is strongly recommended that the selection and validation of appropriate reference targets should always be included in the methodology section of papers presenting gene expression studies. This practice would increase confidence in the accuracy of the results shown, since the variation in expression of the genes of interest is dependent on the stability of the reference genes (Lacroix *et al*., 2014).

Culture depth does not appear to affect the physiological stress response in *Mytilus edulis*. When differences are present between the sites examined in this study, they do not seem to have a chronic or long-lasting effect on mussels; differences in stress response are only temporary and appear to be seasonal. From this study it also appears that gills would be a better and more sensitive tissue to examine for evidence of physiological stress, compared to haemocytes, which did not present any significant stress effect during this study. Finally, it is interesting to point out that the Mouse Island deep site does not show at any time point higher physiological stress than all the other sites; this difference could be explained by the location of this particular site (offshore open water compared to all the other sites examined in this study; Appendix B- Figure B1,2).

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CHAPTER 5

Condition and biochemical profile of blue mussels (*Mytilus edulis* L.) cultured in deep and shallow water sites in Notre Dame Bay, Newfoundland

5.1. Introduction

Newfoundland blue mussel (*Mytilus edulis*) aquaculture is undergoing a period of expansion in production, due to utilization of existing culture sites and development of new sites throughout the Province. Typically, mussel culture sites are situated in sheltered areas nearshore, such as river mouths, estuaries, harbours and shallow bays. However, mussel culture in nearshore zones is subjected to a number of issues of concern. These zones can be affected by land runoff, especially during times of pronounced precipitation and therefore they can be exposed to contaminants of land origin (i.e., bacteria, industrial pollution, fertilizers). Also, user conflicts in coastal zones, i.e., aquaculture versus commercial and recreational activities, have increased (Cheney *et al*., 2010). Production and ecological carrying capacity limits can be exceeded in shallow nearshore locations, and food depletion can limit mussel production (McKindsey *et al*., 2006; Cheney *et al*., 2010; Duarte *et al*., 2012). Moreover, aquaculture-related benthic deposition and impact, due to mussel drop-off from long lines and organic material associated with mussel biodeposits, have become important issues of concern (Hartstein & Rowden, 2004; Fabi

et al., 2009; Frechette, 2012). These concerns about nearshore, shallow water bivalve culture have stimulated interest in developing offshore deep water bivalve culture (Cheney *et al*., 2010). Moving farms to more offshore deep water sites may help to address different issues: exposure to contaminants of land origin can be decreased or eliminated, production and ecological carrying capacity limits can be increased and consequently food depletion avoided and environmental effects of the culture diminished (McKindsey *et al.*, 2006; Cheney *et al*., 2010; Duarte *et al*., 2012). Previous studies have indicated that deep water chlorophyll *a* maximum layers are possible due to thermal and saline stratification; thus taking advantage of chlorophyll *a* maximum layers could avoid phytoplankton shortages due to an expansion of bivalve culture (Ogilvie *et al*., 2004).

Recent studies indicate that benthic deposition in offshore deep water mussel culture sites has minimal effects on the benthic domain and is comparable to reference sites without aquaculture influence (Fabi *et al*., 2009; Frechette, 2012). These observations suggest that deep water offshore mussel culture would have a lower impact on the environment than nearshore culture and therefore increased sustainability (McKindsey *et al*., 2006; Fabi *et al*., 2009; Cheney *et al*., 2010; Duarte *et al*., 2012; Frechette, 2012). Potential improvement in water quality and concentrated food sources, coupled with lower fluctuations in temperature and salinity, suggest possible improvement in culture conditions. Offshore deep water culture could present a more stable environment and therefore could have the potential to improve mussel condition and health, increasing growth by lowering mortality, reducing turbidity and fouling, and improving water exchange (Cheney *et al*., 2010). Therefore, changes in culture technology and

improvements in protocols have the potential to increase the environmental sustainability of mussel aquaculture in Newfoundland and worldwide.

Recent studies have highlighted an improved overall condition for a variety of shellfish species when grown in deep water (Langan & Horton, 2003; Buck, 2007; Yu *et al*., 2010). However, there is a lack of information on how the deep water environment specifically affects condition in the blue mussel (*Mytilus edulis*) compared to the traditional coastal shallow water areas, and how these benefits may be defined. It is important for the industry to understand these relationships and the consequent environmental and production benefits in order to make decisions on the feasibility of deep water sites for mussel culture, since its development may involve considerable effort and expense (Cheney *et al*., 2010).

To evaluate the specific effects of deep water aquaculture on *M. edulis*, blue mussels were grown in deep and shallow water sites under commercial standards and were compared for condition and biochemical parameters for two consecutive years. Condition (condition indices) and biochemical composition responses are useful indicators of nutritional and commercial quality for bivalves; they follow seasonal environmental changes and they are often specific to different bivalve species, varying among and within geographical locations (Orban *et al*., 2002; Hemachandra & Thippeswamy, 2008; Irisarri *et al*., 2015). Condition indices in bivalves are useful tools to determine condition at a single point in time, providing information about the physiological state at a given time, or over a period of time in order to give information about the physiological changes of a population (Lucas & Beninger, 1985). In mussels, biochemical composition varies seasonally and in relation to water temperature, food availability, and reproductive cycle.

When food is abundant reserves are accumulated in the form of lipids, glycogen, and proteins and subsequently utilized for gamete production (Mathieu & Lubet, 1993; Okumus & Stirling, 1998; Freites *et al*., 2002; Orban *et al*., 2002; Kopp *et al*., 2005; Ventrella *et al*., 2008; Karayücel *et al*., 2013). Lipids are the densest form and highest energy yield form in aquatic ecosystems; among them are included the essential fatty acids (EFA), important for ecosystem health and stability. These are often essential nutrients for bivalves and they influence growth, reproduction, and immunity. Included in the EFAs are the omega-3 polyunsaturated fatty acids (PUFAs), in particularly eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) (Parrish, 2013). Lipids also include neutral lipids such as triacylglycerols (TAG) which are used for energy storage, and polar lipids such as phospholipids (PL), a critical constituent of cell membranes (Beninger, 1984; Parrish *et al*., 2009; Martínez-Pita *et al*., 2012). In bivalves, TAGs and PLs are the principal lipid classes detected; TAG has been shown to be the predominant lipid during spring, when energy storage is peaking, while PL is predominant during the rest of the year (Freites *et al*., 2002; Li *et al*., 2007; Prato *et al*., 2010; Martínez-Pita *et al*., 2012).

In *Mytilus edulis* the reproductive cycle can affect both biochemical parameters and condition. In Newfoundland, mussels have been shown to present gametogenesis in spring, with spawning often taking place in July, over a three week period (Thompson, 1984a; Toro *et al*., 2002). However, little is know about differences in reproductive cycle in mussels grown at different depth. The objective of this chapter is to compare cultured blue mussels grown in shallow and deep water culture sites, to highlight differences in condition and biochemical profile between mussels cultured at the two different depths.

5.2. Materials and Methods

5.2.1. Study site, experimental set-up and sampling protocol

This study was conducted between September 2012 and September 2014. During September 2012 mussels from the 2011 year class were collected from three culture sites in Notre Dame Bay, Newfoundland and Labrador, Canada: South Arm, Bulley's Cove and Mouse Island (Appendix B- Figure B1). South Arm included a shallow water site, with headline at 5 m depth, and a deep water site, with headline at 15 m depth. Bulley's Cove included two shallow water sites (5 m) and one deep water site (15 m). Mouse Island included only one deep water site (15 m), due to the frequent presence of pack ice during the spring. Mussels of both sexes were collected in all six sites at an initial time point (September 2012), and after 4 months (January 2013), 8 months (May 2013) and 12 months (September 2013) when experimental mussels were harvested and processed. In September 2013 the Year 1 experiment was concluded and new deployment was completed using 2012 year class seed. In Year 2 mussels were again collected from the 6 sites at an initial time point (October 2013), and following 8 months (May 2014) and 12 months (September 2014). In January 2014 the 4-month time point sampling was suspended, due to extreme winter conditions and the presence of ice preventing access to the sampling sites from December 2013 until May 2014.

Mytilus edulis were cultured using the traditional longline system and harvested using standard commercial protocols. At each time point, five socks were randomly sampled from different areas of the longline (beginning, center and end). After transport to the commercial processing facility each sock was sectioned into 3 parts (top, middle bottom) and the mussels sampled randomly for each analysis (condition measurements, lipid, fatty

acids, and glycogen analysis). Protein content was not included in the analysis due to resources and time restrictions.

5.2.2. Condition analysis

For each time point, 150 mussels were sampled at each of the six study sites; they were transported back on ice to the North Atlantic Fisheries Centre (NAFC) facilities in St. John's for measurement and analysis. For each individual, the valves were separated and the interstitial water drained and the total wet weight was measured to the nearest 0.001 g, after which the meat was carefully dissected from the shell and placed in pre-weighed aluminum trays for dry weight measurement. Meat was dried to a constant weight for 48-72 hours at 80°C (modified from Lutz *et al.,* 1980); shells were allowed to air dry for 48-72 hours at room temperature. Meat and shell dry weight were then measured to the nearest 0.001 g. Condition indices were calculated as the ratio of dry tissue weight to wet tissue weight (total wet weight minus shell weight) and as the ratio of dry tissue weight to dry shell weight × 100 (Lucas & Beninger, 1985; Gallardi *et al*., 2014).

5.2.3. Lipid and fatty acid analyses

5.2.3.1. Lipid classes

A total of 15 mussels were sampled at each time point for lipid extraction, using a modified Folch method (Parrish, 1999). Meat was carefully dissected from the shell and stored in 40 mL glass vials containing 5 mL of chloroform and stored at -80°C until analysis. Lipid classes were determined for 10 individuals, randomly selected from the

original 15 mussels. Samples were allowed to thaw on ice before the addition of 2.5 mL of methanol and subsequent homogenization with a Polytron homogenizer (Brinkmann Instruments). Following homogenization, chloroform: methanol 2:1 solution and chloroform-extracted water (2:1 ratio) were added to the homogenate. The samples were sonicated for 4 min and placed in a -20°C freezer for a further 10 min. The lower, organic layer was then removed using a double pipetting technique (Parrish, 1999). This procedure was repeated at least four times to maximize lipid recovery and all organic layers were combined.

Lipid classes were determined by thin layer chromatography (TLC) with flame ionization detection using an Iatroscan analyzer (MK-6 TLC-F1D) and a three-stage development system to separate lipid classes (Parrish, 1999). The resulting chromatograms were analyzed with PeakSimple software (SRI Instruments, Torrance, CA). Total lipid content was expressed as mg g^{-1} of wet weight and triacylglycerol to sterol (TAG: ST) ratios were calculated.

5.2.3.2. Fatty acids

Fatty acids were determined from the same samples previously used for lipid class analysis. The fatty acid derivatization procedure followed that described by Parrish (1999) with minor modifications. Briefly, 250 µL of the extract was dried under N_2 and then 0.5 mL of hexane and 1.5 mL of 14% boron trifluoride in methanol were added. Samples were then sonicated for 4 min and heated at 85°C for 1.5 h. Subsequently, 0.5 mL of chloroform-extracted water and 2 mL of hexane were added before the upper,

organic layer was removed and placed in 2 mL glass vials, completely dried under N_2 and then re-suspended in 1 mL of hexane. The samples were analyzed as FAME (fatty acids methyl esters) on a HP 6890 Gas Chromatograph FID equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, U.S.A.). The column length was 30 m with an internal diameter of 0.32 mm. The column temperature began at 65°C where it was held for 0.5 min. The temperature was then ramped to 195°C at a rate of 40°C·min^{-1}, held for 15 min then ramped to a final temperature of 220°C at a rate of 2°C·min^{-1}. This final temperature was held for 0.75 min. The carrier gas was hydrogen flowing at a rate of 2 mL·min^{-1}. The injector temperature started at 150°C and ramped to a final temperature of 250°C at a rate of 120°C·min^{-1}. The detector temperature stayed constant at 260°C. Peaks were identified using retention times from standards purchased from Supelco, 37 component FAME mix (Product number 47885-U), Bacterial acid methyl ester mix (product number 47080-U), PUFA 1 (product number 47033) and PUFA 3 (product number 47085-U). Chromatograms were integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2. A quantitative standard purchased from Nu-Chek Prep, Inc (product number GLC490) was used to check the GC column about every 300 samples (or once a month) to ensure that the areas returned were as expected.

5.2.4. Glycogen analysis

A total of 15 mussels were sampled at each time point for glycogen analysis. The mussels were dissected, placed in 20 mL glass vials and quickly frozen on dry ice. Ten randomly chosen samples were then processed using the method described by Gallardi *et al*. (2014).

Briefly, 30% KOH was added to 0.5 g of homogenized mussel tissue (ratio 2:1 by volume). The samples were then heated in a shaking water bath at 100°C for 20 min, vortexed for 30 sec and subsequently chilled on ice for 5 min. After cooling, 200 µL of each sample was transferred to a 4 mL glass vial followed by 200 µL of 95% ethanol. The solution was vortexed again briefly and then placed in a boiling water bath for 15 min followed by the addition of 1.2 mL of lukewarm water. The samples were again briefly vortexed and then allowed to stand at room temperature for 5 min before measuring the glycogen content. This was determined by colorimetric reaction using 25 µL of prepared sample. Ten µL of 80% aqueous phenol and 200 µL of sulphuric acid were added to the samples on 96-well plates. Absorbance was measured using a multi-detection microplate reader (Synergy HT, BIO-TEK) at 490 nm. The concentration of glycogen in the samples was calculated based on a mussel glycogen standard (Sigma, Saint Louis, MO).

5.2.5. Statistical analysis

The collected data were analyzed with Sigmaplot (12.0 and successive versions) statistical and graphical software (Systat software). Data were tested for normality (Shapiro-Wilk test) and equality of variances and associated means ± SE (condition measurements) or ±SD (total lipids, fatty acids and glycogen measurements) were also calculated. Two-way analysis of variance (ANOVA) and the appropriate post-hoc tests (Tukey's test) were conducted on condition, lipids, glycogen and fatty acid variables. When the assumptions of equal variance and normality were not met an ANOVA on ranks was conducted. Significance was set at $\alpha = 0.05$. Multivariate statistical analysis was conducted on lipid classes and fatty acid data using Primer 7 statistical and graphical

software (Clarke & Gorley, 2006). ANOVA tables are presented in Appendix G (Table G12-G13).

5.3. Results

5.3.1. Condition analysis

5.3.1.1. Year 1

In Year 1 of the study, the total wet weight differed between shallow and deep water sites at 4, 8 and 12 months sampling times. Shallow water sites consistently presented a higher total weight than deep water sites ($p<0.001$). Differences in total weight among shallow water sites can be observed at 8 and 12 months (Figure 5.1a). Among deep water sites, Mouse Island mussels gave a lower total wet weight than both South Arm deep and Bulley's Cove deep at 12 months ($p<0.001$) (Figure 5.1a).

Mussel tissue dry weight showed clear differences between shallow and deep sites at 4 and 8 months: the former exhibited a higher weight than all deep water sites ($p<0.001$). At 12 months the dry weight was comparable among shallow and deep water sites, with the highest dry weight being recorded at Mouse Island deep ($p<0.001$). It is interesting to point out that this site presented the same dry weight at 8 and 12 months, while all the other sites lost dry weight between the two sampling times (Figure 5.1b).

The condition index expressed as the dry tissue weight to dry shell weight ratio showed a clear difference between shallow and deep water sites at 4 months. The former showed a higher condition than deep sites ($p<0.001$). At 12 months mussels at Mouse Island deep reached the highest condition ($p<0.001$) with an average of 26.4 compared to

14.9-17.1 for all other sites (Figure 5.2a). Mussels at Mouse Island at 12 months reached a condition comparable to that at 4 months, while all the other sites exhibited lower condition.

At 4 months the dry tissue weight: wet tissue weight ratio showed similar results to the dry weight: shell weight ratio, with the shallow water sites presenting a higher ratio than the deep water sites ($p<0.001$). As with the dry weight: shell weight ratio, at 12 months, mussels at the Mouse Island site presented the higher dry weight: wet weight ratio ($p<0.001$) (Figure 5.2b). It is interesting to note that the two condition measurements in this case present similar results to the dry weight and opposite results to the total wet weight.

Despite the fact that the mussels were graded before socking, shell length showed some differences at the initial time point (Figure 5.3a). Significant differences were found at 4 months, when both Mouse Island deep and Bulley's Cove deep showed a lower shell length than all the shallow sites ($p<0.001$). At 8 months all the deep water sites presented a lower shell length than the shallow water sites ($p<0.001$); at 12 months all the deep water sites exhibited lower shell length than South Arm shallow and Bulley's Cove shallow 1 sites ($p<0.001$), while Mouse Island deep also had a lower shell length than Bulley's Cove shallow 2 ($p=0.016$) (Figure 5.3a). Dry shell weight showed no differences at the initial time point; however, at 4, 8 and 12 months all the shallow water sites showed a consistently higher shell weight than all the deep water sites (p-values from 0.004 to <0.001). Also, Mouse Island deep consistently showed the lowest shell weight among all the study sites (Figure 5.3b).

5.3.1.2. Year 2

In contrast to Year 1 no clear difference between shallow and deep water was noted for total wet weight in Year 2. However, differences were present between single sites at 12 months; Mouse Island presented the lowest total wet weight ($p<0.001$) (Figure 5.1c). Compared to Year 1 the total wet weight of mussels at all sites at 8 and 12 months was lower in Year 2; in Year 1 the average weight at 8 months was 13.88 ±4.34 g (±SD), while in Year 2 it was 7.10 g ±1.98 g. At 12 months the average total wet weight was 21.59 ±5.48 g, while in Year 2 it was 14.40 ±3.84 g (Figure 5.1c).

Similarly, to total wet weight, dry weight in Year 2 did not show clear differences between shallow and deep water sites. However, differences were present between single sites (Figure 5.1d): at 12 months Bulley`s Cove shallow 1 and Mouse Island were the sites with lowest dry weight ($p<0.001$) (Figure 5.1d). Again, in comparison with Year 1 the dry weight at 8 months was lower in Year 2; in Year 1 at 8 months dry weight averaged 1.66 ±0.45 g (±SD) while in Year 2 the average weight was 0.67 ±0.19 g (Figure 5.1d).

The dry weight: shell weight ratio in Year 2 presented a clear difference between shallow and deep water sites at the 12-month sampling time; at deep water sites mussels achieved a higher condition index than at the shallow water ones ($p<0.001$). At 8 months condition was comparable among sites, with the exception of Bulley's Cove shallow 2, which presented a higher condition than all other sites ($p<0.001$) (Figure 5.2c). In Year 2 condition reached at the 8 month sampling was lower than at the same time in Year 1 (28.63 ±4.68 SD and 39.64 ±6.07 respectively).

The dry weight: wet weight ratio presented differences between shallow and deep water sites at 8 and 12 months. At 8 months shallow water sites presented a higher ratio than deep water sites (p-values from 0.033 to <0.001); however, at 12 months this result was reversed, and deep water sites presented a higher ratio than the shallow sites ($p<0.001$). Also, at 12 months shallow water sites presented either a ratio comparable (Bulley's Cove shallow 1 and 2) or lower (South Arm) to the initial time point, while all the deep water sites showed an increased ratio (Figure 5.2d).

As in Year 1, despite grading, some differences in shell length were present at the initial time point (Figure 5.3c). At 12 months mussels at Mouse island deep showed the lowest shell length of all sites ($p<0.001$) (Figure 5.3c). Dry shell weight did not differ among sites at the initial time point. At 8 months mussels at Mouse Island showed a heavier shell than those in South arm shallow, Bulley's Cove shallow 1 and Bulley's Cove deep ($p=0.001$, $p=0.034$ and $p<0.001$, respectively). However, at 12 months Mouse island deep was the site with mussels exhibiting the lowest shell weight ($p<0.001$) (Figure 5.3d); the lower shell weight and lower shell length in Mouse Island at the end of Year 2 may be explained by the period of time that the longline was on the sea bottom at this site, as a result of spring ice dragging the line away from its original location. However, in Year 1 mussels at Mouse Island also presented smaller and lighter shells.

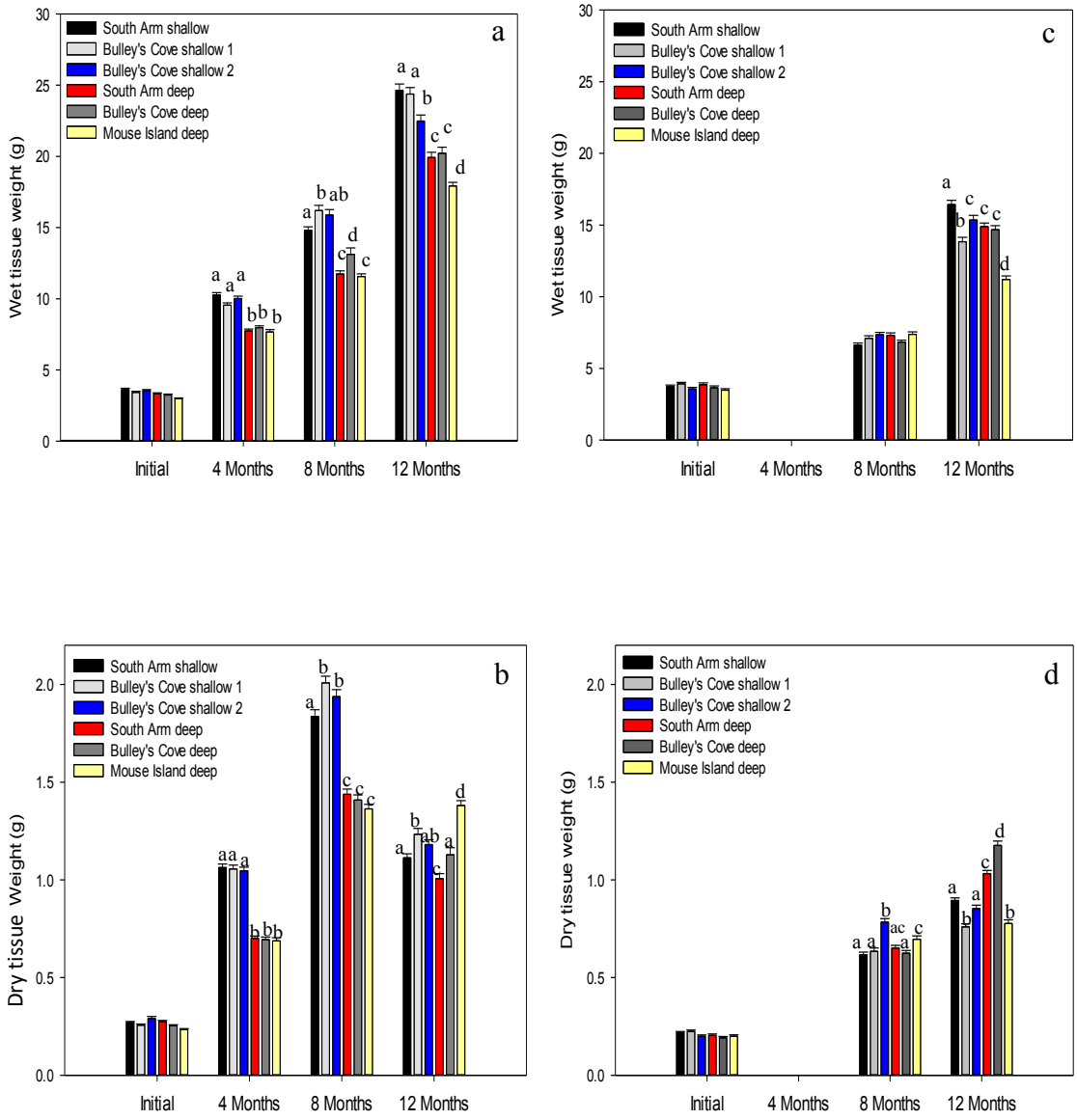

Figure 5.1: Change in mean (± SE) wet tissue weight (a) and dry tissue weight (b) for Year 1 initial time (September 2012), 4 (January 2013), 8 (May 2013) and 12 months (September 2013). Change in mean (± SE) wet tissue weight (c) and dry tissue weight (d) for Year 2 initial time (October 2013), 8 (May 2014) and 12 months (September 2014); (n=150). Letters represent statistically significant differences across sites.

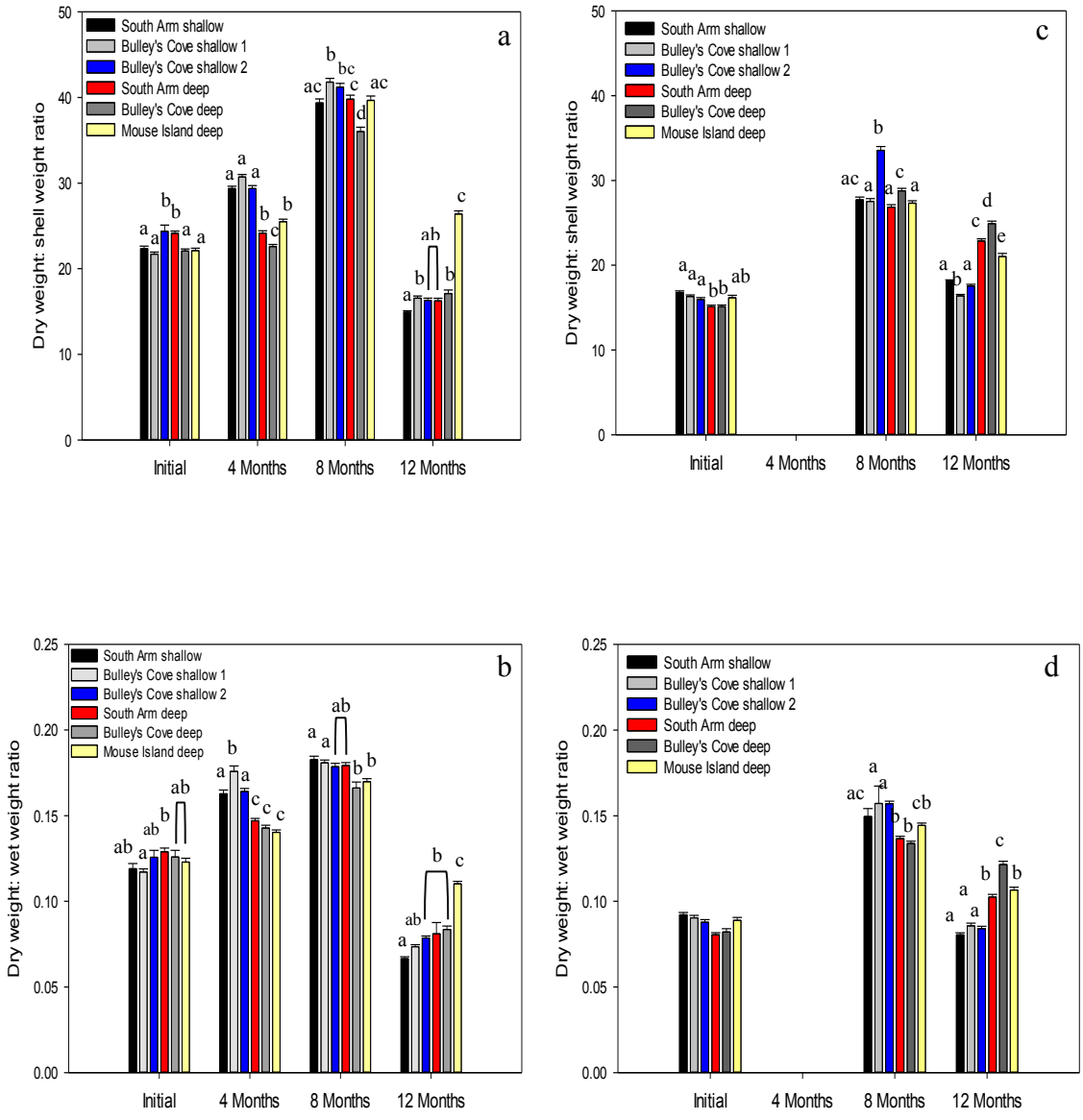

Figure 5.2: Change in mean (± SE) dry weight: shell weight ratio (a) and dry weight: wet weight ratio (b) for Year 1 initial time (September 2012), 4 (January 2013), 8 (May 2013) and 12 months (September 2013). Change in mean (± SE) dry weight: shell weight ratio (c) and dry weight: wet weight ratio (d) for Year 2 initial time (October 2013), 8 (May 2014) and 12 months (September 2014); (n=150). Letters represent statistically significant differences across sites.

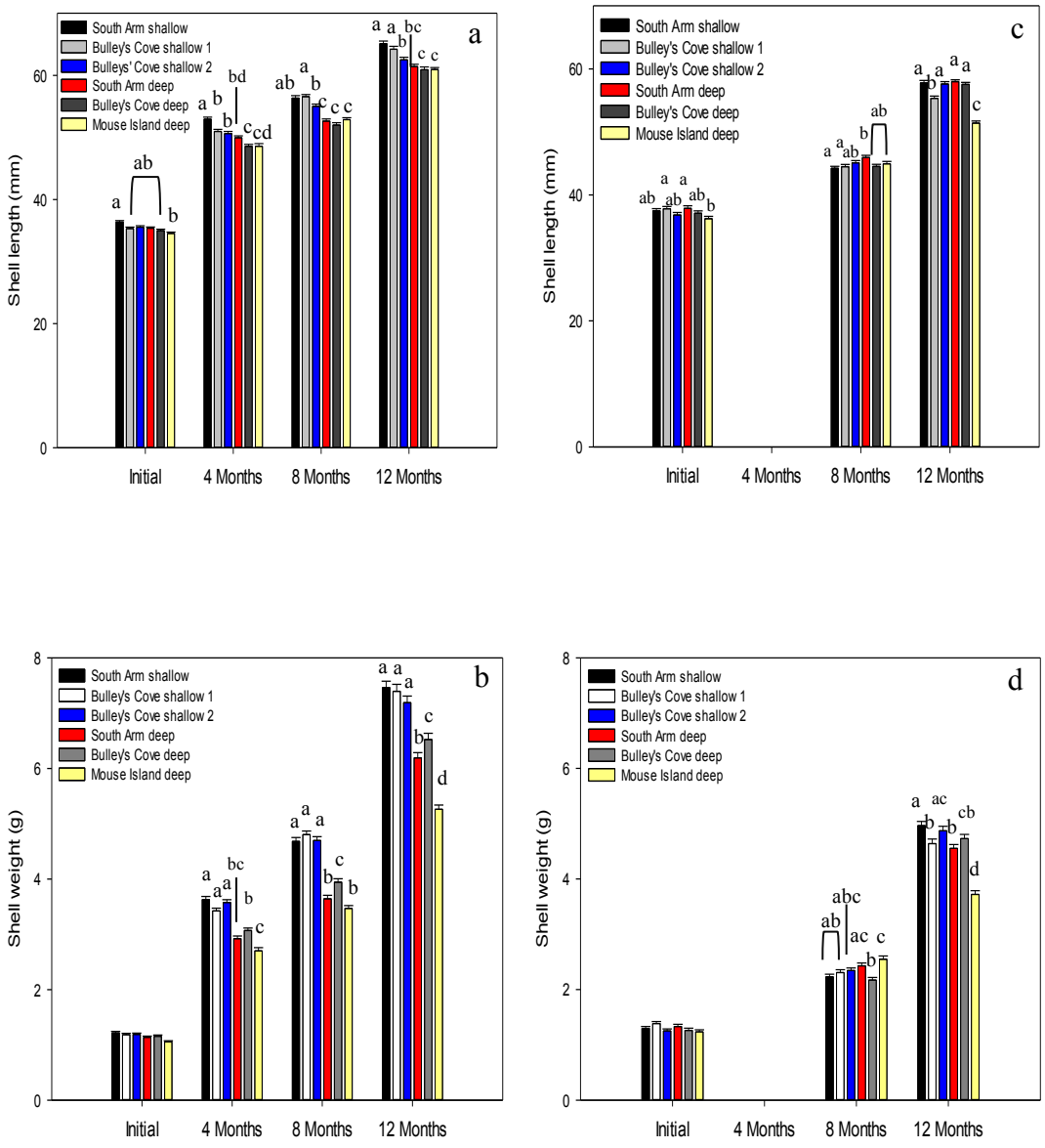

Figure 5.3: Change in mean (± SE) shell length (a) and shell weight (b) for Year 1 initial time (September 2012), 4 (January 2013), 8 (May 2013) and 12 months (September 2013). Change in mean (± SE) shell length (c) and dry shell weight (d) for Year 2 initial time (October 2013), 8 (May 2014) and 12 months (September 2014); (n=150). Letters represent statistically significant differences across sites.

5.3.2. Lipids and fatty acids analyses

5.3.2.1. Lipids

5.3.2.1.1. Total lipids and lipid classes

In Year 1 total lipids in mg g^{-1} of wet tissue weight did not differ among sites or between shallow and deep water; however, differences were found between sampling times. During the winter (4-month time point) total lipids were at their lowest and differed from the initial time point ($p<0.001$), 8 ($p<0.001$) and 12 months ($p=0.013$). The initial time point gave the highest total lipid content compared to all other sampling times ($p<0.001$), while the lipid content between 8 and 12 months remained comparable (Table 5.1).

In Year 2 a similar trend was observed. The initial time point showed the higher total lipid content ($p<0.001$) whereas the 8 and 12 month content did not differ (Table 5.1). The dominant lipid class at all sites during both years was phospholipids; other important lipid classes, as shown from principal components analysis (PCA), included triacylglycerols and acetone mobile polar lipids (Figure 5.4a,b).

In both years, the highest similarity for lipid class proportion among sites is shown at the 12 month sampling time, with 92.7% similarity in Year 1 and 85.8% similarity in Year 2 (Table 5.2). In Year 2 similarity among samples of the same site was lower than that in Year 1; moreover, in Year 2, the similarity decreased between sampling times and among sites when compared to Year 1 (Table 5.2).

5.3.2.1.2. Triacylglycerol: sterol ratio

In both years the triacylglycerol: sterol ratio (TAG:ST) did not differ among sites or between shallow and deep water. In Year 1 differences were found between seasons; spring sampling at 8 months showed the highest ratio compared to all other time points ($p<0.001$) (Table 5.1).

In Year 2 a similar trend was observed with mussels at 8 months again presenting the highest ratio ($p<0.001$), while mussels at the initial time point and at 12 months showed a similar, lower ratio (Table 5.1). It is interesting to observe that the TAG:ST ratio of mussels at all sites at the 8 month sampling (spring) of Year 1 is higher than at the 8 month sampling of Year 2, with an average of 6.41 ±3.74 versus 2.61 ±1.37, respectively.

Table 5.1: Concentration of total lipids, TAG: Sterol ratio, and concentration of glycogen for Year 1 and Year 2 in mussels from shallow and deep water study sites.

Year 1						
	SAS	BCS1	BCS2	SAD	BCD	MID
Time	*Total Lipids ($mg \cdot g^{-1}$ WW)*[1]					
Initial	8.29 ± 5.01^{a}	7.42 ± 4.05^{a}	6.21 ± 3.20^{a}	5.49 ± 2.47^{a}	5.24 ± 1.97^{a}	5.14 ± 3.67^{a}
4 months	0.98 ± 0.43^{b}	1.10 ± 440.7^{b}	1.36 ± 0.51^{b}	1.04 ± 0.58^{b}	1.16 ± 0.72^{b}	1.01 ± 0.66^{b}
8 months	3.52 ± 5.15^{c}	2.58 ± 1.97^{c}	2.44 ± 1.51^{c}	3.38 ± 2.53^{c}	4.12 ± 2.55^{c}	3.04 ± 1.77^{c}
12 months	2.52 ± 1.49^{c}	2.08 ± 0.73^{c}	2.50 ± 1.44^{c}	2.35 ± 1.26^{c}	2.26 ± 1.16^{c}	2.82 ± 1.15^{c}
	TAG/Sterol ratio[1]					
Initial	0.82 ± 0.41^{a}	1.31 ± 0.66^{a}	1.00 ± 0.53^{a}	1.39 ± 0.71^{a}	1.02 ± 0.75^{a}	1.06 ± 0.41^{a}
4 months	2.16 ± 1.77^{b}	3.09 ± 3.41^{b}	2.92 ± 2.77^{b}	1.74 ± 1.29^{b}	2.42 ± 1.87^{b}	5.24 ± 8.21^{b}
8 months	5.45 ± 3.35^{c}	8.41 ± 5.07^{c}	6.79 ± 3.90^{c}	6.66 ± 3.35^{c}	5.13 ± 3.51^{c}	6.02 ± 2.76^{c}
12 months	1.04 ± 0.94^{a}	0.83 ± 0.69^{a}	0.82 ± 0.73^{a}	0.73 ± 1.05^{a}	0.70 ± 0.69^{a}	1.15 ± 1.16^{a}
	Glycogen ($mg \cdot g^{-1}$)[2]					
Initial	$24.8 \pm 9.1^{a\,\alpha}$	$29.7 \pm 6.6^{a\,\alpha}$	21.8 ± 7.1^{a}	$32.5 \pm 7.1^{a\,\alpha}$	$27.3 \pm 17.7^{a\,\alpha}$	$48.1 \pm 23.4^{b\,\alpha}$
4 months	$35.1 \pm 15.9^{A\,\alpha}$	$29.0 \pm 13.2^{AB\,\alpha}$	18.1 ± 6.9^{BC}	$19.9 \pm 11.6^{BC\,\beta}$	$17.9 \pm 6.3^{BC\,\alpha}$	$14.8 \pm 7.7^{C\,\beta}$
8 months	$8.4 \pm 2.7^{\beta}$	$13.6 \pm 5.4^{\beta}$	$17.1 \pm 1.5^{\beta}$	$11.8 \pm 3.6^{\beta}$	$17.9 \pm 1.5^{\alpha}$	$17.4 \pm 1.7^{\beta}$
12 months	$12.3 \pm 5.1^{ab\beta}$	$22.3 \pm 8.6^{a\,\alpha\beta}$	15.4 ± 10.9^{ab}	$15.9 \pm 4.6^{ab\,\beta}$	$6.2 \pm 3.1^{b\,\beta}$	$10.2 \pm 3.9^{b\,\beta}$

Year 2						
	SAS	BCS1	BCS2	SAD	BCD	MID
Time	*Total Lipids ($mg \cdot g^{-1}$ WW)*[1]					
Initial	3.81 ± 4.05^{a}	5.75 ± 4.19^{a}	4.83 ± 3.98^{a}	6.57 ± 8.31^{a}	5.15 ± 3.95^{a}	3.16 ± 2.64^{a}
8 months	1.85 ± 1.24^{b}	2.55 ± 1.97^{b}	1.97 ± 0.84^{b}	1.94 ± 1.09^{b}	2.66 ± 2.68^{b}	2.43 ± 2.29^{b}
12 months	1.73 ± 0.75^{b}	2.97 ± 2.21^{b}	1.94 ± 1.62^{b}	2.08 ± 0.91^{b}	2.22 ± 1.31^{b}	2.16 ± 1.24^{b}
	TAG/Sterol ratio[1]					
Initial	0.33 ± 0.16^{a}	0.73 ± 0.71^{a}	0.79 ± 1.12^{a}	0.67 ± 0.34^{a}	0.87 ± 1.01^{a}	0.62 ± 0.56^{a}
8 months	2.66 ± 1.43^{b}	2.59 ± 1.68^{b}	2.82 ± 1.32^{b}	2.25 ± 1.34^{b}	2.60 ± 1.19^{b}	2.76 ± 1.49^{b}
12 months	0.57 ± 0.50^{a}	0.44 ± 0.29^{a}	0.99 ± 1.14^{a}	1.15 ± 1.01^{a}	1.05 ± 1.08^{a}	1.62 ± 1.90^{a}
	Glycogen ($mg \cdot g^{-1}$)[2]					
Initial	$11.5 \pm 3.8^{a\,\alpha}$	$39.1 \pm 16.1^{b\,\alpha}$	$55.2 \pm 10.2^{c\,\alpha}$	$12.2 \pm 6.6^{a\,\alpha}$	$58.9 \pm 19.4^{c\,\alpha}$	$47.7 \pm 19.1^{bc\,\alpha}$
8 months	$34.6 \pm 13.9^{A\,\beta}$	$16.0 \pm 6.9^{B\,\beta}$	$17.5 \pm 6.8^{B\,\beta}$	$22.7 \pm 7.6^{AB\,\alpha\beta}$	$36.4 \pm 10.5^{A\,\beta}$	$29.2 \pm 9.6^{AB\,\beta}$
12 months	$15.6 \pm 5.2^{a\,\alpha}$	$29.0 \pm 13.1^{a\,\alpha\beta}$	$25.7 \pm 11.6^{a\beta}$	$29.4 \pm 10.3^{a\,\beta}$	$59.2 \pm 17.0^{b\,\alpha}$	$65.3 \pm 19.3^{b\,\gamma}$

Values represent mean ± SD (n= 10; $p<0.05$). [1] Letter superscripts represent statistical significance in time and across sites. [2] Letter superscripts represent statistical significance across sites; Greek letter superscripts represent statistical significance in time. SAS= South Arm shallow; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; SAD= South Arm deep; BCD= Bulley's Cove deep; MID= Mouse Island deep.

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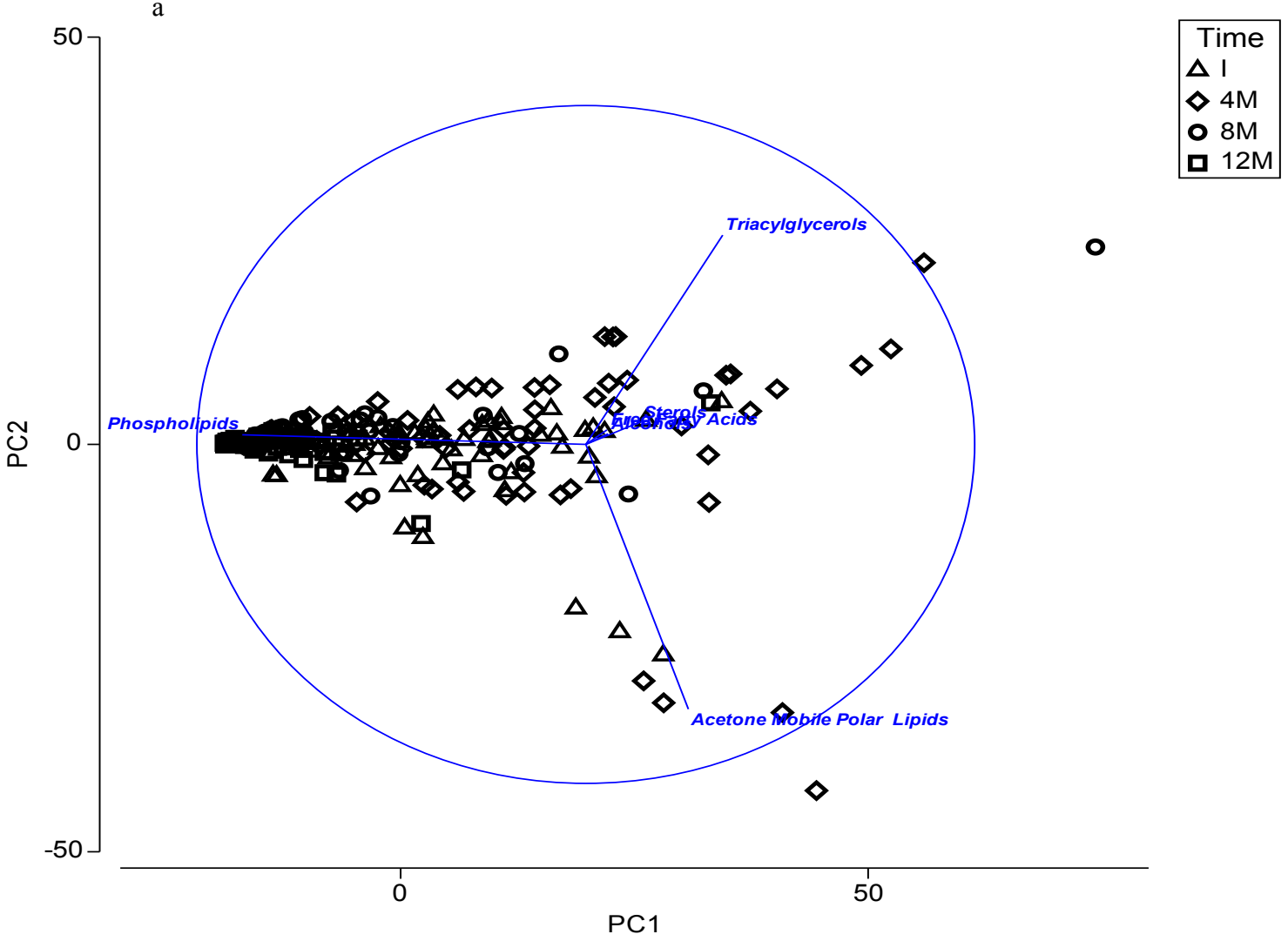
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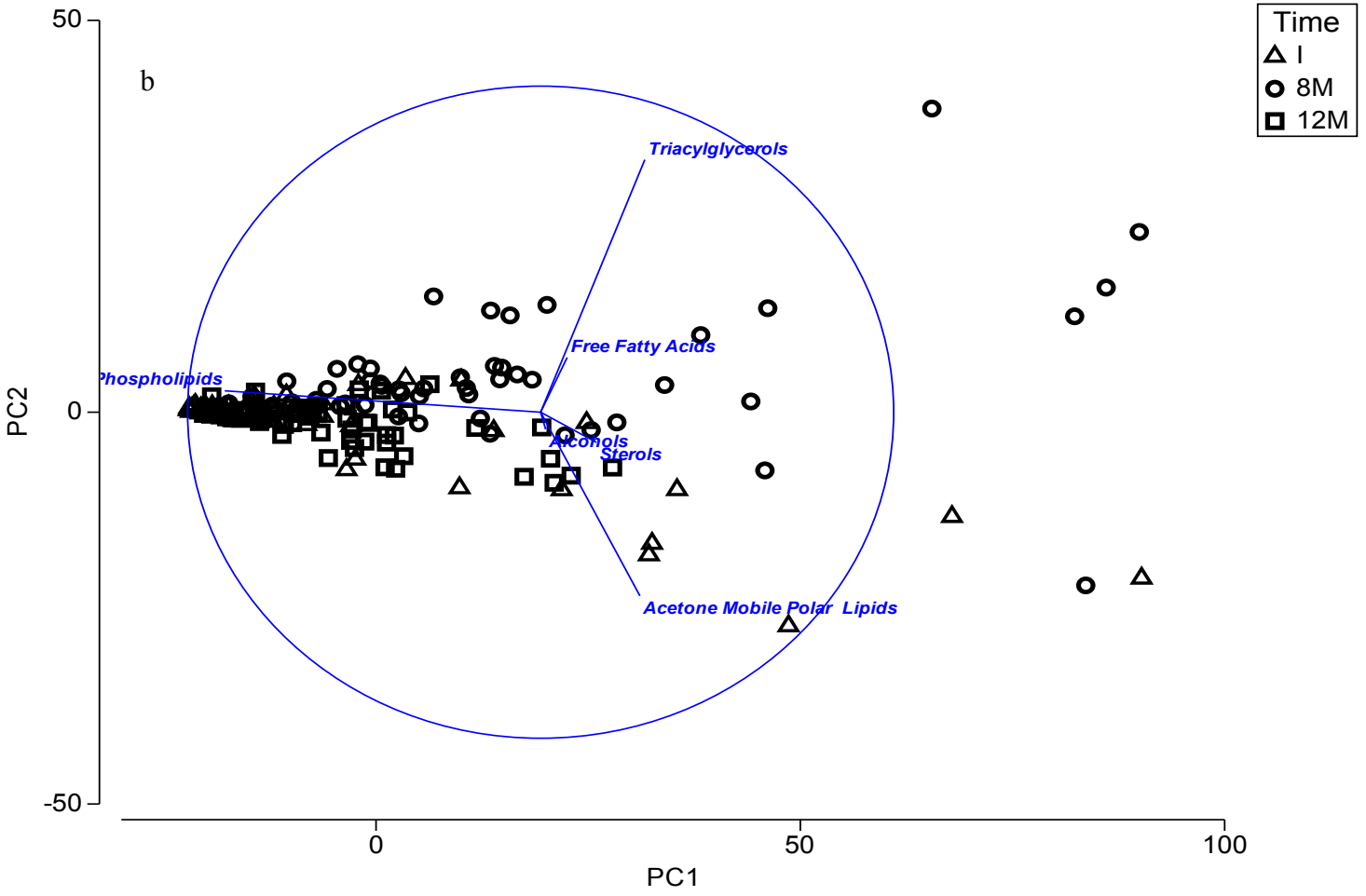


Figure 5.4: Principal components analysis (PCA) of lipid classes expressed as percentage of the total lipids in Year 1 (a) and Year 2 (b). I= initial time, 4M= 4 months, 8M= 8 months, 12M= 12 months sampling time. Blue circle represents correlation circle.

Table 5.2: Lipid class similarity percentages (%) between time points, between sites, and within time points and sites in Year 1 and Year 2.

Similarity (Percentage %)

		Initial	4 Months	8 Months	12 Months		
	Initial	85.01	79.25	82.11	81.38		
Y1	4 Months		75.51	75.13	73.31		
	8 Months			88.11	89.19		
	12 Months				92.75		
	Initial	77.39	n.a.	70.16	81.24		
Y2	4 Months		n.a.	n.a.	n.a.		
	8 Months			73.54	76.18		
	12 Months				85.76		
		SAS	SAD	BCS1	BCS2	BCD	MID
	SAS	86.44	84.37	87.21	83.66	86.55	86.39
	SAD		81.73	85.07	81.76	87.60	87.47
Y1	BCS1			88.59	85.36	87.60	87.47
	BCS2				81.51	84.04	84.57
	BCD					86.72	86.91
	MID						87.10
	SAS	75.76	77.38	76.62	79.63	81.36	75.88
	SAD		78.50	78.58	79.96	82.98	76.58
Y2	BCS1			76.88	78.83	82.14	75.40
	BCS2				81.9	84.74	78.14
	BCD					87.16	79.92
	MID						73.20

SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep. n.a.= Year 2, 4 months was not sampled and therefore excluded from analysis.

5.3.2.2. Fatty acid analysis

5.3.2.2.1. Year 1

In Year 1 of the study, mussels presented the highest docosahexaenoic acid (DHA) content at the initial sampling point and the lowest content at 8 months. However, no differences in DHA content were observed among sites at the initial time point, 8 and 12 months; while at 4 months Mouse Island deep showed a higher DHA content than all the shallow water sites (South Arm shallow and Bulley's Cove shallow 1, $p<0.001$; Bulley's Cove shallow 2, $p=0.039$) (Table 5.3).

Opposite to DHA, the highest mussel eicosapentaenoic acid (EPA) content was found at the 8 month sampling, while the lowest content was observed at the initial time point. Significant differences among sites are present only at 12 months, with Mouse Island showing a higher EPA content than any of the other sites ($p = 0.009$ to <0.001) (Table 5.3).

The total content of omega-3 fatty acids (ω3 FA) presented an increase between the initial time point and the 4 month time point but then remained constant for the rest of the study period. Differences among sampling sites were observed at 12 months, when mussels at Mouse Island deep presented higher total ω3 FA content than those at South Arm shallow ($p=0.001$) and Bulley's Cove shallow 2 ($p=0.004$) (Table 5.3). Bacterial fatty acids in Year 1 were higher at the initial time point than during all the other sampling times (Table 5.3).

Terrestrial fatty acids reached the highest content at the initial sampling point and the lowest at 8 months. They also presented differences among sites; at the initial point

mussels at Mouse Island presented the lower content (p-value from 0.028 to <0.001). At 12 months the content in Mouse Island mussels was lower than at all the shallow water sites (South Arm shallow, p=0.001; Bulley's Cove shallow, 1 p=0.008; Bulley's Cove shallow, 2 p=0.003) (Table 5.3).

Principal coordinates analysis (PCO, vectors with Pearson's correlation >0.8) shows differences between seasons and occasionally between depth. The fatty acids that carry the highest weight in determining differences are essential fatty acids (DHA, EPA, arachidonic acid-ARA), bacterial fatty acids (17:1, 16:1ω7, 18:2ω4), 16:3ω4, 20:2ω6, 20:1ω11, docosapentaeonic acid (ω6DPA) and non-methylene interrupted dienoic fatty acids-NMID (Kelly & Scheibling, 2012; Parrish, 2013) (Figure 5.5a). It is interesting to observe that at 12 months a cluster of deep water samples separates from the shallow water sites and takes an intermediate position between the initial and 8 month samples, possibly suggesting a higher content of EPA in those samples (Figure 5.5a). This result is in agreement with the two-way ANOVA results, where a higher content of EPA was noted in some of the deep water sites, compared to some of the shallow water sites (Table 5.3). Similarity of the fatty acid profile between time points (seasons) is the lowest between initial time and 8 months (60.3%); while the highest similarity is shown between initial time and 4 months (77.9%; Table 5.4). The main fatty acid driving similarity between sites appears to be EPA, while similarity between samples in each season is driven by DHA for the fall (initial and 12 months) and EPA for winter and spring (4 months and 8 months). Differences between seasons are again determined by EPA followed by DHA (Table 5.4).

5.3.2.2.2. Year 2

In Year 2 the mussels presented the highest docosahexaenoic acid (DHA) content at the initial time point and at 12 months, while the lowest content was recorded at 8 months. This time South Arm shallow showed lower DHA content than all the other sites ($p<0.001$) (Table 5.3).

As in Year 1, Year 2 also showed that mussels conteained the highest levels of eicosapentaenoic acid (EPA) at 8 months. This time, musses at the Mouse Island deep sites presented a higher EPA than those at all the shallow water sites (South Arm shallow, $p<0.001$; Bulley's Cove shallow 1, $p=0.023$; Bulley's Cove shallow 2, $p=0.042$). Also, at 8 months, South Arm shallow showed the lowest content of EPA between all the six sites (p-value from 0.002 to <0.001). At 12 months all the deep water sites showed a higher content of EPA than the shallow water sites (p-value ranged from 0.038 to <0.001) (Table 5.3). Moreover, for all the shallow water sites the EPA content at 12 months was comparable to that at the initial time point, while for the deep water sites it was found to be higher at 12 months. The EPA content of mussels in the spring (8 months) in Year 2 was 21.9 % of total fatty acid content, while in Year 1 was 32.1%.

The total content of ω3 fatty acids appeared to be consistent among sampling times; however some differences were observed among sites. At 8 months due to the lower DHA and EPA content, mussels at South Arm shallow also presented significantly less total ω3 FA than at all the other sites ($p<0.001$) (Table 5.3).

Bacterial fatty acids reached their peak at 12 months sampling. No differences among sites were observed at 8 months. At 12 months, the deep water sites showed lower

bacterial fatty acid content than the shallow water sites (p-value ranged from 0.031 to <0.001) with Mouse Island deep mussels presenting the lowest content of all the sites ($p<0.001$) (Table 5.3).

Terrestrial fatty acids in Year 2 did not show any significant difference among sampling time or sites; however, the average terrestrial fatty acids content at 8 months in Year 2 was 2.4% of total fatty acid, while in Year 1 was 1.3%.

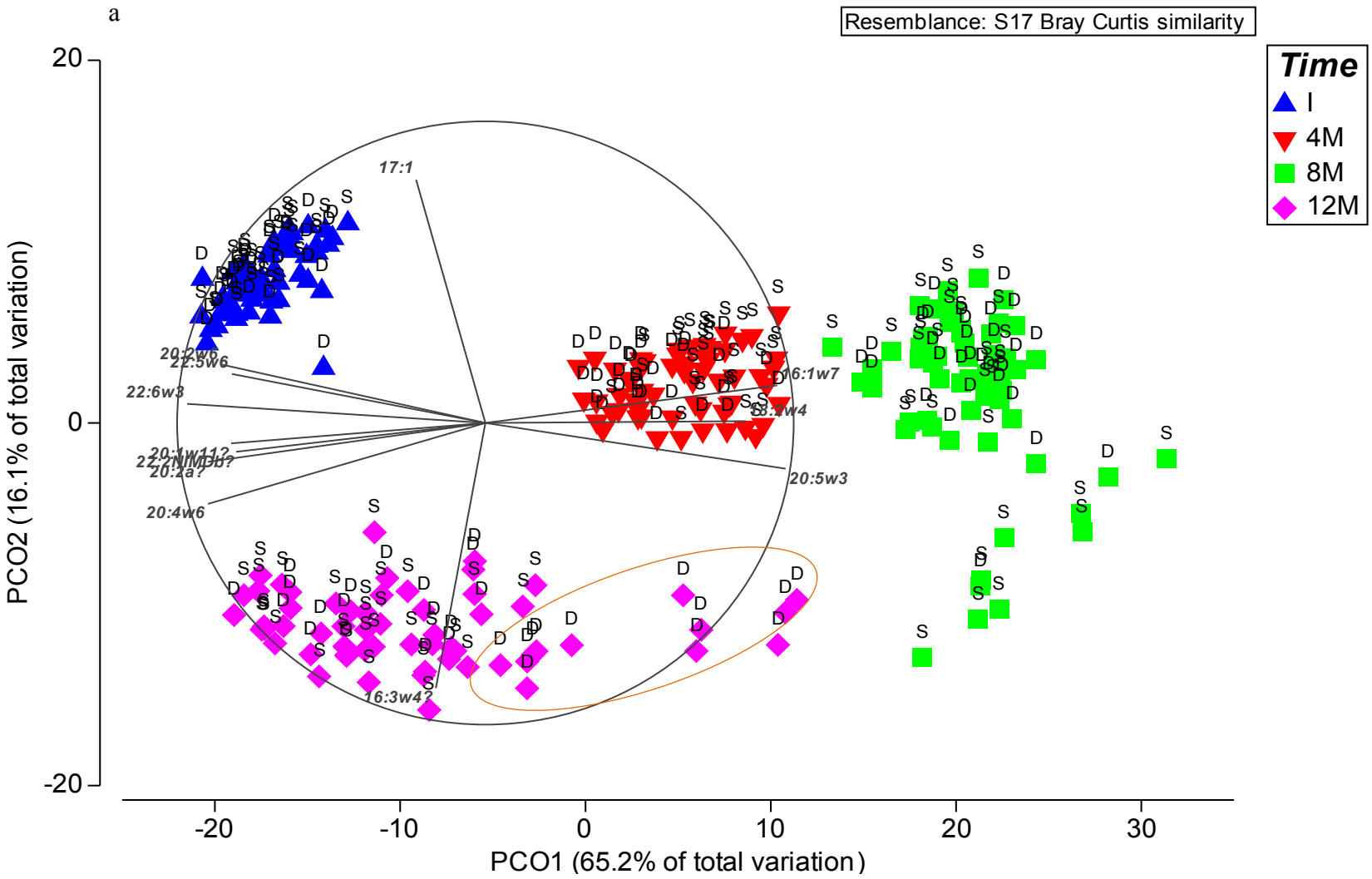
In Year 2 principal coordinates analysis (PCO, vectors with Pearson's correlation >0.8) showed differences between seasons and occasionally between depths. The fatty acids that carried the highest weight in determining the differences are similar to Year 1 (DHA, EPA, ARA, ω6DPA, 17:1, 16:1ω7, 18:2ω4, 16:3ω4, NMID). However, some fatty acids that were not included in Year 1 appear to be important in Year 2; in particular bacterial fatty acids, such as 16:2ω4, 17:0, *i*17:0, 18:1ω7, and γlinolenic acid (18:3ω6) a biomarker for macroalgae (Kelly & Scheibling, 2012; Parrish, 2013). As in Year 1, at 12 months it is possible to observe a cluster of deep water samples separated from the shallow water ones (Figure 5.5b); again they locate in an intermediate position between initial and 8 months sampling. This result is in agreement with the 2-way ANOVA, which showed higher EPA content of mussels in the deep water sites than at all the shallow water sites (Figure 5.5b and Table 5.3). The main fatty acid driving similarity between sites appears to be DHA, while similarity between samples within each season is again driven by DHA for the fall (initial and 12 months) and EPA for spring (8 months). Differences between seasons are determined by DHA more than EPA during Year 2 (Table 5.4). It is therefore interesting to note how the effect of EPA varies between the two years of this study. It is also worth noting that he similarity of samples during the

same season is generally lower in Year 2 compared to Year 1; however, at 12 months the samples were more comparable in Year 2 than in Year 1 (Table 5.4).

Table 5.3: Fatty acid profile (% of total fatty acids) for Years 1 and 2 in mussels from shallow and deep water sites.

			SAS	SAD	BCS1	BCS2	BCD	MID
Y1	*Initial*	DHA	24.1±1.3	22.9±1.2	23.7±0.7	24.0±0.9	23.8±1.3	23.8±3.1
		EPA	10.3±0.5	10.1±1.1	9.8±0.3	9.9±0.4	10.5±1.1	10.2±0.7
		ω3	42.0±1.6	41.5±1.8	43.8±1.4	42.5±1.8	42.5±2.3	41.6±1.4
		Bacterial[1]	10.2±1.5^{ab}	9.5±1.9^{ab}	8.5±1.4^{a}	9.6±1.2^{ab}	10.3±1.8^{ab}	11.5±1.4^{b}
		Terrestrial[2]	3.1±0.3^{a}	4.3±1.0^{c}	4.8±0.8^{bc}	3.7±0.8^{ac}	3.4±1.1^{a}	2.5±0.4^{d}
	4 months	DHA	15.0±1.7^{a}	16.3±1.2^{ab}	15.2±1.1^{a}	16.1±1.1^{a}	16.4±1.0^{ab}	18.5±0.9^{b}
		EPA	23.5±0.8	22.5±1.1	23.4±0.9	22.2±1.1	23.5±0.7	22.2±1.1
		ω3	46.7±1.5	46.2±1.0	47.1±1.9	46.4±0.4	47.5±1.3	47.5±1.8
		Bacterial[1]	5.1±0.7	5.8±0.5	5.3±1.4	5.6±1.3	5.5±1.2	5.7±1.3
		Terrestrial[2]	2.2±0.4^{a}	1.8±0.4^{ab}	2.0±0.4^{ab}	1.7±0.4^{ab}	1.5±0.4^{b}	1.5±0.2^{ab}
	8 months	DHA	8.2±1.2	8.0±1.3	8.2±1.4	9.3±1.9	8.5±1.8	7.9±0.7
		EPA	32.2±2.5	33.1±1.3	32.1±1.2	31.2±1.6	31.1±2.4	32.5±1.2
		ω3	46.1±2.7	46.8±1.9	46.2±1.8	46.3±2.2	44.9±3.7	45.9±1.5
		Bacterial[1]	3.7±1.7^{a}	4.4±1.3^{ab}	3.9±1.9^{a}	4.9±1.2^{ab}	6.3±3.8^{b}	5.1±0.7^{ab}
		Terrestrial[2]	1.4±0.1	1.2±0.2	1.5±0.1	1.5±0.1	1.2±0.1	0.9±0.1
	12 months	DHA	19.9±2.1	19.2±2.1	20.5±1.5	19.9±1.6	19.5±4.1	18.3±3.5
		EPA	15.6±2.2^{a}	18.8±3.3^{b}	17.0±4.0^{abc}	16.1±2.1^{ac}	18.7±4.5^{c}	22.0±4.8^{d}
		ω3	42.7±2.3^{a}	44.9±3.1^{ab}	43.9±4.4^{ab}	43.0±2.4^{a}	44.4±3.4^{ab}	46.8±3.2^{b}
		Bacterial[1]	4.1±0.6^{ab}	5.2±3.1^{ab}	5.5±2.8^{b}	3.8±0.3^{ab}	3.6±0.8^{ab}	3.3±0.7^{a}
		Terrestrial[2]	2.7±0.5^{a}	2.1±0.5^{ab}	2.6±0.7^{a}	2.7±0.5^{a}	2.2±0.5^{ab}	1.8±0.3^{b}
Y2	*Initial*	DHA	24.2±1.9	24.5±1.5	24.6±0.5	23.5±1.1	25.4±2.7	24.7±1.6
		EPA	12.4±1.4	11.8±0.8	11.9±1.1	11.8±0.7	12.7±1.9	12.5±1.1
		ω3	42.8±3.9	43.4±2.1	43.7±5.6	41.0±2.1	44.3±6.1	42.5±1.9
		Bacterial[1]	4.7±0.4^{ab}	5.6±2.5^{a}	4.3±1.0^{ab}	4.8±0.6^{ab}	4.2±0.5^{b}	3.8±0.3^{b}
		Terrestrial[2]	2.2±0.5	3.7±3.1	2.7±0.6	2.3±0.7	2.6±1.1	2.3±0.5
	8 months	DHA	10.3±2.6^{a}	13.6±1.6^{b}	13.7±2.3^{b}	14.4±1.9^{b}	13.7±1.3^{b}	14.0±1.4^{b}
		EPA	19.2±2.7^{a}	22.4±1.1^{bc}	21.6±1.5^{b}	21.7±0.7^{b}	22.8±1.1^{bc}	23.6±0.6^{c}
		ω3	37.3±5.3^{a}	43.9±1.2^{b}	43.1±1.7^{b}	43.1±2.4^{b}	44.2±1.4^{b}	45.6±1.4^{b}
		Bacterial[1]	3.3±0.5	3.6±0.9	3.7±1.7	2.9±0.4	2.9±0.3	2.9±0.3
		Terrestrial[2]	2.4±0.4	2.3±0.3	2.6±0.7	2.6±0.2	2.5±1.1	1.9±0.1
	12 months	DHA	24.0±0.8^{ab}	25.9±1.5^{b}	24.4±1.2^{ab}	23.6±1.6^{a}	25.4±1.5^{ab}	23.5±3.2^{a}
		EPA	12.0±0.7^{a}	13.9±1.5^{b}	11.4±0.8^{a}	11.3±0.7^{a}	14.9±1.3^{b}	14.7±2.9^{b}
		ω3	42.3±1.5^{a}	46.5±1.3^{b}	42.7±1.6^{a}	42.2±1.5^{a}	46.9±1.7^{b}	45.2±3.1^{ab}
		Bacterial[1]	10.5±0.8^{a}	9.1±0.9^{b}	10.5±1.3^{a}	11.1±0.8^{a}	8.7±1.1^{b}	6.8±1.0^{c}
		Terrestrial[2]	2.5±0.2	2.5±0.4	2.5±0.3	2.7±0.4	2.4±0.8	2.1±0.5

Values represent mean ± SD (n= 10). Letters superscripts represent statistical significance among sites ($p<0.05$).
[1]Bacterial: *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1. [2]Terrestrial: 18:2ω6, 18:3ω3. Sites abbreviations as in Table 5.2.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22:6w3
20:1w11?
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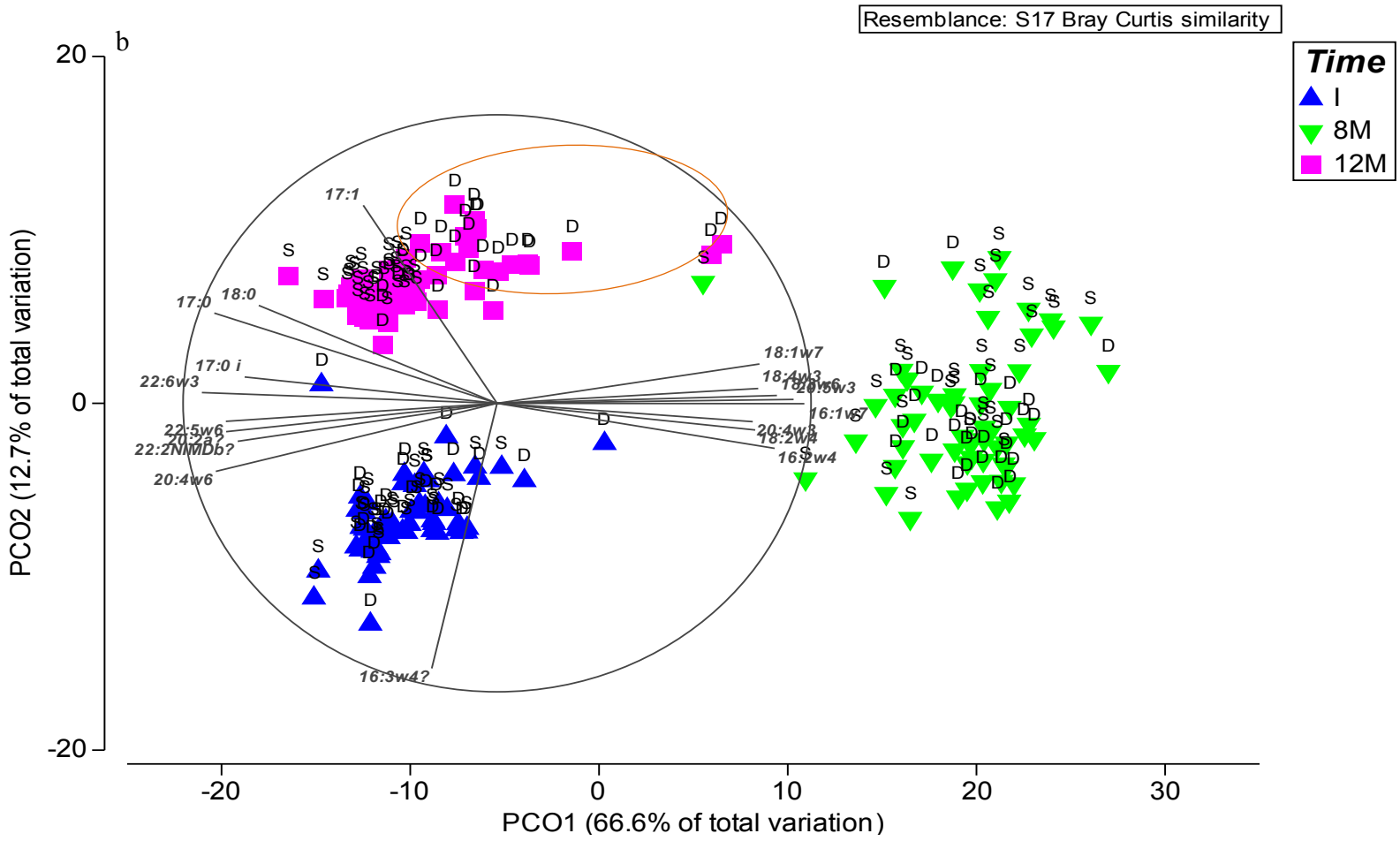

Figure 5.5: Principal coordinates analysis of fatty acids expressed as percentage of the total fatty acids in Year 1 (a) and Year 2 (b). Letters represent shallow (S) and deep (D) water samples. Orange circle highlights deep water samples with an intermediate composition between 8 months and 12 months. I= Initail time, 4M= 4 months, 8M= 8 months, 12M= 12 months.

Table 5.4: Fatty acid profile similarity percentages (%) between time points, between sites, and within time points and sites in years 1 and 2.

Similarity (Percentage %)

		Initial	4 Months	8 Months	12 Months
	Initial	92.5 (DHA)	73.2 (EPA)	60.3 (EPA)	75.7 (EPA)
Y1	4 Months		90.8 (EPA)	77.9 (EPA)	75.1 (EPA)
	8 Months			90.2 (EPA)	65.5 (EPA)
	12 Months				85.8 (DHA)
	Initial	88.7 (DHA)	n.a.	67.5 (DHA)	81.9 (16:3ω4)
Y2	4 Months		n.a.	n.a.	n.a.
	8 Months			89.7 (EPA)	68.0 (DHA)
	12 Months				89.9 (DHA)

		SAS	SAD	BCS1	BCS2	BCD	MID
	SAS	89.9 (EPA)	89.1 (EPA)	89.1 (EPA)	90.0(DHA)	88.2 (EPA)	87.3(EPA)
	SAD		89.9 (EPA)	89.2 (EPA)	89.5(EPA)	88.3(EPA)	88.3(EPA)
Y1	BCS1			89.8 (EPA)	89.7(EPA)	88.1(EPA)	86.6(EPA)
	BCS2				90.9 (EPA)	89.2(EPA)	88.2(EPA)
	BCD					88.2 (EPA)	88.0 (EPA)
	MID						90.1 (EPA
	SAS	90.2 (DHA)	87.0 (DHA)	87.9 (DHA)	88.5 (DHA)	86.5 (EPA)	86.7 (EPA)
	SAD		87.9 (DHA)	87.9 (DHA)	87.9 (DHA)	87.9 (DHA)	87.7 (DHA)
Y2	BCS1			88.9 (DHA)	89.4 (DHA)	87.4 (EPA)	87.6 (EPA)
	BCS2				90.5 (DHA)	87.9 (DHA)	88.2 (DHA)
	BCD					88.4 (DHA)	88.7 (DHA)
	MID						90.8 (DHA)

Fatty acid in brackets indicates the main fatty acid contributing to dissimilarity between groups and to similarity within groups. SAS= South Arm shallow; SAD= South Arm deep; BCS1= Bulley's Cove shallow 1; BCS2= Bulley's Cove shallow 2; BCD= Bulley's Cove deep; MID= Mouse Island deep. N.a.= Year 2-4 months was not sampled and therefore excluded from the analysis.

5.3.3. Glycogen analysis

In Year 1 glycogen content, expressed as mg g^{-1} of wet weight, at the initial time point showed differences between sites (Table 5.1); Mouse island deep had a higher content than all the other sites (p-value from 0.003 to <0.001). At 4 months mussels at South Arm shallow presented a higher glycogen content than those at all the deep water sites plus Bulley's Cove shallow 2 (p-value from 0.004 to <0.001). At 8 months no differences among sites were observed. At 12 months mussels at Bulley's Cove shallow 1 presented a higher glycogen content than those at Bulley's Cove deep (p=0.002) and Mouse Island deep (p=0.048) (Table 5.1).

In Year 2 glycogen content varied among sites; at the initial time point mussels at Bulley's Cove shallow 2 and Bulley's Cove deep present the higher content and they differ from South Arm shallow (p<0.001), Bulley's Cove shallow 1 (p=0.047 and p=0.005, respectively) and South Arm deep (p<0.001). At 8 months mussels at South Arm shallow and Bulley's Cove deep present higher glycogen content than those at Bulley's Cove shallow 1 (p=0.011 and p=0.004, respectively) and Bulley's Cove shallow 2 (p=0.027 and p=0.01, respectively). At 12 months mussels at Bulley's Cove deep and Mouse Island deep present the highest glycogen content (p<0.001) (Table 5.1).

5.4. Discussion

Condition index and meat content of mussels depend on many factors, such as water temperature, salinity, food availability, and stage of the reproductive cycle (Okumus &

Stirling, 1998; Orban *et al*., 2002). In Year 1 of the present study, weight and condition of mussels show that although at the beginning of the year mussels at shallow water sites appear to grow better than those at deep water sites, but this difference is maintained only until winter/spring. Part of this difference can be explained by the higher shell weight of mussels from the shallow water sites compared to that at the deep water sites. At the end of the first year of the study, growth is comparable among sites, with the exception of Mouse Island deep which presented higher dry weight and condition (but lower total weight) than all the other sites. In fact, mussels at Mouse Island deep presented the same dry weight at the 8 and 12 month sampling, while at all the other sites mussels lost dry weight. This suggests a possible spawning event occurring between 8 and 12 months at all the sites but Mouse Island. Mature mussels lose a large portion of their weight during spawning and loss of tissue weight can be used as an estimate for timing of spawning (Bayne & Worral, 1980). A possible cause for this difference in spawning between Mouse Island and the other sites could be a difference in water temperature which, coupled with feeding ratio, can have a major effect on mussel fecundity (Bayne & Worral, 1980). A spawning event would explain not only the differences in weight and condition observed but also those in the water content among sites. Mussels at Mouse Island presented a lower total wet weight in part due to a lighter shell and in part because the mussels retained less fluids than at all the other sites, showing a better physiological state. A high proportion of tissue water content is often related to depleted energy reserves, while a lower water content is often concurrent with accumulation of storage and reserve material (Lucas & Beninger, 1985; Okumus & Stirling, 1998).

It is interesting to notice the difference in shell weight among shallow and deep water sites, with Mouse Island consistently having a lighter shell compared to all the other sites. There is no definitive explanation for this difference; however, it is possible that the deep water sites were less affected by physical forcing, such as waves and currents, compared to the shallow water sites and therefore mussels needed to develop a less thick/strong shell. In blue mussels, shell weight has been shown to increase with increased aerial and wave exposure; and shell growth of suspended mussels has been associated with extreme temperatures and reproductive periods (Seed, 1976; Dame, 1996). Shell shape has also been shown to be affected by environmentally related factors, such as food and density of mussels over time (Alunno-Bruscia *et al*., 2001); moreover, genetic control has been shown to play an important role in shell morphology, particularly in hybrids of two mussel species (Penney *et al*., 2007). In bivalves, shell growth allocation differs from tissue growth and is controlled by the secretion of the shell by the mantle, the metabolism of calcium carbonate ($CaCO_3$) in the shell matrix, and the presence of $CaCO_3$ in the environment. Therefore, the amount of calcium deposited in the shell is a function of the concentration of calcium in the water surrounding the bivalves (Dame, 1996). Increased acidity in the seawater alters the carbonate buffer system resulting in higher dissolution and lower deposition of $CaCO_3$ (Dame, 1996). In this particular case, the difference in depth between shallow and deep water mussels may not have been enough to explain a different calcium carbonate deposition; however, nutrients were not measured in this particular study and therefore a definitive explanation for the difference in shell weight is not possible. I agree, however, with the results of Alunno-Bruscia et al. (2001) study which disregarded the use of the shell length-body mass relationship to detect competition

in field samples, in particular when growth is related to physiological parameters and food availability, as occurs in this study. Since shell is formed through ion (calcium) deposition from the seawater and has a low organic content in mussels (<5%; Alunno-Bruscia *et al*., 2001), its growth may be less susceptible to food availability than soft tissue and has been shown to continue in absence of feeding (Alunno-Bruscia *et al*., 2001). For this reason, a condition index including shell weight may be less readily related to the food supply and the physiological state of mussels. However, shell condition can be a very useful tool when evaluating the action of environmental forces on the mussels. Bourque et al. (2005) found that shells of mussels grown in offshore deep water had a lower weight than the ones grown in lagoons. This result is important to mention since it can lead to higher shell fragility and affect mussel processing, increasing the portion of loss due to broken shells (Bourque *et al*., 2005). It is also important to report that the mussels sampled in this study come from a known hybrid zone between *Mytilus edulis* and *M. trossulus* and it has been shown that *M. edulis* has a stronger and more resistant shell than *M. trossulus* (Penney *et al*., 2007). Although the presence of *M. trossulus* in the location of this study is generally low (~ 3%; Penney *et al*., 2007), it is possible that some of the sites examined presented higher hybridization that influenced the shell weight.

In Year 2 growth and condition differences between shallow and deep water were less pronounced. Mussels at Mouse Island deep presented the lowest wet tissue weight and dry weight among all the sites at the end of Year 2; however, mussel condition, as in all deep water sites, was higher at Mouse island than that of mussels at the shallow water sites. Since Year 2 was characterized by a harsher winter compared to Year 1, we suggest

that, under extreme environmental conditions, there could be a tipping point where deep water becomes a more stable environment than shallow water for the mussels, as has been suggested in previous studies. Finally, it is important to point out that Mouse Island data from Year 2, as in Year 1, presented the lowest shell weight and length at the end of the experiment.

Bivalve biochemical composition is highly variable and the content of lipids and glycogen changes spatially and temporally in relation to the amount and composition of phytoplankton, and in relation to the reproductive state (Abad *et al*., 1995; Orban *et al*., 2002; Kopp *et al*., 2005; Pleissner *et al*., 2012). Glycogen and carbohydrate content in mussels have been shown to be negatively correlated with the gonadal index, highlighting the importance of these nutrients to support gametogenesis (Mathieu & Lubet, 1993). In bivalves, triacylglycerols (TAG) play a role as energy reserves (Gabbott, 1976; Beninger, 1984; Fraser, 1989; Prato *et al*., 2010), and they originate from the conversion of glycogen; in *Mytilus edulis* TAG accumulate during periods of high food availability, reaching a peak during stage III of gametogenesis (Gabbott, 1976; Fraser, 1989; Freites *et al*., 2002; Pernet *et al*., 2007). Following oxidation, TAGs are used, together with other lipids, as an energy source during periods of reduced feeding and during early embryonic development (Gabbott, 1976; Pernet *et al*., 2007; Martínez-Pita *et al*., 2012; Karayücel *et al*., 2013). However, TAG content depends on animal size and therefore to give a useful index it needs to be expressed relative to sterol (ST) content which correlates highly with animal size, contributing a constant proportion in cell structure (Fraser, 1989; Freites *et al*., 2002). In this study, total lipid, triacylglycerol and glycogen content of the mussels do not seem to be influenced by culture depth. With the exception of glycogen, which varied

among single sites but not between shallow and deep water mussels, no differences were found between shallow and deep water. However, our results indicate a marked seasonal pattern. In Year 1, during spring sampling (8 months) a combination of high TAG with low glycogen suggests imminent spawning; this was confirmed by visual observation of the mussels which appeared ripe and ready to spawn at this time. However, in Year 2 this combination of biochemical parameters is not present; this fact, together with the much lower TAG content, is probably the result of a shorter and delayed spring bloom in Year 2, as previously shown in Chapter 3; thus spawning could have been delayed compared to Year 1. It is interesting to observe that in both study years the initial time point and the 12 months time point occurred during the same season (early fall); however the total lipid content differs between these two time points. It is plausible that mussels react differently to physiological and environmental conditions, such as spawning and phytoplankton presence, at a different age and size (Ogilvie *et al*., 2004). The high variability in biochemical parameters, in particular glycogen, between samples and sites can be explained by the high variation in growth rate that *Mytilus edulis* naturally experiences, even within the same population and under identical conditions (Seeds, 1976). It is important to remember that this was a field study and the mussel seed were collected from the wild. Also, the sites examined in this study, as stated previously, are located in a known hybrid zone of *Mytilus edulis* and *M. trossulus* (Bates & Innes, 1995; Penney & Hart, 1999; Murray *et al*., 2010).

Changes in the composition of the diet and in stages of the reproductive cycle are the main factors influencing the fatty acid profile in mussels and other bivalves (Abad *et al*., 1995; Alkanani *et al*., 2007; Ventrella *et al*., 2008; Prato *et al*., 2010; Irisarri *et al*., 2014;

Fernández-Reiriz *et al*., 2015). Fatty acids analysis showed a strong seasonal component, with the highest percentage of DHA during fall while the highest percentage of EPA occurred during the spring sampling. Dominance of DHA in early fall can be explained by the presence of DHA-rich dinoflagellates in the phytoplankton; while dominance of EPA in the spring is explained by the presence of diatoms during the spring bloom (Khan *et al*., 2006; Alkanani *et al*., 2007; Ventrella *et al*., 2008; Irisarri *et al*., 2014; Fernández-Reiriz *et al*., 2015). Interesting differences among sites can be observed in Year 1 where Mouse Island deep mussels showed a higher content of EPA compared to all the other sites at the end of the experiment, and in Year 2, when mussels at all the deep water sites contained higher EPA than at the shallow water ones at the end of the experiment. A similar result was shown previously (Section 5.3.1, Figure 5.2) for mussel condition, highlighting how growth can be related to this particular ω-3 fatty acid.

Total mussel content of ω-3 fatty acids did not show a clear seasonal trend nor appeared to be affected by culture depth; similarly, terrestrial fatty acids content did not vary among sampling times and sites in Year 2. However, in Year 1 mussels at Mouse Island deep consistently showed the lowest terrestrial fatty acid content, suggesting a lower influence of land run-off at this particular deep water site. Again in Year 2, the effects of the extreme winter are evidenced by the higher percentage of terrestrial fatty acids during the spring sampling (8 months) compared to Year 1, possibly due to the input of land ice and snow melt bringing terrestrial markers to the seawater.

Bacterial fatty acids showed seasonal variation, which differed however between Year 1 and 2. While in Year 1 the highest percentage of bacterial fatty acids was found at the time of initial sampling, in Year 2 it is shown at final sampling. It is important to note that

both the initial and 12 months sampling took place in early fall, during both study years. It is, however, interesting to point out the higher percentage of bacterial fatty acids at the 12 months sampling of Year 2 compared to the same of Year 1. This result, associated with the lowest content of EPA in the spring of Year 2 and with the shorter and delayed spring bloom shown in Chapter 3, could be explained by the particularly harsh weather conditions during winter 2014. The presence of bacterial fatty acids can provide an indicator of the bacterial contribution to the bivalve diet and has been shown to vary seasonally. Bacteria represent a minor source of food for most bivalves such as mussels, due to their diameter (<1 μm), too small to be effectively retained by the gills (Trottet *et al*., 2008; Prato *et al*., 2010; Pernet *et al*., 2012; Irisarri *et al*., 2014). However, bacteria can become a significant source of food when they are aggregated or attached to other particles (Dame, 1996; Trottet *et al*., 2008; Pernet *et al*., 2012). A higher content of bacterial fatty acids may be attributed to contamination from runoff, spawning of mussels or mussel consumption of bacteria associated with suspended phytoplankton or other suspended particulates (Khan *et al*., 2006; Irisarri *et al*., 2014). High levels of bacterial lipid biomarkers may be also due to degraded plankton material derived from resuspended sediment, stemming primarily from increased decomposition processes or from a seasonal enhancement of the microbial loop (Budge *et al*., 2001; Pernet *et al*., 2012). Additionally, mussels increase their filtration rate and pseudofaeces production, and increase the amount of material ingested when fed seston with low organic content (Bayne *et al*., 1993; Ogilvie *et al*., 2004). In this particular case, it is possible that the highest content of bacterial fatty acids may be linked to increased mussel filtration and consequent consumption of bacteria present in the water column, due to the shortage of

nutrients from the phytoplankton. This lack of nutrients could be explained by the shorter and delayed spring bloom in Year 2 compared to Year 1, and therefore linked to the extreme winter conditions of Year 2. This being the case, deep water sites (in particularly Mouse Island) showing lower bacterial fatty acid content than shallow water ones at the end of Year 2, could indicate that once again deep water could be a more stable environment for growth during years of particularly extreme environmental conditions. Bivalves have been shown to have high trophic flexibility; during seasonal variations in phytoplankton and non-bloom periods they appear to be able to utilize other food sources such as terrestrial and bacterial inputs (Pernet *et al*., 2012). The increased importance of bacterial fatty acids in Year 2 compared to Year 1 is further confirmed by the principal coordinates analysis (PCO), showing that an increased number of these fatty acids was responsible for the variation among samples and seasons in Year 2.

Previous studies found similar growth in mussels grown at different depths and have not been able to find a clear relationship between depth of culture and mussel growth and health, even when higher chlorophyll *a* was present at depth (Richardson *et al*., 1990; Karayücel & Karayücel, 2000; Ogilvie *et al*., 2004). The relationship between food concentration and bivalve production is complex and can be affected by factors that influence both the food supply to the mussels and the conversion of food to biomass (Ogilvie *et al*., 2004); therefore, the difficulty in finding a clear relationship is due to the complexity of the interrelated factors determining mussel growth and health. Other than food concentration and quality, important factors to consider are water current and velocity. Spatial differences in mussel biochemical composition and condition have been found between inner and outer regions of the same coastal embayment and have been

linked to variation in food quantity and quality. This variability was caused in part by particular environmental conditions such as storm-enhanced current velocity, making more or less phytoplankton available to mussels (Ogilvie *et al*., 2004; Irisarri *et al*., 2015). The phytoplankton population may have a higher turnover rates in shallow water, due to better light conditions; also, the phytoplankton abundance may be sufficient to allow maximum mussel growth at both depths, or phytoplankton cells could be of different nutritional value at different depths (Ogilvie *et al*., 2004). Moreover, changes in mussel energy allocation linked to reproduction stage may interfere with the depth effect (Ogilvie *et al*., 2004). Finally, a chlorophyll *a* maximum layer could affect smaller or younger mussels more than adults, since storage and utilization of food varies with age because of age-related relative allocation between growth and gamete production (Thompson, 1984b; Karayücel & Karayücel, 2000).

This study showed a clear link between mussel condition and biochemical composition, and the environment. The two years of this study were characterized by different weather and environmental characteristics, in particular during the winter/spring seasons, and these differences were reflected in the mussels' health and condition. In the present study, as in previous ones, mussel characteristics varied not only seasonally but also year to year in relation to variability of climatic conditions and the consequent variation in the phytoplankton population (Orban *et al*., 2002). Given this, the difficulty in comparing interannual variations in mussel condition is evident. However, some general conclusions about the effect of water depth on mussel growth and health can be highlighted. Firstly, based on the condition and biochemical parameters determined in Year 1, it is safe to state that mussels grown in shallow and deep water appeared to be

comparable at the time of harvest. When differences between shallow and deep water were present, they lasted only for part of the year and they were mostly seasonal; moreover, the largest differences were in total wet weight, which is influenced by both shell weight and water content of mussel tissues, while the condition was often comparable between shallow and deep water. However, during Year 2 mussels grown in deep water exhibited a better condition and higher ω-3 fatty acid content (EPA) at the time of harvest. Given the more extreme winter and spring conditions of Year 2, these results suggest that in this case, deep water provided a more stable environment for the mussels to grow in and maintain good condition and feeding, while it is plausible that shallow water was more affected by the weather. The exact impact of the environment in shallow water is not clear, however, some suggestions can be made: while chlorophyll *a* content did not seem to vary between shallow and deep water, it is possible that food quality, not quantity, differred, allowing feeding on a more nutritious food supply for deep water mussels (Ogilvie *et al*., 2004). Also, physical forces such as ice movement, waves, and currents may have had a greater effect in shallow water, driving the mussels to allocate more energy to counteract those forces, and leaving less energy for storage of reserves and growth (Ogilvie *et al*., 2004; Irisarri *et al*., 2015). Finally, during the summer of the second year, a sharper and larger increase in water temperature was shown at shallow water sites compared to deep water ones; a temperature rise from 5°C to almost 20°C in the span of a month (early July to early August 2014) may have caused environmental stress and consequent compensation in shallow water mussels.

The Mouse Island deep water site, however, needs to be considered independently from the other sites and analyzed in view of its particular characteristics. During Year 1

this is the only site presenting a different mussel condition and quality (higher content of EPA) at the time of harvest; moreover, mussels at this site show consistently lower shell weight and length, lower bacterial fatty acids and lower terrestrial fatty acids. Therefore, Mouse Island appears to be less affected by land runoff and possibly by physical forces compared to all the other sites analyzed; it also appears to have had more favourable conditions of food supply and/or more energy to allocate to growth during summer of Year 1, leading to a better condition at the end of the study period, possibly suggesting a different reproductive cycle compared to all the other sites. The explanation for these differences can be found in the location of Mouse Island (Appendix B- Figure B1,2). This particular site can be considered offshore and open water, if compared with all the other sites analyzed, thus explaining the lesser effect of land runoff and the possible differences in mussel feeding and/or spawning activity.

5.5. Conclusions

In conclusion, in many instances deep water mussel culture appears to be beneficial; under favourable environmental conditions mussels in shallow and deep water achieved a comparable growth; however, under more extreme weather conditions deep water grown mussels presented better growth. It is therefore safe to suggest to the Newfoundland mussel aquaculture industry that at least some deep water culture, in conjunction with the traditional shallow water one, should be developed. Moreover, taking into consideration the environmental and climatic constraints, an attempt to develop offshore deep water

mussel culture should be made, giving the positive results obtained at the Mouse Island site.

5.6. References

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CHAPTER 6

Effects of extended ambient live holding on cultured blue mussels (*Mytilus edulis* L.) with reference to condition index, lipid profile, glycogen content and organoleptic testing

6.1. Introduction

The Newfoundland mussel aquaculture industry has been steadily growing over the past decade. Since 2000, production has increased from 1,051 to 4,397 tonnes in 2012 and the industry is currently investigating new market opportunities and initiatives including the establishment of organic status in 2013 (NAIA, 2011; DFA, 2013). In Newfoundland, the blue mussel (*Mytilus edulis* L.) is farmed mostly on the northeast coast of the island, although some farms are also found on the south coast. Mussels produced in this province, as in the rest of Atlantic Canada, are grown by suspended longline culture. Market size is 50-75 mm and mussels may be harvested throughout the year, depending on meat quality, yield and shelf-life (Mallet & Myrand, 1995; Brown *et al*., 2000). Fresh product is typically shipped to a variety of local and international markets. However, geography, extreme weather conditions and/or mechanical issues may create delays in the harvest of fresh product or affect the timely shipping of product to market. In such situations, harvested fresh mussels may require stockpiling or extended holding for up to one month in ambient, wet-storage facilities prior to processing. Several factors can affect

condition, physiological stress response, and biochemical characteristics of mussels subject to extended wet holding, including temperature, mussel density, food availability and reproductive effort (Seed, 1976; Karayücel & Karayücel, 2000; Wyatt *et al*, 2013; Wyatt *et al*., 2014). Generally, wet storage under ambient water temperatures has been shown to result in a lower stress response compared to storage in ice or chilled air as long as optimal water flow allows sufficient oxygen concentration and temperature control (Harding *et al*., 2004a; Harding *et al*., 2004b; Barrento *et al*., 2013). However, depending on season, the storage of mussels over extended periods under ambient conditions (i.e., 1-3 months) has been found to affect the overall condition index and lead to mortality (Wyatt *et al*, 2013). These authors found a significant loss in dry tissue weight and an overall gain in wet tissue weight, due to an apparent increase in tissue water content and a significant reduction in condition index after as little as one month holding. Additionally, mussels held for the same period showed a significant increase in their stress response as measured using the Neutral Red Assay and compared to field controls (Wyatt *et al*., 2013; Wyatt *et al*., 2014). Handå et al. (2013) showed that mussels held under starved conditions are able to reduce their oxygen consumption and metabolic rate, thus maintaining weight. However, physiological compensation may not be possible when starvation is combined with other stressors, such as low oxygen and elevated ammonia concentrations. The authors also suggested the need to take into account seasonal effects during long-term storage of mussels. During extended holding, mussel density is a factor that can also play an important role. High densities have been negatively correlated with mussel growth and health, leading to increased mortality in extreme cases, due to competition for food resources and space (Alluno-Bruscia *et al*., 2001; Lauzon-Guay *et*

al., 2005; Cubillo *et al*., 2012). To date no studies have investigated variability in the biochemical composition of mussel tissue and meat palatability following extended holding in processing facilities. The consequences of extended holding under ambient conditions on mussel physiology are an important consideration for Newfoundland mussel growers, that can lead to concrete suggestions for the industry. The specific objectives of the present study were to investigate seasonal changes in biochemical parameters (total lipids, fatty acids and glycogen), condition (weight and condition indices) and palatability (discriminative and descriptive taste panel) and of cultured mussels kept in ambient, wet-holding facilities for up to one month, and to determine if held mussels differed seasonally from freshly harvested mussels.

6.2. Materials and Methods

6.2.1. Study site, experimental set-up and sampling protocol

The present study included three sampling seasons: fall 2011, winter 2012 and spring 2012. Mussels from the 2010 year class were collected from site 13 in Bulley's Cove, Newfoundland and Labrador, Canada, and transported in plastic tubs by boat (30 min trip) to a commercial processing facility in nearby Pleasantview.

The experimental setup and sample collection were similar to that described in Wyatt et al. (2013; 2014). Briefly, *M. edulis* (2010 year class) were cultured using the traditional longline system and harvested using standard commercial protocols, after which they were transported to the processing facility where they were held unprocessed in two

replicate unstacked plastic D332 800 l "cod tubs" modified with false bottoms to avoid dead water spaces (inside dimensions 112 cm long, 95 cm wide, 78 cm high). Each tank contained 10 to 12 socks and the total density, as recommended by industry, was calculated to be approximately 0.362 kg L^{-1} for a total weight of 289 kg. Mussels were held under ambient water conditions in a continuous, flow-through (60 L min^{-1}), non-aerated system with unfiltered water pumped from the bay from a distance of 145 m and an approximate depth of 13 m from the surface. Water temperatures in the holding tanks were recorded using automated temperature loggers (VEMCO Minilogs) placed directly in the tanks and set to record once every four hours. Daily temperature checks at the main intake showed that there was no difference between this and the water temperature in the tanks, so for the purpose of the present study only temperatures in tanks were recorded, summarized and reported as daily averages (Figure 6.1). Water temperatures were not measured at the farm sites. During each season, mussels were sampled at random from the two holding tanks (mussels were moved in the tub in order to obtain samples from both top, center, bottom, and sides of the tub) at Time 0 (Initial), 1 week, 2 weeks and 4 weeks after holding. Simultaneously a sample of fresh mussels was taken from the farm site. This sample is denoted as the field control.

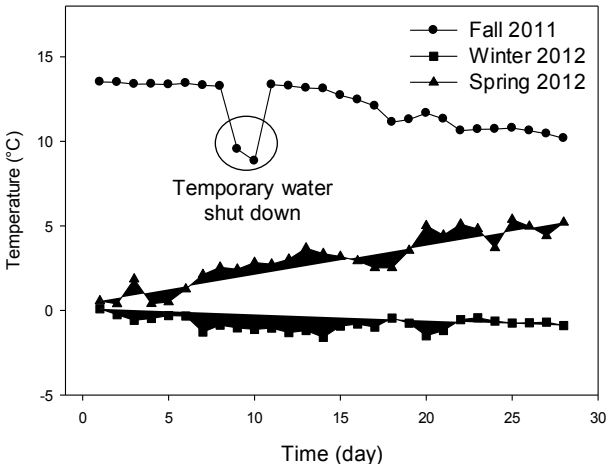

Figure 6.1: Average daily temperature recorded in holding tanks during fall 2011, winter 2012 and spring 2012.

6.2.2. Condition analysis

Three hundred mussels were taken for condition analysis at time 0 (Initial); subsequently 150 held mussels (2 tank replicates) and 150 controls were sampled during subsequent sampling periods. Mussels were placed on ice and transported (5 hours trip) to the North Atlantic Fisheries Centre (NAFC) facilities in St. John's, Newfoundland and Labrador, for measurement and analysis. Mussels were transported on ice to avoid spoiling and maintain appropriate temperature. For each individual, the total wet weight (g) was measured to the nearest 0.001 g, after which the whole mussel meat was carefully dissected away from the shell and placed in pre-weighed aluminum trays for dry weight analysis. Meat was dried to a constant weight for 48-72 h at 80°C (modified from Lutz *et al.,* 1980); shells were allowed to air dry for 48-72 h. Meat and shell dry weight were then

measured to the nearest 0.001 g. Condition indices were calculated as the ratio of dry tissue weight to wet tissue weight (Lucas & Beninger, 1985) and as the ratio of dry tissue weight to dry shell weight × 100 (Lucas & Beninger, 1985; Wyatt *et al*. 2013). At week 2 during the winter season the mussels used as controls were mistakenly harvested from the wrong line. The mussels were of the same year class as holding, but from an initial medium grading size (3/4- 1 inch; 19- 25.4 mm), while the mussels used for holding were initially graded as large (1-1.75 inch; 25.4- 44.45 mm). Therefore, a comparison between held and control mussels at that particular time point may not be significant.

6.2.3. Lipid and fatty acid analyses

6.2.3.1. Lipid classes

A total of 15 mussels were sampled at each time for lipid extraction, using a modified Folch method (Parrish, 1999), as described in Section 5.2.3.1 (Chapter 5). Briefly, meat was dissected from the shell and stored in chloroform at -80°C until analysis. Lipid classes were determined for 10 individuals, randomly selected from the original 15 mussels, with the chloroform: methanol method. Lipid classes were determined by thin layer chromatography (TLC) with flame ionization detection using an Iatroscan analyzer (MK-6 TLC-F1D) and a three-stage development system to separate lipid classes (Parrish, 1999). The resulting chromatograms were analyzed with PeakSimple software (SRI Instruments, Torrance, CA). Total lipid content was expressed as μg g^{-1} of wet weight and triacylglycerol: sterol (TAG: ST) ratios were calculated.

6.2.3.2. Fatty acids

Fatty acids were determined from the same samples previously used for lipid class analysis. The fatty acid derivatization procedure followed that described by Parrish (1999) with minor modifications, as described in Section 5.2.3.2 (Chapter 5). The samples were analyzed as FAME (fatty acids methyl esters) with an Agilent 7890A gas chromatograph (GC) equipped with an auto-injector.

6.2.4. Glycogen analysis

A total of 15 mussels were taken from both holding and control groups at each sampling time for glycogen analysis. Mussels were dissected, sodt tissue placed in 20 mL glass vials and quickly frozen on dry ice. Ten randomly chosen samples were then processed using a method modified from Naimo et al. (1998), as described in Section 5.2.4 (Chapter 5). Glycogen content was measured by colorimetric reaction in 96-well plates. Absorbance was measured using a multi-detection microplate reader (Synergy HT, BIO-TEK) at 490 nm. The concentration of glycogen in the samples was calculated based on a mussel glycogen standard (Sigma, Saint Louis, MO).

6.2.5. Sensory evaluation

6.2.5.1. Sample preparation

A sample of 200 mussels from both holding and control groups were immediately placed on ice, separated by a layer of aluminum foil in order to avoid contact between mussels

and freshwater ice, and transported to the NAFC facility in St. John's, NL, Canada. The following day a sensory evaluation was conducted by 24 untrained panellists. Due to distance between farm and location of analysis, and due to length of the procedure it was not possible to proceed to sensory evaluation the same day than sampling. Prior to the evaluation, mussels were washed with cold seawater and scrubbed in order to remove debris and any remaining byssal threads, after which they were stored on ice until cooking. Samples were steamed for 10 minutes, shucked and placed in randomly numbered food cups. Holding and field control samples were handled using separate pans and utensils. The facility used for the sensory evaluation was a large conference room, divided into eight booths with white poster boards. Each booth was prepared before the arrival of the panellists and supplied with a napkin, a survey form, a pencil, a glass of water (to clean the palate between tastings), some plain crackers (to clean the palate at the end of tasting), and an empty cup for discarded mussels. All the panellists were allowed into the room together and had unlimited time to complete the testing, to avoid time pressures. Both the triangle and the hedonic test were developed with the help of personnel trained in sensory analysis at the Centre for Aquaculture and Seafood Development (CASD), Marine Institute, Memorial University of Newfoundland and Labrador.

6.2.5.1.1. Triangle test (Discriminative testing)

Each of the 24 panellists was presented with three randomly numbered cups, two of which contained the same sampling group, with the remaining cup containing the other, i.e. 2 holding samples and 1 control sample or 2 control samples and 1 holding sample

(Carpenter *et al.*, 2000). The panellists were asked to simply choose the odd sample (the different one) from the three on a pre-printed form, and to provide any additional comments.

6.2.5.1.2. Hedonic test (Descriptive testing)

Each of the 24 panellists was presented with two randomly numbered cups, one of which contained mussels from the holding group and one of which contained mussels from the control group. The panellists were asked to evaluate both samples separately for appearance, odour, texture and flavour using a 7-point scale, as described by Cardello et al. (1982). The 7-point scale was previously described to be the most effective one; a scale < 7 would not give enough choice to the panellists, while a scale larger than 7 (i.e. 10) would cause the majority of the panellists to score in the middle (i.e. 5) (Cardello *et al.*, 1982).

6.2.6. Statistical analysis

The data collected were analyzed with Sigmaplot (12.0 and successive versions) statistical and graphical software (Systat software). Data were tested for normality (Shapiro-Wilk test) and equal variance, and means ± SE or ± SD were calculated. One-way analysis of variance and the appropriate post-hoc tests were conducted with time as factor on condition, lipid, glycogen and hedonic test scores variables for both holding and

control samples. When the assumptions of equivalence and normality were not met an ANOVA on ranks was conducted. Paired comparison and t-test (or Mann-Whitney rank sum test) were also conducted between held and control samples at single time points. This approach was chosen due to the fact that the main objective of the analysis was to test differences between held and control samples at the same time point (paired comparison); a separate objective was to highlight trends in held mussel over time (One-way ANOVA). Significance was set at $\alpha = 0.05$. Triangle test data were analyzed based on the Table presented by Carpenter et al. (2000); significance was set at $\alpha = 0.1$, meaning 13 panellists over the total of 24 that detected the correct odd sample. ANOVA tables are presented in Appendix G (Table G14-G16).

6.3. Results

6.3.1. Condition analysis

During the fall season, the total wet weight of held and control mussels differed only at the one week time point ($p=0.04$). The wet weight for holding increased significantly from week 1 to week 4 (Figure 6.2a). The dry weight was different between held and control mussels at 1 week ($p<0.001$), 2 weeks ($p=0.039$) and 4 weeks ($p=0.03$). For both groups a decline in dry weight from initial to 4 weeks was detected, with held mussels consistently presenting the lower weight (Figure 6.2b). The dry weight: wet weight ratio was different between held and control mussels at 2 weeks ($p<0.001$) and 4 weeks ($p=<0.001$) (Figure 6.2c). Dry weight to shell weight ratio did not differ between held

and control mussels at any point of time; however, an overall decline was also observed in this ratio from the initial time point to 4 weeks, in both held and control mussels (Figure 6.2d).

During the winter season, wet weight presented a more consistent trend in held mussels than in control mussels (Figure 6.3a). Control mussels declined in wet weight from initial time to 1 week and 2 weeks ($p<0.001$), while at week 4 an increase in wet weight was noted ($p<0.001$). Held and control mussels differed in wet weight at each time point in this season: at the initial and 4 week samples control mussels had higher wet weight ($p=0.046$ and $p<0.001$ respectively), while at one week and two weeks held mussels had the higher wet weight ($p<0.001$) (Figure 6.3a). The dry weight remained similar for the entire season in the held mussels, while control mussels showed a declining trend followed by an increase similar to that for wet weight. Held mussels had a higher dry weight than controls at week 1 ($p<0.001$) and week 2 ($p<0.001$), while at week 4 control mussels had a higher dry weight ($p<0.001$) (Figure 6.3b). The dry weight: wet weight ratio was different between holding and control at 2 and 4 weeks: at 2 weeks, held mussels had a higher ratio ($p<0.001$), while at 4 weeks control mussels had the higher ratio ($p=0.007$) (Figure 6.3c). Also the dry weight to shell weight ratio was different between held and control mussels at 1 week ($p<0.001$), 2 weeks ($p<0.001$) and 4 weeks ($p<0.001$). Held mussels had a higher index at week 1 and 2, while the inverse was observed at week 4, when control mussels had the higher index (Figure 6.3d).

During the spring season, held mussels had a higher wet weight than control mussels at each sampling period (initial $p=0.037$, 1 week $p<0.001$, 2 weeks $p<0.001$ and 4 weeks $p<0.001$). The wet weight of control mussel declined after one week ($p<0.001$), while that

of held mussels increased at week 1 ($p<0.001$) and then remained constant until the end of the season (Figure 6.4a). The dry weight differed between held and control mussels at one week ($p<0.001$), when control mussels had a higher weight, and 4 weeks ($p=0.006$), where held mussels had the higher dry weight (Figure 6.4b). The dry weight: wet weight ratio differed between holding and control mussels at week 1 ($p<0.001$), week 2 ($p<0.001$) and week 4 ($p<0.001$) with the held mussels consistently having a lower ratio than control mussels. The ratio for held mussels declined after the initial time point, while in control mussels it increased at one week ($p<0.001$), and then remained constant during the remainder of the season (Figure 6.4c). The dry weight on shell weight ratio gave a similar pattern with a lower index for held mussels at week 1 ($p<0.001$), week 2 ($p<0.001$) and week 4 ($p<0.001$) (Figure 6.4d). Therefore, condition index for held mussels declined slowly from initial to four weeks, while the index for control mussels increased notably, with a peak at one week (Figures 6.4c,d).

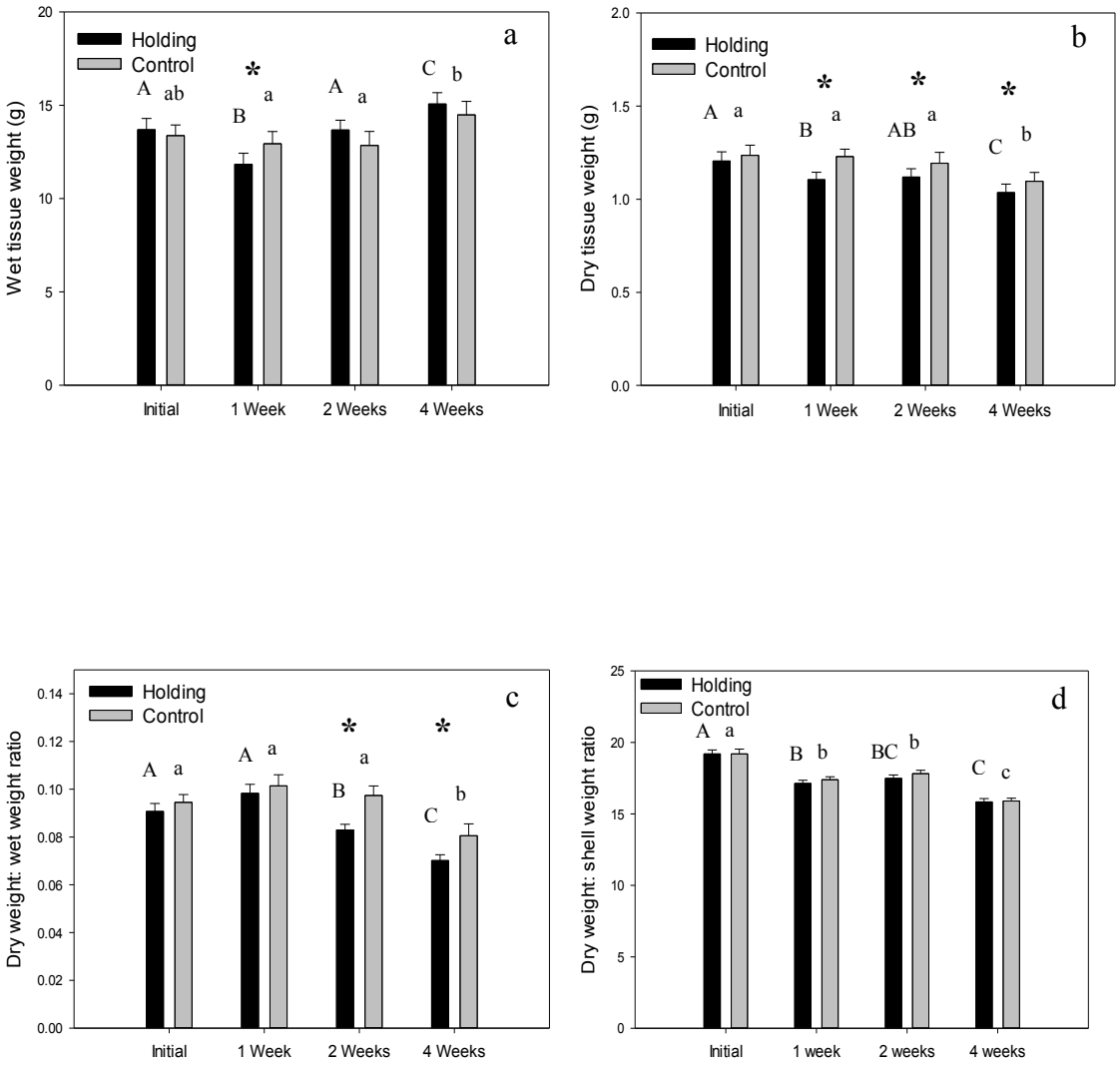

Figure 6.2: Fall 2011 change in mean (± SE) wet soft tissue weight (a), dry soft tissue weight (b), dry weight: wet weight ratio (c) and dry weight: shell weight ratio of held and control mussels (n=150). Upper case letters represent statistical significance in time for held mussels; lower case letters represent statistical significance in time for control mussels. Asterisks represents significant pairwise comparisons at p= 0.05.

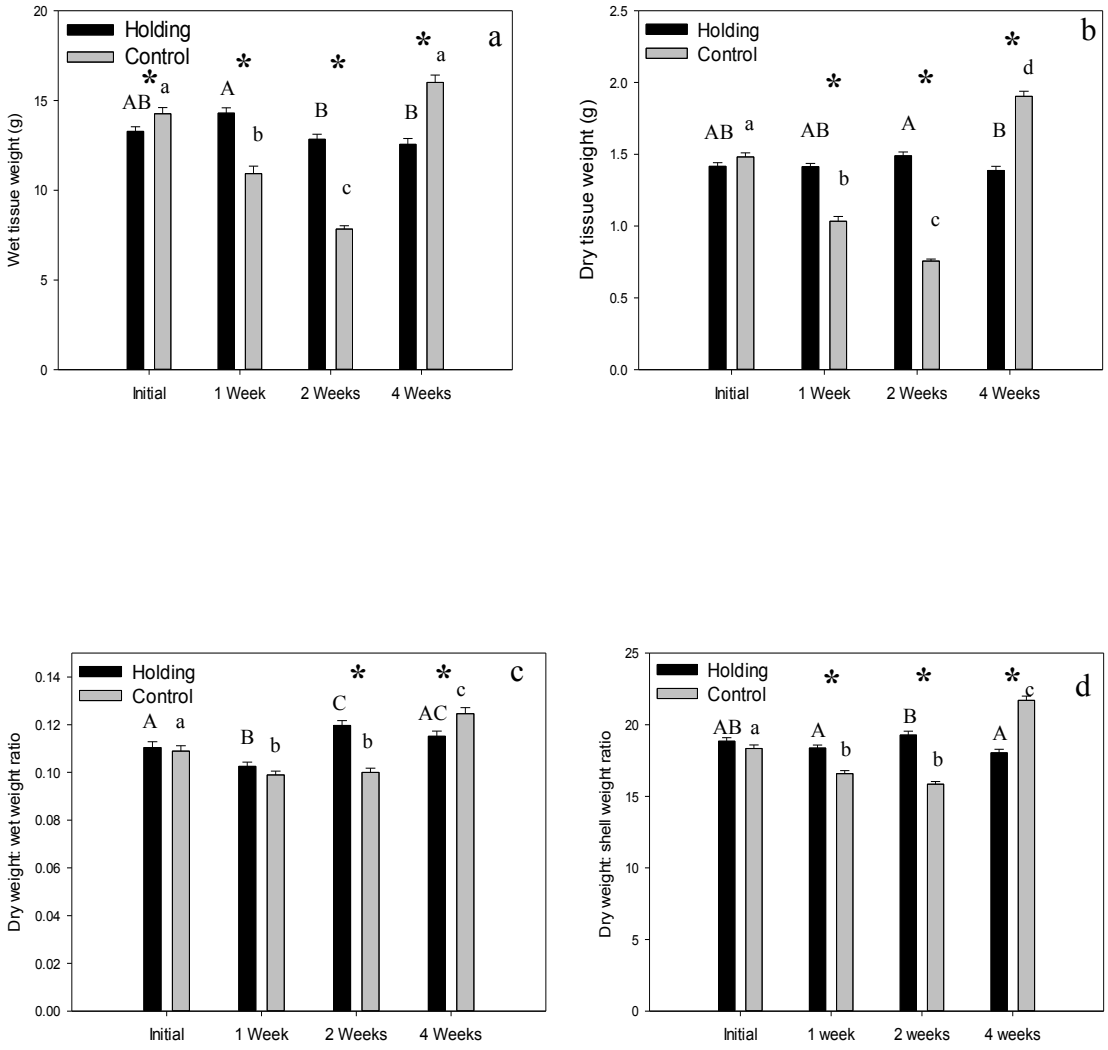

Figure 6.3: Winter 2012 change mean (± SE) wet soft tissue weight (a), dry soft tissue weight (b), dry weight: wet weight ratio (c) and dry weight: shell weight ratio for held and control mussels (n=150). Upper case letters represent statistical significance in time for held mussels; lower case letters represent statistical significance in time for control mussels. Asterisks represents significant pairwise comparisons at p= 0.05.

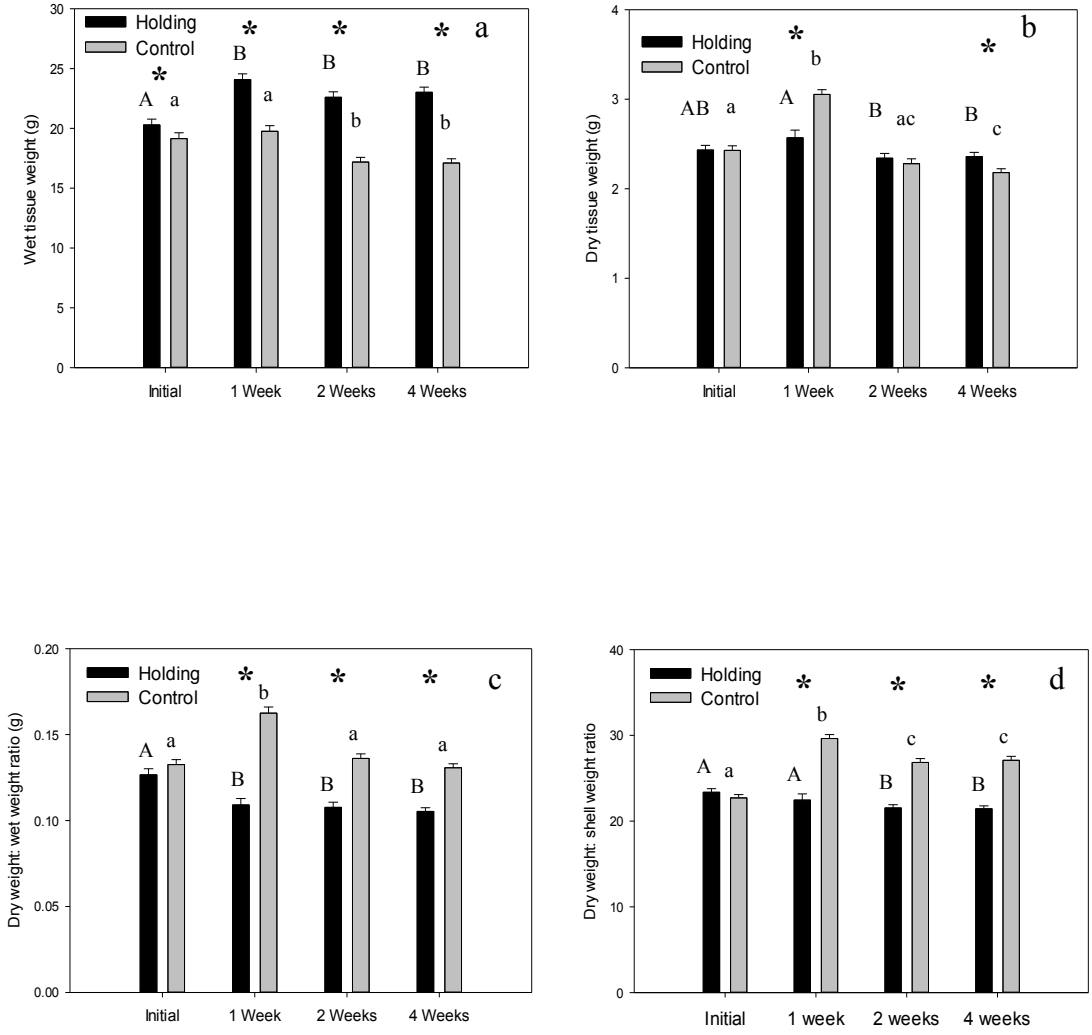

Figure 6.4: Spring 2012 change mean (± SE) wet soft tissue weight (a), dry soft tissue weight (b), dry weight: wet weight ratio (c) and dry weight: shell weight ratio for held and control mussels (n=150). Upper case letters represent statistical significance in time for held mussels; lower case letters represent statistical significance in time for control mussels. Asterisks represents significant pairwise comparisons at p= 0.05.

6.3.2. Lipid and fatty acids analyses

6.3.2.1. Lipid analyses

6.3.2.1.1. Total lipid

Total lipid content was measured by the sum of all lipid classes obtained in the analysis. During the fall season total lipid content increased for held mussels from the initial time to 2 weeks ($p=0.045$). The control mussels peaked in total lipid content at 1 week ($p=0.004$). Held mussels had a significantly higher total lipid content at 2 weeks compared to initial time ($p=0.004$: Table 6.1).

During the winter season the total lipid content for held and control mussels were similar at each sampling point also showing a similar trend over time. However, held mussels showed a peak in total lipid content at 2 weeks ($p=0.049$: Table 6.1).

The spring season revealed the lowest total lipid content of both held and control mussels. The lipid content in control mussels remained comparable during the complete season. Held mussels showed a decline in lipid content at 4 weeks ($p<0.001$). Held and control mussels differed in total lipid content at 1 week, when held mussels had a higher lipid content ($p=0.007$) and at 4weeks, when control mussels had the higher lipid concentration ($p=0.004$: Table 6.1).

Table 6.1: Concentration of total lipids expressed as mg g^{-1} wet soft tissue weight (WW) and fatty acid profile (% of total fatty acids) in mussels kept in holding and control mussels for fall 2011, winter 2012 and spring 2012.

	Initial		1 week		2 weeks		4 weeks	
	Holding	Control	Holding	Control	Holding	Control	Holding	Control
Fall								
Tot lipids (mg g^{-1})	0.32 ± 0.16^{A}	0.35 ± 0.21^{a}	1.14 ± 1.12^{AB}	1.24 ± 0.85^{b}	1.19 ± 0.94^{B}	0.46 ± 0.22^{ab}	0.76 ± 0.67^{AB}	1.25 ± 1.48^{ab}
DHA	20.5±1.1	19.9±2.5	19.8±2.2	21.1±1.6	19.7±1.2	21.5±1.8	20.8±1.6	21.2±1.2
EPA	15.7±1.6	16.4 ± 1.9^{a}	16.7±2.3	14.1 ± 1.7^{bc}	15.9±1.5	15.3 ± 1.6^{ac}	15.5±1.8	13.0 ± 1.4^{b}
Bacterial	5.2±1.1	4.8 ± 0.9^{a}	5.7±1.1	6.1 ± 2.2^{ab}	6.5±1.5	6.1 ± 1.1^{ab}	6.3±1.3	6.3 ± 0.7^{b}
ω3	46.3±0.8	46.5 ± 1.5^{a}	45.8±1.7	44.9 ± 1.6^{ab}	45.3±1.9	45.6 ± 1.4^{ab}	45.4±2.1	44.3 ± 1.2^{b}
Winter								
Tot lipids	0.48 ± 0.35^{A}	0.49±0.41	0.78 ± 0.49^{AB}	0.96±0.48	1.10 ± 0.79^{B}	0.72±0.80	0.59 ± 0.21^{AB}	0.39±0.19
DHA	19.3 ± 1.4^{AB}	19.9 ± 1.3^{a}	19.7 ± 1.7^{A}	17.8 ± 2.0^{ab}	17.7 ± 1.5^{B}	15.6 ± 1.5^{bc}	19.5 ± 1.5^{A}	14.1 ± 2.5^{c}
EPA	19.3±1.4	18.1 ± 2.6^{a}	18.9±0.9	20.3 ± 2.0^{a}	20.3±1.5	23.8 ± 2.3^{b}	20.9±3.0	25.4 ± 1.7^{b}
Bacterial	6.2 ± 0.9^{A}	6.3±0.9	6.2 ± 0.8^{A}	5.9±1.4	6.3 ± 0.8^{A}	6.3±0.8	7.5 ± 1.3^{B}	6.0±1.0
ω3	49.5 ± 1.7^{A}	48.8±2.5	48.3 ± 1.2^{AB}	47.1±1.9	47.6 ± 1.7^{B}	47.0±2.0	47.9 ± 3.0^{B}	47.3±0.8
Spring								
Tot lipids	0.31 ± 0.17^{A}	0.44±0.27	0.42 ± 0.23^{A}	0.23±0.12	0.44 ± 0.16^{A}	0.46±0.25	0.09 ± 0.07^{B}	0.39±0.24
DHA	12.8±2.6	11.4 ± 2.7^{ab}	12.7±2.5	11.2 ± 7.1^{b}	11.8±2.5	9.6 ± 0.9^{b}	11.2±2.2	11.5 ± 1.1^{a}
EPA	26.9±1.7	26.2 ± 2.8^{a}	27.7±2.9	29.6 ± 2.4^{b}	25.6±1.9	29.7 ± 2.7^{b}	25.4±1.8	25.4 ± 0.9^{a}
Bacterial	5.7 ± 1.6^{A}	5.1 ± 1.9^{ab}	4.2 ± 1.4^{AB}	4.3 ± 1.4^{b}	5.4 ± 1.1^{AB}	3.7 ± 1.2^{b}	6.3 ± 1.9^{B}	6.1 ± 0.7^{a}
ω3	46.6 ± 1.6^{A}	45.2 ± 1.6^{ab}	47.2 ± 2.3^{A}	46.7 ± 2.6^{a}	44.1 ± 1.2^{B}	46.1 ± 2.9^{a}	43.6 ± 1.4^{B}	43.1 ± 1.6^{b}

Values represent mean ± SD (n= 10). Upper case letter superscripts represent statistically significant differences in time for held mussels; lower case letter superscripts represent statistically significant differences in time for freshly harvested mussels ($p<0.05$). Grey highlights represent significantly different pairwise comparisons.

6.3.2.1.2. Triacylglycerol: Sterol ratio

During the fall season, held mussels showed a sharp decline in the TAG: ST ratio between 2 and 4weeks ($p=0.015$), while the control mussel ratio is comparable across all time points (Figure 6.5a). During the winter season held mussels again had a slightly higher ratio than control mussels up to week 4, when the ratio declined from the 1 and

2vweek- value ($p=0.01$). Control mussels had a stable ratio during the entire season and at 4 weeks it was significantly higher than in held mussels ($p=0.005$) (Figure 6.5b).

During the spring season the TAG: ST ratio reached its peak level across all three seasons examined. Held mussels had a significantly lower ratio than control mussels at 1 week ($p<0.001$) and 2 weeks ($p=0.036$); but not at 4 weeks. Control mussels showed an increase in the TAG: ST ratio at 2 weeks and this ratio was higher than at the initial time point and at 4 weeks ($p=0.011$) (Figure 6.5c).

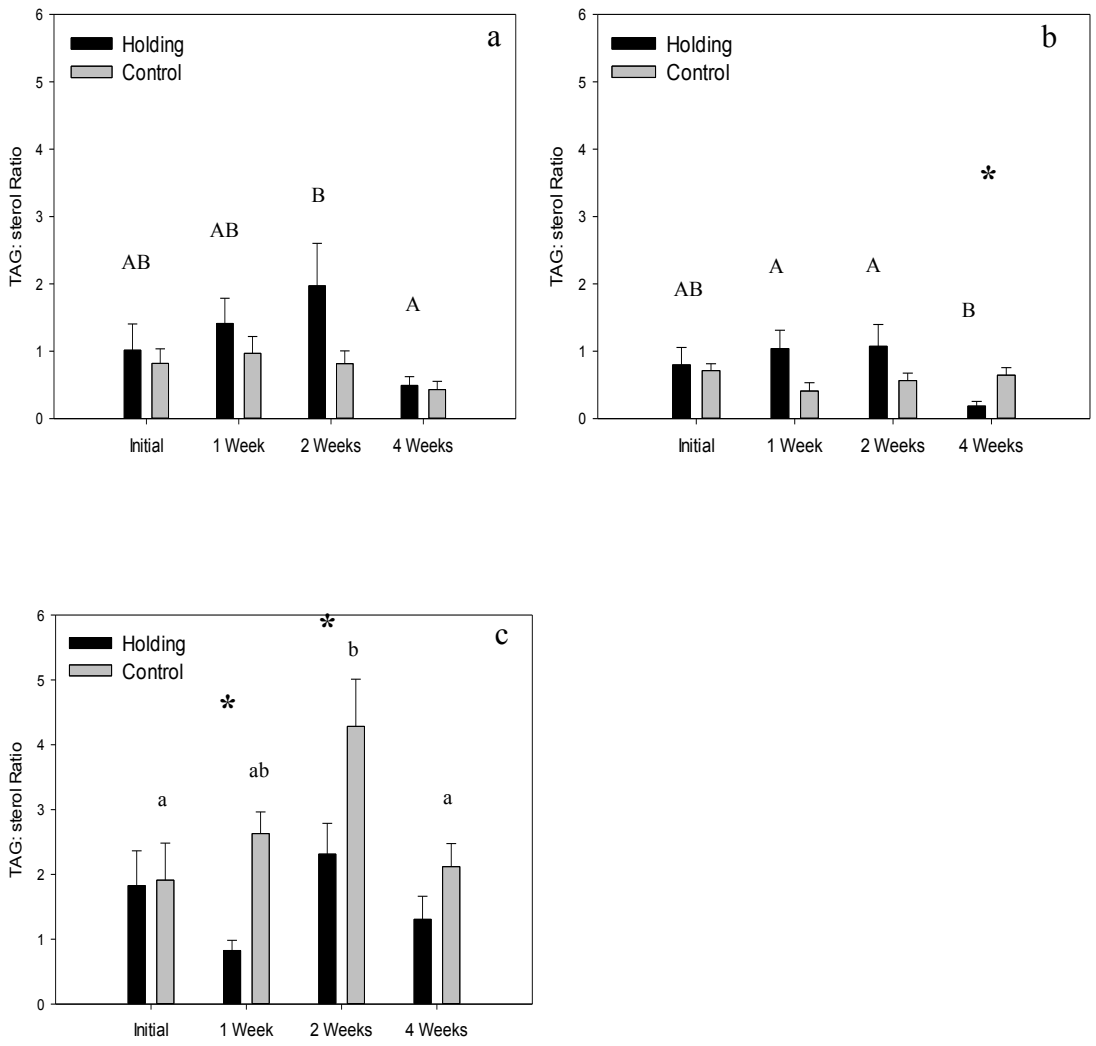

Figure 6.5: Triacylglycerol: sterol ratio for held and control mussels for fall 2011 (a), winter 2012 (b) and spring 2012 (c) seasons (n=10). Bars represent mean ± SE. Upper case letters represent statistical significance over time for held mussels; lower case letters represent statistical significance over time for control mussels. Asterisks represents significant pairwise comparison.

6.3.2.2. Fatty acid analyses

For the fall season, levels of docosahexaenoic acid (DHA) at 2 weeks were lower in held mussels than in control mussels ($p=0.018$) (Table 6.1). During the winter season control mussels showed a declining trend in DHA content starting from 2 weeks. Held and control mussels differed in DHA content at 1 week ($p=0.04$), 2 weeks ($p=0.007$) and 4 weeks ($p<0.001$), with held mussels consistently having the higher DHA level. During the spring season the DHA content of held mussels was similar during the whole season, while DHA content in control mussels was lowest at 2 weeks, followed by an increase at 4 weeks ($p<0.001$). Held mussels showed a higher DHA content than controls at 1 week ($p=0.009$) and 2 weeks ($p=0.041$), similar to what was observed for the winter season (Table 6.1).

During the fall season eicosapentaenoic acid (EPA) content in held mussels was comparable across all sampling times. Control mussels declined in EPA content from initial sampling to 1 week ($p=0.02$) and to 4 weeks ($p<0.001$) (Table 6.1). Held mussels contained more EPA at 1 week ($p=0.01$) and 4 weeks ($p=0.003$). During the winter season control mussels increased in EPA levels from the initial time point to 2 weeks ($p<0.001$). Control mussels presented a higher content of EPA than held mussels at 2 weeks ($p<0.001$) and 4 weeks ($p=0.004$). During the spring season EPA levels in held mussels remained similar across all the sampling times, while control mussels had a significant peak at 1 and 2 weeks followed by a decrease at 4weeks ($p=0.001$). Control mussels were significantly higher than held mussels in EPA ($p<0.001$) at 2 weeks (Table 6.1).

During the fall control mussels increased in bacterial fatty acid proportions from the start to 4 weeks ($p=0.013$), while held mussels did not show this increase (Table 6.1). Held and control mussels did not differ in bacterial fatty acid levels at any time during the season. During the winter bacterial fatty acid levels were comparable between held and control mussels and between time points, with the exception of held mussels at 4 weeks, which had a higher level compared to the other time points and to control mussels at 4 weeks ($p=0.012$). During the spring bacterial fatty acid content in held mussels was higher at 4 weeks than at the initial time point ($p=0.03$). In control mussels bacterial fatty acid content was higher at 4 weeks than at 1 week ($p=0.003$). Held mussels showed a higher bacterial fatty acid content than controls at 2 weeks ($p=0.005$: Table 6.1).

Held and control mussels did not differ in total omega-3 fatty acid content at any sampling point during the three seasons examined. During the fall ω-3 content in control mussels was higher initially than at 4 weeks ($p=0.011$) (Table 6.1). The levels of ω-3 fatty acids during this season ranged from 41 to 49% of total fatty acids. During the winter the total proportion of ω-3 fatty acids remained at a high level during the whole season (44-56% of total fatty acids). However, a slight but significant decline occurred in held mussels from the initial time to 2 weeks and 4 weeks ($p=0.01$). During the spring season, held mussels declined in total ω-3 fatty acid levels from the initial time point to 2 weeks ($p=0.009$) and 4 weeks ($p=0.001$), while control mussels declined from 1 week to 4 weeks ($p=0.007$: Table 6.1). The level of total ω-3 fatty acids during the spring season varied between 40 and 51% of total fatty acids.

6.3.3. Glycogen analysis

During the fall, mussels kept in holding reached the lowest concentration of glycogen at 2 weeks, and it was significantly lower than at 4 weeks ($p=0.016$) (Table 6.2). At 2 weeks held mussels had also a lower glycogen concentration than controls ($p<0.001$). However, mussels in holding reached a level comparable to control mussels at week 4 (Table 6.2).

During the winter, mussels did not differ significantly between holding and control at any time point. However, glycogen content increased at 2 weeks for held mussels ($p=0.004$) and 4 weeks for controls ($p=0.001$) compared to values at 1 week and initial (Table 6.2). During the spring, control mussels had a higher glycogen content than held mussels at 1 week ($p=0.016$). However, held mussels did show an increase in glycogen content from the initial time point to 4 weeks: glycogen content at 2 and 4 weeks was significantly higher than at the initial time point ($p<0.001$: Table 6.2).

Table 6.2: Glycogen content for mussels kept in holding and freshly harvested mussels during fall 2011, winter 2012 and spring 2012.

Time	Glycogen content in $mg \cdot g^{-1}$					
	Fall 2011		*Winter 2012*		*Spring 2012*	
	Holding	Control	Holding	Control	Holding	Control
Initial	14.23 ± 4.65^{AB}	16.82 ± 5.09^{ab}	13.71 ± 5.22^{A}	14.76 ± 6.98^{ab}	31.75 ± 8.89^{A}	34.82 ± 11.02
1 week	15.25 ± 5.38^{AB}	15.27 ± 3.81^{a}	13.03 ± 4.17^{A}	12.48 ± 3.63^{a}	40.32 ± 8.81^{AB}	48.57 ± 11.64
2 weeks	10.27 ± 3.37^{B}	19.92 ± 4.60^{ab}	21.08 ± 7.73^{B}	21.00 ± 6.08^{bc}	50.30 ± 15.90^{BC}	66.36 ± 39.15
4 weeks	20.10 ± 8.34^{A}	23.17 ± 8.89^{b}	19.45 ± 4.18^{AB}	24.24 ± 8.79^{c}	59.52 ± 18.10^{C}	42.23 ± 21.70

Values represent mean ± SD (n= 10). Upper case letter superscripts represent statistical significance in time for held mussels; lower case letter superscripts represent statistical significance in time for freshly harvested mussels ($p<0.05$). Grey highlights represent significantly different pairwise comparisons.

6.3.4. Sensory evaluation

6.3.4.1. Triangle test

Over the three seasons the time points presenting the highest number of panellists correctly detecting the odd samples included fall- 2 weeks, winter- initial and winter- 2 weeks (Table 6.3). However, it is noteworthy that the results of the triangle test were not statistically significant at any time point during the three seasons. Therefore, it appears that mussels held for up to one month and field control mussels do not differ in term of quality and taste.

Table 6.3: Triangle test results obtained with mussels kept in holding and freshly harvested mussels during fall 2011, winter 2012 and spring 2012

	Correct (# panellists)	Incorrect (# panellists)	Total panellists
Fall			
Initial	6	18	24
1 Week	12	12	24
2 Weeks	8	16	24
4 Weeks	10	14	24
Winter			
Initial	12	12	24
1 Week	11	13	24
2 Weeks	12	12	24
4 Weeks	9	15	24
Spring			
Initial	7	17	24
1 Week	8	16	24
2 Weeks	10	14	24
4 Weeks	7	17	24

6.3.4.2. Hedonic test

During the fall season held mussels and controls did not differ significantly in appearance, texture and flavour. Odour seemed to present higher variability between sampling times and between held and control mussels. However, only at one week was the difference between the two groups significant: mussels in holding had a higher score than freshly harvested mussels ($p= 0.007$: Table 6.4).

During the winter, appearance, odour and texture scores did not differ significantly between held and control mussels (Table 6.4). In terms of appearance, at 2 weeks the field control mussels had a low score which was significantly different to that at 4 weeks ($p=0.022$). Similarly, the odour for the control group at 2 weeks obtained a lower score, different from the initial time point ($p=0.003$), and that obtained at 4 weeks ($p=0.003$). Flavour did differ between held and control mussels at 2 weeks, with held mussels scoring higher ($p=0.036$: Table 6.4).

During the spring, there was no difference in appearance, texture or flavour between held and control mussels (Table 6.4). The former scored lower than controls for odour at 2 weeks ($p=0.049$). Flavour results for the held group declined throughout the season, although this decline was not significant (Table 6.4).

Table 6.4: Hedonic test scores obtained with mussels kept in holding, and freshly harvested mussels during fall 2011, winter 2012 and spring 2012

Characteristic	Score (7-point scale)							
	Initial		1 week		2 weeks		4 weeks	
	Holding	Control	Holding	Control	Holding	Control	Holding	Control
Fall 2011								
Appearance	5.0±0.9	4.7±1.3	5.0±1.5	5.0±1.6	5.0±1.3	4.9±1.1	5.1±1.1	5.4±0.9
Odour	4.7±1.3^{AB}	4.9±1.4	5.3±1.3^{A}	4.3±1.1	4.3±1.2^{B}	4.7±1.1	4.5±0.8^{AB}	4.4±1.4
Texture	4.5±1.3	4.5±1.5	4.0±1.3	3.7±1.3	4.1±1.5	3.7±1.2	4.2±1.3	4.1±1.6
Flavour	4.9±0.9	4.7±1.1	4.7±1.2	4.3±1.2	4.2±1.1	4.3±1.3	4.6±1.2	4.2±1.4
Winter 2012								
Appearance	4.2±1.6	4.2±1.4^{ab}	4.7±1.6	3.9±1.4^{ab}	3.9±1.4	3.1±1.6^{b}	3.9±1.7	4.4±1.6^{a}
Odour	4.9±1.1	5.0±1.2^{a}	4.8±1.3	4.5±1.3^{ab}	4.6±1.5	3.7±0.9^{b}	4.3±1.3	4.7±1.6^{a}
Texture	4.2±1.1	4.3±1.4	4.2±1.2^{ab}	4.7±0.9	4.6±1.5	3.9±1.2	4.4±1.2	4.7±1.2
Flavour	4.8±1.0	4.8±0.9	4.9±1.0	4.3±0.9	5.0±1.5	4.1±1.2	4.2±1.3	4.6±1.2
Spring 2012								
Appearance	4.8±1.0	4.5±1.2	3.9±1.4	4.7±1.3	4.5±1.1	5.1±1.3	4.2±1.3	4.1±1.2
Odour	4.7±1.3	4.7±1.3	4.7±1.1	4.6±1.2	4.4±1.1	5.0±1.0	4.2±0.8	4.3±1.2
Texture	4.5±1.2	4.7±1.3	4.4±0.9	3.9±1.5	3.7±1.4	4.1±1.6	4.5±1.1	3.9±1.1
Flavour	5.0±1.1	5.0±0.9	4.7±1.2	4.4±1.3	4.4±1.4	5.1±1.1	4.2±0.8	4.4±1.2

Values represent mean ± SD (n= 24). Upper case letter superscripts represent statistical significance over time for held mussels; lower case letter superscripts represent statistical significance over time for freshly harvested mussels ($p<0.05$). Grey highlights represent significantly different pairwise comparisons

6.4. Discussion

The present study assessed the change in condition and biochemical composition of cultured mussels held in an ambient commercial wet storage facility during fall, winter, and spring in Newfoundland and compared it to freshly harvested field controls.

In order to measure the change in condition a combination of dry weight: wet weight ratio and dry weight: shell weight ratio was used (Lucas & Beninger, 1985). These ratios

describe the change in tissue water content and loss in dry tissue weight indicating variation in the animal's energy balance (Lucas & Beninger, 1985). In the spring and fall mussels maintained in the holding facility exhibited an increase in wet tissue weight leading to a decline in the dry weight: wet weight ratio. This suggests a loss of dry tissue weight and an increase in water content. A high proportion of tissue water content is often related to depleted energy reserves (Lucas & Beninger, 1985). These results are in agreement with those described by Wyatt et al. (2013), where depleted condition occurred after 2-3 months. In the present study, depleted energy reserves seemed to occur after only two weeks in holding during the fall season and as early as one week in the spring season. The results of the dry tissue weight to shell weight ratio analysis showed a similar trend only during spring.

During winter a different pattern was observed for held mussels. In this case results indicated only a slight decline in wet weight at the end of the trial (four weeks), together with an increase in the dry weight: wet weight ratio. Field control samples taken during the same period declined in wet weight and dry weight during the first two weeks of the experiment. This is also reflected in the condition ratios. This trend seems to indicate that holding during the winter season at colder temperatures may present more stable environmental conditions for the mussels possibly causing less physiological stress and improved condition. Wyatt et al. (2014) observed a similar effect during holding up to three months under similar winter conditions. However, the marked differences between held and control mussels at week 2 during the winter season may be explained by the fact that during that period the mussels used as controls were mistakenly harvested from a different line. The mussels were of the same year class as those in holding, but from an

initial medium grading size (3/4- 1 inch; 19- 25.4 mm), while the mussels used for holding were initially graded as large (1-1.75 inch; 25.4- 44.45 mm).

Different studies have investigated meat yield, condition, biochemical composition and general nutritional quality of cultured mussels in relation to seasonal variability (Okumus & Stirling 1998; Orban *et al*., 2002). The biochemical composition of *M. edulis* has been shown to follow seasonal changes especially in relation to the reproductive cycle, since gametogenesis requires essential lipids and fatty acids, and sufficient energy resources (Okumus & Stirling 1998; Orban *et al*., 2002). However, when food levels are below the maintenance ration, mussels reach a state of metabolic imbalance and must utilize their body reserves to meet the metabolic energy demand (Gabbott, 1976). During the fall and winter, mussels kept in holding showed a comparable or even higher total lipid content than control mussels. This suggests a good metabolic balance and therefore appropriate nutrient availability with the environmental conditions maintained in the storage facility. In contrast, during the spring season, a sharp decline in lipid content of held mussels at 4 weeks was noted, suggesting a metabolic imbalance. This results could be due to increasing seasonal water temperatures combined with the possible negative effect of holding densities. Mussels subjected to a temperature increase appear also to have high energy requirement and therefore a higher metabolic rate and feeding needs (Widdows & Bayne, 1971). During the spring, the total lipid content was found to be the lowest for both held and field control mussels. This also seems to suggest a seasonal phenomenon related to the reproductive cycle and the natural stress response associated to it (Gabbott, 1976; Harding *et al*., 2004a).

Triacylglycerols (TAG) play a role as energy reserves (Gabbott, 1976; Fraser, 1989) accumulating during feeding and declining during starvation and may thus give useful information about animal condition (Fraser, 1989). However, TAG content depends on animal size and therefore to give an applicable index it needs to be expressed relative to sterol (ST) content which correlates highly with animal size (Fraser, 1989). Sterols are membrane lipids and they have been shown to vary in relation to temperature changes (Pernet *et al*., 2007). However, during the course of this experiment, sterol content either did not change significantly over time or its changes were not correlated to water temperature. Therefore, the effect of temperature on sterol content can be considered negligible in the present study. During the fall and winter trials held mussels often presented a TAG: ST ratio comparable to or higher than control mussels with the only exception found at the 4 weeks time point in winter. Alternatively, during spring the ratio was constantly lower in held mussels and may denote depletion of energy reserves and the consequent use of stored lipids. This seems to reflect the results of condition analysis. Triacylglycerols also appeared to follow a seasonal pattern, as noted in the high levels found in the field controls during spring. Interestingly, spring was also the season in which mussels attained the lowest levels of total lipids. The greatest proportion of total lipid appeared to be contributed by TAG suggesting that field control mussels are accumulating reserves in this season. Triacylglycerols originate from the conversion of glycogen; in *Mytilus edulis* they accumulate during winter, reaching a peak during stage III of gametogenesis (Gabbott, 1976). Following oxidation TAGs are used as an energy source during early embryonic development (Gabbott, 1976). They have been shown to be mobilized from the digestive gland to the mantle tissue to sustain gamete production

with temperature increase (Tremblay *et al*., 2011). The high levels of TAG during spring may suggest that the mussels were in stage III of gametogenesis and therefore ready to spawn. During the same season, the lower content of TAG in held mussels may suggest higher standard metabolism compared to control mussels and higher energy maintenance requirements. Therefore, the energy available to allocate to other functions and to accumulate as reserve is reduced (Gabbott, 1976; Tremblay *et al*., 2011).

Food quality is key to mussel growth and health. Microalgae can be considered one of the major fatty acid sources for bivalves (Alkanani *et al*., 2007; Ventrella *et al*., 2008). During this experiment no major differences were observed for fatty acid content between held and control mussels. It is noteworthy however, that in the fall there was a lower content of DHA (22:6ω3) in held mussels compared to controls. This corresponded to a higher content of EPA (20:5ω3). This pattern is inverted in the winter and spring experiments, when held mussels had a higher content of DHA, while control mussels had a higher content of EPA. Previous studies in Notre Dame Bay, Newfoundland, observed seasonal variation in the fluctuation of DHA and EPA content and also occasionally a reciprocal relationship of these two fatty acids (Khan *et al*., 2006; Alkanani *et al*., 2007). This trend is thought to reflect a diatom versus a dinoflagellate based diet (Alkanani *et al*., 2007). In the present study it is possible that a difference in phytoplankton composition existed between the part of the bay where the field controls were sampled and the area near where the water supply for the holding tanks was sourced. Generally however, the sum of ω-3 fatty acids was constant during all three seasons examined as well as between held and field control mussel groups. Bacterial fatty acids were only observed to increase in held mussels at the 4 week time point during the winter season

and at the 2 week time point during the spring. This increase may suggest a rise in bacterial levels in the holding water. However, this increase could not be associated with any particular event and therefore could be due to mussel natural individual variation in bacterial content related to food digestion. Carbohydrates play a dominant role in bivalve metabolism as nutrient stores and as a source of energy to support gametogenesis (Mathieu & Lubet, 1992). The seasonal cycles for storage and utilization of glycogen reserves reflect the interaction between food supply, temperature, growth and reproductive cycle. The seasonal changes in lipid content of *M. edulis* show an inverse correlation with the changes in glycogen content (Gabbott, 1976). In the present study, with the exception of the 2 week time point during the fall and the 1 week time point during the spring the glycogen level was comparable between held and field control mussels. At the 2 week time point in the fall a lower content of glycogen was observed for held mussels. This could be due to a temporary (48 h) water shut-down in the holding facility, resulting in the reduction or absence of food and increased environmental stress during which time the mussels may have started to use internal energy reserves. Currently, we do not have an explanation for the difference at the 1 week time point in the spring. The glycogen content during spring shows an inverse correlation with the total lipid content peak and a positive correlation with TAG content. This may be related to the storage of lipids and reproductive effort (Gabbott, 1976).

The organoleptic survey from the present study revealed that mussel quality was comparable between held and field control mussels, with the exception of a few isolated cases. The triangle test is used to determine a non-specific sensory difference between two treatments (Carpenter *et al*., 2000). During this test the panellists were not able to

discriminate between held and control mussels at any point in time for the three seasons examined. The hedonic test is a descriptive method where the assessors are asked to select a category on a scale (Carpenter *et al*., 2000). This type of test is suggested as a practical tool for evaluating freshness of seafood during the complete production chain (Hyldig & Green-Petersen, 2004). The results of this study indicated a difference between held and control mussels only in two isolated cases for odour and one for flavour; in two of these cases the held mussels scored higher than the field control and therefore were considered "preferable" by the panellists. It is possible that these isolated cases were due to individual variation and they do not appear to be linked to the holding condition. Moreover, the nutritional characteristics of the mussels, in particular ω-3 fatty acid content, remained unaffected by the holding condition. Therefore, it is reasonable to state that the final quality of the mussels after one month in ambient wet holding appears unaltered with reference to the recorded observations of the tasting panel and of the fatty acid analysis. It is however interesting to note that at 4 weeks sampling time during the winter and 2 weeks in spring control mussels scored higher than held mussels for each characteristic examined. Although the difference in score was not significant, it corresponded to a higher content in bacterial fatty acids in held mussels and higher content of TAG in control mussels; it is thus possible to hypothesise that both bacterial fatty acids and TAG levels were involved in the determination of the perceived sensory characteristics of blue mussels.

6.5. Conclusions

In conclusion, results from the current study suggest that while physiological and biochemical changes are evident in mussels stored up to one month (i.e., loss in condition/dry weight; lower TAG: ST ratio) when compared directly to field controls, generally there appears to be no overall significant effect on the quality of the product based on lipid/fatty acid and glycogen content. This also appears to be reflected in the taste panel results. Season seems to be the most important factor to consider in an ambient facility. Erring on the side of caution and with careful consideration of changes in seasonal environmental parameters, results from the present study lead to recommend that the length of the holding period under the conditions investigated here should be limited to a maximum of two weeks. This should be reduced further at the discretion of the farm manager if environmental conditions change.

It is important to note that the design of the holding and storage facilities will vary depending on the needs of the farmer and the region. Closed contained facilities and/or those with environmental control still need to be monitored so as to consistently maintain the marketed product at optimum quality. It would be interesting to investigate and compare the variation in biochemical and organoleptic parameters observed in the present study with those from mussels held in an environmentally controlled facility.

6.6. References

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CHAPTER 7

Summary

7.1. Overall conclusions

The present study investigated the effects of the culture environment on *Mytilus edulis* condition and quality. Particular attention was given to culture depth and post-harvest practices, focusing on environmental characteristics, mussel condition, biochemical content, physiological stress response, and final product quality. Moreover, an extensive review of bivalve aquaculture effects on the environment was incorporated into the thesis, to emphasize the importance of investigating environment-cultured mussel interactions in both directions. Research in the field of aquaculture has the responsibility to not only enhance culture, but also to find a balance between production and environmental sustainability.

The effect of culture depth was investigated over two years of sampling of mussel and environmental conditions, comparing deep and shallow water culture sites (Chapter 3, 4 & 5). Taking into consideration the substantial dissimilarities between the two years examined, the comparison between shallow and deep water cultured blue mussel sites showed differences, at times pronounced, particularly for temperature, salinity and chlorophyll *a* concentration. Deep water sites appeared to supply, at least in part, a more stable environment for mussel growth (Chapter 3). However, no noticeable effect on *M. edulis* physiological stress responses were observed either in shallow or in deep water grown mussels (Chapter 4). In contrast, condition and biochemical profile of cultured

blue mussels seemed to be affected by culture depth, in particular in Year 2 of the experiment, when unusual extreme weather conditions were present during winter. Deep water cultured mussels showed better condition and fatty acid (ω-3) profile at the time of harvest, supporting the hypothesis that deep water supplies a more stable environment for *M. edulis* growth. The deep water Mouse Island site often represented an exception when compared with all the other sites analysed: in Year 1, it showed higher condition, higher ω-3 fatty acid content, lower shell weight, and lower terrestrial fatty acid content than the other sites. This could possibly be due to its location in a channel further offshore from the head of the inlet and at greater depth than the other sites. In my opinion, in order to see more marked differences between shallow and deep water cultured mussels, the distance from the head of the bay should be considered. In Newfoundland, open water mussel culture away from bays and inlets may not be feasible due to extreme weather and ice conditions during certain periods of the year. However, sites such as Mouse Island which is still close to shore but further away from the head of the inlet could constitute a good alternative location to develop deep water mussel culture.

Post-harvest, extended ambient live holding appeared to affect cultured mussel condition, in particular when it was protracted for longer than two weeks; the dry soft tissue weight: wet soft tissue weight ratio proved to be the most effective measure of condition during this study. However, the perceived final quality of the mussels by consumers was not altered by holding. In addition, the nutritional content of the held mussels, based on the content of omega-3 fatty acids, did not decrease during live holding (Chapter 6).

To improve the results of the present study, growth (new tissue deposition) could have been measured, in order to validate the results obtained with the condition indices calculation. Also, an analysis of both protein and ash content could have been performed; these analyses would have completed the biochemical profile of the mussels, in both the culture depth and the live holding studies. Moreover, the collection and analysis of discrete water samples (i.e. nutrients, phytoplankton composition and size structure) could have helped in highlighting differences in environment between shallow and deep water, and between held and freshly harvested mussels. Finally, the nutritional profile of held and freshly harvested mussels could have been analysed and linked to the taste panel results, in order to have a better understanding of the effect of live holding on the final product quality.

7.2. Importance of the study

The present research highlights how the environment-mussel relationship is affected by local conditions and their seasonality. This is the first comprehensive study to investigate the potential of deep water mussel aquaculture in Newfoundland; it is also the first study attempting to relate mussel palatability and condition during extended ambient holding. Therefore, the results of this study constitute important new knowledge available for the aquaculture industry, in Newfoundland, and in other parts of the world. The analysis of the environmental conditions in South Arm, New Bay, Notre Dame Bay, is the first example of a comprehensive study in this particular inlet; to date, only an extensive but

very localized study of Charles Arm, an inlet of South Arm, has been published (Penney *et al*., 2001).

The data collected for this study also have the potential to be further analysed to present a better and more complete picture of the hydrography and oceanography of South Arm, Notre Dame Bay. For instance, salinity data obtained with the moored instruments may be corrected and analysed to show continuous seasonal profiles of salinity at all sites. Temperature, salinity and density data obtained with the CTD profiles could be further analysed and plotted to show seasonal snap-shots of the bay. Finally, the flux of organic particles can be calculated from the current and chlorophyll *a* data.

7.3. Future directions

Generally, the results of this study suggest that deep water blue mussel aquaculture does deserve attention, and its development in Newfoundland is recommended. However, the current knowledge would benefit from further studies highlighting a better understanding of the relationship between the local hydrography and the phytoplankton dynamics, consequently linking *M. edulis* growth and condition to the physical environment. For instance, a more frequent (i.e., monthly) sampling of mussels would be beneficial in order to better link the continuous environmental data to the mussels' condition and biochemical profile. Also, monthly collection of water samples would increase the information about phytoplankton composition and size structure, and aid calibration of the moored instruments. Moreover, the effect of the mussels' reproductive cycle could be studied in relation to culture depth, in order to understand if the mussel response to depth

is affected by seasonal spawning and to understand if such response changes between male and female subjects.

In Newfoundland, future research may also focus more closely on the environmental gain and increased sustainability that it is possible to achieve with deeper water and offshore mussel culture. Farm deposition and benthic impact could be evaluated comparing both shallow versus deep water sites, and inshore versus offshore sites. Future studies could also be extended to include a more in depth analysis of nutrients and phytoplankton, not only limited to chlorophyll *a* data. Investigations on benthic impact and nutrient depletion for offshore mussel culture are currently ongoing in other provinces of Canada (McPhee *et al.*, 2015).

The post-harvest practice of extended, ambient live-holding could also be investigated further, including continuous monitoring of environmental parameters, such as dissolved oxygen and chlorophyll *a*, both in holding and in the field. These additional parameters would help in evaluating exactly when holding conditions start to differ from those in the farm, and to what extent. Moreover, it would be beneficial to investigate if and how the condition of holding affects the final product shelf-life, during the transport to the market and in the sale location (retail). Finally, as a general note on Newfoundland blue mussel aquaculture, future research should concentrate more in-depth on the mutual relationship between the environment and mussels, developing studies on the environmental effect of the culture, in particular concentrating on ecological carrying capacity.

7.2. References

McPhee, D., Donaghy, T., Duhaime, J., and Parson, G.J. (Eds.). (2015). Canadian Aquaculture R&D Review 2015, Shellfish: Mussels. Aquaculture Association of Canada Special Publication, 24, p. 82-83.

Penney, R.W., McKenzie, C.H., and Mills, T.J. (2001). Assessment of the particulate food supply available for mussel (*Mytilus* spp.) farming in a semi-enclosed, northern inlet. Estuarine, Coastal and Shelf Sciences, 53, 107-121.

Appendix A: Chapther 2 integrative tables

Table A1: Main effects of bivalve aquaculture on the environment and their direct consequences. Grey highlight represents effects that can be considered both negative and positive depending on the situation.

	Effect	Consequences
Water column and nutrients	Phytoplankton modification	Bloom modification
	Reduced turbidity	Increased light penetration
	Increased NH4+	Increased primary production
	Metals concentration	
Sediment and benthic habitat	Increased deposition	Anaerobic sediment Increased bacteria and meiofauna Decreased suspension-feeders Increased deposit feeders
	Modification of topography and hydrography	Habitat creation/modification
	Removal of calcium carbonate	Increased acidification Decreased positive feed-back
Other marine species	Nutrient and habitat modification	Increased crustaceans & some fish Seagrass displacement Disturbance for mammals and birds Creation of new habitat for birds
	Food competition	Decreased zooplankton & larval fish
Introduction	Introduction of nonnative species	Diseases introduction Pest introduction

Table A2: Evaluation measures and prevention methods for the main effects of bivalve aquaculture on the environment. Evaluation and prevention measures may be employed separately or in conjunction under an ecosystem-based management plan.

Effect	Evaluation measure	Prevention	
Phytoplankton modification	Environmental indicators (water)	Ecological carrying capacity models	**Ecosystem based management for bivalve aquaculture**
Nutrient modification	Environmental indicators (water)	Ecological carrying capacity models	
Increased deposition	Environmental indicators (sediment)	Ecological carrying capacity models	
Benthic fauna modification	Environmental indicators (sediment)	Ecological carrying capacity models	
Habitat modification	Environmental indicators (sediment)	Ecological carrying capacity models	
Effects on marine mammals and birds		Environmental risk assessment BMP & codes of conduct	
Introduction of nonnative species, diseases and pests	Quarantine Hatchery Testing	Environmental risk assessment BMP & codes of conduct Legislation	

Appendix B: Maps of study area

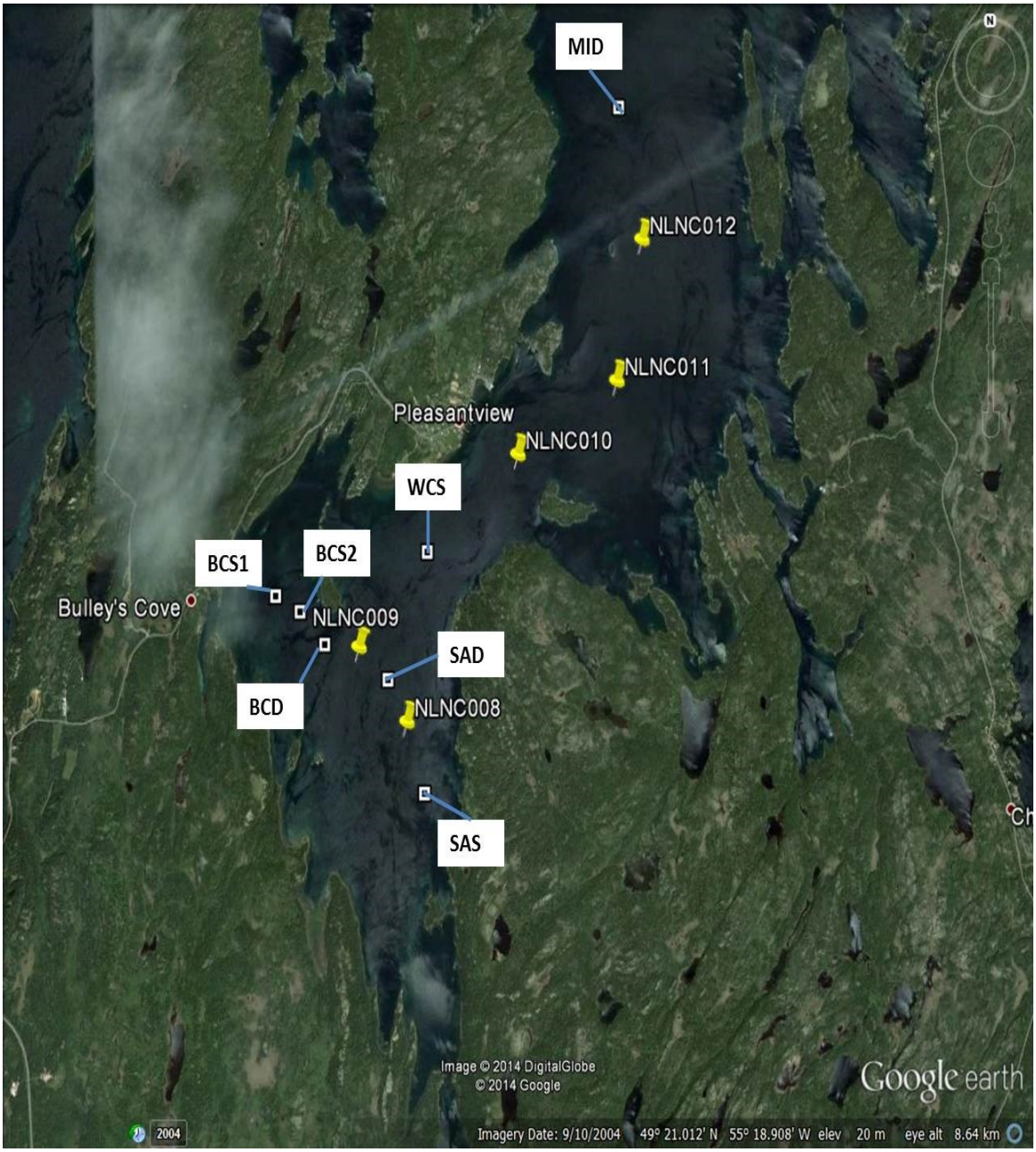


Figure B1: Map of study sites for Chapters 3, 4 and 5 (Pleasantview, Notre Dame Bay, NL, Canada): South Arm shallow (SAS), South Arm deep (SAD), Bulley's Cove shallow (BCS1 and BCS2), Bulley's Cove deep (BCD), Mouse Island deep (MID). Yellow pins represent stations where CastAway-CTD profiles were taken in order to allow better profiling of the bay.

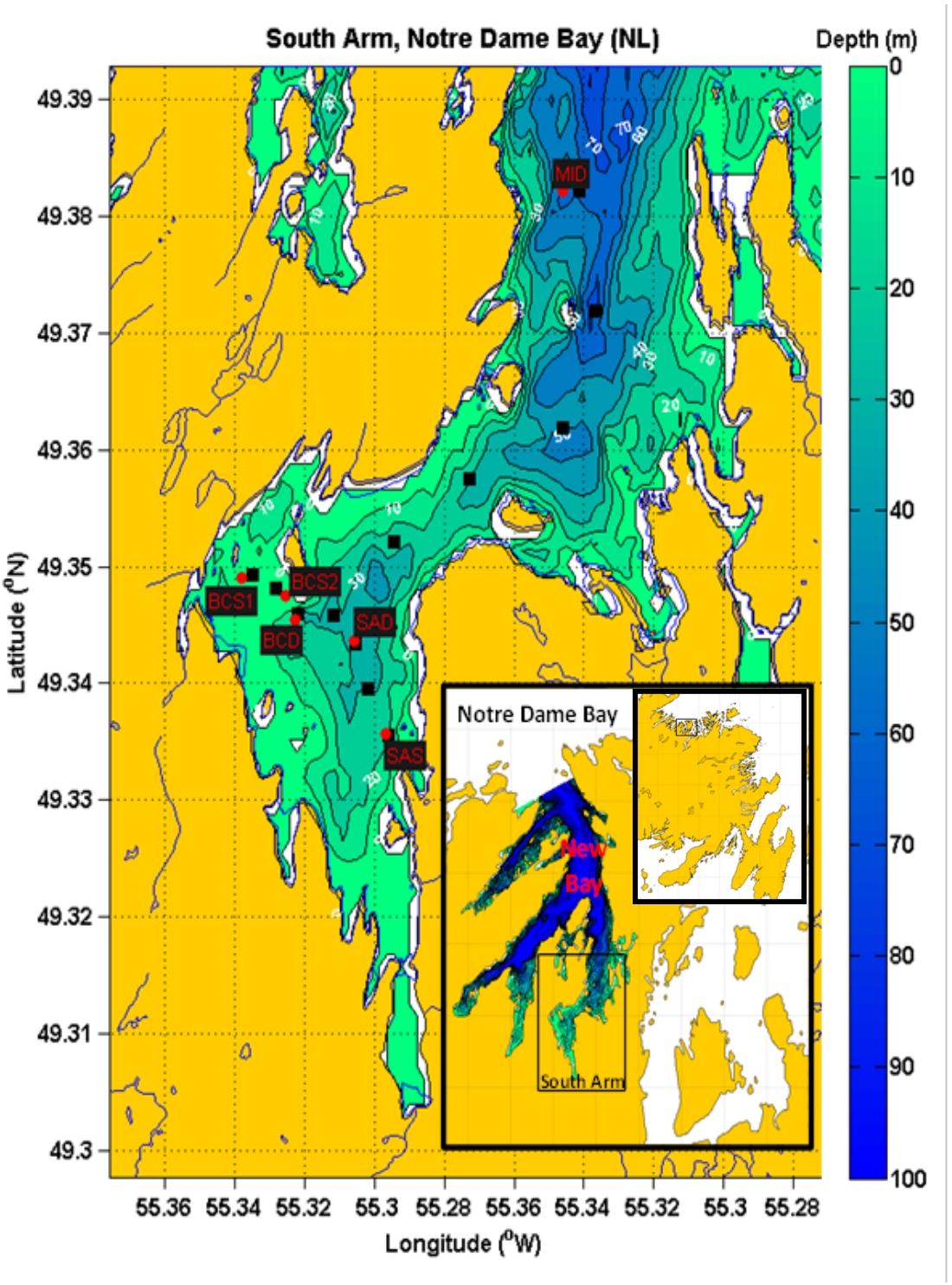


Figure B2: Study area for Chapters 3 (South Arm, Notre Dame Bay, NL, Canada): names in red represent studied site; South Arm shallow (SAS), South Arm deep (SAD), Bulley's Cove shallow (BCS1 and BCS2), Bulley's Cove deep (BCD), Mouse Island deep (MID).

Appendix C: Statistics for sondes results

Table C1: Monthly temperature (C°) statistics for Year 1 and 2 of the study.

Year 1

						SAS							
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	11.8	6.7	2.2	-0.4	-1.2	-0.8	0	3.5	8.3	12.3	14.9	13.9	6.5
SD	1.9	1.6	0.9	0.6	0.2	0.2	0.7	1.2	1.5	2	1.2	0.5	5.8
min	9.3	3.5	0.9	-1.5	-1.4	-1.2	-0.7	0.9	5.3	6.7	10.3	12.9	-1.5
max	14.6	9.3	3.7	0.9	-0.8	-0.3	1.8	5.5	11.4	16.4	17.7	14.8	17.7
						SAD							
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	NaN	6.3	2.5	0.1	-1	-0.8	-0.6	1.7	4.8	8.8	11.1	11.9	4.6
SD	NaN	1.1	0.9	0.8	0.2	0.1	0.2	1.5	1	1.5	2.4	1.7	4.5
min	NaN	3.8	1.1	-1.4	-1.3	-1	-0.9	-0.2	2.9	3.1	6.5	7.5	-1.4
max	NaN	8.8	3.9	1.6	-0.5	-0.6	0.1	5.3	7	12.4	14.9	14.8	14.9
						BCS1							
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	11.5	6.8	2.3	-0.2	-1.1	-0.6	-0.1	3.2	7.7	13.1	14.4	13.8	6.6
SD	1.7	1.5	0.9	0.8	0.2	0.2	0.6	1.4	1.7	2.3	1.4	0.6	5.7
min	9.3	3.7	0.9	-1.5	-1.4	-1	-0.6	0.8	4.1	6.6	9.1	12.1	-1.5
max	14.8	9.3	3.6	1.3	-0.6	-0.3	1.8	5.7	11.3	17.3	16.8	14.9	17.3
						BCS2							
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	11.6	6.8	2.3	-0.2	-1	-0.6	0	3.6	8.3	12.6	15.1	14.1	6.6
SD	1.7	1.5	1	0.6	0.2	0.2	0.7	1.3	1.7	2.1	1.2	0.5	5.7

min	9.3	3.7	0.9	-1.4	-1.4	-1	-0.8	0.9	4.7	7.3	10.3	12.8	-1.4
max	14.8	9.4	3.8	1.2	-0.4	-0.3	2	6.1	11.5	16.2	17.6	14.9	17.6
						BCD							
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	11.1	6.2	2.4	0.2	-1	-0.8	-0.6	1.5	4.3	NaN	10.7	11.7	4.7
SD	1.6	1.1	0.9	0.9	0.3	0.1	0.2	1.4	0.9	NaN	2.4	1.7	4.8
min	8.8	4	1	-1.5	-1.4	-1	-0.9	-0.3	2.7	NaN	6.5	6.9	-1.5
max	14.1	8.8	4.1	1.6	-0.5	-0.5	-0.1	4.7	6.5	NaN	14.5	14.6	14.6
						MID							
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	11.3	NaN	2.5	0.5	-0.9	-1	NaN	NaN	4.5	7.8	NaN	12.2	5
SD	1.7	NaN	0.8	0.6	0.3	0.1	NaN	NaN	0.9	1.6	NaN	1.7	4.7
min	8.9	NaN	1.3	-1	-1.2	-1.1	NaN	NaN	2.7	3.1	NaN	7.4	-1.3
max	14.5	NaN	4	1.5	-0.2	-0.8	NaN	NaN	6.5	10.3	NaN	14.1	14.5
						Year 2							
						SAS							
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	NaN	5.6	0.6	0.3	NaN	NaN	NaN	NaN	5.3	NaN	NaN	13.8	5.4
SD	NaN	1.5	1.4	0.7	NaN	NaN	NaN	NaN	1.2	NaN	NaN	0.7	5.4
min	NaN	2.9	-1.3	-1.3	NaN	NaN	NaN	NaN	2.2	NaN	NaN	12.5	-1.3
max	NaN	8.3	2.9	1.3	NaN	NaN	NaN	NaN	7.8	NaN	NaN	14.9	15.2
						SAD							
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	10.1	5.8	1.3	0.4	NaN	NaN	NaN	1.4	3.5	7.8	11.4	13.2	5.7
SD	0.8	1.7	1.2	0.8	NaN	NaN	NaN	1.1	1.3	2.9	1.4	0.9	4.7
min	8.5	2.7	-1.1	-1.4	NaN	NaN	NaN	0.3	1.4	1.9	7.5	10.4	-1.4
max	11.3	8.6	3	1.5	NaN	NaN	NaN	4.1	5.8	12.1	14.7	14.6	14.7

BCS1													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	10.1	5.8	1.3	NaN	NaN	NaN	NaN	4.4	4.8	NaN	14.7	13.9	8.4
SD	0.9	1.6	1.2	NaN	NaN	NaN	NaN	2	1.3	NaN	1.9	0.6	5
min	8.1	3	-0.9	NaN	NaN	NaN	NaN	1.3	2.3	NaN	11	12.1	-0.9
max	11.5	8.4	3	NaN	NaN	NaN	NaN	8.1	6.4	NaN	19.2	15	19.2
BCS2													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	10.1	5.8	0.8	0.1	-0.4	-0.5	-0.1	2.3	4.5	8.8	13.1	13.7	4.6
SD	0.9	1.6	1.4	0.8	0.2	0	0.4	2	1.3	3.3	1.5	0.7	5.2
min	8.3	2.9	-1.3	-1.3	-1	-0.5	-0.5	0.1	2	1.8	8.8	11.8	-1.3
max	11.4	8.3	2.9	1.3	-0.1	-0.4	1.2	7	6	13.1	18	14.9	18
BCD													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	9.9	5.7	1.5	NaN	NaN	NaN	NaN	1.2	2.6	4.6	9.8	12.4	5.6
SD	0.9	1.7	1.1	NaN	NaN	NaN	NaN	0.9	0.9	2.4	1.9	1.3	4.2
min	8.4	2.6	-0.6	NaN	NaN	NaN	NaN	0.3	1.2	0.8	5.1	8.9	-1.4
max	11.2	8.4	2.9	NaN	NaN	NaN	NaN	3	5	8.5	13.2	14.4	14.4
MID													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	10.1	5.7	1.2	-0.2	-0.8	-0.9	-0.7	0.7	NaN	NaN	NaN	13.5	3.1
SD	0.9	1.7	1.3	0.7	0.2	0.1	0.3	0.8	NaN	NaN	NaN	0.8	5.1
min	8.2	3	-1.2	-1.5	-1.3	-1.1	-1	-0.2	NaN	NaN	NaN	11.9	-1.5
max	11.6	8.2	3.2	1.2	-0.5	-0.7	-0.1	2.5	NaN	NaN	NaN	14.8	14.8

NaN= not a number (i.e. missing data).

Table C2: Monthly dissolved oxygen (%) statistics for Year 1 and 2 of the study.

Year 1

SAS													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	96.6	97.3	99.2	98.5	98.3	108.9	109.8	108.5	108.6	109.3	107.3	100.7	103.7
SD	2.7	2.5	1	1.8	2.7	2.5	3.6	4	2.3	1.1	2.5	1	5.8
min	93.3	92.8	97.3	94.3	94.8	104.7	102.4	100.6	102	106.3	101	99.1	92.8
max	103.1	102.3	101.8	101.3	107.5	115.2	116.8	114.8	112.3	111.2	111.6	102.9	116.8
SAD													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	NaN	95.8	99.3	98.6	101.9	105.7	105.6	109.6	109.5	108.2	104	97.1	103.4
SD	NaN	1.6	0.9	0.7	2.5	5.4	3.6	2.8	0.9	1.6	2.7	2	5.5
min	NaN	92.7	97.7	97.1	97.5	98.1	99.2	104.1	104.9	104.7	100.1	93.1	92.7
max	NaN	99.8	101.4	100	106.1	113.3	112.7	116.4	110.8	111.3	109.6	100.1	116.4
BCS1													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	97.4	97.6	99.9	98.4	99.5	111.2	105.8	111.3	108	110.3	103.6	100.9	103.6
SD	1.9	2.3	1.2	1.4	4	1.6	4.2	4	1.3	2.7	1.7	1.3	5.8
min	92.7	93.6	97.8	95	94.9	105.8	97.9	104.2	104.6	102.6	100.2	98	92.7
max	103.8	103	102.6	100.8	111.4	114.8	117.1	118	110.1	113.7	107.5	103.1	118
BCS2													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	97.8	98.1	99.3	97	104.6	109.9	109.3	109	108.6	109	106.9	100.9	103.7
SD	1.7	2.5	2	1.6	3.7	1.9	3.9	3.8	2.2	1.3	2.5	1.1	5.6
min	94.5	92.7	95.9	95.5	99	105.8	101.6	101.3	103.2	106	100.7	99.1	92.7
max	103.6	102.7	103.2	102.2	113.3	113.5	116	115.4	112.4	112.3	112.4	103.4	116

BCD													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	95.3	94.3	95.3	94.1	97.1	98.9	99.4	105.3	106.3	NaN	102.1	95.5	98.6
SD	1.7	1.4	1.1	0.7	2.1	5.2	2.9	2.2	0.9	NaN	2.7	2.6	4.9
min	91.4	91	93.4	92.6	92.4	90.7	93.3	100.4	104.1	NaN	97.3	90.4	90.4
max	99.7	98.4	97.4	95.9	101.2	105.7	104.1	110.1	107.9	NaN	108	100.3	110.1

MID													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	98.2	NaN	99.7	102.2	99.6	99.3	NaN	NaN	110.8	107.3	NaN	99.3	102.7
SD	1	NaN	3.3	2.2	1.4	3.4	NaN	NaN	1.7	1	NaN	2.3	5.1
min	95.1	NaN	96	97	96.7	95.4	NaN	NaN	105.1	105.2	NaN	94.8	94.8
max	100.6	NaN	104.4	104.6	102.4	106.5	NaN	NaN	112.7	109.6	NaN	102.7	112.7

Year 2

SAS													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	NaN	97.1	97.4	94.3	NaN	NaN	NaN	NaN	106.5	NaN	NaN	104.4	100
SD	NaN	1.3	1.6	0.8	NaN	NaN	NaN	NaN	3.3	NaN	NaN	2.1	6
min	NaN	94	93.9	92.5	NaN	NaN	NaN	NaN	99.5	NaN	NaN	98.1	92.5
max	NaN	99.2	99.9	95.8	NaN	NaN	NaN	NaN	113.1	NaN	NaN	107.2	121.5

SAD													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	95.6	100.7	105.1	102.4	NaN	NaN	NaN	111.5	110.1	114.9	117	102.3	106.7
SD	1.8	4	1.4	0.8	NaN	NaN	NaN	2.3	2.2	3.1	6.8	2.1	7.4
min	91.7	92.8	102.1	100.2	NaN	NaN	NaN	103.4	106.6	109.6	103	97.1	91.7
max	100.5	106.8	107.1	103.9	NaN	NaN	NaN	113.6	114.4	120.9	123.6	105.6	123.6

BCS1													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	97.3	97.5	97.8	NaN	NaN	NaN	NaN	109.2	107.7	NaN	110.9	101.9	103.7
SD	2.5	1.2	1.2	NaN	NaN	NaN	NaN	2.6	3.3	NaN	5.7	2.3	6.8
min	92.7	93.3	95.4	NaN	NaN	NaN	NaN	102	101.6	NaN	100.1	95.6	92.7
max	100.9	99.7	99.8	NaN	NaN	NaN	NaN	112.6	112.3	NaN	119.3	105.7	119.3
BCS2													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	96.1	97.1	97.8	95.1	92.7	88.5	108.6	112.9	109.7	113.4	113.4	103.2	102.5
SD	2	1.5	1.4	0.8	1.9	1.1	13.1	2.3	3.3	2.1	4.7	2.1	9.7
min	92.4	94.9	94.8	92	89.4	85.7	86.7	104.5	103.7	106.5	102.5	97.3	85.7
max	99.3	99.9	100.2	96.5	96.9	90.7	123.9	116.3	115.5	116.8	119.7	106.6	123.9
BCD													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	92.8	93.4	92.9	NaN	NaN	NaN	NaN	108	107	111.1	111.7	98.7	101.4
SD	1.8	1.9	1.5	NaN	NaN	NaN	NaN	1.1	1.6	2.7	4.9	2.5	8.6
min	89	87.7	89.9	NaN	NaN	NaN	NaN	106.5	104.1	105.5	101.6	93.7	87.7
max	96.9	96.5	94.9	NaN	NaN	NaN	NaN	109.9	110.1	115.5	117.2	103.8	117.2
MID													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	98.6	96.6	94.3	93.5	93.5	91.2	98.8	107.5	NaN	NaN	NaN	101.4	97.7
SD	2.3	1.9	1	1.1	1.5	0.7	8.1	2.5	NaN	NaN	NaN	1.2	6
min	93.8	92.4	92.9	91.6	91	90.1	89.8	102.4	NaN	NaN	NaN	98.7	89.8
max	101.6	99.6	96	95.5	95.5	92.9	112.5	112.1	NaN	NaN	NaN	103.8	112.5

NaN= not a number (i.e. missing data).

Table C3: Monthly chlorphyll *a* (μg L^{-1}) statistics for Year 1 and 2 of the study.

Year 1

	SAS												
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	1.7	1.7	2.7	1.3	7.1	4.8	1.8	0.8	0.8	1.8	1.9	2.1	2.1
SD	0.9	1.2	1.6	0.5	4.2	3	1.4	0.5	0.4	0.6	0.4	0.3	2
min	0.2	0	0.3	0.6	2.7	1	0.2	0.3	0.3	0.7	1.1	1.4	0
max	3.2	4.8	5.5	3.3	18.5	12.5	6.7	2.6	1.6	3.2	2.8	2.7	18.5
	SAD												
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	NaN	1.3	2.8	1.9	7.6	10.7	3.2	1.1	1.3	2.4	3	2	2.8
SD	NaN	1.1	1.2	0.5	4.6	6.1	2.1	1.6	0.4	0.8	0.4	0.6	3.1
min	NaN	0.5	1.1	1.2	2.2	2.1	1.1	0.1	0.7	0.8	1.8	1.1	0.1
max	NaN	4.9	5.3	3.6	17.5	26.3	12.4	9.5	2.2	3.6	4	4	26.3
	BCS1												
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	1.7	2.3	3	1.7	6.7	5.3	2.3	1	1.4	1.4	2.6	2.4	2.3
SD	0.4	1.2	1.3	0.5	3.4	2.6	1.9	0.5	0.4	0.4	0.4	0.3	1.8
min	0.9	0.8	0.8	1	2	1.7	0.2	0.5	0.9	0.8	1.7	1.8	0.2
max	2.8	5.5	5.7	3.7	13.2	10.8	8.2	2.7	2.6	2.5	3.5	2.9	13.2
	BCS2												
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	2	2.9	2.7	1.4	5.9	5.1	2.4	0.5	0.8	1.3	1.9	1.8	2.2
SD	0.3	1.5	1.4	0.6	3.1	3.1	1.4	0.5	0.2	0.6	0.3	0.3	1.9
min	1.2	0.9	0.5	0.6	2.1	0.9	0.7	0	0.4	0.5	1.2	1.2	0
max	2.8	6.1	5.4	2.9	11.6	12.4	6.3	2.3	1.4	3.7	2.7	2.3	12.4

BCD

	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	1.3	1.1	2.2	1	6.1	10.1	3.8	1.7	1.9	NaN	2.9	1.9	2.6
SD	0.6	1.1	1.3	0.6	4	3.2	2.7	2.5	1.4	NaN	0.5	0.7	2.8
min	0.4	0.2	0.2	0.1	1.8	3.5	1.2	0.2	0.8	NaN	1.9	0.8	0.1
max	2.7	4.7	5.2	2.8	15.3	16.2	16	16	9.8	NaN	4.1	3.5	16.2

MID

	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	1.3	NaN	2.1	1.1	3.2	4.1	NaN	NaN	1.4	2.1	NaN	1.8	2
SD	0.4	NaN	0.8	0.4	1.9	2	NaN	NaN	0.4	0.7	NaN	0.4	1.3
min	0.4	NaN	0.6	0	0.1	1.3	NaN	NaN	0.9	1.2	NaN	1.1	0
max	2.6	NaN	4	1.8	6.7	10.4	NaN	NaN	3.1	4.1	NaN	2.7	10.4

Year 2

SAS

	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	NaN	1.2	1.1	0.9	NaN	NaN	NaN	NaN	1.4	NaN	NaN	4.7	1.7
SD	NaN	0.6	0.3	0.2	NaN	NaN	NaN	NaN	0.3	NaN	NaN	2.9	1.7
min	NaN	0.3	0.5	0.6	NaN	NaN	NaN	NaN	0.6	NaN	NaN	1.8	0.3
max	NaN	2.5	1.7	1.3	NaN	NaN	NaN	NaN	2	NaN	NaN	10.8	10.8

SAD

	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	1	1.1	1.3	1	NaN	NaN	NaN	0.5	0.5	1.2	2.3	2.6	1.3
SD	0.4	0.3	0.3	0.3	NaN	NaN	NaN	0.2	0.3	0.5	0.6	0.4	0.8
min	0.5	0.7	0.7	0.5	NaN	NaN	NaN	0.2	0	0.6	1.2	1.6	0
max	2	2	1.9	1.8	NaN	NaN	NaN	1	1.2	2.5	5.5	3.3	5.5

BCS1													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	1.6	1.5	1.4	NaN	NaN	NaN	NaN	0.7	0.4	NaN	3.1	2.4	1.7
SD	0.4	0.5	0.3	NaN	NaN	NaN	NaN	0.4	0.3	NaN	2.1	0.5	1.3
min	0.8	0.7	0.8	NaN	NaN	NaN	NaN	0.1	0	NaN	0.5	1.5	0
max	2.1	2.4	2.1	NaN	NaN	NaN	NaN	1.4	1.1	NaN	11.9	3.2	11.9

BCS2													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	1	1.1	1.3	1.1	3.3	0.7	4.2	1.4	1.1	1.2	2.3	2.2	1.7
SD	0.5	0.4	0.2	0.3	5.3	0.3	3.8	0.3	0.3	0.3	0.6	0.3	2.1
min	0	0.3	0.8	0.6	0.5	0.5	1.1	0.8	0.7	0.8	0.8	1.6	0
max	1.7	2.5	1.8	2.2	28.3	1.5	22	2	2	1.9	3.4	2.9	28.3

BCD													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	1	0.9	0.9	NaN	NaN	NaN	NaN	0.3	0.6	1.5	2.7	2.6	1.3
SD	0.4	0.5	0.3	NaN	NaN	NaN	NaN	0.2	0.4	0.7	0.8	0.6	1
min	0.5	0.3	0.3	NaN	NaN	NaN	NaN	0	0	0.9	0.9	1.4	0
max	2.1	2.3	1.4	NaN	NaN	NaN	NaN	0.9	1.3	3.5	4	4	4

MID													
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	YEAR
mean	0.7	0.8	1.1	0.6	0.3	NaN	6.7	1.4	NaN	NaN	NaN	1.7	1.6
SD	0.4	0.3	0.2	0.4	0.2	NaN	5.9	1.9	NaN	NaN	NaN	0.4	2.8
min	0	0	0.6	0	0	NaN	0	0.3	NaN	NaN	NaN	1	0
max	1.4	1.8	1.5	1.4	0.7	NaN	21.3	8.3	NaN	NaN	NaN	3	21.3

Appendix D: Comparison of chlorophyll *a* measurements

Table D1: Comparison between chlorophyll *a* concentration in μg L^{-1} detected by moored sondes and measured in discrete water samples.

	Chlorophyll *a* (μg L^{-1})					
Site	**Sondes**	**Water Samples**	**Difference**	**Ratio***	**Ratio Max**	**Ratio Min**
South Arm Shallow	1.49±0.67	0.56±0.23	0.954±0.72	2.63±1.07	4.10	0.90
South Arm Deep	1.48±0.85	0.44±0.22	1.143±0.63	3.34±0.44	3.80	2.60
Bulley's Cove S 1	1.58±0.72	0.66±0.30	0.951±0.71	2.54±1.11	4.30	1.40
Bulley's Cove S 2	1.25±0.62	0.73±0.22	0.932±0.31	2.47±0.49	3.10	1.60
Bulley's Cove Deep	1.48±1.04	0.52±0.22	0.966±0.89	2.89±1.32	4.90	1.70
Mouse Island Deep	1.46±0.98	0.40±0.19	0.932±0.96	2.90±1.60	5.00	1.20

Values represent mean ± SD (n= 5-7). *Ratio= Sonde reading: water sample result.

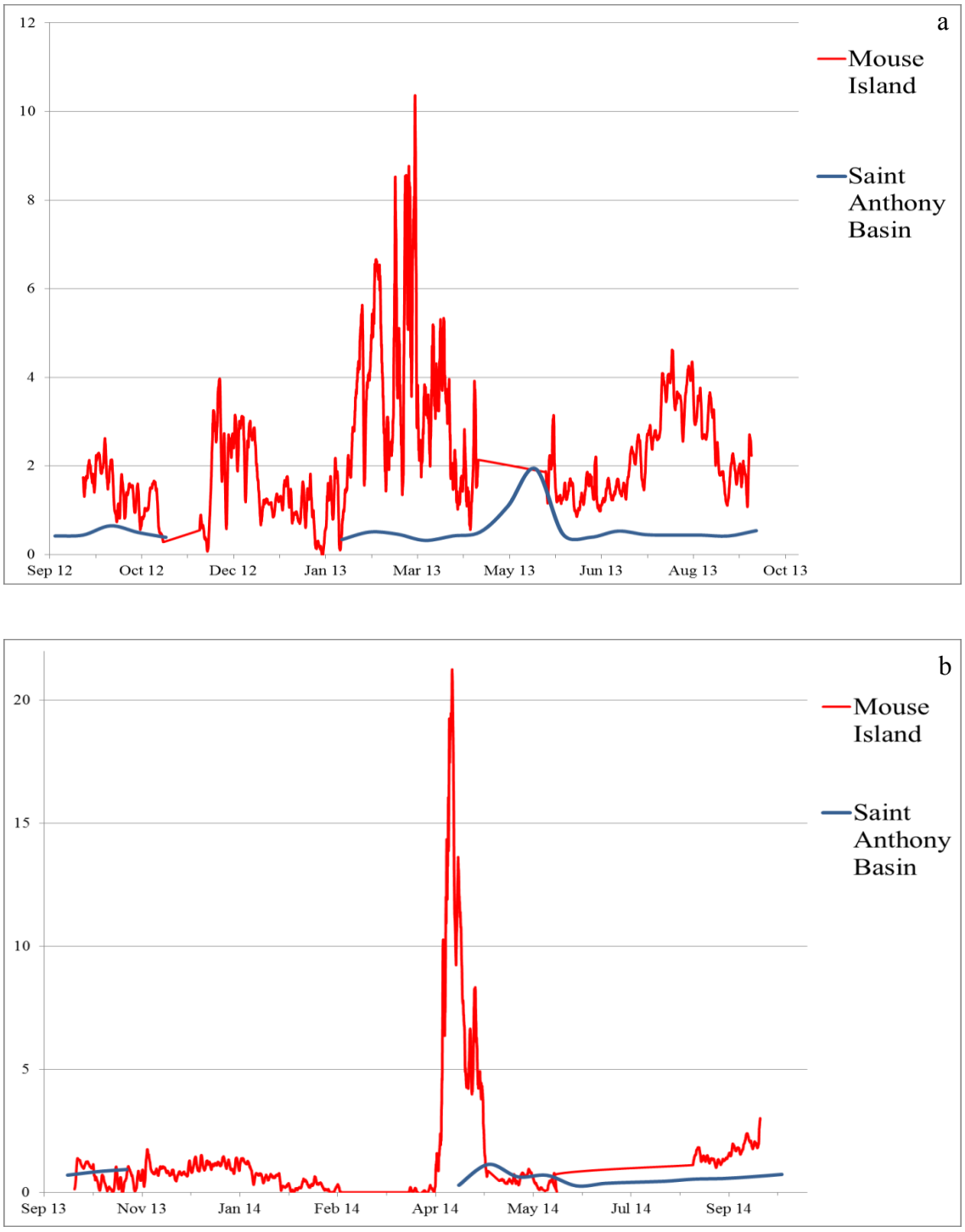


Figure D1: Chlorophyll *a* measurement for (a) Year 1 (September 2012- September 2013) and for (b) Year 2 (October 2013- September 2014) obtained with moored instruments at Mouse Island Deep (red line) site and MODIS for Saint Anthony Basin (blue line; Pepin *et al.*, 2015).

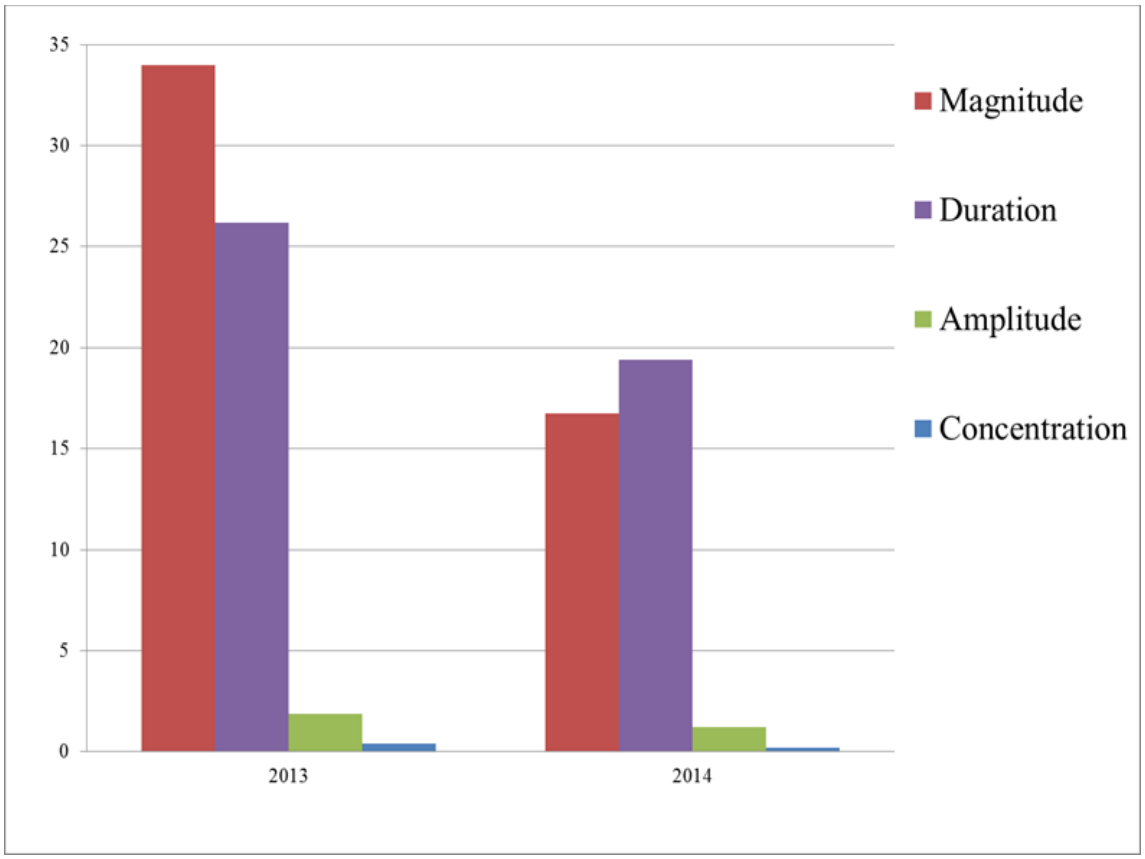
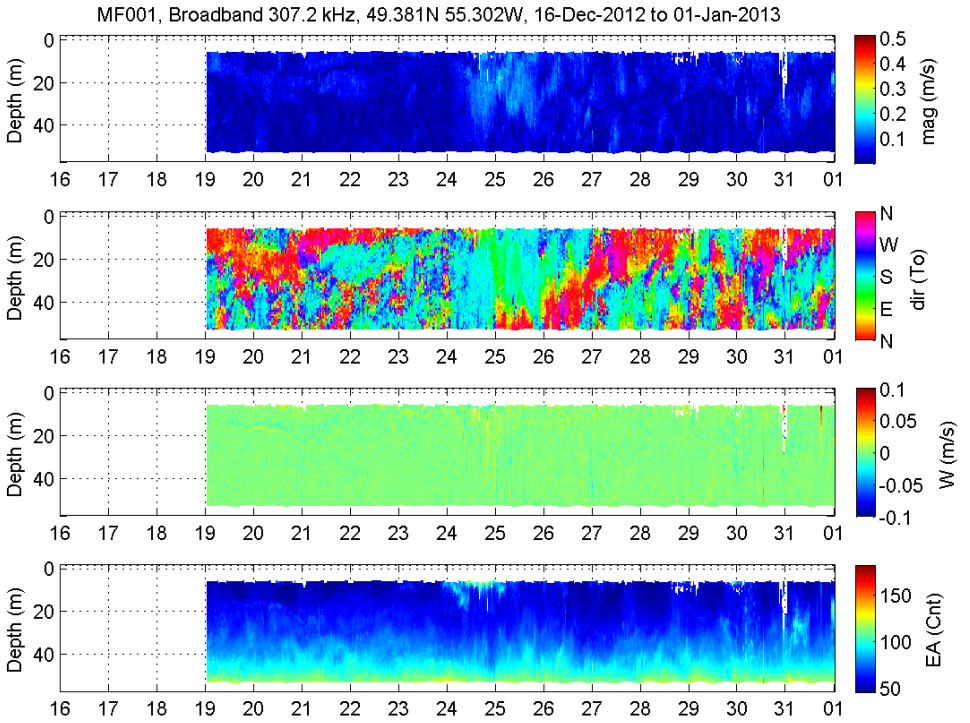
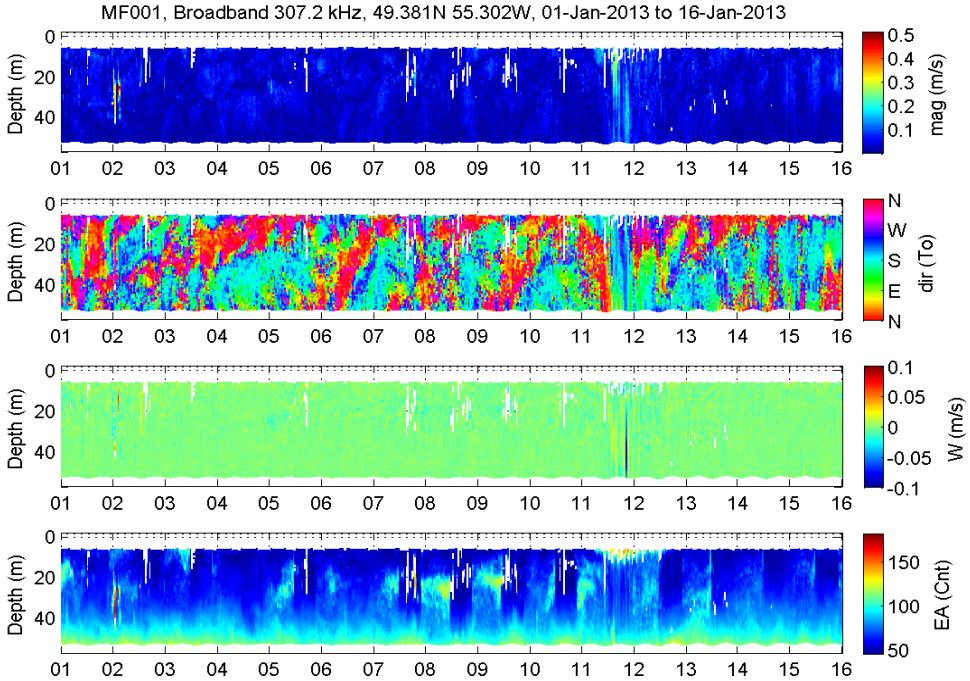
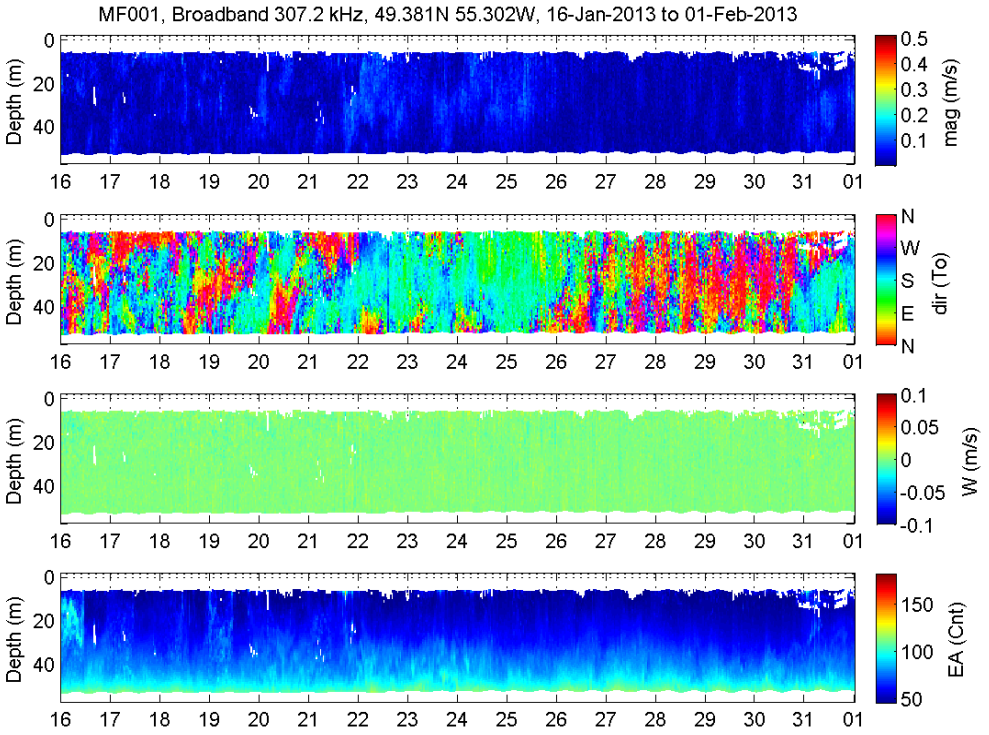


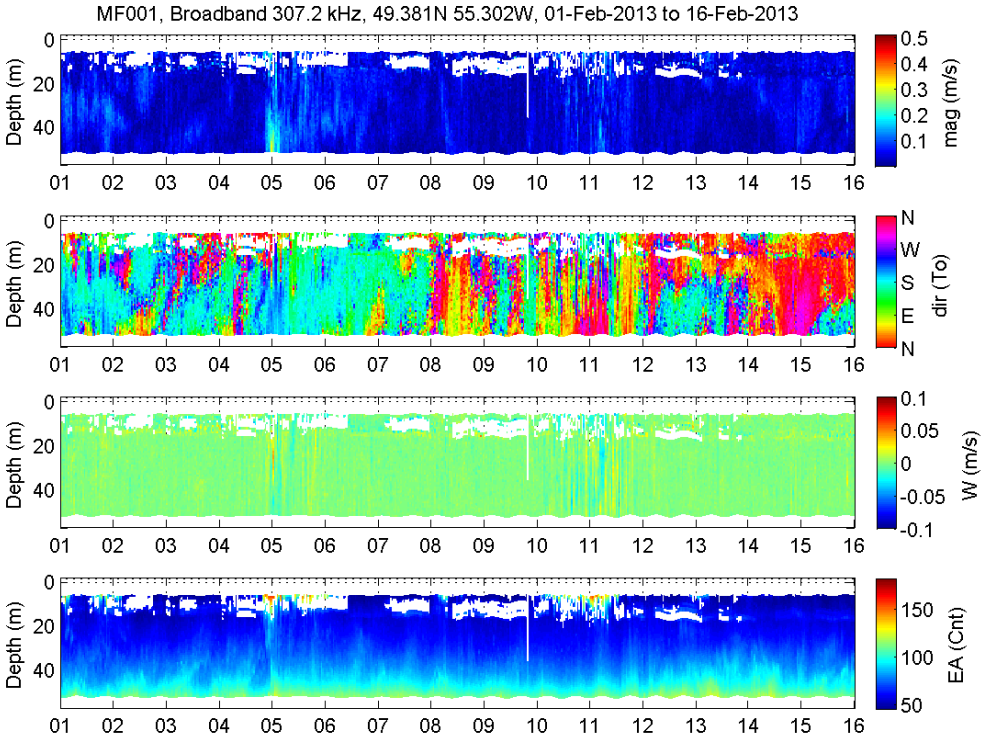
Figure D2: Comparison of offshore chlorophyll *a* data between Year 2013 and 2014 for magnitude (mg m^{-3} d^{-1}), duration (days), amplitude of the spring peak (mg m^{-3}) and concentration (mg m^{-3}; Pepin *et al*., 2015).

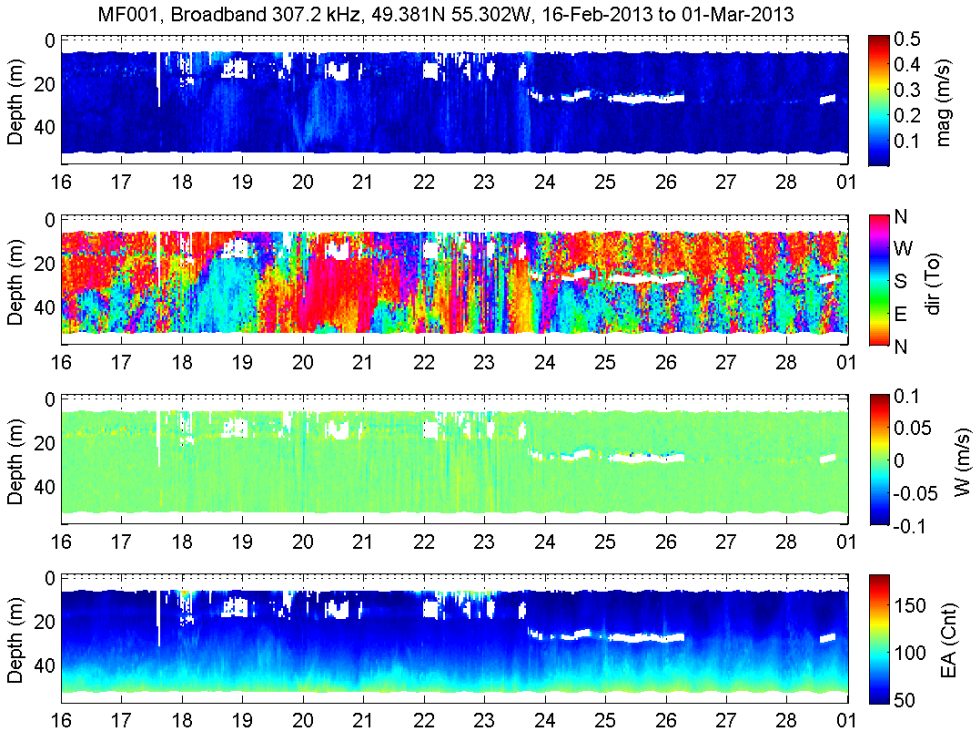
Appendix E: ADCP by-weekly results

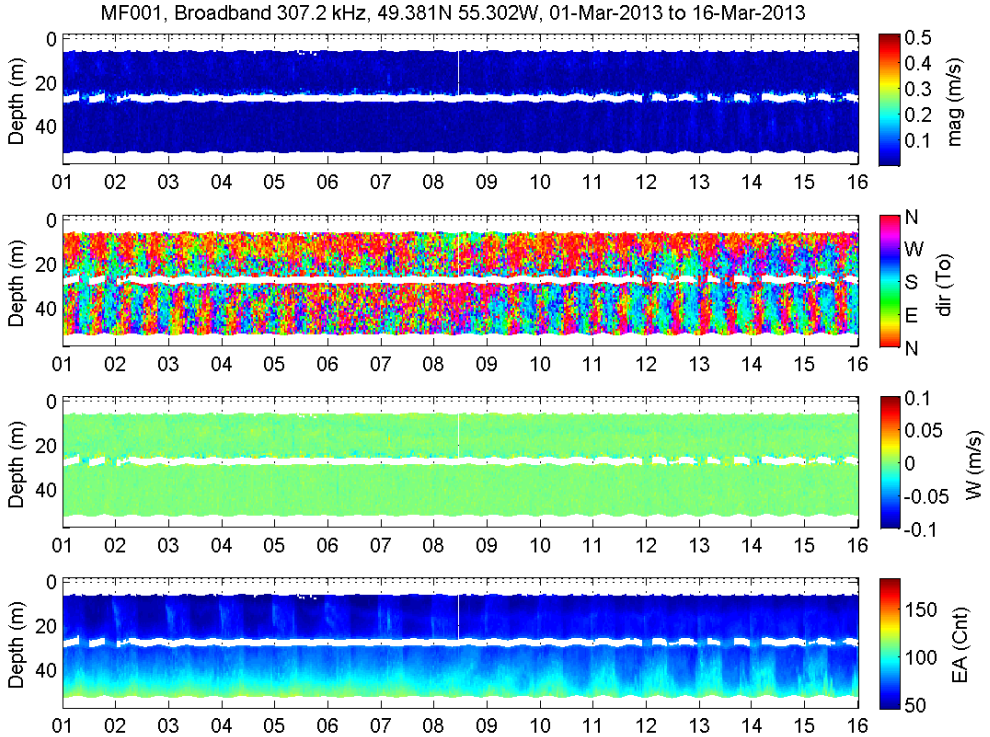


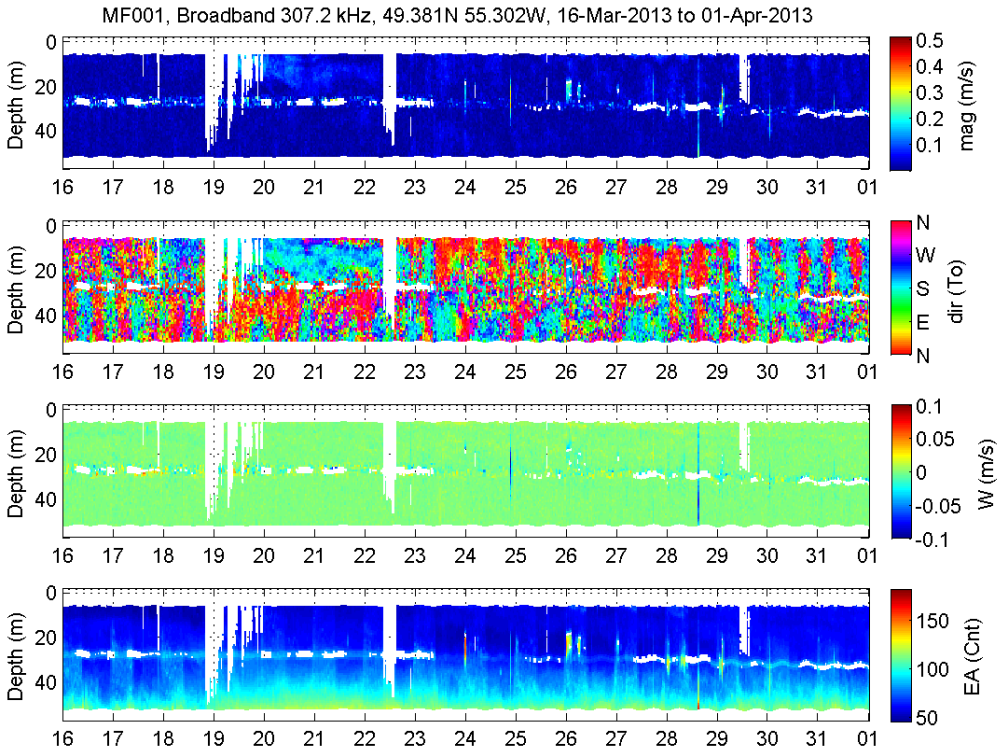


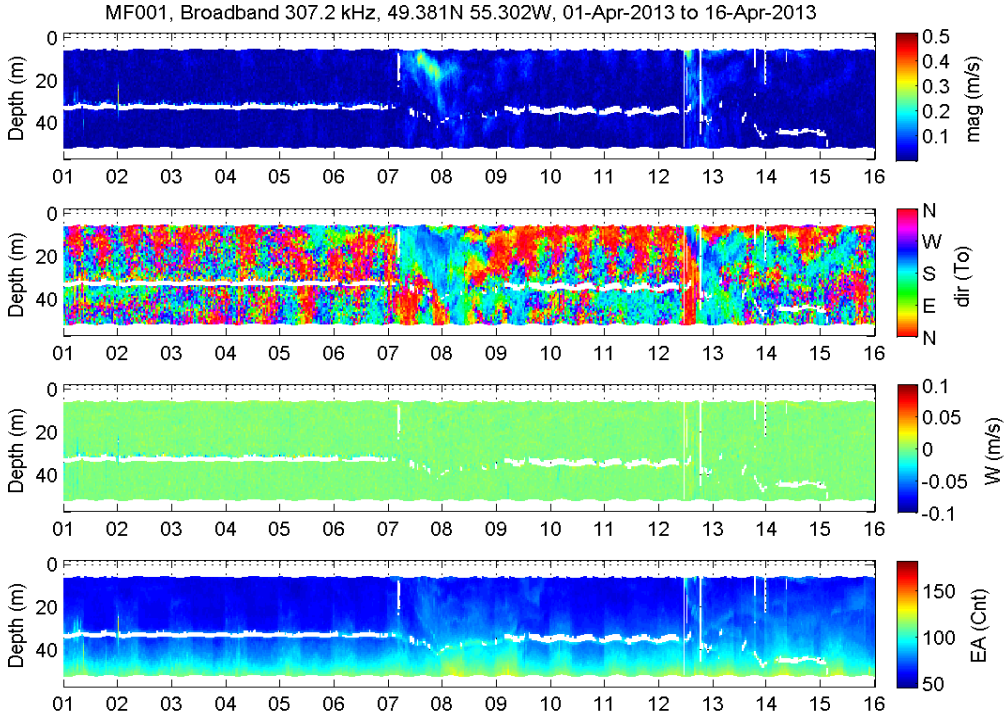

MF001, Broadband 307.2 kHz, 49.381N 55.302W, 16-Jan-2013 to 01-Feb-2013
Depth (m)
0
20
40
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 01
mag (m/s)
0.5
0.4
0.3
0.2
0.1
dir (To)
N
W
S
E
N
W (m/s)
0.1
0.05
0
-0.05
-0.1
EA (Cnt)
150
100
50

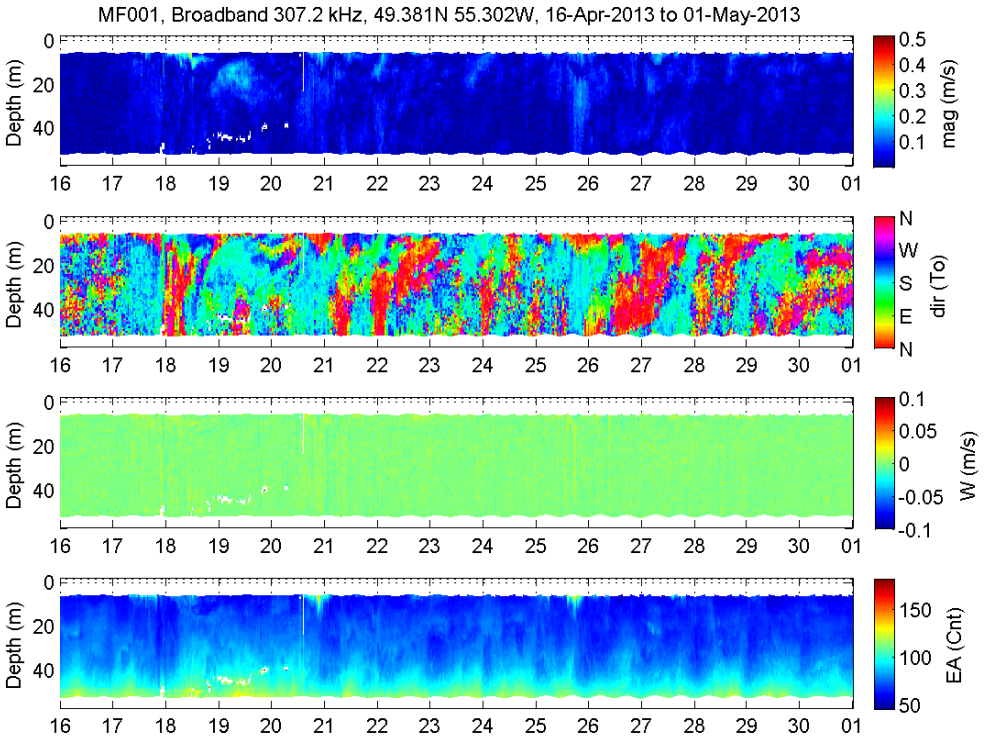

MF001, Broadband 307.2 kHz, 49.381N 55.302W, 01-Feb-2013 to 16-Feb-2013
Depth (m)
0
20
40
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
mag (m/s)
0.5
0.4
0.3
0.2
0.1
dir (To)
N
W
S
E
N
W (m/s)
0.1
0.05
0
-0.05
-0.1
EA (Cnt)
150
100
50

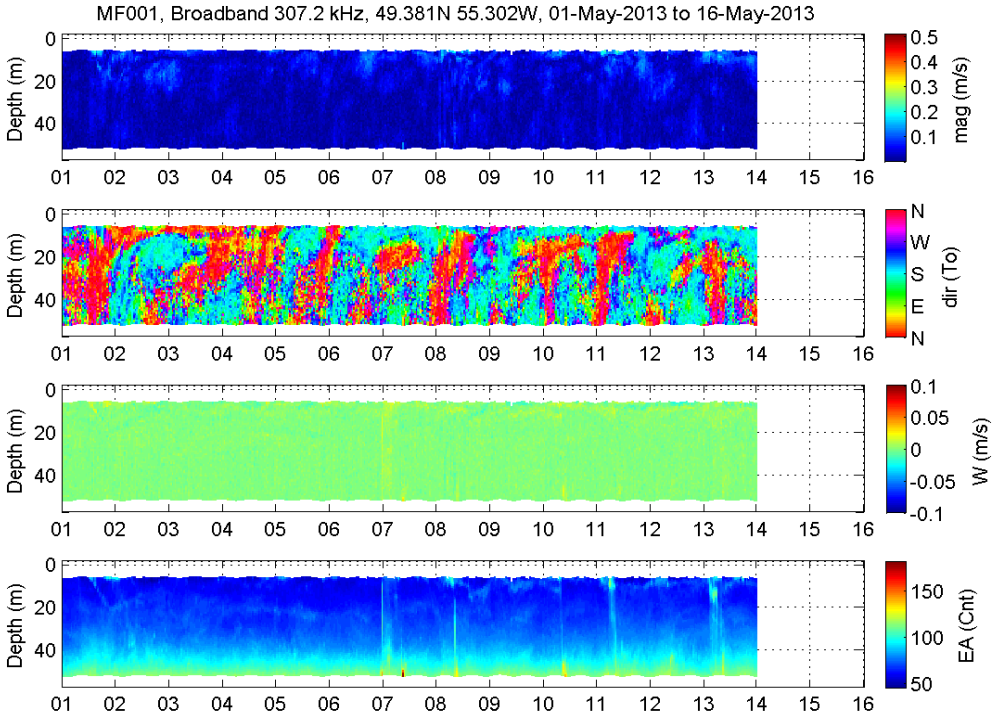

MF001, Broadband 307.2 kHz, 49.381N 55.302W, 16-Feb-2013 to 01-Mar-2013
Depth (m)
0
20
40
16 17 18 19 20 21 22 23 24 25 26 27 28 01
0.5
0.4
0.3
0.2
0.1
mag (m/s)
N
W
S
E
N
dir (To)
0.1
0.05
0
-0.05
-0.1
W (m/s)
150
100
50
EA (Cnt)

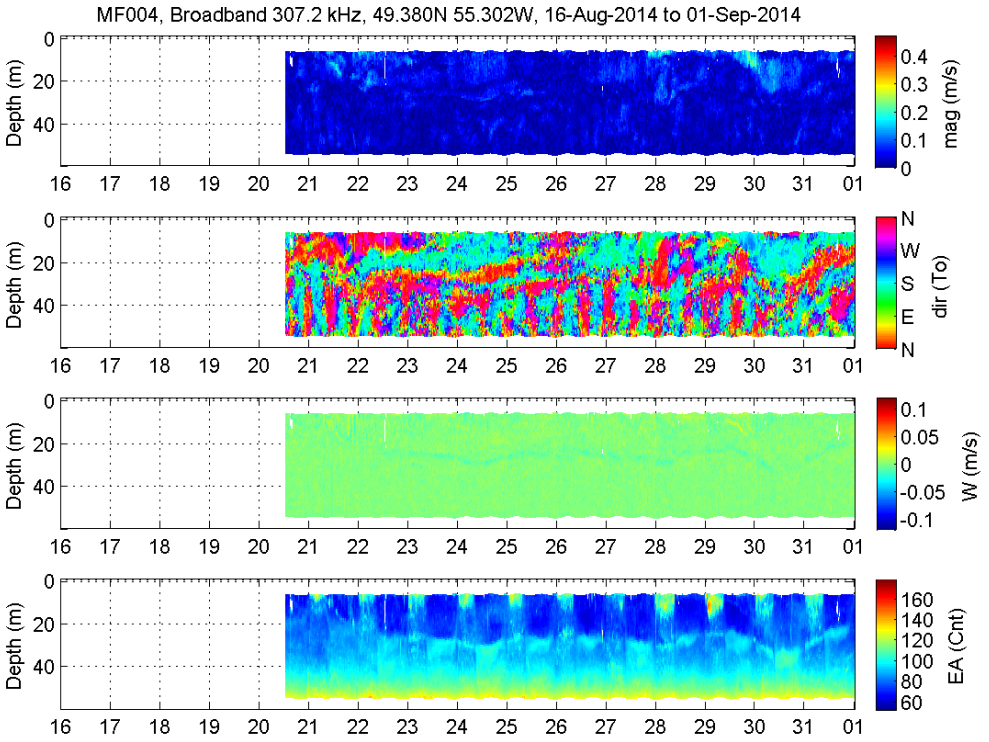

MF001, Broadband 307.2 kHz, 49.381N 55.302W, 01-Mar-2013 to 16-Mar-2013
Depth (m)
0
20
40
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.5
0.4
0.3
0.2
0.1
mag (m/s)
N
W
S
E
N
dir (To)
0.1
0.05
0
-0.05
-0.1
W (m/s)
150
100
50
EA (Cnt)

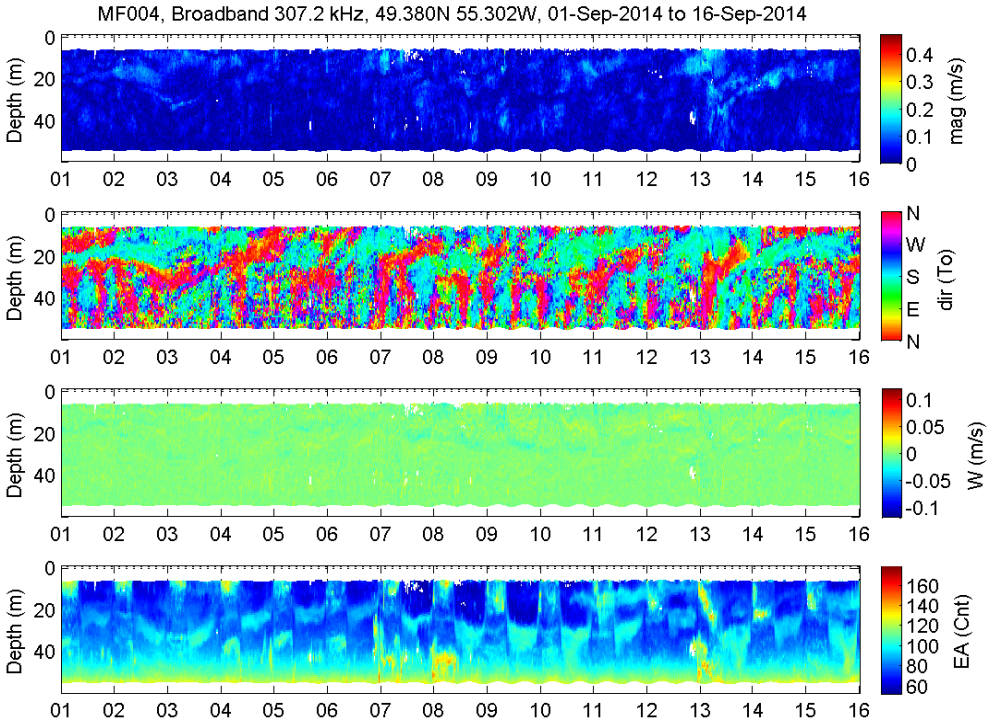

MF001, Broadband 307.2 kHz, 49.381N 55.302W, 16-Mar-2013 to 01-Apr-2013
Depth (m)
0
20
40
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 01
0.5
0.4
0.3
0.2
0.1
mag (m/s)
N
W
S
E
N
dir (To)
0.1
0.05
0
-0.05
-0.1
W (m/s)
150
100
50
EA (Cnt)

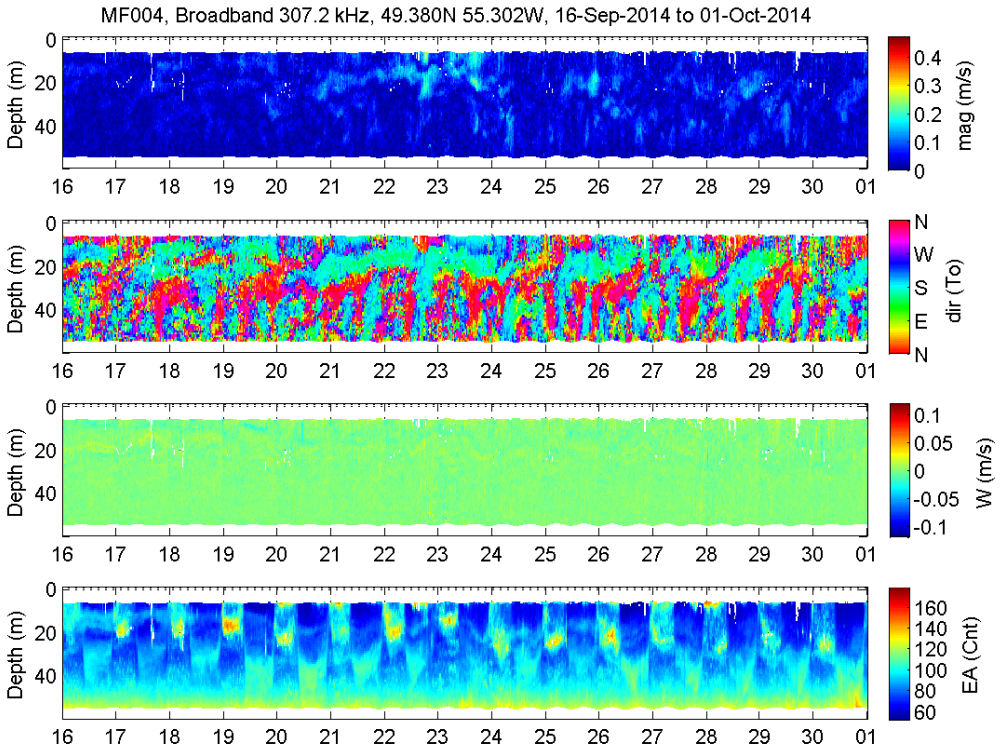

MF001, Broadband 307.2 kHz, 49.381N 55.302W, 01-Apr-2013 to 16-Apr-2013
Depth (m)
0
20
40
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.5
0.4
0.3
0.2
0.1
mag (m/s)
N
W
S
E
N
dir (To)
0.1
0.05
0
-0.05
-0.1
W (m/s)
150
100
50
EA (Cnt)

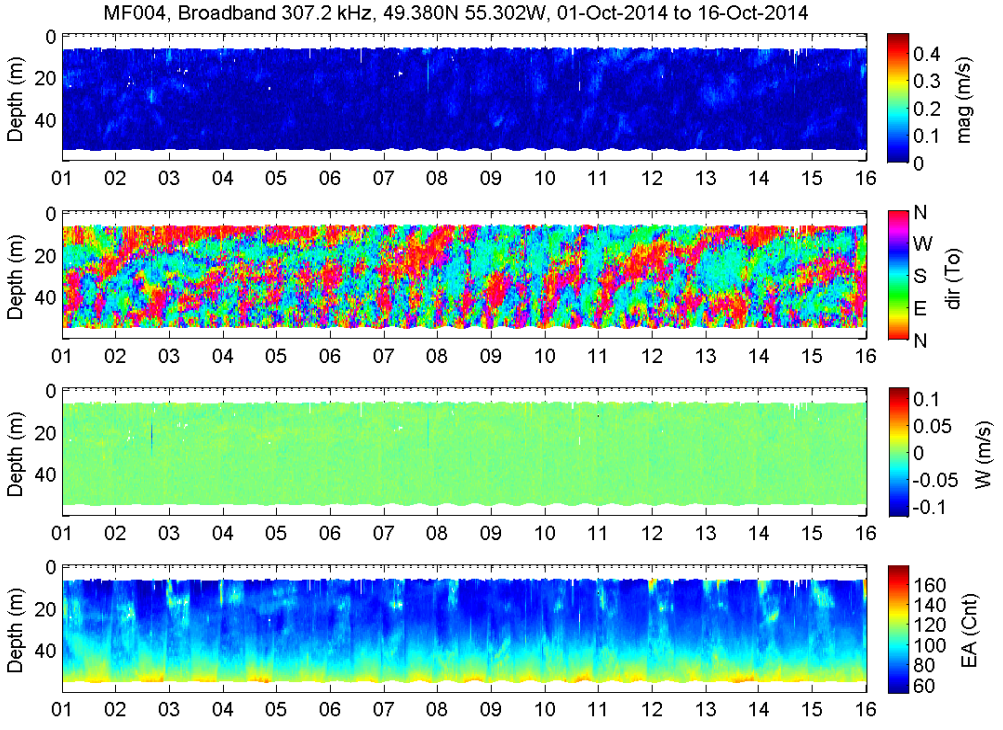

MF001, Broadband 307.2 kHz, 49.381N 55.302W, 16-Apr-2013 to 01-May-2013
Depth (m)
mag (m/s)
dir (To)
W (m/s)
EA (Cnt)
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 01

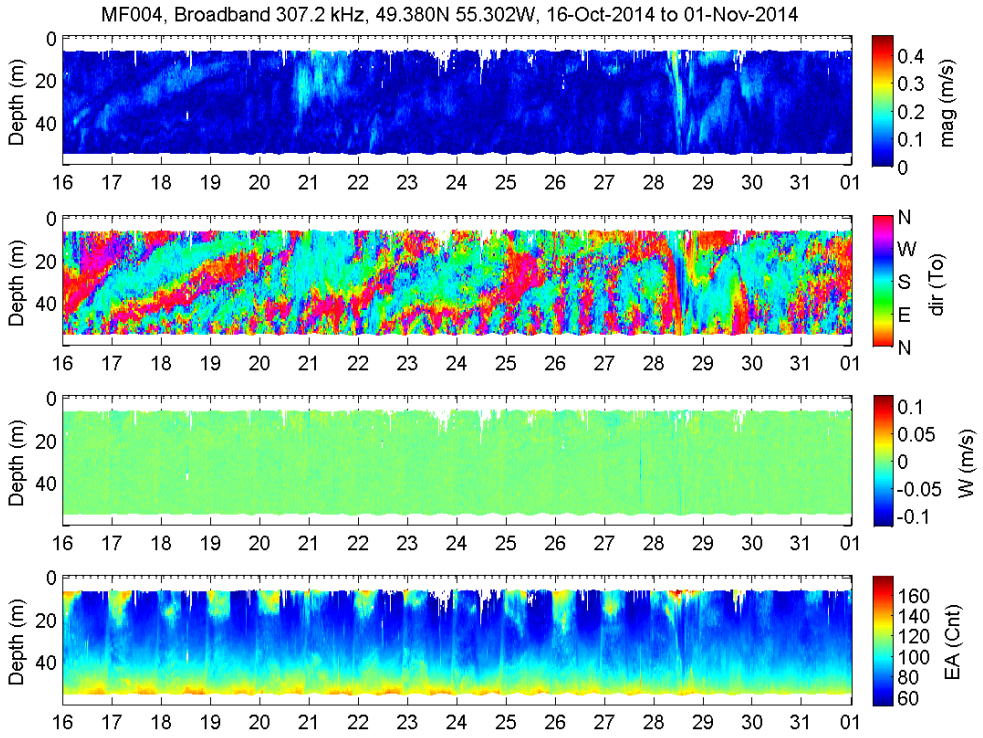

MF001, Broadband 307.2 kHz, 49.381N 55.302W, 01-May-2013 to 16-May-2013
Depth (m)
mag (m/s)
dir (To)
W (m/s)
EA (Cnt)
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16

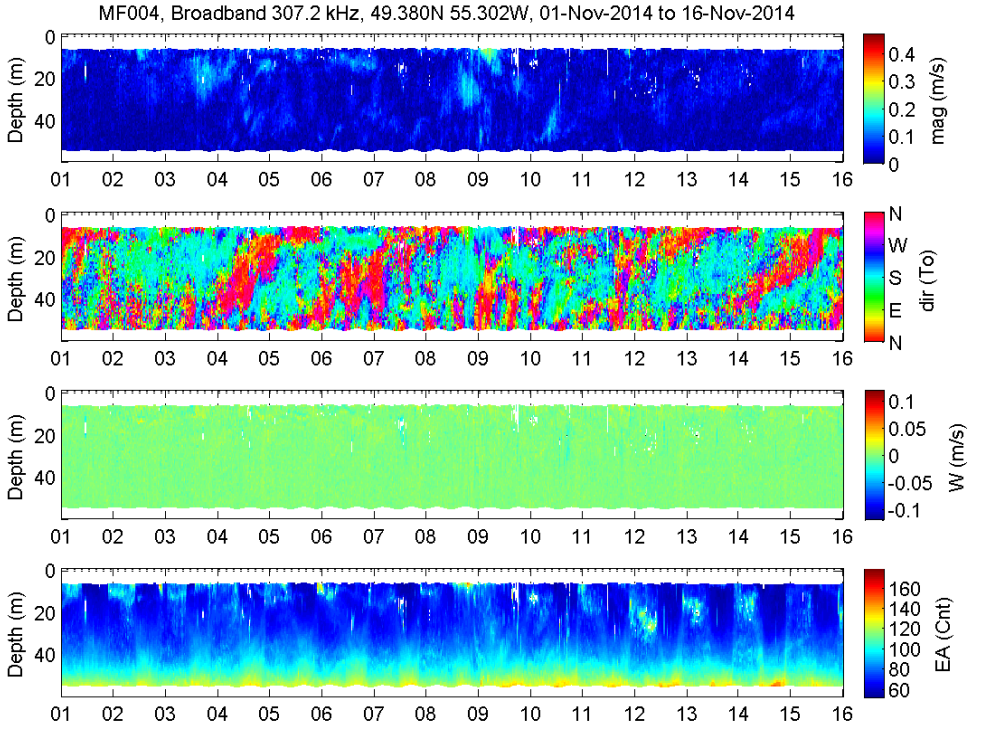

MF004, Broadband 307.2 kHz, 49.380N 55.302W, 16-Aug-2014 to 01-Sep-2014
Depth (m)
0
20
40
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 01
0.4
0.3
0.2
0.1
0
mag (m/s)
N
W
S
E
N
dir (To)
0.1
0.05
0
-0.05
-0.1
W (m/s)
160
140
120
100
80
60
EA (Cnt)


MF004, Broadband 307.2 kHz, 49.380N 55.302W, 01-Sep-2014 to 16-Sep-2014
Depth (m)
0
20
40
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.4
0.3
0.2
0.1
0
mag (m/s)
N
W
S
E
N
dir (To)
0.1
0.05
0
-0.05
-0.1
W (m/s)
160
140
120
100
80
60
EA (Cnt)


MF004, Broadband 307.2 kHz, 49.380N 55.302W, 16-Sep-2014 to 01-Oct-2014
Depth (m)
0
20
40
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 01
0.4
0.3
0.2
0.1
0
mag (m/s)
N
W
S
E
N
dir (To)
0.1
0.05
0
-0.05
-0.1
W (m/s)
160
140
120
100
80
60
EA (Cnt)


MF004, Broadband 307.2 kHz, 49.380N 55.302W, 01-Oct-2014 to 16-Oct-2014
Depth (m)
0
20
40
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.4
0.3
0.2
0.1
0
mag (m/s)
N
W
S
E
N
dir (To)
0.1
0.05
0
-0.05
-0.1
W (m/s)
160
140
120
100
80
60
EA (Cnt)


MF004, Broadband 307.2 kHz, 49.380N 55.302W, 16-Oct-2014 to 01-Nov-2014
Depth (m)
0
20
40
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 01
0.4
0.3
0.2
0.1
0
mag (m/s)
N
W
S
E
N
dir (To)
0.1
0.05
0
-0.05
-0.1
W (m/s)
160
140
120
100
80
60
EA (Cnt)


MF004, Broadband 307.2 kHz, 49.380N 55.302W, 01-Nov-2014 to 16-Nov-2014
Depth (m)
0
20
40
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.4
0.3
0.2
0.1
0
mag (m/s)
N
W
S
E
N
dir (To)
0.1
0.05
0
-0.05
-0.1
W (m/s)
160
140
120
100
80
60
EA (Cnt)

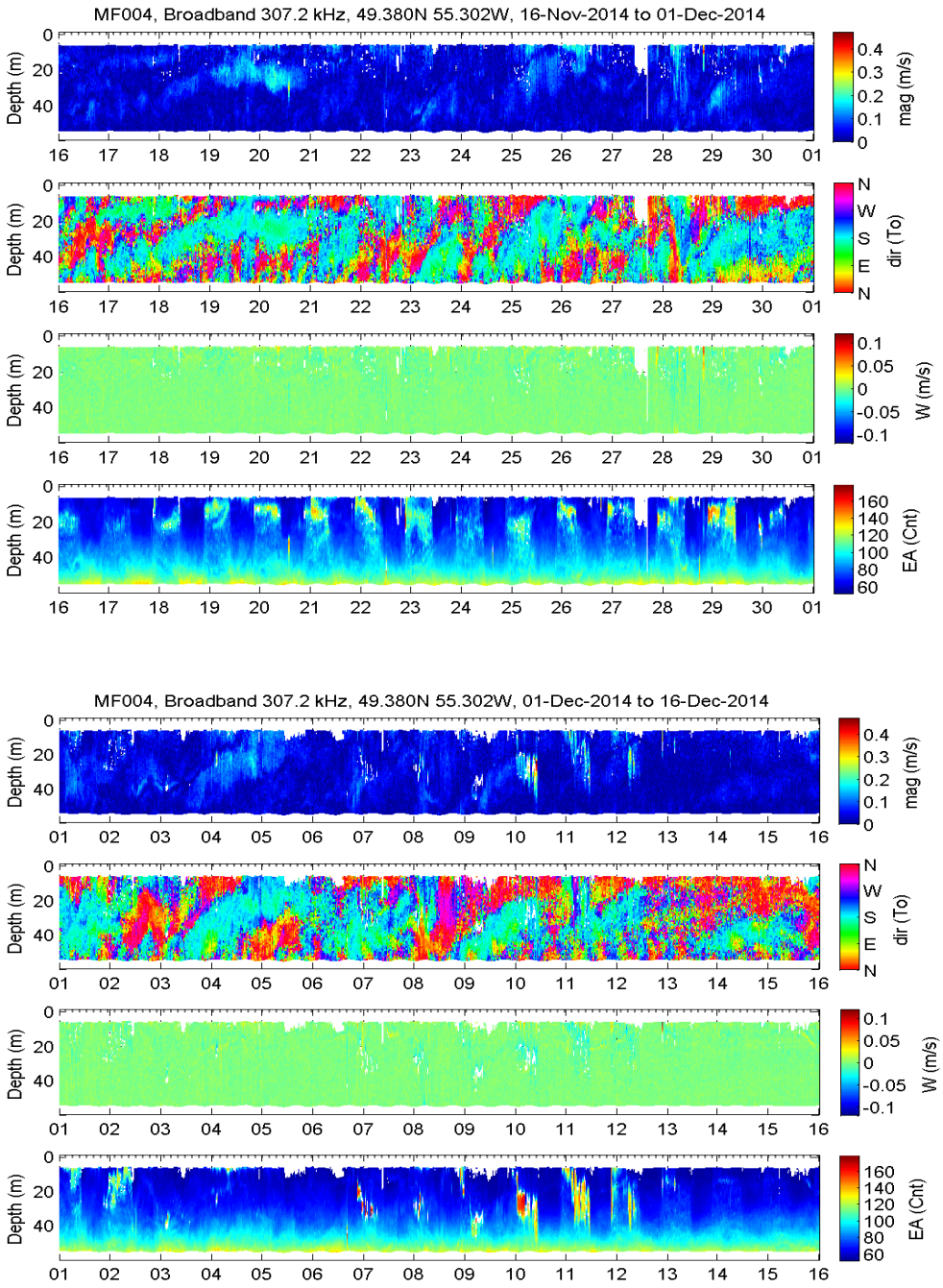
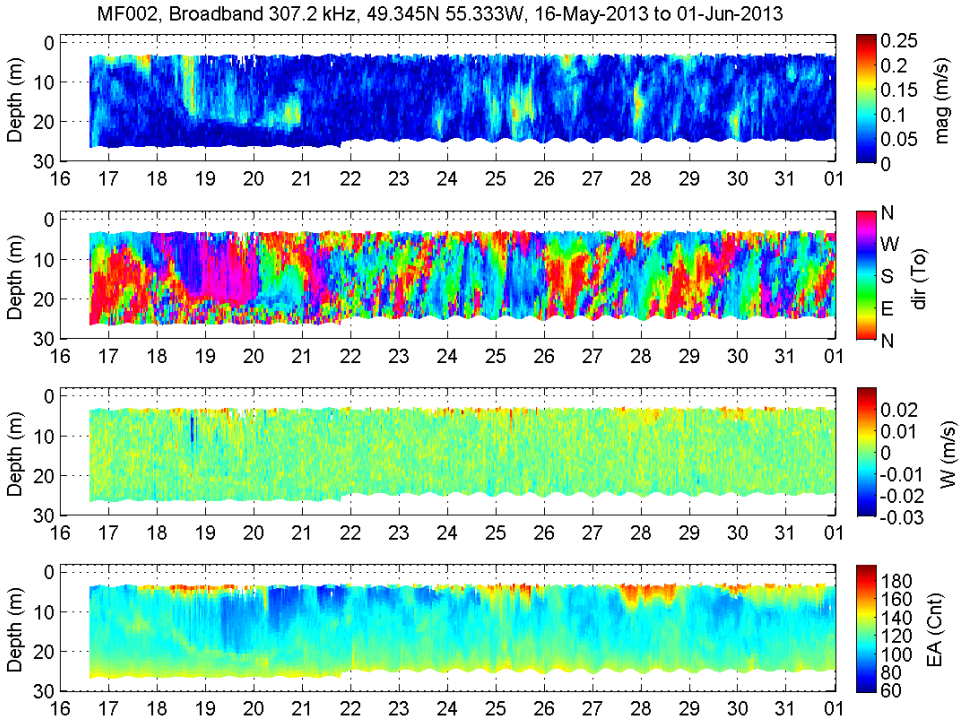
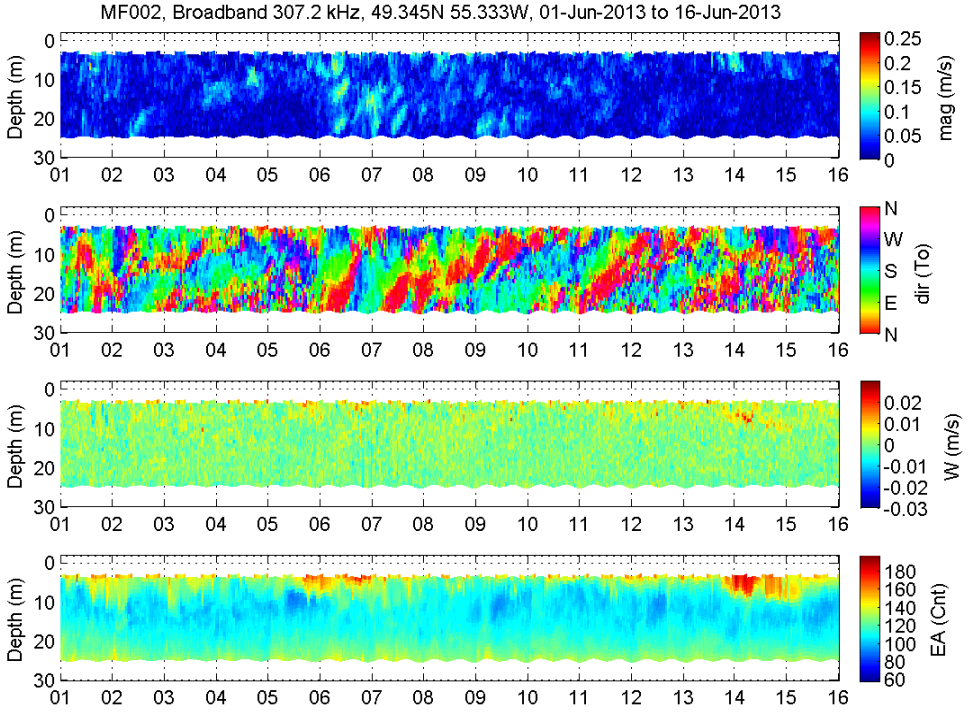
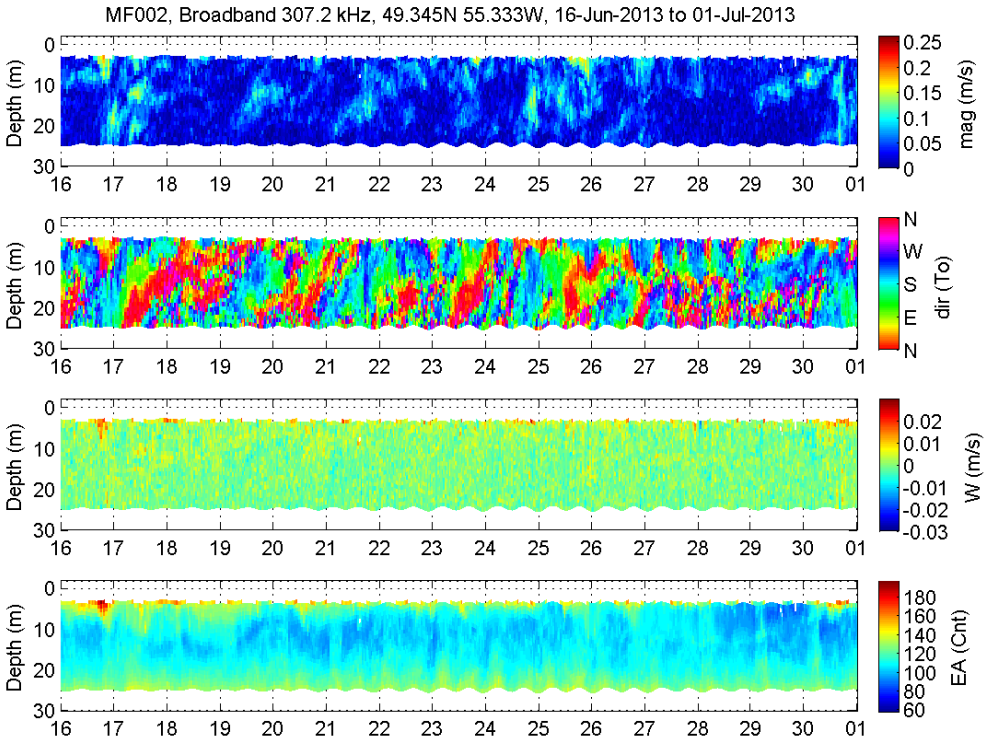
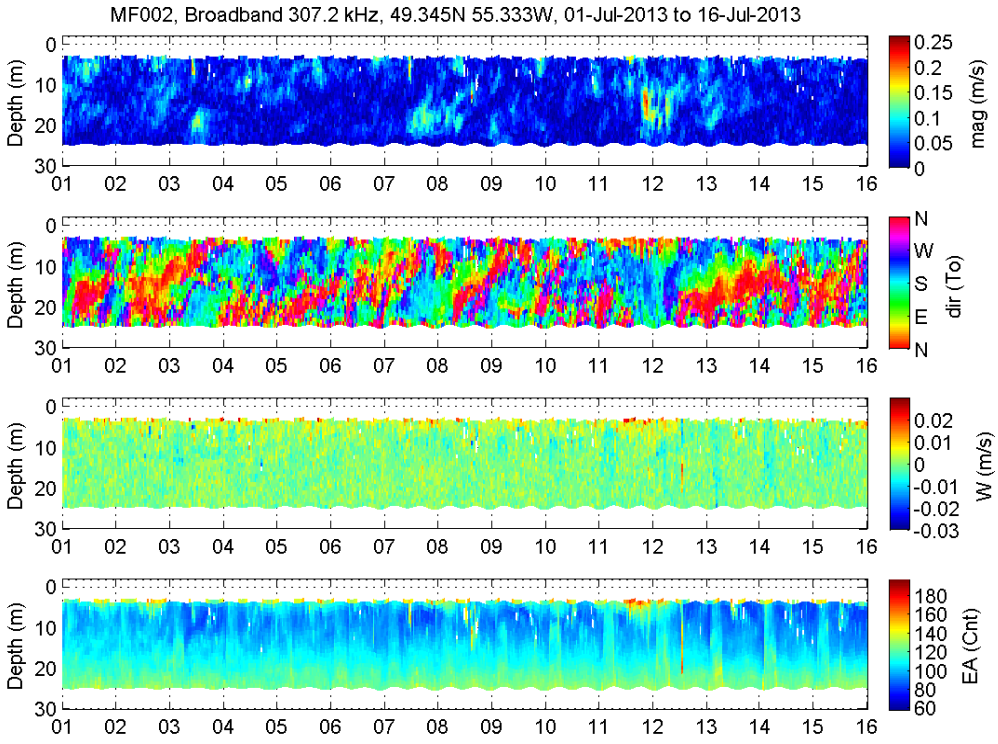


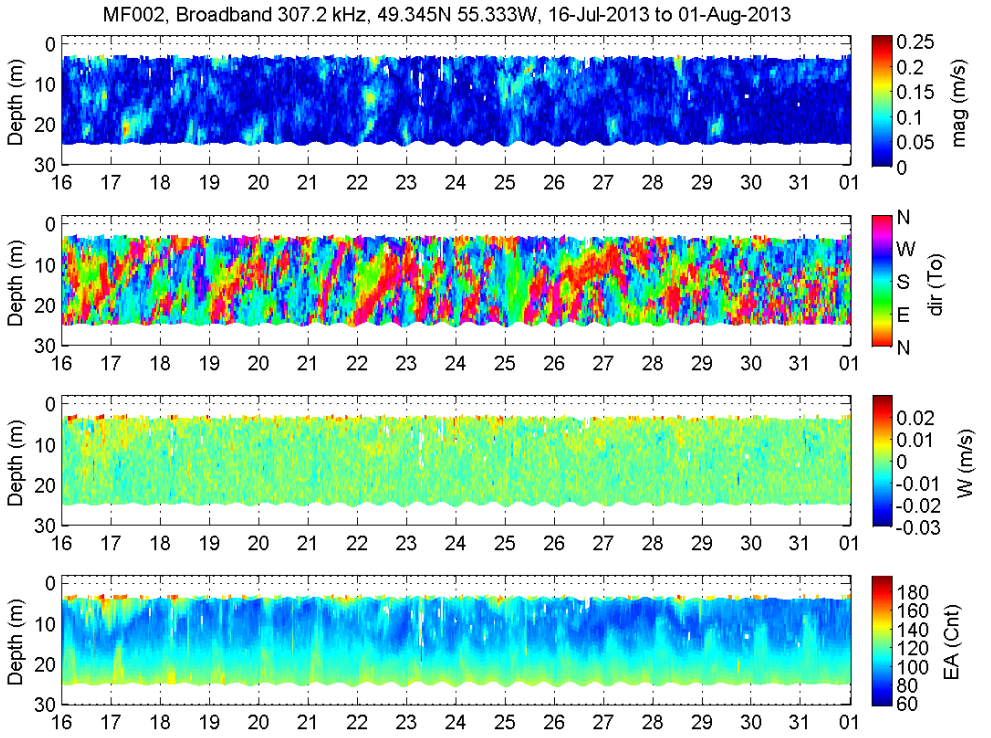
Figure E1: Mouse Island ADCP results for current speed (mag, m s^{-1}), direction, vertical velocity (W, m s^{-1}) and back scatter strength (EA, cnt= units based on voltage) for the period December 2012- May 2013 and August- December 2014.

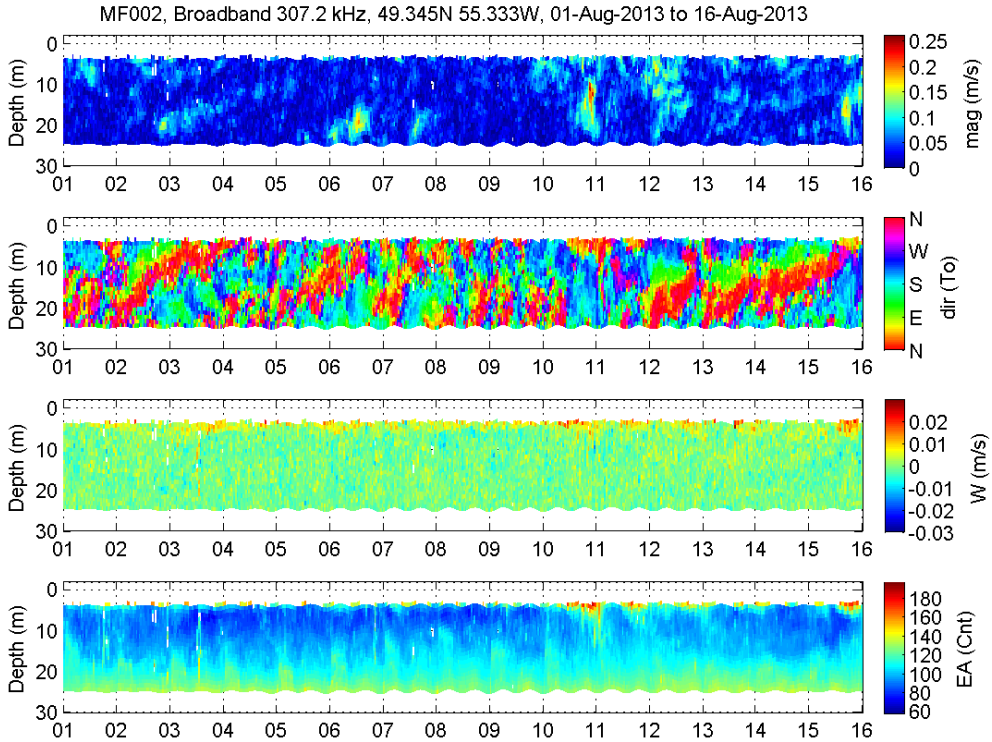

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 16-May-2013 to 01-Jun-2013
Depth (m)
mag (m/s)
dir (To)
W (m/s)
EA (Cnt)

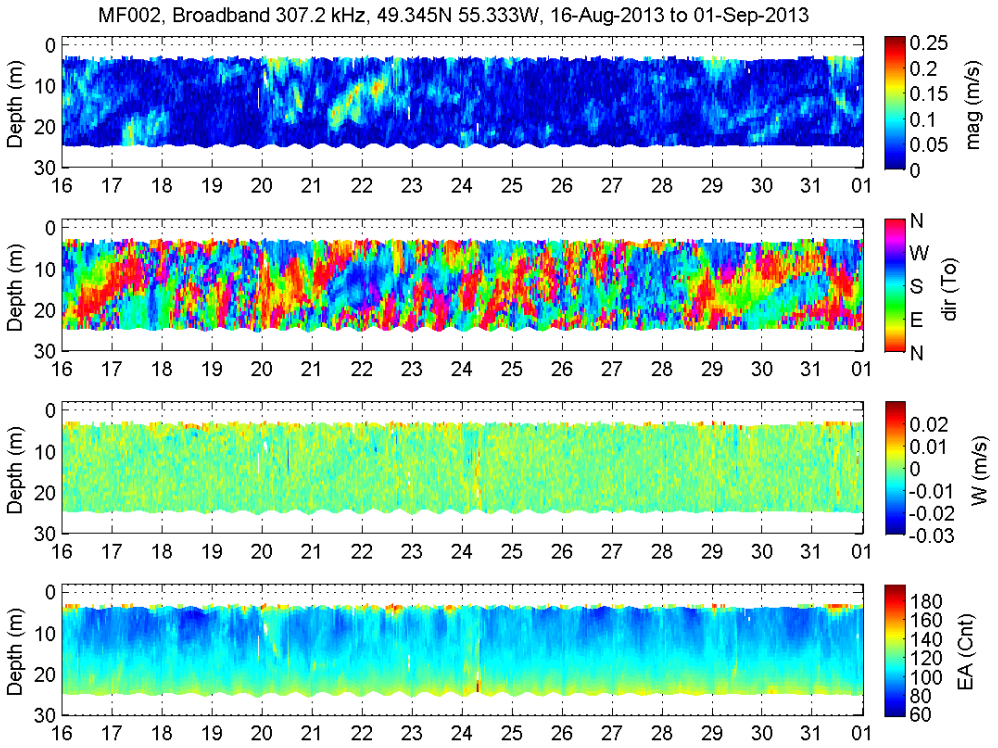

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 01-Jun-2013 to 16-Jun-2013
Depth (m)
mag (m/s)
dir (To)
W (m/s)
EA (Cnt)

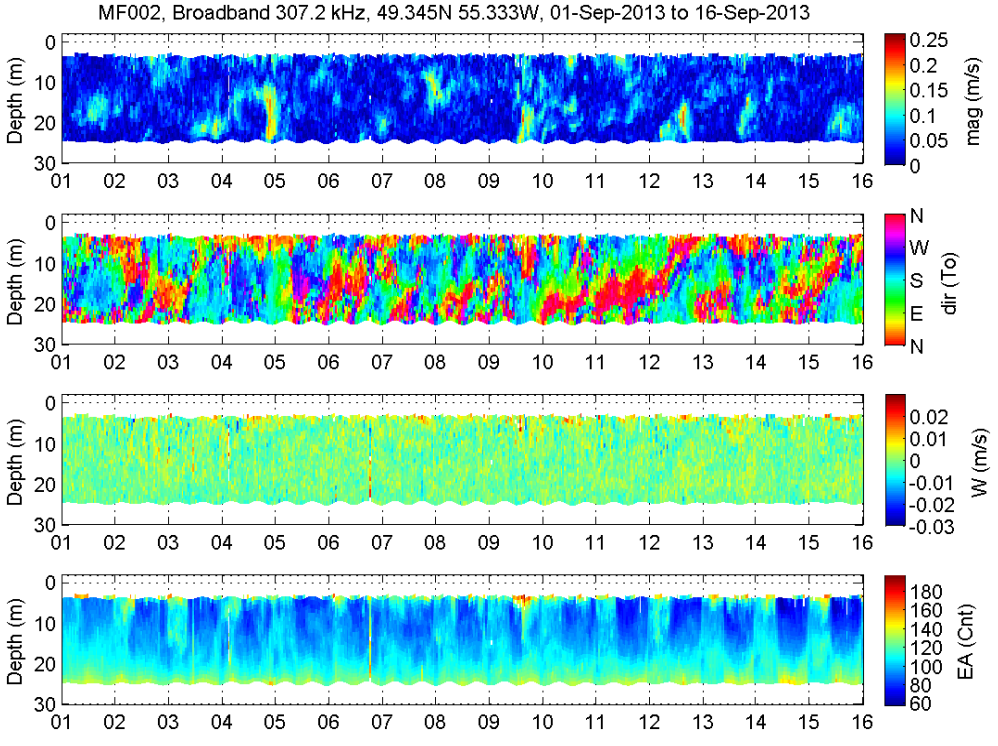

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 16-Jun-2013 to 01-Jul-2013
Depth (m)
0
10
20
30
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 01
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.02
0.01
0
-0.01
-0.02
-0.03
W (m/s)
180
160
140
120
100
80
60
EA (Cnt)

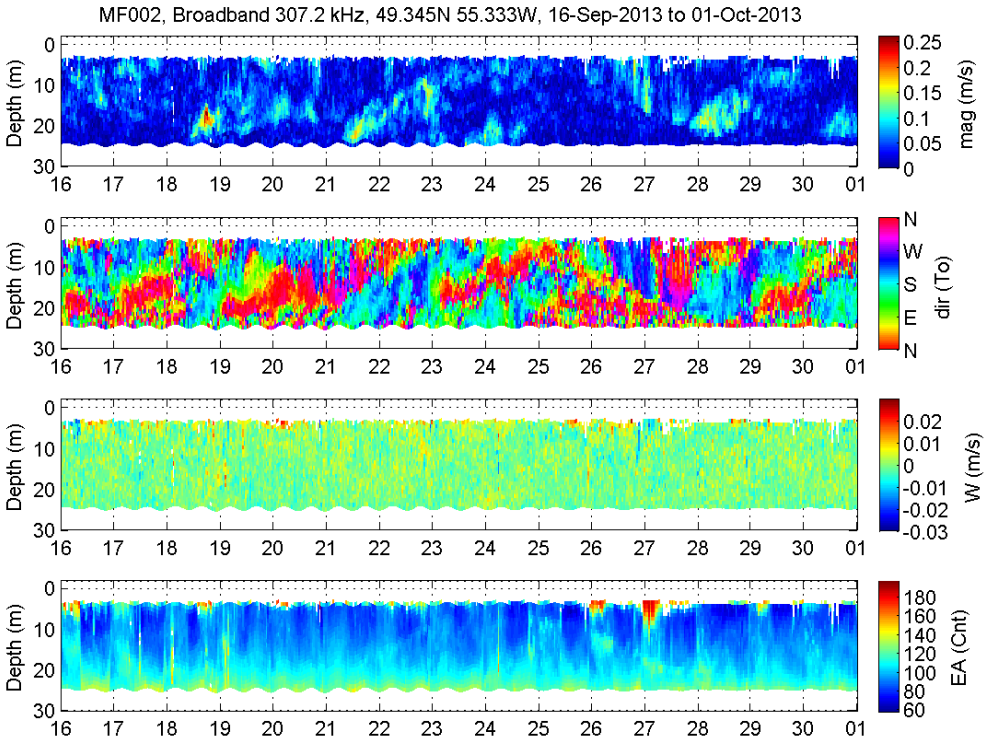

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 01-Jul-2013 to 16-Jul-2013
Depth (m)
0
10
20
30
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.02
0.01
0
-0.01
-0.02
-0.03
W (m/s)
180
160
140
120
100
80
60
EA (Cnt)

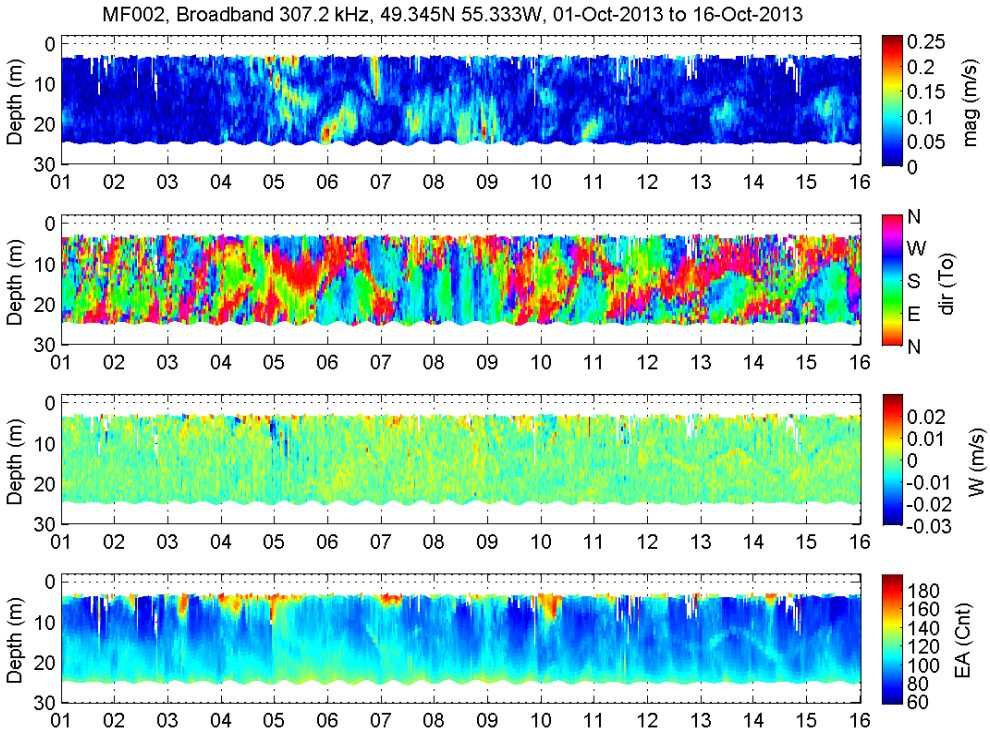

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 16-Jul-2013 to 01-Aug-2013
Depth (m)
0
10
20
30
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 01
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.02
0.01
0
-0.01
-0.02
-0.03
W (m/s)
180
160
140
120
100
80
60
EA (Cnt)

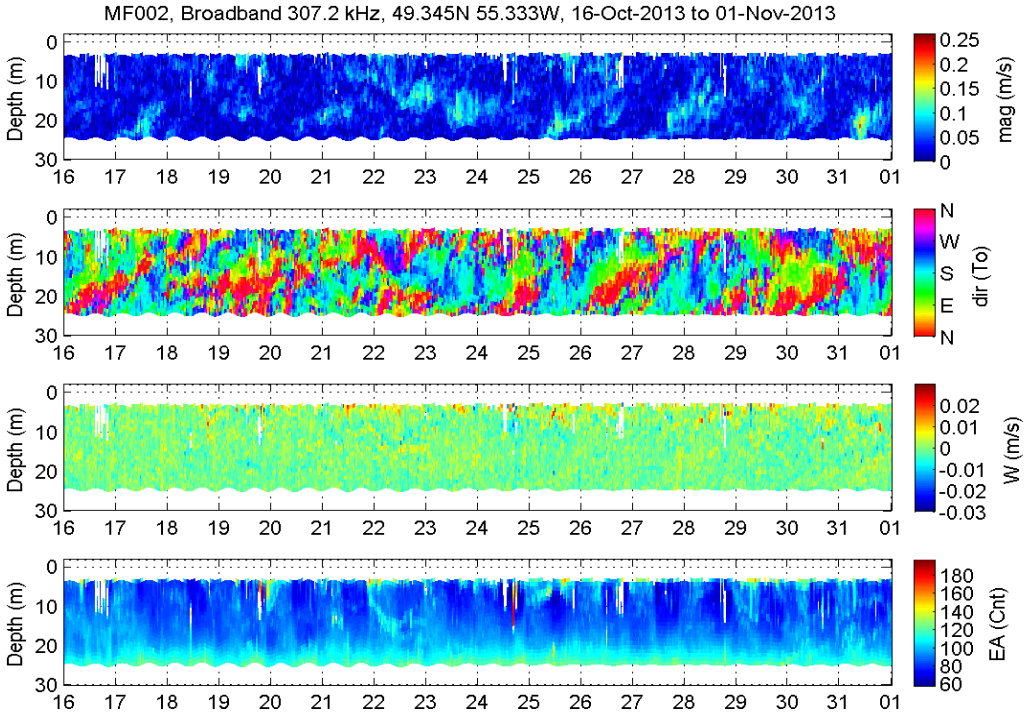

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 01-Aug-2013 to 16-Aug-2013
Depth (m)
0
10
20
30
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.02
0.01
0
-0.01
-0.02
-0.03
W (m/s)
180
160
140
120
100
80
60
EA (Cnt)

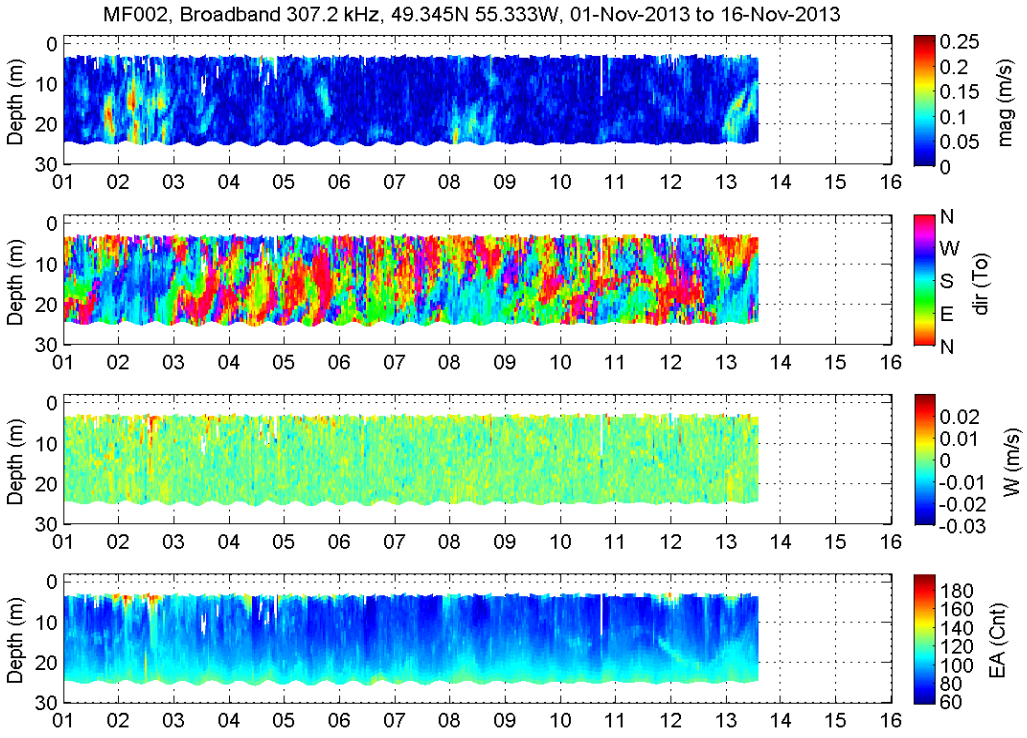

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 16-Aug-2013 to 01-Sep-2013
Depth (m)
mag (m/s)
dir (To)
W (m/s)
EA (Cnt)

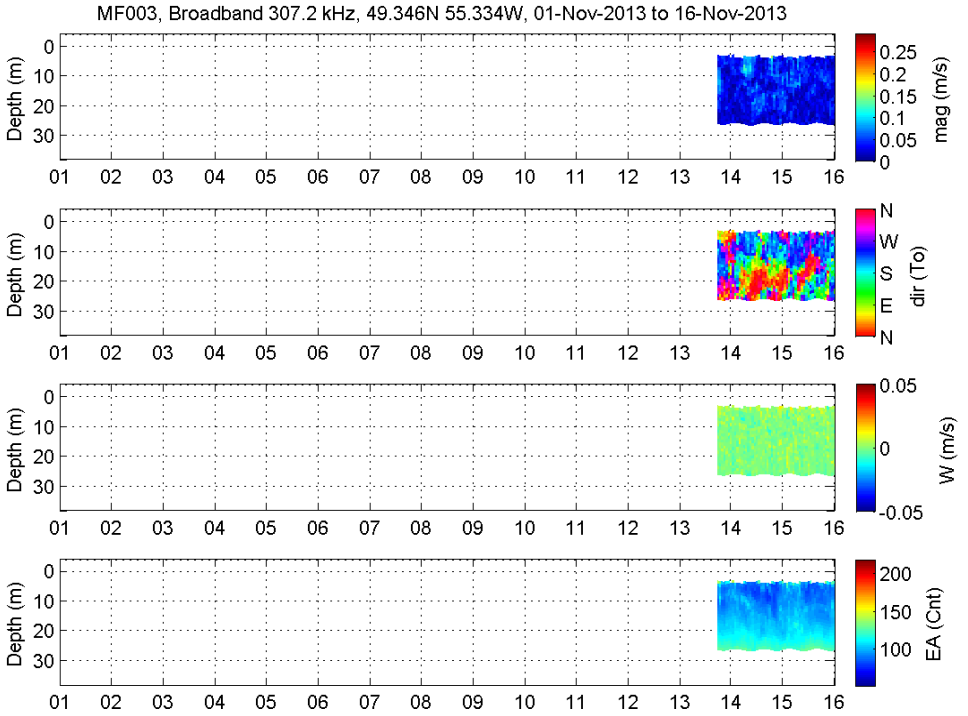

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 01-Sep-2013 to 16-Sep-2013
Depth (m)
mag (m/s)
dir (To)
W (m/s)
EA (Cnt)

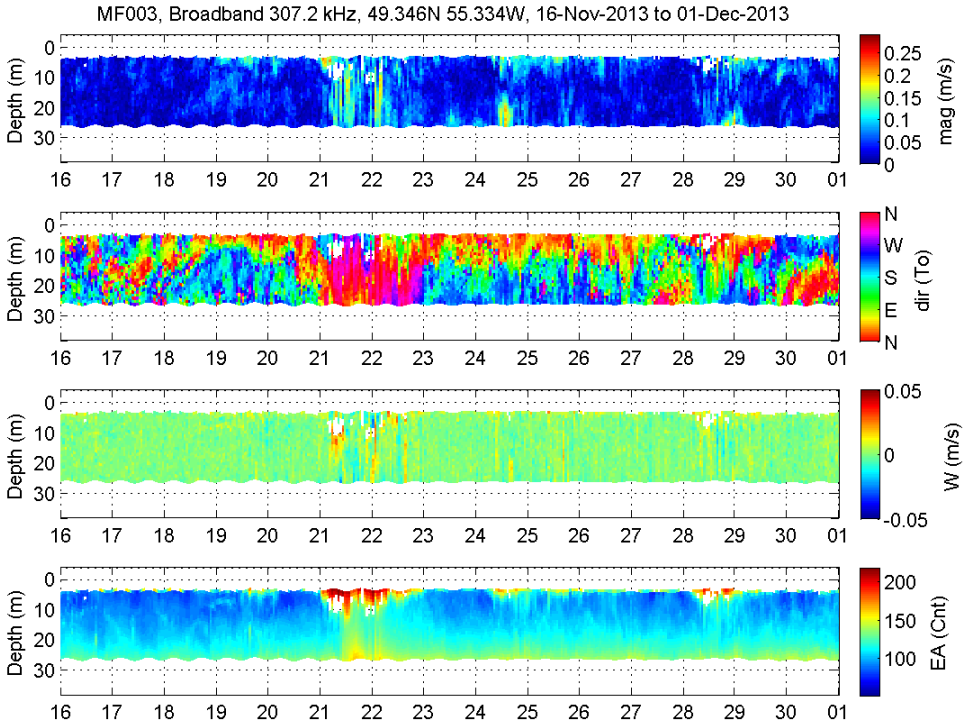

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 16-Sep-2013 to 01-Oct-2013
Depth (m)
0
10
20
30
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 01
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.02
0.01
0
-0.01
-0.02
-0.03
W (m/s)
180
160
140
120
100
80
60
EA (Cnt)

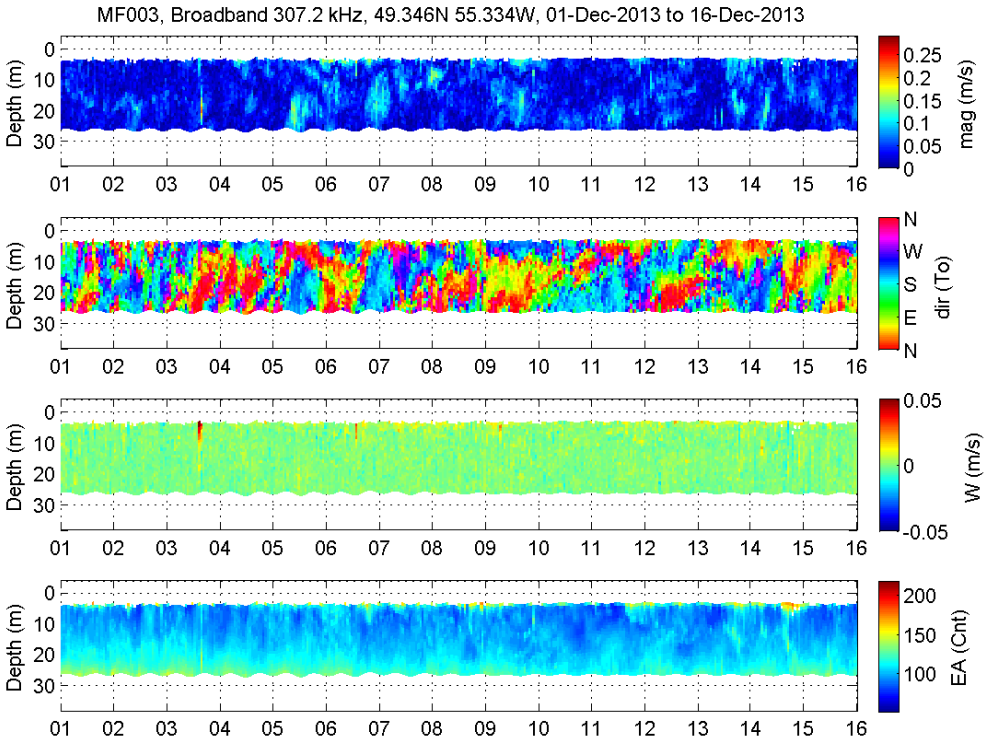

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 01-Oct-2013 to 16-Oct-2013
Depth (m)
0
10
20
30
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.02
0.01
0
-0.01
-0.02
-0.03
W (m/s)
180
160
140
120
100
80
60
EA (Cnt)

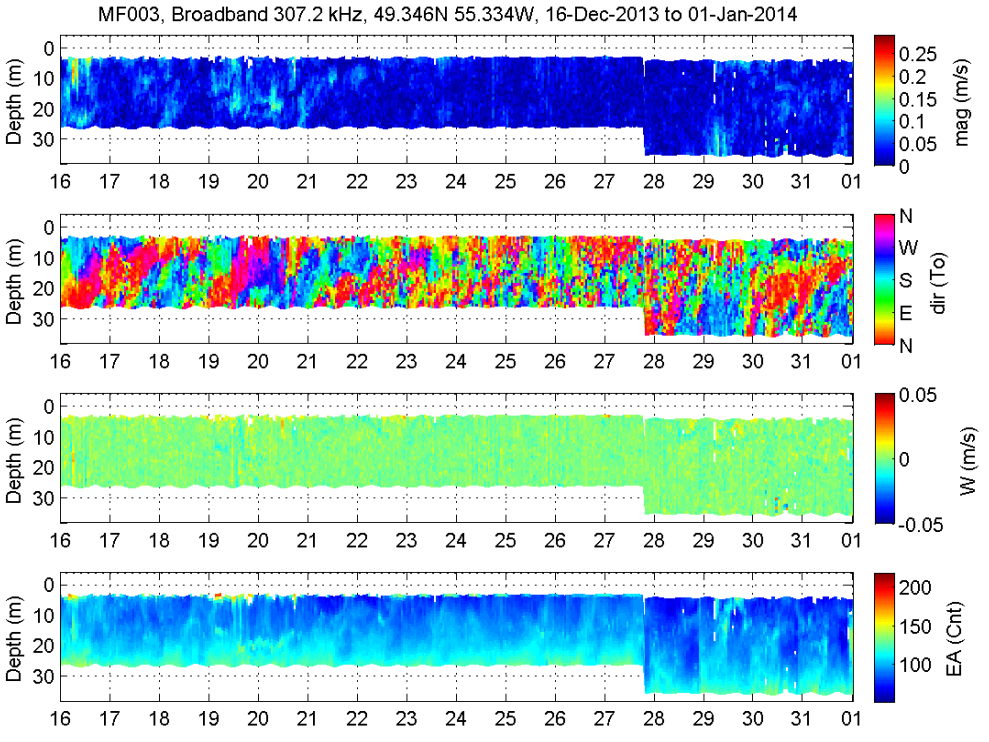

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 16-Oct-2013 to 01-Nov-2013
Depth (m)
mag (m/s)
dir (To)
W (m/s)
EA (Cnt)
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 01

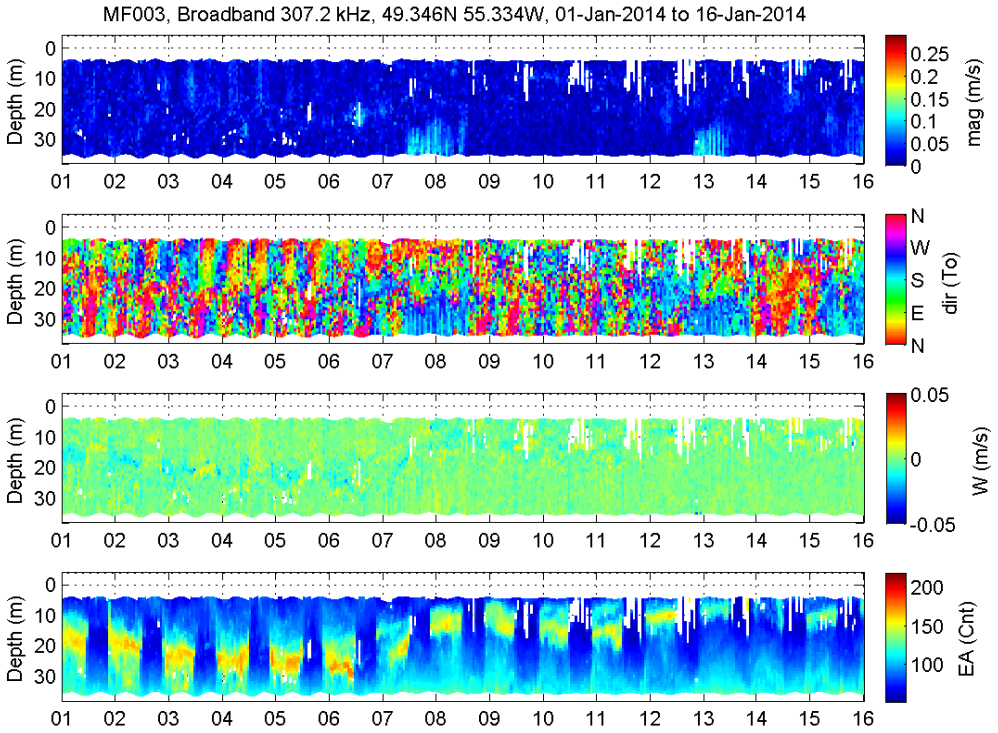

MF002, Broadband 307.2 kHz, 49.345N 55.333W, 01-Nov-2013 to 16-Nov-2013
Depth (m)
mag (m/s)
dir (To)
W (m/s)
EA (Cnt)
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16

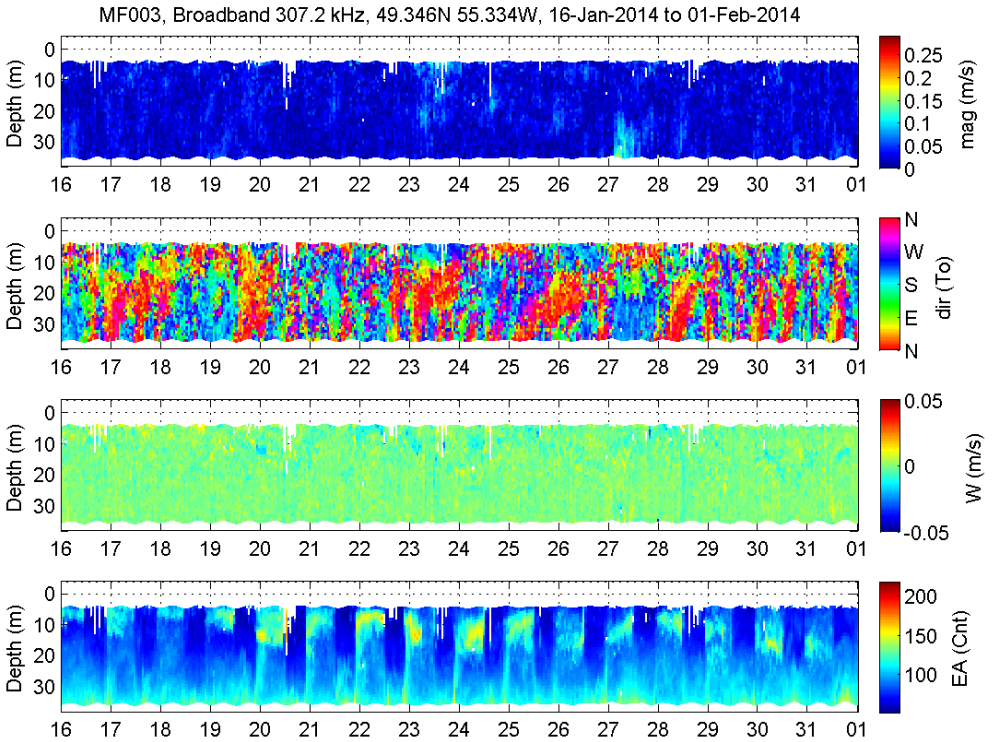

MF003, Broadband 307.2 kHz, 49.346N 55.334W, 01-Nov-2013 to 16-Nov-2013
Depth (m)
0
10
20
30
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.05
0
-0.05
W (m/s)
200
150
100
EA (Cnt)

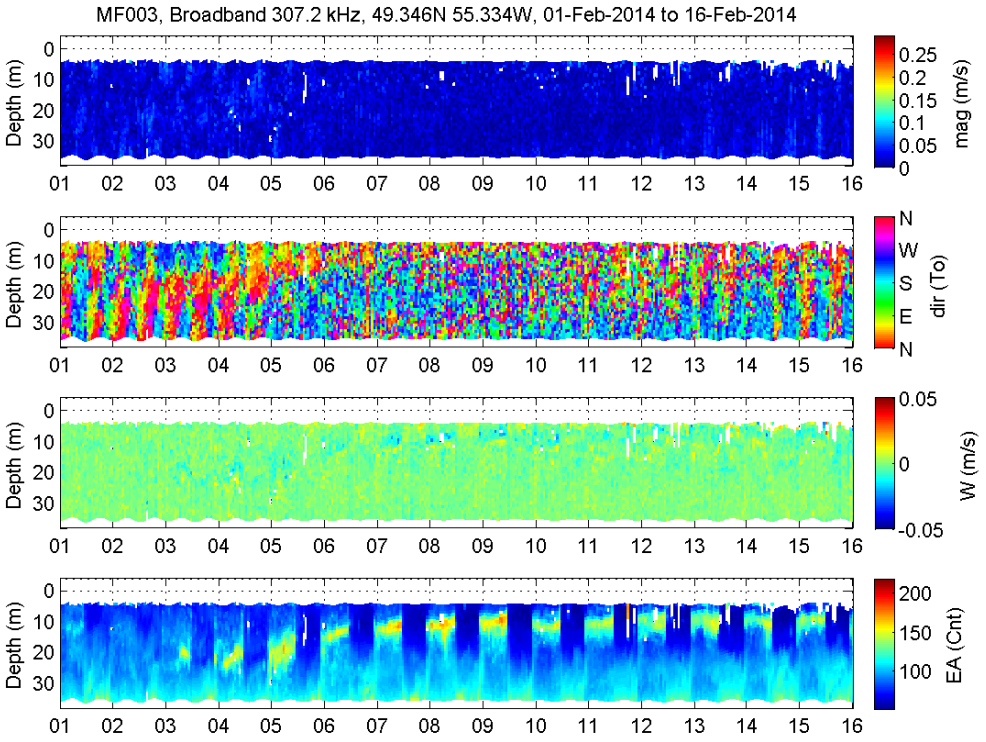

MF003, Broadband 307.2 kHz, 49.346N 55.334W, 16-Nov-2013 to 01-Dec-2013
Depth (m)
0
10
20
30
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 01
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.05
0
-0.05
W (m/s)
200
150
100
EA (Cnt)

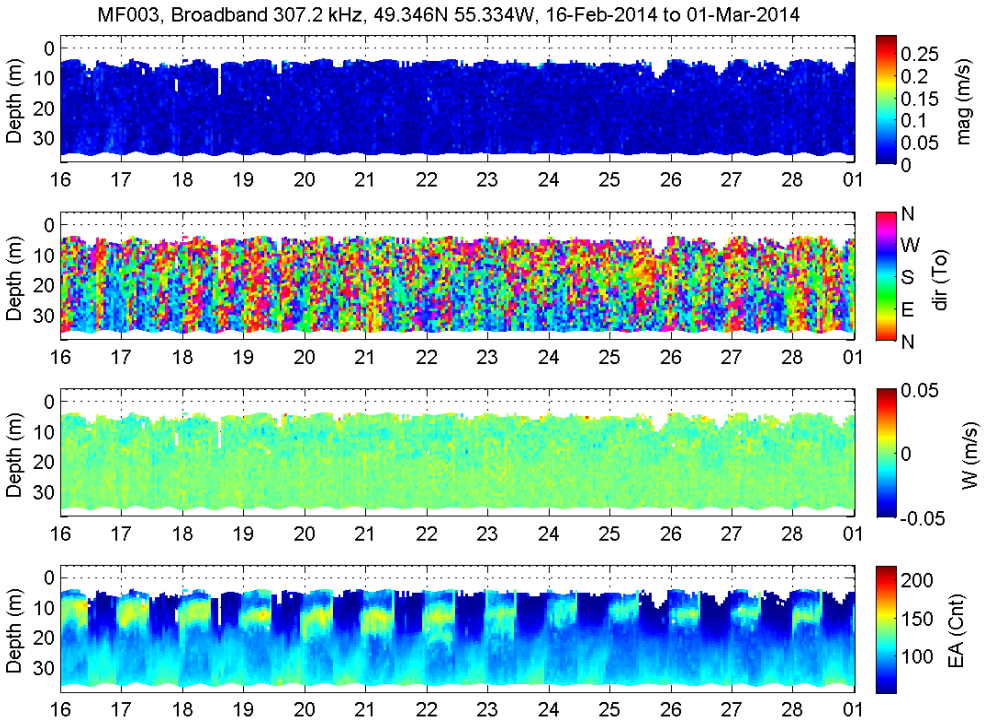

MF003, Broadband 307.2 kHz, 49.346N 55.334W, 01-Dec-2013 to 16-Dec-2013
Depth (m)
0
10
20
30
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.05
0
-0.05
W (m/s)
200
150
100
EA (Cnt)

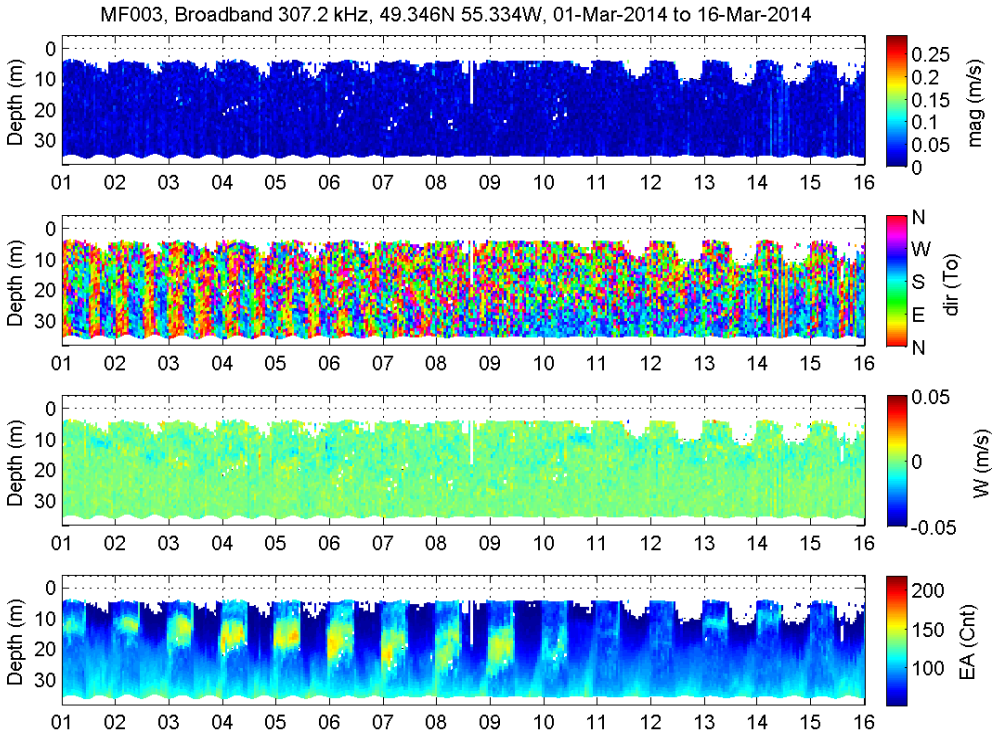

MF003, Broadband 307.2 kHz, 49.346N 55.334W, 16-Dec-2013 to 01-Jan-2014
Depth (m)
0
10
20
30
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 01
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.05
0
-0.05
W (m/s)
200
150
100
EA (Cnt)

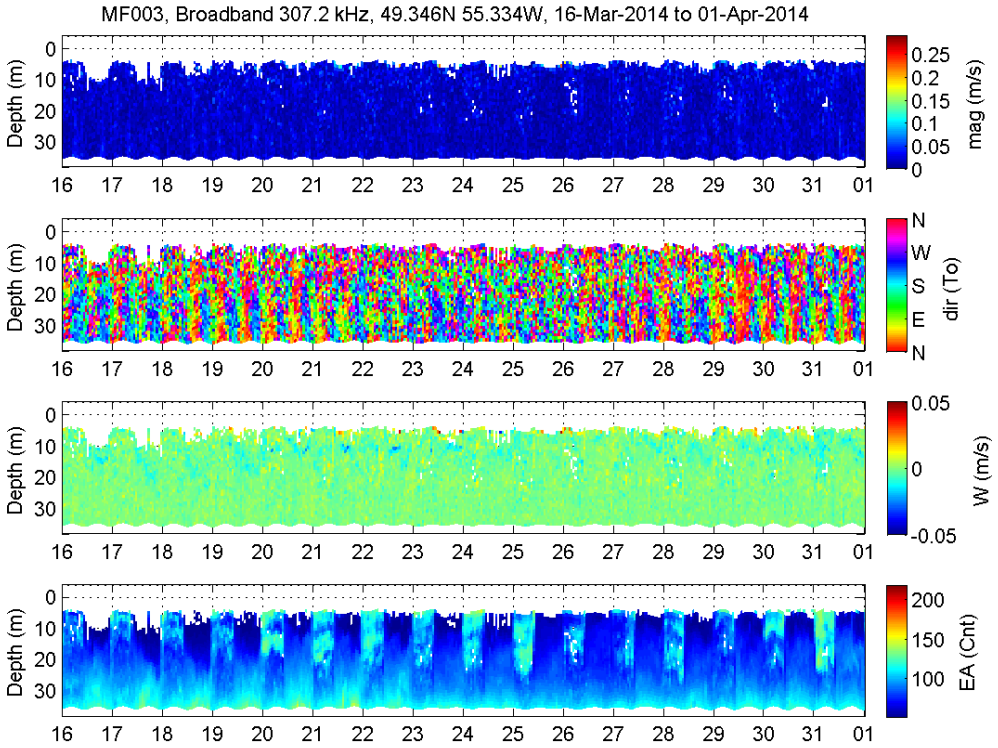

MF003, Broadband 307.2 kHz, 49.346N 55.334W, 01-Jan-2014 to 16-Jan-2014
Depth (m)
0
10
20
30
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.05
0
-0.05
W (m/s)
200
150
100
EA (Cnt)


MF003, Broadband 307.2 kHz, 49.346N 55.334W, 16-Jan-2014 to 01-Feb-2014
Depth (m)
0
10
20
30
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 01
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.05
0
-0.05
W (m/s)
200
150
100
EA (Cnt)


MF003, Broadband 307.2 kHz, 49.346N 55.334W, 01-Feb-2014 to 16-Feb-2014
Depth (m)
0
10
20
30
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.05
0
-0.05
W (m/s)
200
150
100
EA (Cnt)


MF003, Broadband 307.2 kHz, 49.346N 55.334W, 16-Feb-2014 to 01-Mar-2014
Depth (m)
0
10
20
30
16 17 18 19 20 21 22 23 24 25 26 27 28 01
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.05
0
-0.05
W (m/s)
200
150
100
EA (Cnt)


MF003, Broadband 307.2 kHz, 49.346N 55.334W, 01-Mar-2014 to 16-Mar-2014
Depth (m)
0
10
20
30
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.05
0
-0.05
W (m/s)
200
150
100
EA (Cnt)


MF003, Broadband 307.2 kHz, 49.346N 55.334W, 16-Mar-2014 to 01-Apr-2014
Depth (m)
0
10
20
30
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 01
0.25
0.2
0.15
0.1
0.05
0
mag (m/s)
N
W
S
E
N
dir (To)
0.05
0
-0.05
W (m/s)
200
150
100
EA (Cnt)

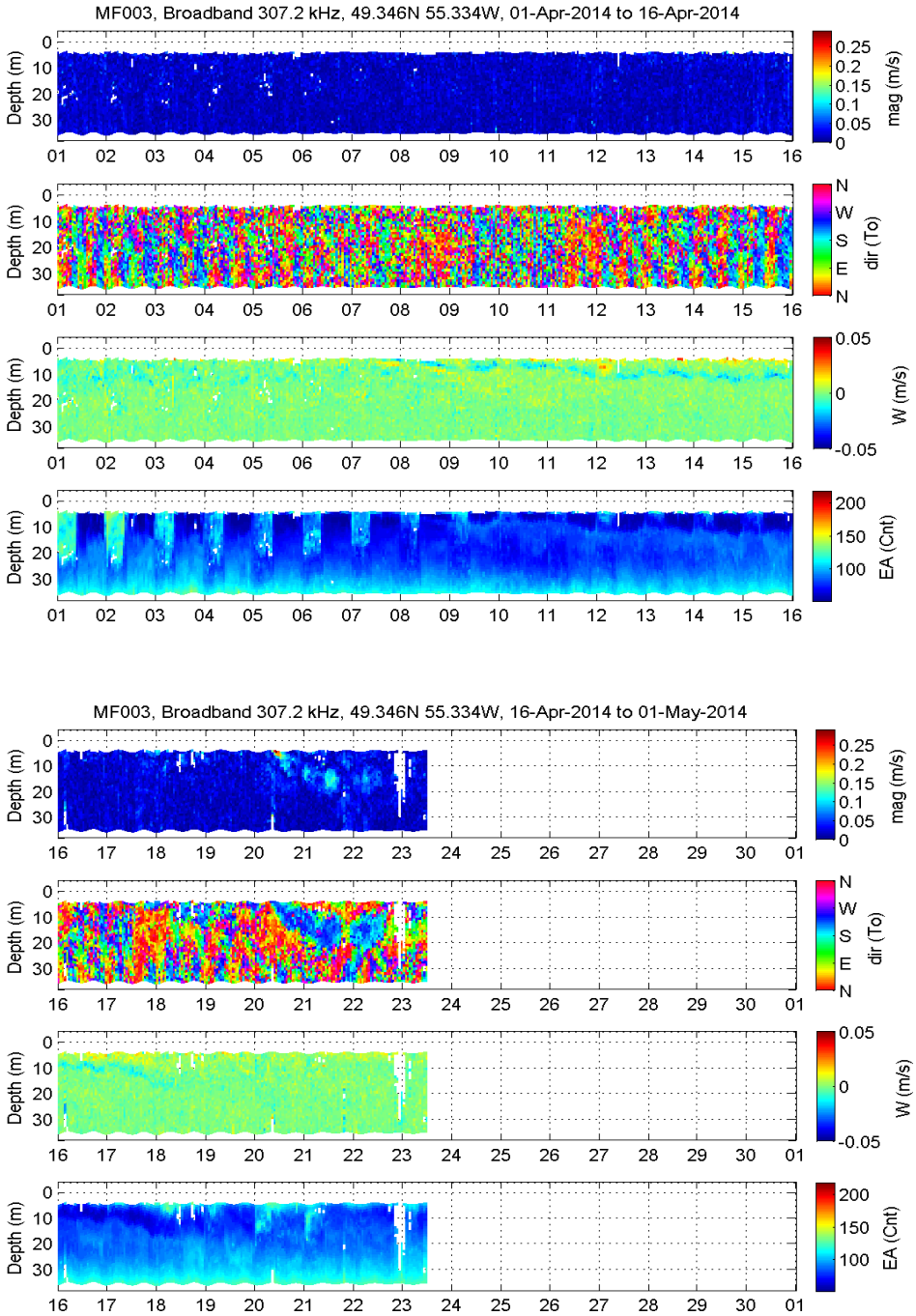


Figure E2: South Arm ADCP results for current speed (mag, m s-1), direction, vertical velocity (W, m s-1) and back scatter strength (EA, cnt= units based on voltage) for the period May 2013- May 2014.

Appendix F: Major fatty acid and lipid class structures

Figure F1: 22:6ω3 Docosahexaenoic acid (DHA)

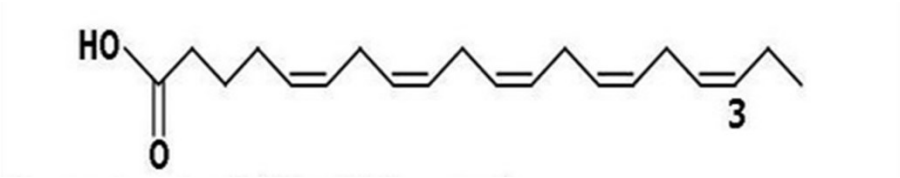


Figure F2: 20:5ω3 Eicosapentaenoic acid (EPA)

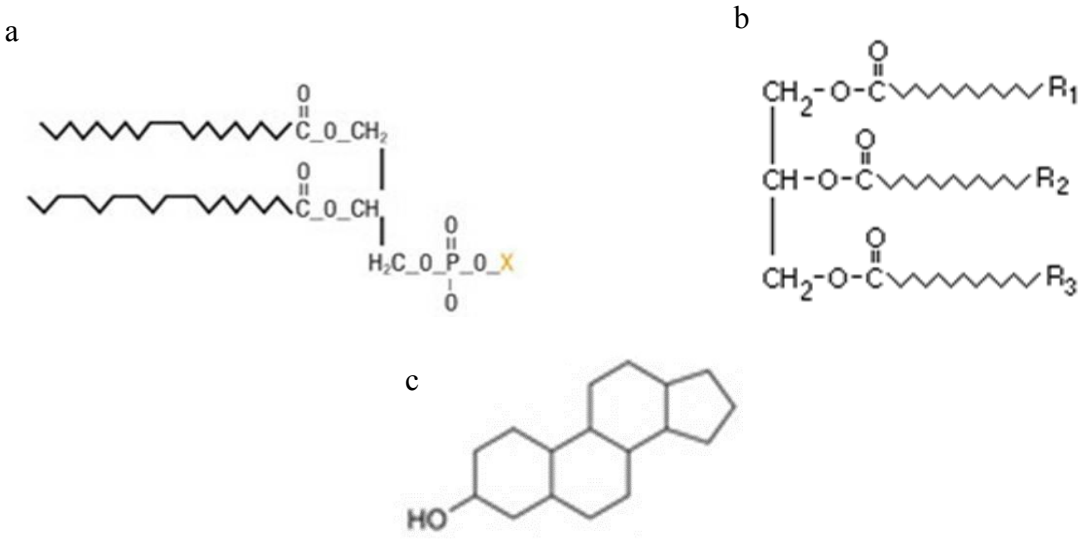


Figure F3: Main lipid class structures (a) phospholipids (b) triacylglycerols (c) sterols.

Appendix G: ANOVA tables for Chapter 4, 5 and 6

Table G1: Physiological stress response on gill tissue, Year 1 – Initial time (Chapter 4; Figures 4.2).

Year 1- Initial time

Defensin- ANOVA on ranks				
Source of Variation	DF		H	P
	5		16.619	0.005
HSP 70- ANOVA on ranks				
Source of Variation	DF		H	P
	5		15.735	0.008
GSTp- ANOVA on ranks				
Source of Variation	DF		H	P
	5		8.199	0.146
SOD- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.421	3.876	0.006
Residual	42	0.109		
Total	47			

Table G2: ANOVA tables for physiological stress response on gill tissue, Year 1– 4 Months (Chapter 4; Figures 4.3).

Year 1- 4 Months

Defensin-ANOVA on ranks				
Source of Variation	DF		H	P
	5		4.865	0.433
HSP 70- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.245	0.579	0.716
Residual	54	0.423		
Total	59			
GSTp - One way ANOVA				
Source of Variation	DF		H	P
	5		13.469	0.019
SOD- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	2.466	5.867	<0.001
Residual	48	0.42		
Total	53			

Table G3: ANOVA tables for physiological stress response on gill tissue, Year 1– 8 Months (Chapter 4; Figures 4.4).

Year 1- 8 Months

Defensin- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	33.717	1.907	0.122
Residual	31	17.68		
Total	36			
HSP 70- ANOVA on ranks				
Source of Variation	DF		H	P
	5		15.415	0.009
GSTp- ANOVA on ranks				
Source of Variation	DF		H	P
	5		3.202	0.699
SOD- ANOVA on ranks				
Source of Variation	DF		H	P
	5		3.763	0.584

Table G4: ANOVA tables for physiological stress response on gill tissue, Year 1– 12 Months (Chapter 4; Figures 4.5).

Year 1- 12 Months

Defensin- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	33.674	2.068	0.095
Residual	33	16.286		
Total	38			
HSP 70- ANOVA on ranks				
Source of Variation	DF		H	P
	5		6.400	0.269
SOD- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.0627	0.461	0.802
Residual	32	0.136		
Total	37			

Table G5: ANOVA tables for physiological stress response on haemocytes, Year 1– Initial time (Chapter 4; Figures 4.6).

Year 1- Initial time

Defensin- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.585	0.782	0.587
Residual	9	0.784		
Total	14			
HSP 70- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.237	2.115	0.155
Residual	9	0.112		
Total	14			
GSTp- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	20.741	1.172	0.393
Residual	9	17.693		
Total	14			
SOD- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.106	0.281	0.912
Residual	9	0.376		
Total	14			

Table G6: ANOVA tables for physiological stress response on haemocytes, Year 1– 4 Months (Chapter 4; Figures 4.7).

Year 1- 4 Months

Defensin- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.161	0.257	0.927
Residual	11	0.268		
Total	16			
HSP- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	115.216	0.854	0.538
Residual	12	134.943		
Total	17			

GSTp- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	2.854	0.519	0.757
Residual	11	5.503		
Total	16			
SOD- ANOVA on ranks				
Source of Variation	DF		H	P
	5		7.573	0.181

Table G7: ANOVA tables for physiological stress response on haemocytes, Year 1– 8 Months (Chapter 4; Figures 4.8).

Year 1- 8 Months

Defensin- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.161	0.257	0.927
Residual	11	0.628		
Total	16			
HSP 70- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	3.415	1.317	0.321
Residual	12	2.594		
Total	17			
GSTp- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	2.854	0.519	0.757
Residual	11	5.503		
Total	16			
SOD- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	3.125	1.413	0.288
Residual	12	2.212		
Total	17			

Table G8: ANOVA tables for physiological stress response on haemocytes, Year 1– 12 Months (Chapter 4; Figures 4.9).

Year 1- 12 Months

Defensin Y1- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	2.822	1.087	0.419
Residual	11	2.596		
Total	16			
HSP 70- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.0954	1.029	0.447
Residual	11	0.0926		
Total	16			
SOD- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.0252	0.584	0.713
Residual	11	0.0431		
Total	16			

Table G9: ANOVA tables for physiological stress response on gill tissue Year 2– Initial time (Chapter 4; Figures 4.10).

Year 2- Initial time

Defensin- ANOVA on ranks				
Source of Variation	DF		H	P
	5		1.682	0.891
HSP 70- One way ANOVA				
Source of Variation	DF	MS	F	P
Between groups	5	0.223	1.215	0.315
Residual	54	0.184		
Total	59			
GSTp- ANOVA on ranks				
Source of Variation	DF		H	P
	5		5.480	0.36
SOD- One way ANOVA				
Source of Variation	DF		H	P
	5		7.572	0.181

Table G10: ANOVA tables for physiological stress response on gill tissue Year 2– 8 Months (Chapter 4; Figures 4.11).

Year 2- 8 Months

Defensin- ANOVA on ranks			
Source of Variation	DF	H	P
	5	4.045	0.543
HSP 70- ANOVA on ranks			
Source of Variation	DF	H	P
	5	30.588	<0.001
GSTp- ANOVA on ranks			
Source of Variation	DF	H	P
	5	2.603	0.761
SOD- ANOVA on ranks			
Source of Variation	DF	H	P
	5	10.716	0.057

Table G11: ANOVA tables for physiological stress response on gill tissue Year 2– 12 Months (Chapter 4; Figures 4.12).

Year 2- 12 Months

Defensin- ANOVA on ranks			
Source of Variation	DF	H	P
	5	5.101	0.404
HSP 70 - ANOVA on rank			
Source of Variation	DF	H	P
	5	22.260	<0.001
GSTp- ANOVA on rank			
Source of Variation	DF	H	P
	5	2.463	0.782
SOD- ANOVA on rank			
Source of Variation	DF	H	P
	5	1.954	0.855

Table G12: ANOVA tables for condition for Year 1 and Year 2 (Chapter 5; Figures 5.1-5.3).

Year 1					**Year 2**				
Total wet weight					Total wet weight				
Source of Variation	DF	MS	F	P	Source of Variation	DF	MS	F	P
Time	3	54008.663	4911.0	<0.001	Time	2	26892.089	4482.8	<0.001
Site	5	1315.539	119.6	<0.001	Site	5	144.424	24.1	<0.001
Time x Site	15	203.083	18.5	<0.001	Time x Site	10	175.352	29.2	<0.001
Residual	3576	10.998			Residual	2682	5.999		
Total	3599	58.621			Total	2699	26.086		
Dry meat weight					Dry meat weight				
Source of Variation	DF	MS	F	P	Source of Variation	DF	MS	F	P
Time	3	309.473	4176.0	<0.001	Time	2	116.526	3914.5	<0.001
Site	5	9.778	131.9	<0.001	Site	5	0.997	33.5	<0.001
Time x Site	15	3.811	51.4	<0.001	Time x Site	10	1.762	59.2	<0.001
Residual	3576	0.074			Residual	2682	0.030		
Total	3599	0.361			Total	2699	0.124		
Dry weight/shell weight					Dry weight/shell weight				
Source of Variation	DF	MS	F	P	Source of Variation	DF	MS	F	P
Time	3	77968.096	3816.9	<0.001	Time	2	37846.692	3386.8	<0.001
Site	5	1270.575	62.2	<0.001	Site	5	468.431	41.9	<0.001
Time x Site	15	1281.577	62.7	<0.001	Time x Site	10	1107.217	99.1	<0.001
Residual	3576	20.427			Residual	2682	11.175		
Total	3599	92.395			Total	2699	44.119		

Dry weight/wet weight				
Source of Variation	DF	MS	F	P
Time	3	1.511	1502.7	<0.001
Site	5	0.005	4.7	<0.001
Time x Site	15	0.030	22.8	<0.001
Residual	3576	0.001		
Total	3599	0.002		

Dry weight/wet weight				
Source of Variation	DF	MS	F	P
Time	2	0.914	677.7	<0.001
Site	5	0.003	2.5	0.029
Time x Site	10	0.027	19.9	<0.001
Residual	2682	0.001		
Total	2699	0.002		

Shell weight				
Source of Variation	DF	MS	F	P
Time	3	4718.814	6583.9	<0.001
Site	5	128.940	179.9	<0.001
Time x Site	15	18.455	25.8	<0.001
Residual	3576	0.717		
Total	3599	4.902		

Shell weight				
Source of Variation	DF	MS	F	P
Time	2	2537.651	5175.4	<0.001
Site	5	6.706	13.7	<0.001
Time x Site	10	13.225	27.0	<0.001
Residual	2682	0.490		
Total	2699	2.429		

Shell length				
Source of Variation	DF	MS	F	P
Time	3	116370.600	6669.0	<0.001
Site	5	1258.433	72.1	<0.001
Time x Site	15	93.929	5.4	<0.001
Residual	3576	17.449		
Total	3599	116.480		

Shell lenght				
Source of Variation	DF	MS	F	P
Time	2	82763.085	4263.3	<0.001
Site	5	491.840	25.3	<0.001
Time x Site	10	310.081	16.0	<0.001
Residual	2682	19.417		
Total	2699	82.707		

Table G13: ANOVA tables for biochemical parameters for Year 1 and Year 2 (Chapter 5; Tables 5.1 and 5.3).

Year 1					Year 2				
Total lipids					Total lipids				
Source of Variation	DF	MS	F	P	Source of Variation	DF	MS	F	P
Time	3	291144994	51.718	<0.001	Time	2	142999458	14.847	<0.001
Site	5	3606856.61	0.641	0.669	Site	5	8290264.04	0.861	0.509
Time x Site	15	5959362.8	1.059	0.397	Time x Site	10	5158183.1	0.536	0.863
Residual	216	5629525.6			Residual	162	9631459.7		
Total	239	91911787.6			Total	179	10834237.7		
TAG/sterols ratio					TAG/sterols ratio				
Source of Variation	DF	MS	F	P	Source of Variation	DF	MS	F	P
Time	3	308.104	50.294	<0.001	Time	2	65.327	51.607	<0.001
Site	5	8.762	1.159	0.331	Site	5	1.015	0.801	0.55
Time x Site	15	6.445	0.853	0.618	Time x Site	10	0.784	0.62	0.796
Residual	203	7.558			Residual	161	1.266		
Total	226	12.475			Total	178	1.953		
Glycogen					Glycogen				
Source of Variation	DF	MS	F	P	Source of Variation	DF	MS	F	P
Time	3	3818.46	42.838	<0.001	Time	2	2571.009	16.428	<0.001
Site	5	245.888	2.759	0.019	Site	5	5167.132	33.016	<0.001
Time x Site	15	561.225	6.296	<0.001	Time x Site	10	1988.778	12.707	<0.001
Residual	216	89.137			Residual	162	156.505		
Total	239	168.857			Total	179	425.806		

DHA				
Source of Variation	DF	MS	F	P
Time	3	2541.435	768.687	<0.001
Site	5	2.532	0.766	0.575
Time x Site	15	7.565	2.288	0.005
Residual	216	3.306		
Total	239	35.417		

DHA				
Source of Variation	DF	MS	F	P
Time	2	2497.405	765.502	<0.001
Site	5	14.945	4.581	<0.001
Time x Site	10	10.291	3.154	0.001
Residual	162	3.262		
Total	179	31.849		

EPA				
Source of Variation	DF	MS	F	P
Time	3	5034.321	1126.485	<0.001
Site	5	16.905	3.783	0.003
Time x Site	15	16.387	3.667	<0.001
Residual	216	4.469		
Total	239	68.613		

EPA				
Source of Variation	DF	MS	F	P
Time	2	1731.261	856.433	<0.001
Site	5	31.644	15.654	<0.001
Time x Site	10	10.07	4.982	<0.001
Residual	162	2.021		
Total	179	22.62		

Omega -3				
Source of Variation	DF	MS	F	P
Time	3	248.981	46.647	<0.001
Site	5	6.749	1.264	0.28
Time x Site	15	9.32	1.746	0.044
Residual	216	5.338		
Total	239	8.675		

Omega-3				
Source of Variation	DF	MS	F	P
Time	2	39.065	4.405	0.014
Site	5	84.71	9.553	<0.001
Time x Site	10	29.408	3.316	<0.001
Residual	162	8.868		
Total	179	12.471		

Bacterial				
Source of Variation	DF	MS	F	P
Time	3	408.829	148.546	<0.001
Site	5	3.29	1.195	0.312
Time x Site	15	7.937	2.884	<0.001
Residual	216	2.752		
Total	239	8.186		

Bacterial				
Source of Variation	DF	MS	F	P
Time	2	647.273	614.583	<0.001
Site	5	15.366	14.59	<0.001
Time x Site	10	7.805	7.411	<0.001
Residual	162	1.053		
Total	179	9.051		

Terrestrial				
Source of Variation	DF	MS	F	P
Time	3	61.98	232.29	<0.001
Site	5	4.845	18.159	<0.001
Time x Site	15	1.469	5.505	<0.001
Residual	216	0.267		
Total	239	1.213		

Terrestrial				
Source of Variation	DF	MS	F	P
Time	2	0.977	1.144	0.321
Site	5	1.851	2.168	0.06
Time x Site	10	1.181	1.383	0.192
Residual	162	0.854		
Total	179	0.901		

Table G14: ANOVA tables for condition for fall, winter and spring seasons (Chapter 6; Figure 6.2- 6.4).

Fall

Holding					Control				
Total wet weight					Total wet weight				
Source of Variation	DF	MS	F	P	Source of Variation	DF	MS	F	P
Between Groups	3	264.188	20.612	<0.001	Between Groups	3	85.166	4.911	0.002
Residual	596	12.817			Residual	596	17.344		
Total	599				Total	599			
Dry meat weight					Dry meat weight				
Source of Variation	DF	MS	F	P	Source of Variation	DF	MS	F	P
Between Groups	3	0.727	9.512	<0.001	Between Groups	3	0.618	6.502	<0.001
Residual	596	0.0764			Residual	596	0.0951		
Total	599				Total	599			
Dry weight/shell weight ratio					Dry weight/shell weight ratio				
Source of Variation	DF	MS	F	P	Source of Variation	DF	MS	F	P
Between Groups	3	275.109	28.627	<0.001	Between Groups	3	285.742	32.401	<0.001
Residual	596	9.61			Residual	596	8.819		
Total	599				Total	599			
Dry weight/wet weight ratio					Dry weight/wet weight ratio				
Source of Variation	DF	MS	F	P	Source of Variation	DF	MS	F	P
Between Groups	3	0.0217	62.195	<0.001	Between Groups	3	0.0123	18.247	<0.001
Residual	596	0.000348			Residual	596	0.000677		
Total	599				Total	599			

Winter

Holding				
Total wet weight				
Source of Variation	DF	MS	F	P
Between Groups	3	87.018	6.705	<0.001
Residual	596	12.978		
Total	599			
Dry meat weight				
Source of Variation	DF	MS	F	P
Between Groups	3	0.287	2.771	0.041
Residual	596	0.104		
Total	599			
Dry weight/shell weight ratio				
Source of Variation	DF	MS	F	P
Between Groups	3	44.602	5.009	0.002
Residual	596	8.904		
Total	599			
Dry weight/wet weight ratio				
Source of Variation	DF	MS	F	P
Between Groups	3	0.00809	11.826	<0.001
Residual	596	0.000684		
Total	599			

Control				
Total wet weight				
Source of Variation	DF	MS	F	P
Between Groups	3	1968.513	106.783	<0.001
Residual	596	18.435		
Total	599			
Dry meat weight				
Source of Variation	DF	MS	F	P
Between Groups	3	38.174	311.47	<0.001
Residual	596	0.123		
Total	599			
Dry weight/shell weight ratio				
Source of Variation	DF	MS	F	P
Between Groups	3	1017.332	119.653	<0.001
Residual	596	8.502		
Total	599			
Dry weight/wet weight ratio				
Source of Variation	DF	MS	F	P
Between Groups	3	0.0212	32.686	<0.001
Residual	596	0.000649		
Total	599			

Spring

Holding				
Total wet weight				
Source of Variation	DF	MS	F	P
Between Groups	3	594.862	12.205	<0.001
Residual	596	48.738		
Total	599			
Dry meat weight				
Source of Variation	DF	MS	F	P
Between Groups	3	1.607	2.817	0.038
Residual	596	0.571		
Total	599			
Dry weight/shell weight ratio				
Source of Variation	DF	MS	F	P
Between Groups	3	122.235	3.413	0.017
Residual	596	35.81		
Total	599			
Dry weight/wet weight ratio				
Source of Variation	DF	MS	F	P
Between Groups	3	0.0143	9.521	<0.001
Residual	596	0.0015		
Total	599			

Control				
Total wet weight				
Source of Variation	DF	MS	F	P
Between Groups	3	1046.77	25.061	<0.001
Residual	596	41.769		
Total	599			
Dry meat weight				
Source of Variation	DF	MS	F	P
Between Groups	3	23.026	59.849	<0.001
Residual	596	0.385		
Total	599			
Dry weight/shell weight ratio				
Source of Variation	DF	MS	F	P
Between Groups	3	1232.277	41.15	<0.001
Residual	596	29.946		
Total	599			
Dry weight/wet weight ratio				
Source of Variation	DF	MS	F	P
Between Groups	3	0.033	26.166	<0.001
Residual	596	0.00126		
Total	599			

Table G15: ANOVA tables for biochemical parameters for fall, winter and spring seasons (Chapter 6; Tables 6.1 and 6.2; Figure 6.5).

Fall

Holding

Total lipids- ANOVA on ranks

Source of Variation	DF	H	P
	3	8.027	0.045

TAG/sterol- ANOVA on ranks

Source of Variation	DF	H	P
	3	10.502	0.015

Glycogen- ANOVA on ranks

Source of Variation	DF	H	P
	3	10.305	0.016

DHA- ANOVA on ranks

Source of Variation	DF	H	P
	3	5.361	0.147

EPA- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	2.668	0.803	0.5
Residual	36	3.321		
Total	39			

Control

Total lipids- ANOVA on ranks

Source of Variation	DF	H	P
	3	13.532	0.004

TAG/sterol One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	0.437	1.239	0.313
Residual	30	0.353		
Total	33			

Glycogen- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	122.356	3.481	0.026
Residual	36	35.147		
Total	39			

DHA- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	4.372	1.322	0.282
Residual	36	3.308		
Total	39			

EPA- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	21.08	7.821	<0.001
Residual	36	2.695		
Total	39			

Omega-3 - One way ANOVA				
Source of Variation	DF	MS	F	P
Between Groups	3	2.016	0.665	0.579
Residual	36	3.03		
Total	39			

Bacterial- One way ANOVA				
Source of Variation	DF	MS	F	P
Between Groups	3	3.335	2.114	0.116
Residual	36	1.578		
Total	39			

Omega-3 - One way ANOVA				
Source of Variation	DF	MS	F	P
Between Groups	3	8.886	4.283	0.011
Residual	36	2.074		
Total	39			

Bacterial- ANOVA on ranks			
Source of Variation	DF	H	P
	3	10.760	0.013

Winter

Holding

Total lipids- ANOVA on ranks			
Source of Variation	DF	H	P
	3	7.834	0.05

TAG/sterol- ANOVA on ranks			
Source of Variation	DF	H	P
	3	11.411	0.010

Glycogen- One way ANOVA				
Source of Variation	DF	MS	F	P
Between Groups	3	163.716	5.375	0.004
Residual	36	30.461		
Total	39			

DHA- One way ANOVA				
Source of Variation	DF	MS	F	P
Between Groups	3	8.456	3.612	0.022
Residual	36	2.341		
Total	39			

Control

Total lipids- ANOVA on ranks			
Source of Variation	DF	H	P
	3	6.395	0.094

TAG/sterol- ANOVA on ranks			
Source of Variation	DF	H	P
	3	4.746	0.191

Glycogen- One way ANOVA				
Source of Variation	DF	MS	F	P
Between Groups	3	296.062	6.725	0.001
Residual	36	44.025		
Total	39			

DHA- One way ANOVA				
Source of Variation	DF	MS	F	P
Between Groups	3	62.501	17.636	<0.001
Residual	36	3.544		
Total	39			

EPA- ANOVA on ranks

Source of Variation	DF	H	P
	3	7.758	0.051

Omega-3 - ANOVA on ranks

Source of Variation	DF	H	P
	3	11.407	0.010

Bacterial- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	4.297	4.402	0.01
Residual	36	0.976		
Total	39			

EPA- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	110.706	23.56	<0.001
Residual	36	4.699		
Total	39			

Omega-3 - One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	6.441	1.703	0.184
Residual	36	3.783		
Total	39			

Bacterial- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	0.283	0.253	0.859
Residual	36	1.117		
Total	39			

Spring

Holding

Total lipids- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	252412.5	9.072	<0.001
Residual	36	27824.29		
Total	39			

TAG/sterol- ANOVA on ranks

Source of Variation	DF	H	P
	3	7.045	0.070

Control

Total lipids- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	108515.2	2.085	0.119
Residual	36	52052.21		
Total	39			

TAG/sterol- One Way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	11.334	4.286	0.011
Residual	35	2.644		
Total	38			

Glycogen- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	1451.822	7.882	<0.001
Residual	36	184.197		
Total	39			

DHA- ANOVA on ranks

Source of Variation	DF	H	P
	3	3.197	0.362

EPA- ANOVA on ranks

Source of Variation	DF	H	P
	3	3.965	0.265

Omega-3 - One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	32.816	11.821	<0.001
Residual	36	2.776		
Total	39			

Bacterial- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	7.232	2.867	0.05
Residual	36	2.522		
Total	39			

Glycogen- ANOVA on ranks

Source of Variation	DF	H	P
	3	7.708	0.052

DHA- ANOVA on ranks

Source of Variation	DF	H	P
	3	13.177	0.004

EPA- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	50.861	9.181	<0.001
Residual	36	5.54		
Total	39			

Omega-3 - One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	24.142	4.7	0.007
Residual	36	5.137		
Total	39			

Bacterial- ANOVA on ranks

Source of Variation	DF	H	P
	3	14.202	0.003

Table G16: ANOVA tables for hedonic test for fall, winter and spring seasons (Chapter 6; Tables 6.4).

Fall

Holding

Appearance- ANOVA on ranks

Source of Variation	DF	H	P
	3	0.350	0.950

Odour- ANOVA on ranks

Source of Variation	DF	H	P
	3	8.916	0.030

Texture- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	1.292	0.72	0.543
Residual	92	1.794		
Total	95			

Flavour- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	2.125	1.677	0.177
Residual	92	1.267		
Total	95			

Control

Appearance- ANOVA on ranks

Source of Variation	DF	H	P
	3	3.505	0.320

Odour- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	2.01	1.24	0.3
Residual	92	1.621		
Total	95			

Texture- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	3.694	1.879	0.139
Residual	92	1.966		
Total	95			

Flavour- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	1.344	0.818	0.487
Residual	92	1.643		
Total	95			

Winter

Holding

Appearance- ANOVA on ranks

Source of Variation	DF		H	P
	3		6.043	0.110

Odour- ANOVA on ranks

Source of Variation	DF		H	P
	3		4.553	0.208

Texture- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	0.861	0.543	0.654
Residual	92	1.586		
Total	95			

Flavour- ANOVA on ranks

Source of Variation	DF		H	P
	3		5.442	0.143

Control

Appearance- ANOVA on ranks

Source of Variation	DF		H	P
	3		9.638	0.022

Odour- ANOVA on ranks

Source of Variation	DF		H	P
	3		14.147	0.003

Texture- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	3.194	2.301	0.082
Residual	92	1.389		
Total	95			

Flavour- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	2.122	1.782	0.156
Residual	92	1.191		
Total	95			

Spring

Holding

Appearance- One way ANOVA

Source of Variation	DF	MS	F	P
Between Groups	3	3.611	2.434	0.07
Residual	92	1.484		
Total	95			

Odour- ANOVA on ranks

Source of Variation	DF		H	P
	3		1.693	0.638

Control

Appearance- ANOVA on ranks

Source of Variation	DF		H	P
	3		7.087	0.069

Odour- ANOVA on ranks

Source of Variation	DF		H	P
	3		5.634	0.131

Texture- ANOVA on ranks				
Source of Variation	DF		H	P
	3		5.406	0.144
Flavour- One way ANOVA				
Source of Variation	DF	MS	F	P
Between Groups	3	2.556	2.033	0.115
Residual	92	1.257		
Total	95			

Texture- ANOVA on ranks				
Source of Variation	DF		H	P
	3		4.977	0.173
Flavour- One way ANOVA				
Source of Variation	DF	MS	F	P
Between Groups	3	3.399	2.6	0.057
Residual	92	1.308		
Total	95			