IMPORTANCE OF EELGRASS (*ZOSTERA MARINA*) TO FISHES IN NORTH SHORE ESTUARIES ON PRINCE EDWARD ISLAND

A Thesis

Submitted to the Graduate Faculty

In Partial Fulfilment of the Requirements

For the Degree of

Master of Science

In the Department of Biology

Faculty of Science

University of Prince Edward Island

Canadian Rivers Institute

Christina Pater

Charlottetown, P.E.I.

December, 2010

Library and Archives
Canada

Published Heritage
Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-82253-1
Our file Notre référence
ISBN: 978-0-494-82253-1

NOTICE:

The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

CONDITIONS OF USE OF THESIS

Chair of the Department of Biology

Faculty of Science

University of Prince Edward Island

Charlottetown, P. E. I.

Canada C1A-4P3

SIGNATURE PAGES

iii-iv

REMOVED

ABSTRACT

Seagrass beds are known to support an abundant and diverse ichthyofauna and are generally considered essential habitats within estuarine and coastal environments. On Prince Edward Island (PEI) anthropogenic activities within watersheds are causing eutrophication of estuaries resulting in macroalgal (primarily sea lettuce, *Ulva lactuca*) proliferation and degradation and loss of eelgrass (*Zostera marina*) habitat. This project aimed to: 1) examine the impacts of sea lettuce on eelgrass and the eelgrass fish community using 1 m^2 enclosures and a 12 m^2 exclosure respectively; 2) investigate diel and seasonal patterns of eelgrass habitat use to increase our understanding of eelgrass habitat importance to a variety of estuarine fish species; 3) determine how the fish community composition varies with eelgrass habitat condition; and, 4) explore the effects of eelgrass habitat loss on an eelgrass dependant species, northern pipefish (*Syngnathus fuscus*).

To examine how sea lettuce was affecting eelgrass, 1 m^2 experimental enclosures were constructed to measure changes in eelgrass density and above-ground biomass in response to sea lettuce canopy cover. To explore how the eelgrass fish community was being impacted by sea lettuce, bi-weekly beach seines were conducted at three sites in the Midgell River estuary from July 20th to September 12th, 2009. One site was sea lettuce habitat, the second was mixed eelgrass and sea lettuce, and the third site was an exclosure in which sea lettuce was removed from an eelgrass bed. Treatments containing macroalgal canopies were found to significantly reduce the measured characteristics (shoot density, blade density, leaf area, and above-ground biomass) of eelgrass. The exclosure was effective at maintaining a monospecific eelgrass habitat. Fish communities did not differ significantly among the three sites, although fourspine stickleback (*Apeltes quadracus*) were captured in higher numbers in the eelgrass habitat.

Beach seines were conducted at over 24 h periods twice monthly from June to August 2008 in the Midgell River estuary to better understand the dynamics of eelgrass habitat use within the estuarine environment. Fish were sampled by carrying out two seines, separated by no more than 5 m every four hours. Significantly more individuals occurred in eelgrass habitats throughout the night (22:00-4:00 h) than during the day (6:00-20:00 h). Atlantic silverside (*Menidia menidia*) young-of-the-year (YOY), mummichog (*Fundulus heteroclitus*), and American eel (*Anguilla rostrata*) were captured significantly more often at night, while ninespine stickleback (*Pungitius pungitius*) were captured more frequently during the day. Diel variation differed between months; however, within a given month day catches were generally dominated by *Gasterosteus* spp. YOY, ninespine stickleback, threespine stickleback (*G. aculeatus*), and northern pipefish YOY. Night catches generally had higher numbers of mummichog and Atlantic silverside YOY.

Ten sites in each of eight different estuaries along the north shore of PEI were chosen to examine the relationship between fish community structure and the condition of the eelgrass habitat, based on shoot density, canopy height, percent cover, above-ground biomass, and epiphyte biomass. Fish were sampled by beach seining ten sites in each estuary in June and August 2009. Measures of eelgrass were recorded at the same time as seining occurred using a 0.5 m^2 viewing frame and core sampler. Generally, eelgrass complexity did not affect the adult community in June or August as they responded differences in water parameters (temperature, salinity, dissolved oxygen). In contrast, young-of-the-year (YOY) fishes responded to both water parameters and increased eelgrass complexity. For example, young-of-the-year northern pipefish, winter flounder, *Gasterosteus* spp., and cunner were sampled in higher numbers in areas of higher shoot density.

Northern pipefish, an eelgrass dependent species, were used to assess potential impacts of continued eelgrass habitat degradation. Pipefish were sampled every ten days from May to October, 2009 in an eelgrass and sea lettuce dominated habitat within the Stanley River estuary. Five consecutive seines in each habitat type were conducted to assess population differences. Males, females, and YOY were all significantly more abundant in the eelgrass habitat than in the sea lettuce. Male *S. fuscus* showed no significant difference in brood pouch somatic index (BPSI) between the sea lettuce and eelgrass habitats. Although, brood sizes of males sampled from sea lettuce were significantly smaller than eelgrass males, this was related to their smaller size. Gonadosomatic index (GSI) of females did not differ between the sea lettuce and eelgrass habitats.

Generally, the overall capacity of estuarine environments to support a diverse and abundant fish community is being affected by structural changes in the eelgrass habitat. Taken together, these results provide further evidence that eelgrass beds are an important habitat for estuarine fish species.

ACKNOWLEDGEMENTS

Really all this page needed was "thank-you" in bold italicized letters, but I guess it would be best to properly acknowledge everyone who helped with this project. First, thanks to my supervisor Dr. Kevin Teather for the number of days he spent out in the heat (and rain!) in the field, but particularly for his time and guidance, especially with the written portion of this thesis. Second, thanks to my committee members Dr. Mike van den Heuvel and Dr. Simon Courtenay for their ideas and suggestions during the numerous discussions about eelgrass and estuarine fish communities, and for their help with statistics. Lastly, thank-you to my "stats guru" Dr. Donna Giberson for all her help sorting out the fish community data.

I must say thank-you to (arguably) the most important people who helped out during this project (and who without this project would not have been possible), my field assistants. During the 2009 field season I could not have collected near the amount of data that I did without the help of Jenelle MacKenzie and Dan Watts. Jenelle thanks for keeping me company on those long drives exploring sites, putting up with me for the entire month of June, listening to the many rants, acquiring a "sewing thumb" when the enclosures needed to be put together, and learning how to load and navigate a canoe. Dan thanks for the encouragement all those days we used the boat, the time you spent coming out to sample the enclosures and catch pipefish in September and October (and starting the boat when I could no longer feel my fingers!), for all the down-to-earth conversations we had, and for introducing me to country music. Essentially, thanks for everything. Both of you were so much fun to work with, dry humour and all! Not to forget, a huge thank-you goes out Marla MacAusland and Sean McNeill who helped shorten the days workload by coming out to sample during the summer.

During the 2008 field season I must acknowledge a number of people who helped out, primarily Allison Schein, Jen van der Lee, Joubin Saffary, and Alex Campbell. I can't thank any of you enough for all your time and dedication during both regular sampling and 24 h samples. An energetic thanks goes out to Kirk Roach and Vanessa Lutz who helped out with the 24 h sampling. Lastly thanks to everyone else who volunteered their time to come out and help sample: Megan Finley, Marla MacAusland, Rosalyn Ridlington-Abbott, Andrey Malyshev, and John Greenan, your help was much appreciated.

I would also like to mention my acquaintances in the aquatic lab. Thanks for all the conversations, whether scientific or frivolous, lending an ear to listen, and joining in with all the complaining that generally accompanies a Master's project.

Lastly, a huge thank-you goes out to my parents. Dad, your jack-of-all-trades abilities really came in handy. Thanks for brainstorming with me when we were trying to get the pop traps working properly, and for putting all those grommets in the material, even though they were a flop in the end! Thanks for all the materials supplied during the enclosure project set up, the supports and extensions and for coming out in the field several times to "manicure" both the large and small enclosures and to sample pipefish. Thanks for not complaining too much when I needed you to make more stakes when the tide/waves carried them away, or when Kevin came out with us! Mom thanks for helping sew the material on the pop traps and assemble the enclosures and for running all those little errands when I was busy doing field work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: GENERAL INTRODUCTION

1.1 Estuaries

Estuaries are highly productive ecosystems that provide habitat for many fishes and shellfishes (Orth and Heck 1980, Orth et al. 1984), play critical roles in the life cycle of many marine fishes (Houde and Rutherford 1993), and filter and trap sediments and nutrients (Flemer and Champ 2006). Yet, anthropogenic activities are negatively impacting the estuarine environment which has generated concern about sustainability and continued prosperity (Deegan 2002). An important habitat within estuaries are seagrass beds, but they too have been noticeably impacted, particularly due to macroalgal over-growth (Valiela et al. 1997; Hauxwell et al. 2001). Seagrasses serve as a refuge from predatory species (Orth and Heck 1980) and provide a rich food supply (Orth et al. 1984; Lubbers et al. 1990). Consequently, many fishes utilize seagrass beds as nursery habitat (Deegan et al. 1997; Heck et al. 2003; Lazzari and Stone 2006). In fact, over half of all economically important fish species use estuaries at some point during their life cycle (Houde and Rutherford 1993). Continued degradation of this habitat may have unforeseeable and irreversible consequences (Cummins et al. 2004).

Historically, human settlements have been structured around coastal areas; therefore as the human population continues to increase, the ecological sustainability of these environments may be in jeopardy due to habitat destruction, resource exploitation, and eutrophication (Niemi et al. 2004; Flemer and Champ 2006). In particular, anthropogenic activities have contributed largely to sediment and nutrient additions to estuaries (Meade 1982). Perhaps of greater concern are increasing nutrient amounts, particularly nitrogen, from anthropogenic sources within the watershed. These sources include urbanization, industrial effluent, aquaculture, sewage, and chemical fertilizers

(Nixon 1995; Paerl 1997). Although the effects of all of the above are important, the main issue facing Prince Edward Island (PEI) estuaries deals with the impacts from agricultural fertilizers and therefore will be the focal point of this introduction.

Two nutrients of particular concern from agricultural runoff are nitrogen and phosphorous. Phosphorous that does not adhere to plant surfaces during fertilizer application attaches to soil particles, and during a large rain event are washed into streams and rivers and subsequently into estuaries (Carpenter et al. 1998). Nitrogen (either from fertilizers or ploughed crops) typically leaches into the soil and into groundwater (Paerl 1997), and will eventually enter surface water by springs located within the estuary, or be transported into the estuary via streams and rivers (Valiela et al. 1990; Paerl 1997). Flushing greatly affects the impacts of nutrients within the estuarine ecosystem, and is highly dependent on the tidal influence and amount of freshwater entering the estuary (McLusky and Elliott 2004). On PEI, one factor that makes a large difference to water residence times is the location of the estuary. Estuaries along the south shore have larger tidal ranges than estuaries located on the north shore. Therefore residence times in north shore estuaries are longer (due to lower tidal amplitudes), which may intensify the impacts of nutrient inputs (Raymond et al. 2002).

Inflowing nutrients contribute heavily to estuarine productivity. Increases in organic matter within an aquatic ecosystem, known as eutrophication (Nixon 1995), are not necessarily desirable. While certain levels of nutrients are essential, excess nutrients lead to disruptions of ecologic functions that result in undesirable consequences for most organisms (McLusky and Elliott 2004; Niemi et al. 2004). Eutrophication is now viewed as one of the greatest threats to estuarine health (National Research Council 2000; Bricker et al. 2008). Bricker et al. (2008) found that 65% of estuaries (84 of 139 surveyed) in the

United States were experiencing moderate to high eutrophic conditions. Systems that were once highly productive are becoming unbalanced and deteriorating in habitat quality (Pinckney et al. 2001).

A particularly noticeable response to increased nutrient loading in PEI estuaries has been the over-growth of macroalgae, predominantly sea lettuce (*Ulva lactuca*). Excessive amounts of algae can lead to more serious problems such as loss of seagrasses and low dissolved oxygen concentrations (Deegan et al. 2002; Bricker et al. 2008) and may ultimately lead to decreased diversity and abundance of fishes as well as other biota (Valiela et al. 1997; Breitburg 2002). Consequently, the impacts of eutrophication are reported in Island estuaries each summer: large mats of macroalgae, anoxic waters, and fish kills. Increasing prevalence of eutrophication and subsequent anoxic events have generated increased concern about the impacts on both fish communities and vegetation structure and abundance within estuarine systems on PEI.

Seagrasses are important and necessary habitats for numerous fish species (Orth et al. 1984; Wyda et al. 2002). The structure of seagrass beds (density, height) functions to decrease foraging efficiency of larger predatory species (Orth and Heck 1980), presumably by decreasing visual ability, and provides an abundant food supply for smaller fish species (Orth et al. 1984; Lubbers et al. 1990). Consequently, many fish spawn in and utilize seagrass beds as nursery habitat (Deegan et al. 1997; Heck et al. 2003; Lazarri and Stone 2006). Joseph et al. (2006) found juvenile white hake (*Urophycis tenuis*) and cunner (*Tautogolabrus adspersus*) only in eelgrass habitats. Heck et al. (1989) concluded that eelgrass beds act as nursery areas for both white hake and winter flounder (*Pseudopleuronectes americanus*). Lazzari (2002), when comparing sandy sites with those covered by eelgrass, found positive correlations between species richness and abundance and eelgrass presence. Similar trends have been noted in a number of other studies (see Heck et al. 1989; Gray et al. 1996; Mattila et al. 1999). Fish species richness and abundance appears to be largely related to the complexity of seagrass beds; as seagrass beds deteriorate in quality and therefore habitat complexity, species richness and abundances tend to decline (Hughes et al. 2002a; Wyda et al. 2002). The focus of this thesis is to better understand the relationship between eelgrass bed complexity and associated fish communities in PEI estuaries.

1.2 Eelgrass

Eelgrass is the most common seagrass of temperate coastal areas and estuaries (Homziak et al. 1982). Often, eelgrass forms a continuous stand or may be interspersed in clumps among sandy areas or lastly, may consist of an assortment of eelgrass plants, various algae and sandy regions. Collectively, all of these areas are referred to as an eelgrass bed and will influence the way in which various species identify and utilize this environment (Orth et al. 1984). Eelgrass grows by a continuous cycle of leaf formation and subsequent leaf shedding (Borum 1985), and the lifespan of a leaf varies depending on the growing conditions; in eastern Canada the average lifespan of an eelgrass leaf is about 100 days (Garbary D., St.FX University Antigonish, NS pers. comm.). Eelgrass can regulate dissolved oxygen levels, reduce sediment suspension, regulate nutrient and chlorophyll levels in the water column (Nixon and Oviatt 1972; Stevenson 1988), while roots and rhizomes stabilize sediments and blades of eelgrass plants slow water currents (Ward et al. 1984; Pihl et al. 2006). The physical attributes (height, shoot density and biomass) of the eelgrass plants contribute to habitat complexity relative to surrounding areas (Bell and Westoby 1986; Cummins et al. 2004). Larger, taller plants characteristically represent increased complexity, which in turn supports a greater diversity and abundance of organisms (Orth et al. 1984; Heck et al. 1989). Hence, the enhanced ecological benefits provided by eelgrass plants make them essential components of the estuarine ecosystem (Keser et al. 2003).

1.3 Eelgrass decline

There has been considerable attention drawn to the increasing loss and fragmentation of eelgrass habitat worldwide. Eelgrass-dominated ecosystems do not thrive in anthropogenically-affected areas, as many are sensitive to changes in water and habitat quality (Orth and Moore 1983). Globally, Short and Wyllie-Echeverria (1996) estimated that seagrass loss exceeded 12,000 km^2 between the mid-1980s to mid-1990s. Eelgrass has been in decline in large or small scales in the Atlantic Canada for some time. Seymour et al. (2002) documented a 95% decline in eelgrass in Antigonish Harbour, Nova Scotia between 2000 and 2001, while Locke and Hanson (2004) noted an 88% decline in eelgrass in Rustico Bay and a 61% decline in Bedeque Bay, Prince Edward Island between 2002 and 2003. Declines of this magnitude are not standard, changes typically progress throughout many years to decades. Reasons for the decline of eelgrass cannot be attributed to a single causal factor. Eutrophication (Valiela et al. 1997; Hauxwell et al. 2001; Bricker et al. 2008), damage by green crab (*Carcinus maenas*) during burrowing and foraging activities (Davis et al. 1998), and the impacts of aquaculture (Hidu et al. 1991) are important causal factors.

1.3.1 Effects of nutrient enrichment

Nutrient availability and temperature control net production rates of macroalgae while light allows for seasonal patterns in growth (Valiela et al. 1997). In eutrophic areas, when temperature and light regimes are optimal, macroalgal growth is stimulated which results in the formation of algal blooms (Hauxwell et al. 2001; Deegan et al. 2002; Peralta et al. 2002; Hauxwell et al. 2003 among others). When macroalgal blooms collapse and die-back, bacteria decompose the algae using large amounts of oxygen causing hypoxic (< 2 mg/L) and anoxic events (< 0.2 mg.L) (Valiela et al. 1997; McGlathery 2001). Low water oxygen concentrations are physiologically stressful on seagrass plants. Greve et al. (2003) found that during hypoxic events eelgrass leaf bundles detached from the rhizomes and the bed suffered considerable plant die-backs. This stemmed from degradation of the meristem tissue. Meristems support cell division and growth (Brix and Sorrell 1996) and therefore have high oxygen demands and are vulnerable to low oxygen conditions as they receive most of their oxygen via diffusion (Greve et al. 2003). Coupled with lowering water oxygen concentrations, macroalgae lose buoyancy and sink to the bottom of the water column as they die-back. Large blanket-like coverings are formed which smothers rooted seagrass plants (Deegan et al. 2002).

Valiela et al. (1997) showed that macroalgae were capable of using up to 5000 μmol of nitrogen per gram per day, while seagrasses utilized a maximum of 250 μmol of nitrogen per gram per day. Macroalgae, therefore, have a physiological advantage over seagrasses enhancing the likelihood that they will shade out and replace seagrass communities. Hauxwell et al. (2001) found that macroalgal canopy height increased linearly with nitrogen loading into the watercourse. To test the impacts of macroalgal canopies on eelgrass, they established a series of enclosures with differing macroalgal cover. Enclosures with canopies exceeding 9 cm suffered a substantial decrease to a complete loss of eelgrass. Most notably, eelgrass in enclosures with a canopy greater than 12 cm suffered a complete loss of recruitment. Increased epiphyte loads on seagrass blades can also reduce available light concentration, decreasing photosynthetic ability and seagrass biomass (Neckles et al. 1993; Cebrián et al. 1999). Philippart (1995) performed a field experiment by artificially shading monospecific beds of *Zostera noltii* to simulate the effects of different levels of epiphyte densities. By subjecting plants to different light intensities (100%, 45%, 30%, and 0%), the author found that above-ground biomass decreased linearly with increased shading. Further, light deprived seagrass leaves were less capable of providing photosynthetic material to the generative parts (namely rhizomes) of the plant therefore resulting in decreased productivity. Shading may therefore be particularly detrimental to many seagrass populations as they rely on the energy stores in rhizomes for maintenance and growth from year to year (Loques et al. 1988; Philippart 1995). Light limitations imposed by macro and epiphytic algae may therefore be implicated as a major cause of reduced densities, growth, and production of seagrasses (Hauxwell et al. 2001; Nelson and Lee 2001; Hauxwell et al. 2003).

1.3.2 Effects of green crab and aquaculture

Damage by aquacultural activities and behaviour of the invasive green crab (*C. maenas*) may also contribute to the problem of seagrass habitat degradation and loss. Garbary and Munro (2004) suggested that there is considerable evidence that high numbers of green crab in eelgrass beds have a negative impact on eelgrass abundance, primarily due to their foraging behaviour. As crabs forage they work the top few cm of the sediment and ultimately uproot plants by breaking the stem near the rhizome and/or damaging the bundle sheath (Davis et al. 1998). Similarly, aquacultural activities can directly damage eelgrass plants. In areas of benthic oyster aquaculture, eelgrass plants are said to impede harvesting and are viewed as a nuisance. As the oysters are raked from the benthos, eelgrass plants can be uprooted (Garbary and Munro 2004). Further, areas of high density suspension oyster aquaculture damage eelgrass plants by minimizing available light to the blades (Skinner, M., UNB, Fredericton, NB, pers. comm.). Impacts caused by mussel aquaculture are more indirect. Mussel spat is known to collect on the tips of eelgrass blades, sometimes in high densities (Hidu et al. 1991), presumably this weighs down the blades and could, coupled with macroalgal canopies, minimize available light to the blades.

1.4 Eelgrass and fish communities

Many studies indicate the positive relationship between eelgrass beds and fish abundance and species richness in estuaries (Hughes et al. 2002a; Lazzari 2002; Wyda et al. 2002; Lazzari et al. 2003; Lazzari and Stone 2006). Generally, areas of higher eelgrass complexity tend to have more diverse fish assemblages when compared to areas of lower complexity. As observed in studies conducted by Hughes et al. (2002a) and Wyda et al. (2002), lower abundances and species richness in areas of low vegetation quality suggest that habitat degradation strongly affects different fish species that associate with eelgrass beds. Further, Wyda et al. (2002) found that in areas of high macroalgal biomass, fish abundance and species richness were low compared to areas of high eelgrass biomass suggesting that macroalgae is not providing an alternative habitat for most fishes. The structural integrity and multifaceted complexity produced by eelgrass beds provides necessary habitat within the estuarine environment contributing to greater faunal diversity in estuaries (Orth and Heck 1980; Heck et al. 1989; Hughes et al. 2002a; Wyda et al. 2002).

1.5 Diel sampling of fish communities

Studies that propose differences in fish communities with respect to habitat associations usually restrict sampling to day hours neglecting possible nocturnal differences in habitat usage. Several studies suggest that some estuarine fish species exhibit notable diel activity patterns (Lubbers et al. 1990; Rountree and Able 1993; Gray et al. 1998; Mattila et al. 1999). Night sampling of eelgrass habitats can be used to increase awareness of the importance of this habitat type (Gray et al. 1998). As noted by Rountree and Able (1993), fish community surveys that employ only day sampling efforts tend to underestimate diversity, abundance and biomass of the population. Diel movement and activity patterns as a result of foraging activities or predator avoidance may dictate diel species abundances (Rountree and Able 1993; Mattila et al. 1999). Diel sampling endeavours have recognized similarities in species composition of seagrass beds during both day and night, but noted that more individuals occurred in seagrass habitats throughout the night hours (e.g. Lubbers et al. 1990).

1.6 Importance of eelgrass to northern pipefish

Recently there has been increased interest in using fishes as indicators of estuarine habitat quality (Deegan et al. 1997; Hughes et al. 2002b). Deegan et al. (1997) focused on seagrass habitat degradation and predicted that as estuaries become increasingly impacted by anthropogenic activities the proportion of tolerant species within the estuary would increase and the number of sensitive species would decrease. Northern pipefish (*Syngnathus fuscus*) are generally considered a sensitive species known to associate with high quality seagrass habitats (Hughes et al. 2002a, Wyda et al. 2002). It has been suggested that pipefish depend on eelgrass habitat because their body form highly resembles the blades of the eelgrass plants (Connolly 1994; Vincent et al. 1995). Hughes et al. (2002a) found that *S. fuscus*, was strongly affected by the loss of eelgrass. As eelgrass habitat deteriorated pipefish populations vanished from the area inferring a strong dependence on eelgrass habitats within estuarine environments. To date, little is known about the effects of eelgrass habitat loss on pipefish populations. Continued degradation to estuarine habitats calls for a more in-depth knowledge of pipefish ecology and life history, which could be useful if the need for conservation arises (Orth et al. 1984; Ripley and Foran 2006).

1.7 Objectives

The overall objective of this study was to provide a clearer understanding of the significance of eelgrass beds to estuarine fishes on PEI. The first objective is to explore the impacts of sea lettuce on eelgrass and test the effectiveness of an exclosure in estuarine sampling by looking at community differences between different habitats. To look at the impacts of sea lettuce on eelgrass ten exclosure treatments (five with a macroalgal canopy and five without) were used. To look at how the exclosure may function to assess fish community differences, three areas were sampled: one with sea lettuce, a second of mixed sea lettuce and eelgrass, and a third: an exclosure from which sea lettuce had been removed from an eelgrass bed. It was predicted that eelgrass density would decrease in treatments with macroalgal cover, and that the fish community composition would differ between the exclosure and sea lettuce habitat.

Second, diel abundance trends of estuarine fishes within an eelgrass habitat were evaluated to examine the temporal (diel and monthly) patterns of fish within an eelgrass habitat to better understand eelgrass habitat usage within the estuarine environment. It was thought that the abundance of different fish species might fluctuate throughout the diel periods.

The third objective was to determine the fish community composition and explore fish community dynamics in different quality eelgrass habitats. While the association between complex eelgrass habitats and diverse and abundant fish communities have been documented in the literature, little information is available concerning the relationship between fish communities and eelgrass bed condition on a finer scale. Therefore, this project aims to look at fish community responses to variation in eelgrass bed condition by quantifying differences in eelgrass complexity through the combined characteristics of density, percent cover, above-ground biomass, canopy height, and epiphyte biomass. Individual fish species were expected to respond differently to the various measures of eelgrass, while the community is expected to have more fish and fish species associated with higher measures of complexity.

The last objective was to examine population structure, adult sex ratios, growth rates of adult and young-of-the-year (YOY), and reproductive characteristics of northern pipefish in both an eelgrass- and sea lettuce-dominated habitat. Pipefish are known to be closely associated, if not dependent, on eelgrass habitat. Therefore gathering basic population and reproductive parameters in an eelgrass habitat as well as a lower quality sea lettuce habitat will give necessary in formation on pipefish ecology. It was thought that pipefish would be found in higher numbers in the eelgrass habitat and might have decreased reproductive output in the sea lettuce habitat.

1.8 Literature cited

Bell, J. D. Westoby, M. 1986. Importance of local changes in leaf height and density to fish and decapods associated with seagrasses. *Journal of Experimental Marine Biology and Ecology* **104**: 249-274.

Borum, J. 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. *Marine Biology* **87**: 211-218.

Breitburg, D. 2002. Effects of hypoxia, and the balance between hypoxia and enrichment, on coastal fish and fisheries. *Estuaries* **25**: 767-781.

Bricker, S.B. Longstaff, B., Dennison, W., Jones, A., Boicourt, K., Wicks, C., Woerner, J. 2008. Effects of nutrient enrichment in the nation's estuaries: a decade of change. *Harmful Algae* **8**: 21-32.

Brix, H., Sorrell, B.K. 1996. Oxygen stress in wetland plants: Comparison of de-oxygenated and reducing root environments. *Functional Ecology* **10**: 521-526.

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., and Smith, V. H. 1998. Nonpoint pollution of surface waters with phosphorous and nitrogen. *Ecological Applications.* **8**: 559-568.

Cebrián, J., Enriquez, S., Fortes, M., Agawin, N., Vermaat, J.E., Duarte, C.M. 1999. Epiphyte accrual on *Poisdonia oceanica* (L.) Delile leaves: implications for light absorption. *Botanica Marina* **42**: 123-128.

Connolly, R.M. 1994. A comparison of fish assemblages from seagrass and unvegetated areas of a southern Australian estuary. *Australian Journal of Marine and Freshwater Research* **45:** 1033-1044.

Cummins, S.P., Roberts, D.E., Zimmerman, K.D. 2004. Effects of the green macroalga *Enteromorpha intestinalis* on macrobenthic and seagrass assemblages in a shallow coastal estuary. *Marine Ecology Progress Series* **266**: 77-87.

Davis, R.C., Short, F.T., Burdick, D.M. 1998. Quantifying the effects of green crab damage to eelgrass transplants. *Restoration Ecology* **6:** 297-302.

Deegan, L.A. 2002. Lessons learned: the effects of nutrient enrichment on the support of nekton by seagrass and salt marsh ecosystems. *Estuaries* **25**: 727-742.

Deegan, L.A., Finn, J.T., Ayvazian, S.G., Ryder-Kieffer, C.A., Buonaccorsi, J. 1997. Development and validation of an estuarine biotic integrity index. *Estuaries* **20**: 601-617.

Deegan, L.A., Wright, A., Ayvazian, S.G., Finn, J.T., Golden, H., Rand-Merson, R., Harrison, J. 2002. Nitrogen loading alters seagrass ecosystem structure and support of higher trophic levels. *Aquatic Conservation: Marine and Freshwater Ecosystems* **12**: 193-212.

Flemer, D.A., Champ, M.A. 2006. What is the future fate of estuaries given nutrient over-enrichment, freshwater diversion and low flows? *Marine Pollution Bulletin* **52**: 247-258.

Garbary, D.J, Munro, J. 2004. Eelgrass decline: Reality and causation. Pages 23-26 in: Status and conservation of eelgrass (*Zostera marina*) in Eastern Canada. A.R. Hanson, Ed. Technical Report Series No. 412. Canadian Wildlife Service, Atlantic Region. 40pp.

Gray, C.A., Chick, R.C., McElligott, D.J. 1998. Diel changes in assemblages of fishes associated with shallow seagrass and bare sand. *Estuarine, Coastal and Shelf Science*. **46**: 849-859.

Gray, C.A., McElligott, D.J., Chick, R.C. 1996. Intra- and inter-estuary differences in assemblages of fishes associated with shallow seagrass and bare sand. *Marine and Freshwater Research*. **47**: 723-735.

Greve, T.M., Borum, J., Pedersen, O. 2003. Meristematic oxygen variability in eelgrass (*Zostera marina*). *Limnology and Oceanography* **48**: 210-216.

Hauxwell, J., Cebrian, J., Furlong, C., Valiela, I. 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology* **82**: 1007-1022.

Hauxwell, J., Cebrian, J., Valiela, I. 2003. Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. *Marine Ecology Progress Series* **247**: 59-73.

Heck, K.L., Able, K.W., Fahay, M.P., Roman, C.T. 1989. Fishes and decapod crustaceans of Cape Cod eelgrass meadows: species composition, abundance patterns and comparison with unvegetated substrates. *Estuaries* **12**: 59-65.

Heck, K.L., Hays, G., Orth, R.J. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress Series* **253**: 123-136.

Hidu, H., McAlice, B.J., Podniesinski, G., Short, F., Kindblom, L. 1991. Recruitment and commercial seed procurement of the blue mussel *Mytilus edilus* in Maine. *Journal of the World Aquaculture Society* **22**: 134-152.

Homizak, J. Fonseca, M.S., Kenworthy, W.J. 1982. Macrobenthic community structure in a transplanted seagrass (*Zostera marina*) meadow. *Marine Ecology Progress Series* **9**: 211-221.

Houde, E., Rutherford, E. 1993. Recent trends in estuarine fisheries: predictions of fish production and yield. *Estuaries* **16**: 161-176.

Hughes, J., Deegan, L.A., Wyda, J.C., Weaver, M.J., Wright, A. 2002a. The effects of eelgrass habitat loss on estuarine fish communities of southern New England. *Estuaries* **25**: 235-249.

Hughes, J.E., Deegan, L.A., Weaver, M.J., Costa, J.E. 2002b. Regional application of an index of estuarine biotic integrity based on fish communities. *Estuaries* **25**: 250-263.

Joseph, V., Locke A., Godin J.J. 2006. Spatial distribution of fishes and decapods in eelgrass (*Zostera marina* L.) and sandy habitats of a New Brunswick estuary, eastern Canada. *Aquatic Ecology* **40**: 111-123.

Keser, M., Swenarton, J.T., Vozarik, J.M., Foertch, J.F. 2003. Decline in eelgrass (*Zostera marina* L.) in Long Island Sound near Millstone Point, Connecticut (USA) unrelated to thermal input. *Journal of Sea Research* **49**: 11-26.

Lazzari, M.A. 2002. Epibenthic fishes and decapod crustaceans in northern estuaries: a comparison of vegetated and unvegetated habitats in Maine. *Estuaries* **25**: 1210-1218.

Lazzari, M.A., Sherman, S., Kanwit, J.K. 2003. Nursery use of shallow habitats by epibenthic fishes in Maine nearshore waters. *Estuarine, Coastal and Shelf Science* **56**: 73-84.

Lazzari, M.A., Stone, B.Z. 2006. Use of submerged aquatic vegetation as habitat by young-of-the-year epibenthic fishes in shallow Maine nearshore waters. *Estuarine, Coastal and Shelf Science* **69**: 591-606

Locke, A., Hanson, M.J. 2004. Changes in eelgrass in southern Gulf of Saint Lawrence estuaries. Pages10-12 in: Status and conservation of eelgrass (*Zostera marina*) in Eastern Canada. A.R. Hanson, Ed. Technical Report Series No. 412. Canadian Wildlife Service, Atlantic Region. 40pp.

Loques, F., Caye, G., Meinesz, A. 1988. Flowering and fruiting of *Zostera noltii* in Golfe Juan (French Mediterranean). *Aquatic Botany* **32**: 341-352.

Lubbers, L., Boynton, W.R., Kemp, W.M. 1990. Variations in structure of estuarine fish communities in relation to abundance of submersed vascular plants. *Marine Ecology Progress Series* **65**: 1-14.

Mattila, J. Chaplin, G., Eilers R., Heck, K.L., O'Neal J.P., Valentine J.F. 1999. Spatial and diurnal distribution of invertebrate and fish fauna of a *Zostera marina* bed and nearby unvegetated sediments in Damariscotta River, Maine (USA) *Journal of Sea Research* **41**: 321-332.

McGlathery, K.J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *Journal of Phycology* **37**: 453-456.

McLusky, D.S., Elliott, M. 2004. The Estuarine Ecosystem: ecology, threats and management. Oxford University Press Inc: New York. 214pp.

Meade, R.H. 1982. Sources, sinks and storage of river sediment in the Atlantic drainage of the United States. *Journal of Geology* **90**: 235-252.

National Research Council. 2000. Clean Coastal Waters: Understanding and Reducing the Effects of Nutrient Pollution. National Academy Press, Washington, D.C. 405pp.

Neckles, H.A., Wetzel, R.L., Orth, R.J. 1993. Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. *Oecologia* **93**: 285-295.

Niemi, G., Wardrop, D., Brooks, R., Anderson, S., Brady, V., Paerl, H., Rakocinski, C., Brouwer, M., Levinson, B., McDonald, M. 2004. Rationale for a new generation of indicators for coastal waters. *Environmental Health Perspectives* **112**: 979-986.

Nelson, T.A., Lee, A. 2001. A manipulative experiment demonstrates that blooms of the macroalga *Ulvaria obscura* can reduce eelgrass shoot density. *Aquatic Botany* **71**: 149-154.

Nixon, S.W. 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia* **41**: 199-219.

Nixon, S.W., Oviatt, C.A. 1972. Preliminary measurements of midsummer metabolism in beds of eelgrass, *Zostera marina*. *Ecology* **53**: 150-153.

Orth, R.J., Heck, K.L. 1980. Structural components of eelgrass (*Zostera marina*) meadows in the lower Chesapeake Bay- Fishes. *Estuaries* **3**: 278-288.

Orth, R.J., Heck, K.L., Van Montfrans, J. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries* **7**: 278-286.

Orth, R.J., Moore, K.A. 1983. Chesapeake Bay: an unprecedented decline in submerged aquatic vegetation. *Science* **222**: 51-53.

Paerl, H.W. 1997. Coastal eutrophication and harmful algal blooms: importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. *Limnology and Oceanography* **42**: 1154-1165.

Peralta, G., Pérez-Lloréns, J.L., Hernández, I., Vergara, J.J. 2002. Effects of light availability on growth, architecture and nutrient content of the seagrass *Zostera noltii* Hornem. *Journal of Experimental Marine Biology and Ecology* **269**: 9-26.

Philippart, C.J.M. 1995. Effects of shading on growth, biomass and population maintenance of the intertidal seagrass *Zostera noltii* Hornem. in the Dutch Wadden Sea. *Journal of Experimental Marine Biology and Ecology* **188**: 199-213.

Pihl, L., Baden, S., Kautsky, N., Ronnback, P., Soderqvist, T., Troell, M., Wennhage, H. 2006. Shift in fish assemblage structure due to loss of seagrass *Zostera marina* habitats in Sweden. *Estuarine, Coastal and Shelf Science* **67**: 123-132.

Pinckney, J.L., Paerl, H.W., Tester, P., Richardson, T.L. 2001. The role of nutrient loading and eutrophication in estuarine ecology. *Environmental Health Perspectives* **109**: 699-706.

Raymond, B.G., Crane, C.S., Cairns, D.K. 2002. Nutrient and chlorophyll trends in Prince Edward Island estuaries. pp. 142-153 *In:* Cairns DK. (Ed.). 2002. Effects of land use practices on fish, shellfish, and their habitats on Prince Edward Island. Canadian Technical Report of Fisheries and Aquatic Science No. 2408.

Ripley, J.L., Foran, C.M. 2006. Population structure, growth rates, and seasonal abundance of two *Syngnathus* pipefish species. *Estuaries and Coasts* **29**: 1161-1171.

Rountree, R.A., Able, K.W. 1993. Diel variation in decapod crustacean and fish Assemblages in New Jersey polyhaline marsh creeks. *Estuarine, Coastal and Shelf Science* **37**: 181-201.

Seymour, N.R., Miller, A.G., Garbary, D.J. 2002. Decline of Canada geese (*Branta canadensis*) and common goldeneye (*Bucephala clangula*) associated with a collapse of eelgrass (*Zostera marina*) in a Nova Scotia estuary. *Helgoland Marine Research* **56**: 198-202.

Short, F.T., Wyllie-Echeverria, S. 1996. Natural and human-induced disturbance of seagrasses. *Environmental Conservation* **23**: 17-27.

Stevenson, J.C. 1988. Comparative ecology of submersed grass beds in freshwater, estuarine, and marine environments. *Limnology and Oceanography* **33**: 867-893.

Valiela, I., Costa, J., Foreman, K., Teal, J.M., Howes, B., Aubrey, D. 1990. Transport of groundwater-borne nutrients from watersheds and their effects on coastal waters. *Biogeochemistry* **10**:177-197.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* **42**: 1105-1118.

Vincent, A.C.J., Berglund, A., Ahnesjö, I. 1995. Reproductive ecology of five pipefish species in one eelgrass meadow. *Environmental Biology of Fishes* **44**: 347-361.

Ward, L.G., Kemp, W.M., Boynton, W.R. 1984. The influence of waves and seagrass communities on suspended particles in an estuarine embayment. *Marine Geology*: **59**: 85-103.

Wyda, J.C., Deegan, L.A., Hughes, J.E., Weaver, M.J. 2002. The response of fishes to submerged aquatic vegetation complexity in two ecoregions of the Mid-Atlantic Bight: Buzzards Bay and Chesapeake Bay. *Estuaries* **25**:86-100.

CHAPTER 2: ASSESSING THE IMPACTS OF SEA LETTUCE (*ULVA LACTUCA*) ON EELGRASS (*ZOSTERA MARINA*) AND THE EELGRASS FISH COMMUNITY ASSEMBLAGE: TESTING AN EXPERIMENTAL APPROACH

2.1 Abstract

Eutrophication of estuaries has resulted in increased macroalgal production and fragmented eelgrass (*Zostera marina*) habitat. Continued changes in eelgrass habitat structure may reduce its ability to maintain diverse and abundant fish communities. 1 m^2 enclosures were used throughout a seven week period to demonstrate the impacts of macroalgal (*Ulva lactuca*) canopies on eelgrass growth using macroalgal free ("clear") and macroalgal cover ("canopy") treatments. Treatments containing macroalgae significantly reduced the shoot density, blade density, leaf area and above-ground biomass of the eelgrass. Bi-weekly beach seines were conducted in three sites in the Midgell River estuary from July 20th to September 12th, 2009, to test the applicability of an exclosure to examine the impacts of macroalgae on eelgrass fish communities, A 12 m^2 exclosure was used to exclude sea lettuce from an eelgrass bed and then sampling took place within the exclosure and in an adjacent sea lettuce dominated and eelgrass dominated site. The fish communities did not differ significantly between the eelgrass and sea lettuce sites. Most fish species occurred in higher numbers in the eelgrass habitat, except the the fourspine stickleback (*Apeltes quadracus*), which was significantly more abundant in eelgrass. Macroalgal canopies have negative impacts with respect to the capacity of eelgrass to thrive. Although no significant differences in the fish community were found, the exclosure proved to be an effective method to create a monospecific eelgrass habitat to assess potential differences in fish communities between different habitats.

2.2 Introduction

Increasing coastline development and watershed activities have resulted in declining water quality and eutrophication of estuarine and coastal ecosystems. Of particular importance is the increase in macroalgal production and subsequent decline of rooted vegetation such as seagrasses (Valiela et al. 1997; Deegan et al. 2002). Eelgrass (*Zostera marina*), the most common temperate seagrass, has declined in many areas as a result of such changes in water quality and vegetation abundance (Orth and Moore 1983; Valiela et al. 1992; Short et al. 1995; Deegan et al. 1997; Hughes et al. 2002a). Beds of eelgrass are a valuable habitat for nearshore fish communities, providing abundant food resources, refuge from predators, and extensive nursery habitats (Orth and Heck 1980; Heck et al. 1989), especially when compared to unvegetated habitats (Orth et al. 1984; Mattila et al. 1999; Lazzari 2002).

In general, research has shown that deterioration in eelgrass habitat leads to decreases in fish abundance and diversity. In the Buttermilk and Waquoit Bays of Massachusetts Deegan et al. (1997) found that a decrease in eelgrass abundance was accompanied by lower fish species abundance and diversity. A similar conclusion was made by Wyda et al. (2002) with respect to areas where eelgrass had been replaced by macroalgal mats. Macroalgae was not deemed a sufficient substitute for the structural complexity offered by eelgrass beds, even though macroalgae may provide increased habitat structure when compared to unvegetated areas (Sogard and Able 1991; Wyda et al. 2002).

While macroalgal dominated environments may increase habitat structure, there are many negative impacts imposed by macroalgae on seagrasses. Macroalgae are known to impose light limitations (Hauxwell et al. 2001; Deegan et al. 2002), which leads to diminished plant growth and density (Short et al. 1995), and altered water and sediment chemistry (Valiela et al. 1992), both of which are physiologically stressful on seagrasses, resulting in increased shoot mortality (Greve et al. 2003). Further, the decomposition of macroalgae may stimulate hypoxic and anoxic conditions (Valiela et al. 1997; McGlathery 2001), stimulating increased shoot mortality (Greve et al. 2003). These damaging effects to seagrass species and subsequent changes in the physical complexity of the aquatic plant community may have important implications with respect to nearshore fish communities.

Positive correlations between various measures of seagrass structure and abundance and diversity of fishes have been documented (e.g. Bell and Westoby 1986; Sogard et al. 1987; Hughes et al. 2002b, Wyda et al. 2002). However, while a few studies make reference to differences in the fish community in the presence of macroalgae (Sogard and Able 1991; Wyda et al. 2002), no study has experimentally examined differences in fish communities in variable habitats along a small spatial scale. The primary purpose of this study was to successfully erect an exclosure (to create an exclusively eelgrass habitat) and test its applicability in estuarine sampling. The eelgrass ("exclosure") habitat and two adjacent areas which represent a macroalgal dominated ("sea lettuce") and eelgrass/macroalgal mixed habitat ("mixed") were then sampled to assess potential differences in fish communities. In addition, the impact of canopy cover (light limitation) on eelgrass growth was examined over a seven week period using 1 m x 1 m clear and canopy treatment enclosures.

2.3 Methods

2.3.1 Study site

This study was conducted in the mouth of the Midgell River estuary (semi-diurnal tidal cycle 0.0 m to 0.9 m) where it joins St. Peter's Bay on the north shore of Prince Edward Island (Figure 2.1). The study site was located on the northeast side in an area of

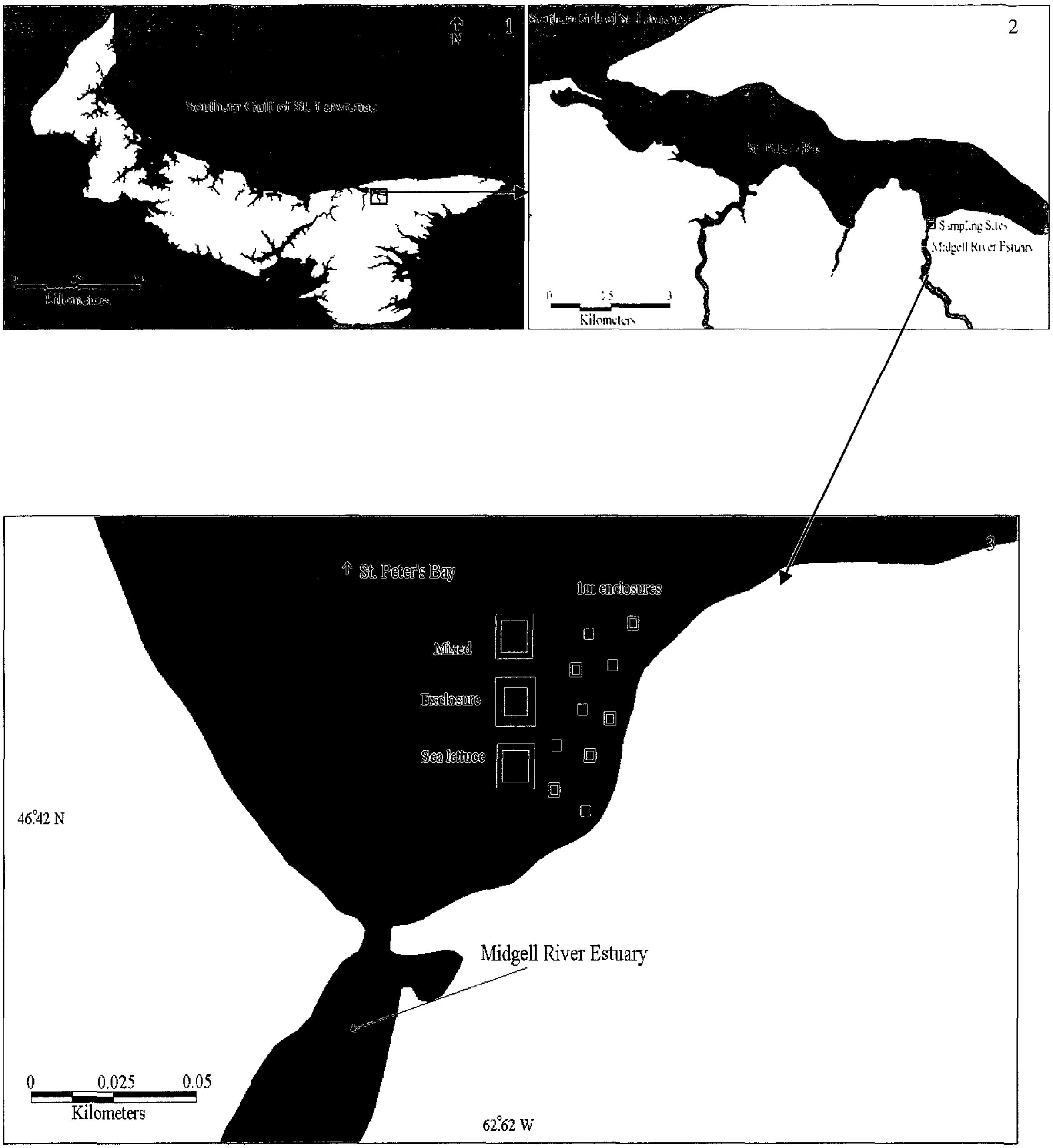

Figure 2.1. Location of Midgell River estuary and associated sampling locations. The 12 m exclosure location is shown by the larger bold open box with respective mixed and sea lettuce sampling locations labelled. Smaller boxes represent 1 m enclosures; open boxes are clear (macroalgal free) treatments, while closed boxes are canopy (containing macroalgae) treatments.

mixed eelgrass (*Zostera marina*) and sea lettuce (*Ulva lactuca*) habitat. Closer to Midgell River the eelgrass bed is often mixed with mats of sea lettuce that diminish as the eelgrass bed continues further into the bay. The larger 12 m^2 exclosure was situated at the far limits of the sea lettuce mats with the sea lettuce habitat situated before the exclosure and the eelgrass mixed habitat directly after. The ten 1 m^2 enclosures were placed closer to shore in front of the larger exclosure.

2.3.2 Experimental design

2.3.2.1 Impacts of a sea lettuce canopy on eelgrass

To assess the impacts of sea lettuce canopy cover on eelgrass beds, ten 1 m x 1 m enclosures were built using 14 gauge gill netting (10 cm stretched openings) secured with 1.3 cm PVC pipes in the four corners. The netting was attached through holes drilled into the piping so that water would enter into the pipes and add weight. Standard size window screening was used (15 cm width strips, 1 mm mesh) along the top of the netting to help keep floating algae from entering. Once the structures were erected, two wooden poles were placed in an "X" shape diagonally from one corner to the other and attached in the middle to maintain 1 m x 1 m dimensions. On July 3rd, 2009 all ten enclosures were erected in an eelgrass bed and all shoots within the 1 m x 1 m area were counted. A 20-30 cm thick canopy of sea lettuce, which covered the entire surface area, was placed in five randomly chosen enclosures ("canopy treatment"). Sea lettuce was excluded from the remaining five ("clear treatment") during the seven week experiment which ended on August 22nd.

2.3.2.2 Impacts of sea lettuce on the fish community

Three sites, established in a linear array with approximately 3 m between adjacent sites, were used to test impacts of sea lettuce on fish communities, one with minimal eelgrass and large mats of sea lettuce ("sea lettuce"), one which had eelgrass and sea lettuce ("mixed"), and one of eelgrass from which sea lettuce had been cleared ("exclosure"). The exclosure was 12 m x 12 m (1.2 m height) built using a 14 gauge gill net. The netting had 10 cm stretched openings to restrict macroalgae from entering, but still allow fish and water movement into and out of the area. The netting was fastened to 1.2 m aluminum poles (at 30 cm intervals) placed in the four corners and to 1.7 m wooden stakes placed every two meters along the sides. All wooden and aluminum posts were driven approximately 30 and 15 cm into the ground, respectively. Polypropylene rope (0.6 cm) was woven along the top of the netting and attached through all posts to keep the net sturdy and prevent entrance of floating algae and other debris when the tide rose. Long (~ 35 cm) pieces of heavy duty galvanized metal (cattle fencing) were bent into hook shapes and placed along the bottom of the net approximately every 2 m to prevent it from lifting. When the net was first erected most (> 90%) of the sea lettuce that was occupying the area was removed and a few days later the remainder was taken out. Initially (during the month of July) the net was maintained free of sea lettuce on a weekly basis, but since the net was efficient at keeping sea lettuce out this practice was discontinued on August 1st.

2.3.3 Fish sampling regime

Fish communities in all three areas (exclosure, mixed, sea lettuce) were sampled once every two weeks starting July 20th, 2009 and ending Sept 12th (with the exception of

Aug 22nd sampled one week early because of the threat of a hurricane). Fish were captured using a 10 m x 1.5 m x 0.3 mm beach seine with 1.2 m bag. An additional lead line was attached to the net to increase capture efficiency in the eelgrass beds (Jenkins et al. 1997). The net was walked out perpendicular to shore until both ends were into the eelgrass bed (or sea lettuce area). The seine net was then pulled in a pentagonal manner, i.e. walking parallel to shore for 8 to 10 m and then together (4 to 5 m) to enclose the sample that was marked out with stakes to obtain a quantitative sample area (Figure 2.2). This was done opposite to the direction of the tide to ensure that the capture bag in the seine would not fold in. Most samples were collected within 4 h of low tide. Once the sample was enclosed the fish were encouraged to the bag, the wings were pulled until the bag was reached and the capture bag was emptied into a large container in the sample area. This method was required to sample within the enclosure and therefore followed at all sites to ensure comparability. All captured fish were identified, sorted, counted, and released live. Fish were separated into adults and young-of-the-year (YOY). YOY were classified by the following standards: mummichog ≤ 3 cm, Atlantic silverside ≤ 8 cm, flounder ≤ 3 cm, stickleback species ≤ 1.5 cm (Weldon et al. 2005).

Water temperature, salinity, and dissolved oxygen were measured with a YSI 650 Multiparameter Display System equipped with a model 600QS-O-M sonde (YSI Inc. Yellow Springs, Ohio, USA) at approximately ¾ the depth of the water column in the middle of the pentagonal sampling area. The sampling area was determined by measuring the distance between the two stakes on the inner and outer side of the pentagon (a), from the end of each side to the tip (b), and across the top of the sides (c). Lastly, water depth was taken (nearest cm) at each of the stakes (Figure 2.2).

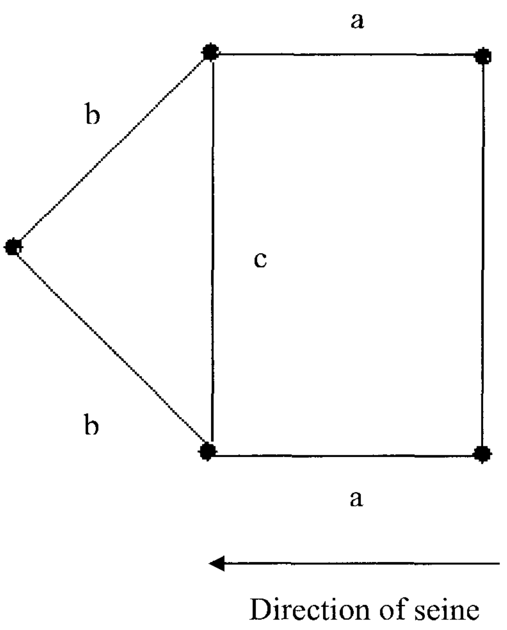

Figure 2.2. Pentagonal sampling manner in eelgrass habitat used to explore fish community differences. “a” represents distance walked on both sides (8-10 m), “b” represents distance walked to tip (4-5 m), “c” indicates distance across sides (8-10 m).

On August 22nd, the coverage of eelgrass in each of the habitats was determined by walking along three transects within the pentagonal area. One transect was 1 m inside the inshore stake, one in the middle of the pentagon and one 1 m inside the outer stake. Using a 0.5 m x 0.5 m viewing frame the percent cover of the eelgrass bed canopy was estimated (within 10%) approximately every meter along each of the three transects. Sea lettuce biomass was also determined on August 22nd by collecting all of the plant material trapped in the collection bag of the seine net and drying at 60°C for 48 h.

2.3.4 Eelgrass sampling and analysis

The ten smaller enclosures were left for seven weeks and taken down on Aug 22nd. The sea lettuce canopy was carefully removed from the enclosures and eelgrass shoots from within both the enclosures and exclosures were removed, placed in plastic Ziploc® freezer bags, taken back to the lab, and frozen (-20°C) for counting and measuring at a later date. Eelgrass samples were thawed and shoot density, blade density, canopy height, leaf area, above-ground biomass, and epiphyte biomass of each of the enclosure samples was determined. Shoot density of the samples was determined by counting the number of shoots attached to a root system within the 1 m^2 area, and blade density was the number of blades attached to the shoot systems. The length (nearest cm) and width (nearest mm) of each blade was then measured to determine total leaf area of the sample (i.e. total length x total width x blade density). Average canopy height of the eelgrass was chosen to be the mean length of the longest blade of each eelgrass shoot within the sample (Sogard et al. 1987). Epiphytes were scraped off the eelgrass blades using the edge of a glass microscope slide into distilled water and filtered using vacuum filtration onto pre-combusted and pre-weighed glass fiber filters (Whatman Type GF/A, 4.7cm). Epiphyte and above-ground eelgrass biomass was determined by drying samples at 60°C for 48 hours (Neckles et al. 1993; Jaschinski and Sommer 2008).

2.3.5 Data analysis

One-way analysis of variance (ANOVA) using Statistica® v.8 software (StatSoft, Inc, 2007) was used to see whether there were differences in the total abundance, abundance of individual fish species, species diversity (Shannon-Weiner index) or environmental factors among the three locations (exclosure, mixed, or sea lettuce), or if eelgrass characteristics (shoot density, blade density, canopy height, above-ground biomass, leaf area, and epiphyte biomass) differed between the two enclosure treatments (canopy and clear). Probability plots and Levene's test were used to examine normality and homogeneity of variance, respectively. Data were log (x+1) transformed to satisfy the assumptions of parametric statistics when needed. Nonparametric Kruskal Wallis tests were used to look for differences in species richness between the three sampling sites.

Multivariate statistics were used to determine if there were temporal differences in the fish community and if the fish community differed among any of the three sampling locations. In both cases analyses were run in two ways: once with adults and young-of-the-year (YOY) and again excluding YOY (as YOY often behave differently than adults). Fish abundances were standardized (by dividing catch by area sampled) to number/100m^2 (absolute abundance) and then fourth-root transformed to down weight the effects of the dominant species and increase the effects of the rare species (Clarke and Warwick 2001). The fourth-root transformed abundance data were then used to generate Bray-Curtis resemblance matrices, most commonly used for abundance samples to detect differences between them (Clarke and Gorley 2006). Non-metric multi-dimensional scaling (nMDS) plots were created from the Bray-Curtis resemblance matrices to visually show differences in the fish communities; samples that are more similar will group closer on the plot than ones that differ.

To test for significant differences in the fish communities ANOSIM (analysis of similarity) analyses were used. Bray-Curtis similarity matrices are used to calculate ANOSIM values (through pairwise testing, 999 permutations) and determine whether the fish assemblages differ. A global R value (and associated p value) is computed in which an R value of 1 denotes a completely different community composition, while a value of 0 represents a community which is identical in composition (Clarke and Warwick 2001). SIMPER (similarity percentages) tests, which are based on the transformed abundance data, were used to examine the contribution of each species to dissimilarity in the community between selected samples.

2.4 Results

2.4.1 Impacts of a sea lettuce canopy on eelgrass

The eelgrass within the five enclosures which contained the sea lettuce canopy decreased in density and vigour over the seven weeks of study. Shoot density within the five canopy treatment enclosures decreased significantly from the beginning to end of the sampling period, while there was no significant difference in the cleared enclosures (ANOVA, $F_{1,8} = 13.780$, $p = 0.006$ and $F_{1,8} = 3.607$, $p = 0.094$, respectively) (Figure 2.3). Comparing the differences between the clear and canopy treatments, eelgrass shoot density, blade density, above-ground biomass, leaf area, and epiphyte biomass were significantly higher in the clear treatment at the end of the experiment. Canopy height did not differ significantly (Table 2.1).

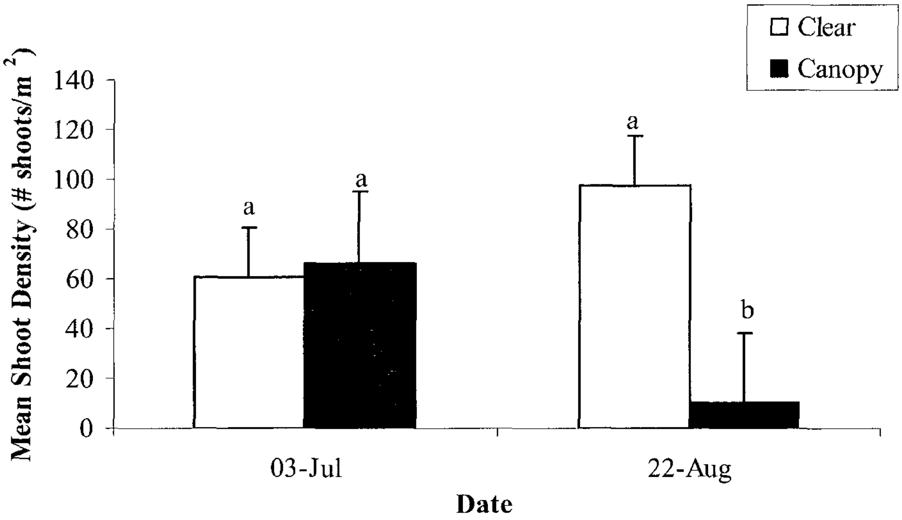

Figure 2.3. Mean eelgrass shoot density from each of the treatments (clear and canopy, n = 5 for each) from the beginning to the end of the sampling period. Bars sharing common letters are not significant ($p<0.05$, Tukey's). Error bars are standard error.

Table 2.1. Summary of the mean eelgrass parameters from each enclosure treatment (canopy and clear, n = 5 for each) at the end of the seven week experiment (July 3rd – August 22nd).

	Treatment	
	Canopy (± SE)	Clear (± SE)
Shoot density (no per m^2)	10.60 (3.83)	97.60* (17.44)
Blade density (no per m^2)	29.20 (10.71)	344.00* (58.62)
Canopy height (cm)	26.76 (4.41)	27.48 (0.78)
Above-ground biomass (g DW)	0.85 (0.32)	10.65* (1.75)
Epiphyte biomass (g DW)	0.002 (0.0001)	0.08* (0.01)
Leaf area (mm^2)	238.8 (90.49)	3039.96* (457.73)

Differences between canopy and clear were tested with a one-way analysis of variance (ANOVA) and significant results are indicated. $^*p<0.05$.

2.4.2 Environmental data

Only minor differences in temperature, salinity, dissolved oxygen, and depth were observed between the three fish sampling sites (Table 2.2). On any given sample date, water temperature, salinity, dissolved oxygen and water depth did not differ more than 1.1 °C, 2.84 ppt, 2.87 mg/L, or 17 cm respectively and there were no significant differences in any of the water parameters measured. Sea lettuce biomass was highest in the sea lettuce location at 194.79 g dry weight (DW) per 100 m^2 area. The exclosure had the lowest biomass, 6.32 g DW, and the mixed site was intermediate, 24.56 g DW. Eelgrass percent cover was highest in the exclosure (70%) while intermediate in the mixed location (61.25%), and lowest in the sea lettuce location (20.94%). Both sea lettuce biomass and eelgrass percent coverage were obtained only once (on August 1^{st}) and therefore differences between sites could not be tested statistically.

2.4.3 Fish community

A total of 8,112 fish from 10 species were captured in the six sampling periods (Tables A.1 - A.2, Appendix A). Of the entire community (adults and young-of-the-year (YOY)), five species comprised slightly over 98% of the total abundance: fourspine stickleback (*Apeltes quadracus*), threespine stickleback (*Gasterosteus aculeatus*), ninespine stickleback (*Pungitius pungitius*), mummichog (*Fundulus heteroclitus*), and northern pipefish (*Syngnathus fuscus*). When YOY are excluded from the analysis only four species, fourspine stickleback, ninespine stickleback, mummichog, and threespine stickleback make up 98% of the community. However, if we include adult northern pipefish, the same five species contribute to more than 99% of the community composition. Two species were exclusive to a particular habitat, although represented by

Table 2.2. Comparison of the mean (± SE) water parameters in each of the three sampling locations from all six sampling periods (July 20th - September 12th, 2009, n = 6 per sampling location). Differences between all three sampling locations were tested with a one-way analysis of variance (ANOVA) and no significant results were found.

Environmental Parameter	Exclosure	Mixed	Sea lettuce
Temperature (°C)	22.74 ± 1.61	23.01 ± 1.57	23.47 ± 1.66
Salinity (ppt)	21.37 ± 1.67	21.57 ± 1.50	22.5 ± 1.23
Dissolved oxygen (mg/L)	9.03 ± 0.71	9.35 ± 0.58	8.91 ± 0.78
Avg. water depth (cm)	50.5 ± 4.73	59.5 ± 3.74	47.5 ± 3.77

only one individual: an American eel (*Anguilla rostrata*) was captured in the sea lettuce site, while an Atlantic tomcod (*Microgadus tomcod*) was captured in the exclosure.

2.4.4 Impacts of sea lettuce on the fish community

Temporal variation was assessed by splitting the six sampling days into mid-summer ($n = 3$) and late-summer periods ($n = 3$). The fish community (adults and YOY) showed little over-lap, differing significantly between the time periods (two-way ANOSIM, Global $R = 0.358$, $p = 0.008$) (Figure 2.4A), but not between any of the sites (Global $R = -0.119$, $p = 0.827$). Similar patterns were observed when YOY were excluded from the analysis (Global $R = 0.259$, $p = 0.075$) (Figure 2.4 B). Temporal variation in the fish community (adults and YOY), was the result of more *Gasterosteus* spp. YOY in mid-summer and more northern pipefish YOY, ninespine stickleback (adults and YOY), and Atlantic silverside YOY in late-summer (SIMPER analysis, Table 2.3A). When YOY are excluded from the analysis, temporal community differences were largely due to higher numbers of northern pipefish and blackspotted stickleback in mid-summer and ninespine, fourspine and threespine stickleback, and mummichog in late-summer (SIMPER analysis, Table 2.3B).

There were no significant differences in the fish communities (adults only and with YOY excluded) found at the three study sites (Global $R = -0.023$, $p = 0.547$ and Global $R = 0.026$, $p = 0.341$, respectively) (Table 2.4, Figure 2.5). There were no significant differences in total abundance among any of the three sites, although there were fewer fish sampled in the sea lettuce site (ANOVA, $F_{2,15} = 1.506$, $p = 0.253$, Figure 2.6). Looking at individual species' abundances, only the fourspine stickleback showed a significant difference; greater numbers were sampled in the mixed habitat (ANOVA, $F_{2,15}$

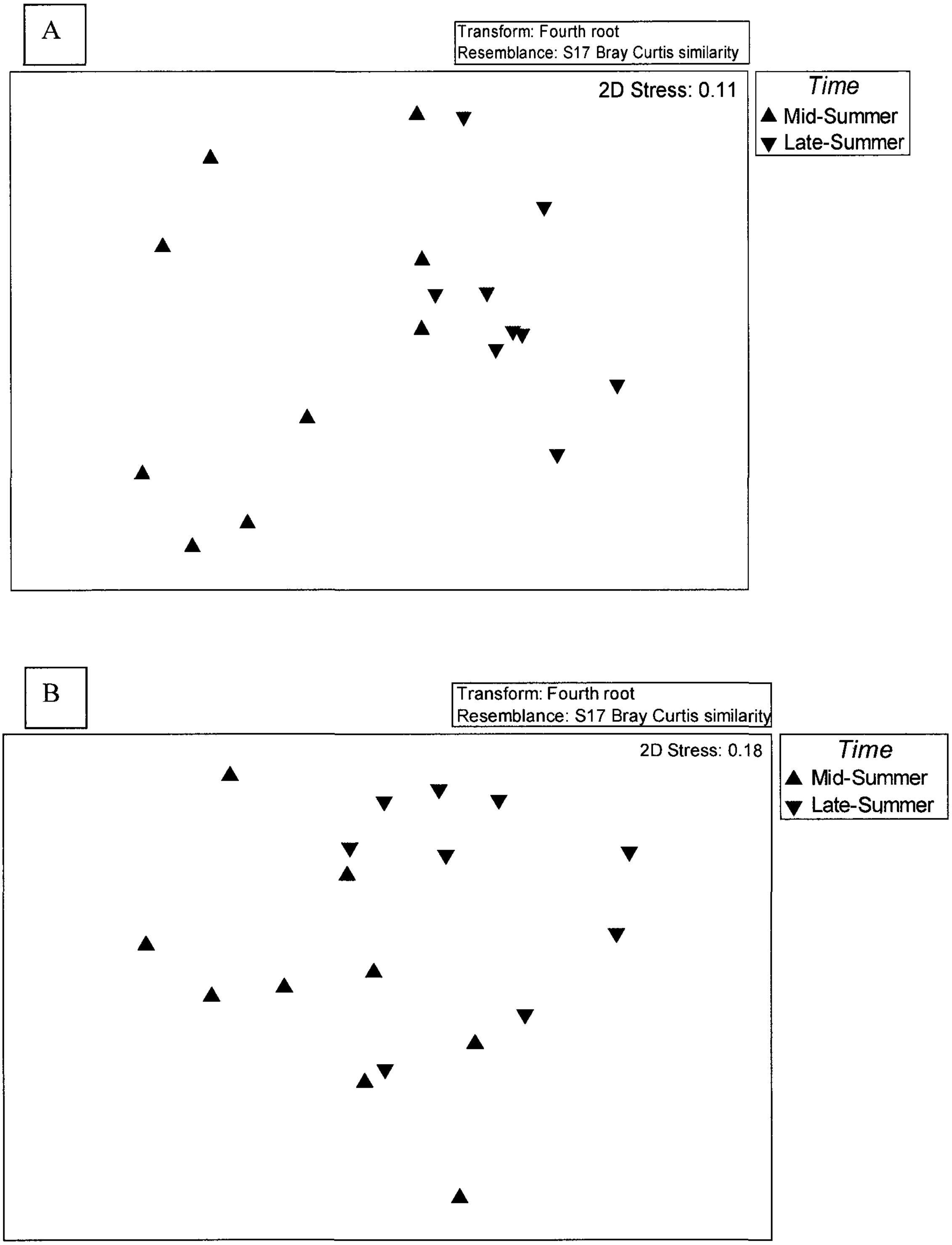

Figure 2.4. Non-metric multi-dimensional scaling (nMDS) plot of the fish community with A) adults and young-of-the-year (YOY) and B) YOY excluded showing temporal differences in the two sampling periods (mid-summer and late-summer). Each point represents one beach seine, n = 9 per sampling period.
Note: Stress is the representation of the dimensionality of the data in 2-D space.

Table 2.3. SIMPER results of temporal differences in the fish community based on the two sampling periods showing fish species contributing more than 8% to the dissimilarity in the community with A) adults and young-of-the-year (YOY) combined and B) YOY excluded. Abundances are back-transformed from the fourth root data used in the analysis.

Temporal Comparison: mid-summer vs. late-summer	**Average dissimilarity**	**Species contributing**	**Average abundance/ 100 m^2 mid-summer**	**Average abundance/ 100 m^2 late-summer**	**Contribution to dissimilarity (%)**
A)	32.24 %	Mummichog YOY*	0.16	33.73	14.23
		Pipefish YOY	0.07	29.98	14.09
		Gasterosteus YOY**	87.68	27.50	10.23
		Ninespine	8.35	67.85	10.03
		Ninespine YOY	59.73	266.39	9.28
		Silverside YOY	1.22	5.06	8.32
B)	27.75 %	Ninespine	8.35	67.85	26.98
		Pipefish	1.00	0.03	17.71
		Fourspine	50.82	136.81	17.47
		Threespine	0.66	1.41	17.32
		Mummichog	5.20	7.59	8.27
		Blackspotted	0.01	0.00	8.05

*YOY designates young-of-the-year fishes

**Threespine and blackspotted stickleback YOY are difficult to distinguish as very small individuals therefore all young of this type are designated *Gasterosteus* YOY.

Note: threespine, fourspine, ninespine, and blackspotted are all stickleback species.

Table 2.4. Summary of one-way ANOSIM test results for differences in the fish community in the three locations with (A) adults and YOY and (B) YOY excluded.

(A)

Pairwise groups	**Global R value***	**Significance level**
Locations combined	-0.023	0.547
Exclosure vs. Mixed	-0.041	0.600
Exclosure vs. Sea lettuce	-0.098	0.846
Mixed vs. Sea lettuce	0.061	0.225

(B)

Pairwise groups	**Global R value**	**Significance level**
Locations combined	0.026	0.341
Exclosure vs. Mixed	0.046	0.331
Exclosure vs. Sea lettuce	-0.154	0.961
Mixed vs. Sea lettuce	0.217	0.05

*Global R 1.00-0.750 = well separated communities, 0.750-0.5 = some overlap in community structure but remaining different, 0.5-0.250 = overlap, still somewhat different, 0.250-0 = lots of overlap, little difference (Clarke and Warwick 2001).

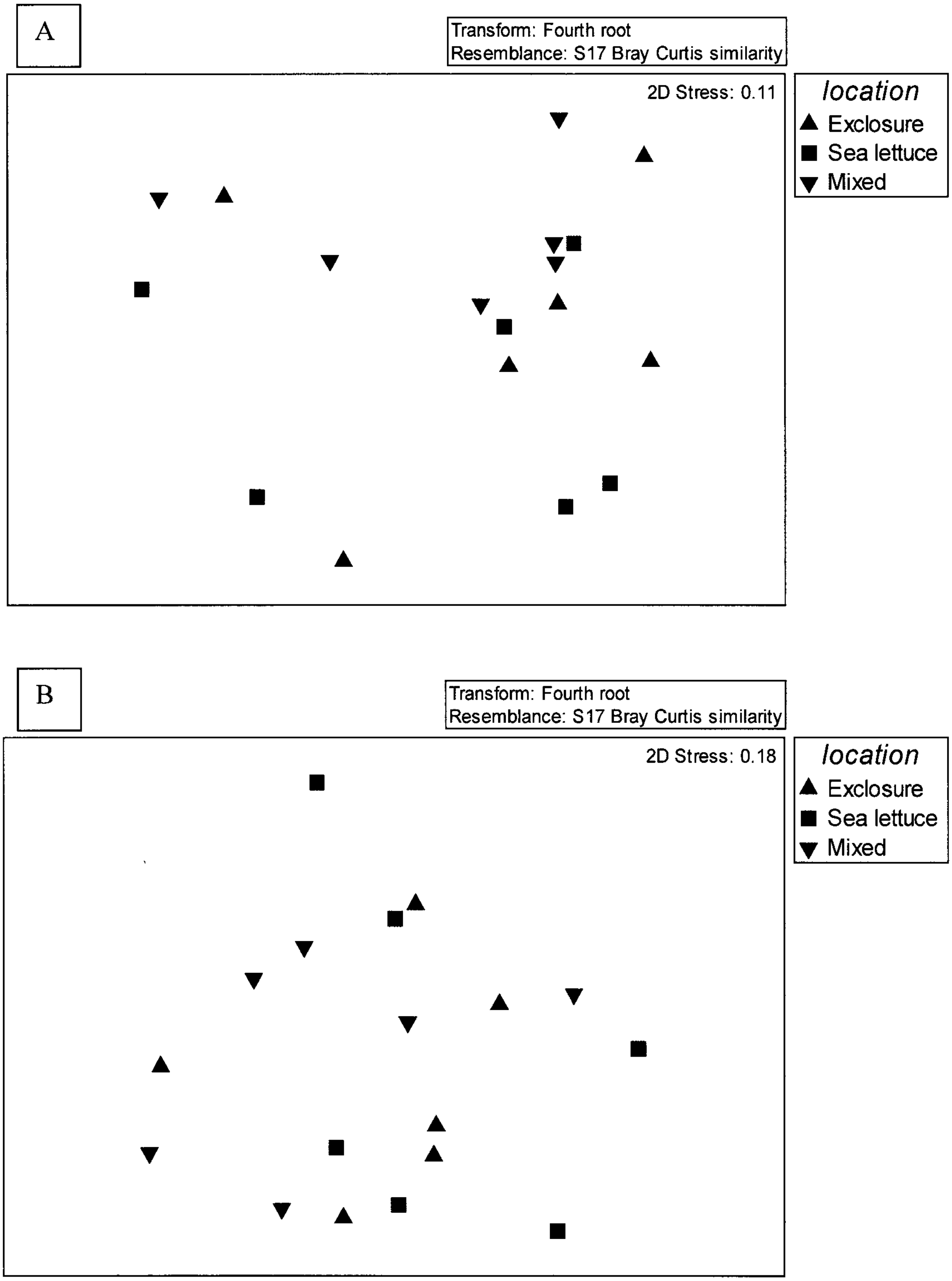

Figure 2.5. Non-metric multi-dimensional scaling (nMDS) plot of the fish community with A) adults and young-of-the-year (YOY) and B) YOY excluded in all three sampling locations (exclosure, mixed, and sea lettuce) throughout the sampling period. Each point represents one beach seine, n = 6 in each location.

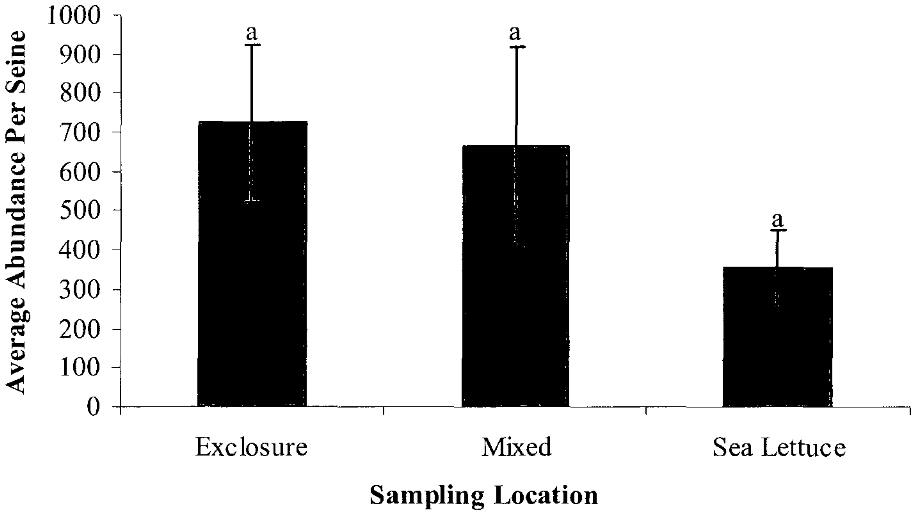

Figure 2.6. Average abundance of fish per beach seine haul in all three sampling locations (exclosure, mixed, and sea lettuce) throughout the sampling period. N = 6 seines in each location. No significant differences were found between sites. Error bars are standard error.

= 3.877, p = 0.044). Generally, all species except for the mummichog showed higher numbers in the exclosure or mixed areas. *Gasterosteus* spp. YOY, fourspine stickleback YOY, Atlantic silverside YOY, and northern pipefish YOY were captured in higher numbers in the exclosure, while threespine, fourspine, and ninespine (adults and YOY) stickleback were sampled in higher numbers in the mixed habitat (Figure 2.7). There were no differences in species richness (Kruskal Wallis, p = 0.435) or species diversity (ANOVA, $F_{2,15} = 0.655$, p = 0.534) between the three habitat types.

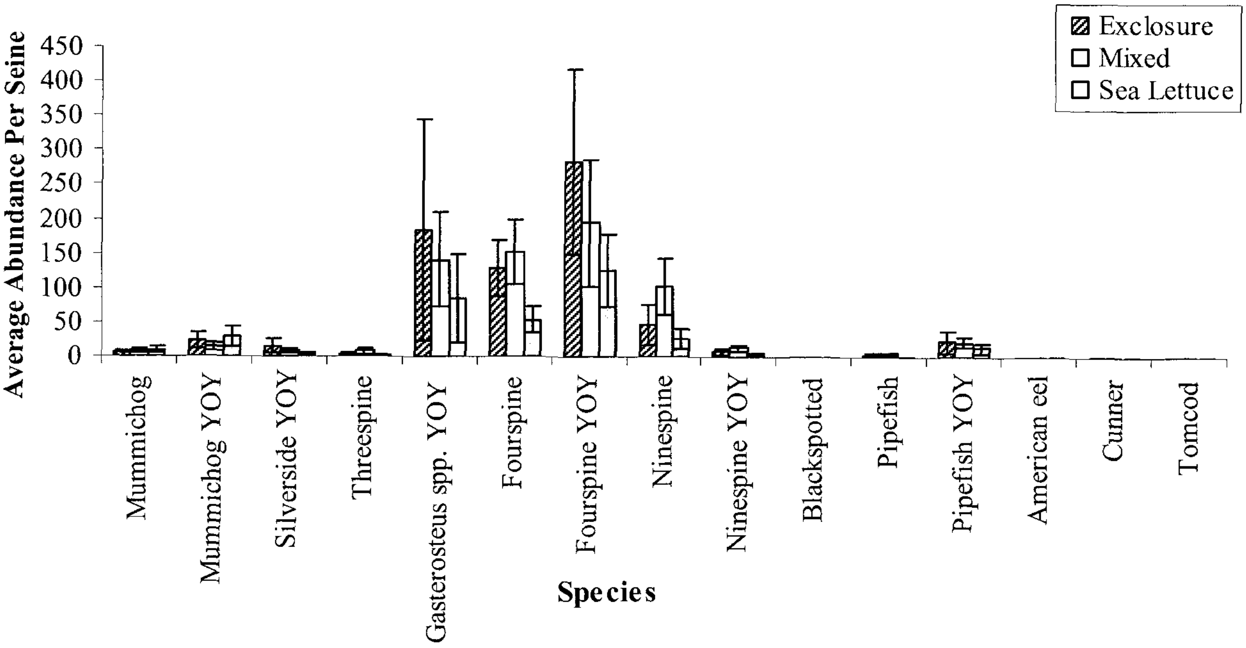

Figure 2.7. Average abundance per seine haul of each fish species captured in each of the sampling locations (exclosure, mixed, and sea lettuce) throughout the sampling period. N = 6 in each location.

Note: threespine, fourspine, ninespine, and blackspotted are all stickleback species.

2.5 Discussion

2.5.1 Impacts of a sea lettuce canopy on eelgrass

The 20-30 cm *Ulva* canopy treatment used in this study significantly reduced most of the measured eelgrass characteristics. This was consistent with Hauxwell et al. (2001) who set up a series of 1 m x 1 m enclosures with varying macroalgal canopies and concluded that a canopy which was greater than 9 cm thick resulted in the total loss of eelgrass in only a four month period, with the greatest loss occurring in the first seven weeks. The authors predicted that a canopy height of 12 cm thick would result in a loss rate of 45 eelgrass shoots per square meter per month. In two of our enclosures shoot loss rate was 76 and 86 shoots/m^2 in the seven weeks of the experiment and all enclosures suffered at least a 79% loss (range 79%-99%). Hauxwell et al. (2001) also found a linear relationship between macroalgal canopy height and above-ground production: as canopy height increased, production rates decreased. Brun et al. (2003) found that increased shading by *Ulva rigida* decreased starch content and reserves in the roots of *Zostera noltii* up to 60%. As a result, the seagrass plants suffered decreased leaf, root, and rhizome growth rates and a net production loss. Such alterations of nutrient storage will have profound effects on both short term production and annual regeneration. Although starch reserves can help seagrasses survive short periods (days-weeks) of shading (Peralta et al. 2002; Hauxwell et al. 2003), long term light reduction (weeks-months) severely impacts survival and increases the rate of seagrass habitat fragmentation and total loss (Gordon et al. 1994; Hauxwell et al. 2001; Brun et al. 2003), a phenomenon we have experimentally shown in the Midgell River estuary and which others have shown elsewhere.

2.5.2 Impacts of sea lettuce on the fish community

The main purpose of this component of the study was to determine if exclosures could be used to document the impacts of macroalgae on the fish community. The observed temporal variation was expected; as spawning proceeded and young-of-the-year (YOY) continued to emerge the fish assemblage changed. It was anticipated that the fish communities would show significant variation between the sea lettuce and the exclosure site and less so between the mixed habitat and exclosure. Dense beds of eelgrass have been shown to support a diverse and abundant fish assemblage when compared to lower quality habitats (Heck et al. 1989; Mattila et al. 1999; Hughes et al. 2002b; Wyda et al. 2002 among others). Hughes et al. (2002b) found that sites with low eelgrass shoot density generally had fewer fish and lower species richness when compared to sites with more eelgrass. Wyda et al. (2002) made similar conclusions reinforcing the idea that as eelgrass habitat is lost, not only do the number of fish decrease, but the composition of the community changes as well. In the present study no differences in the fish community between the eelgrass and sea lettuce sites were found; however differences in individual species abundance were noted (see below). Although the sea lettuce site had noticeably less eelgrass it is likely that the presence of some eelgrass continued to support the fish assemblage. Hughes et al (2002b) stated that areas of low shoot density continue to support more fish when compared to sites in which eelgrass has disappeared.

Sites of lower quality can still provide food resources and predator refuge. Conceivably, isopods, amphipods, and other invertebrates associated with the blades and shoots would persist. Also, sea lettuce harbours large quantities of some organisms such as amphipods (*Gammarus* sp.) (Schein 2009), which have been found to feed on and live within macroalgae (Deegan et al. 2002). Presumably, the proximity of sites would have allowed fish to forage in sea lettuce and take refuge in eelgrass.

The presence of eelgrass blades would continue to reduce the visual ability of predators. For example, Orth and van Montfrans (1982) used three densities of vegetation (high 1,600 blades m^2, intermediate 800 blades m^2, and low 400 blades m^2) to test the predation susceptibility of juvenile crab and found that there was no significant difference between predator success in any of the density variations. The shelter provided by seagrass habitats significantly reduces mortality from predation (e.g. Heck and Orth 2006) and therefore it is assumed that as seagrass density increases, predator efficiency decreases. Initial experiments by Nelson (1979) found that foraging efficiency of pinfish (*Lagodon rhomboides*) decreased with increasing seagrass density, but the relationship between predation success and seagrass density was not linear. In fact, a stepwise decrease with increasing seagrass complexity was a better representation of this relationship. Subsequent studies by Heck and Thoman (1981) and Adams et al. (2004) supported these findings. Also, Harris et al. (2004) looked at predation rates of bluefish (*Pomatomus saltatrix*) on Atlantic silverside (*M. mendidia*) and tautog (*Tautoga onitis*) by comparing eelgrass and sea lettuce/*Gracillaria* spp. habitats to bare sand and found that eelgrass significantly increased survivability of both fish species, while survival rates in the macroalgae did not differ significantly from those in bare substrate. Although the amount of eelgrass in our sea lettuce site was notably lower than in the exclosure or mixed site, the outcome of these studies suggests that our site may have continued to provide adequate foraging opportunities and protection from visual predators.

Lack of significant differences in the fish communities between the exclosure and sea lettuce site may have been the result of proximity and habitat complexity. Kingsford

(1995) found that drift algae increases habitat complexity of near-shore coastal waters by providing a substrate for larval and juvenile fish and invertebrates. Habitat complexity likely played a role in determining the fish community composition given the mixture of macrophytes in the sampling areas of this study. The sea lettuce habitat was chosen based on the low abundance of eelgrass; ultimately it may not have completely lacked the benefits known to be provided by eelgrass. However, as stated by Hughes et al. (2002b), lower quality habitats provide superior functions to the fish community relative to areas in which eelgrass have been completely lost, but such benefits are temporary as the eelgrass within low quality sites will most likely disappear as eelgrass habitat degradation continues. The sea lettuce site in the present study is most likely representative of an intermediate stage in which the fish communities would be expected to exhibit the predicted response (i.e. lower abundances and species richness) as eelgrass continues to disappear.

In spite of the lack of significant fish community differences between sites, initial signs of macroalgal impacts were detected as shown in abundance and individual species differences. In the exclosure, notably more fishes were captured per seine haul throughout the sampling period when compared to the sea lettuce site. The mixed site also had noticeably more fish. As eelgrass disappears from an area, fish tend to concentrate in surrounding vegetated habitats (Pihl et al. 2006). This was particularly noticeable for fourspine stickleback which was captured in higher numbers in the mixed site. Meng et al. (2004) evaluated fish abundances in a variety of habitat types including eelgrass and sea lettuce and reported that fourspine stickleback, although captured in both habitats, were found in much higher numbers in the eelgrass. While comparing vegetated and unvegetated sites Orth and Heck (1980), Heck et al. (1989), and Lazzari and Tupper

(2002) found fourspine stickleback were restricted to the eelgrass habitat. Szedlmayer and Able (1996) confirmed that fourspine stickleback show eelgrass specific habitat relations; this was apparent in our study as fourspine stickleback were captured in significantly higher numbers in eelgrass.

In summary, the impacts of a sea lettuce canopy on eelgrass were experimentally demonstrated in this study. Eelgrass declined in response to macroalgal canopies demonstrating that the role of macroalgae in eelgrass loss is substantial. The exclosure proved successful in modifying aquatic vegetation and would was a viable resource for assessing differences in fish communities. The observation that fish communities did not differ significantly between treatments is likely the result of a small sample size and proximity of the sites. Further, it is likely that the presence of some eelgrass within the sea lettuce site allowed fish the protection and foraging advantages they require. Sampling in an area which was dominated by sea lettuce with no available eelgrass may have resulted in differences in community structure.

2.6 Literature Cited

Adams, A.J., Locascio, J.V., Robbins, B.D. 2004. Microhabitat use by a post-settlement stage estuarine fish: evidence from relative abundance and predation among habitats. *Journal of Experimental Marine Biology and Ecology* **299**: 17-33.

Bell, J. D., Westoby, M. 1986. Importance of local changes in leaf height and density to fish and decapods associated with seagrasses. *Journal of Experimental Marine Biology and Ecology* **104**: 249-274.

Brun, F.G., Vergara, J.J., Navarro, G., Hernández, I., Pérez-Lloréns, J.L. 2003. Effects of shading by *Ulva rigida* canopies on growth and carbon balance of the seagrass *Zostera noltii*. *Marine Ecology Progress Series* **265**: 85-96.

Clarke, K.,R., Gorley, R., N. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E: Plymouth, UK.

Clarke, K., R., Warwick, R., M. 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E: Plymouth, UK.

Deegan, L.A., Finn, J.T., Ayvazian, S.G., Ryder-Kieffer, C.A., Buonaccorsi, J. 1997. Development and validation of an estuarine biotic integrity index. *Estuaries* **20**: 601-617.

Deegan, L.A., Wright, A., Ayvazian, S.G., Finn, J.T., Golden, H., Rand-Merson, R., Harrison, J. 2002. Nitrogen loading alters seagrass ecosystem structure and support of higher trophic levels. *Aquatic Conservation: Marine and Freshwater Ecosystems* **12**: 193-212.

Gordon, D.M., Grey, K.A., Simpsom, C.J. 1994. Changes in the structure and productivity of a *Posidonia sinuosa* meadow during and after imposed shading. *Aquatic Botany* **47**: 265-275.

Greve, T.M., Borum, J., Pedersen, O. 2003. Meristematic oxygen variability in eelgrass (*Zostera marina*). *Limnology and Oceanography* **48**: 210-216.

Harris, L.A., Buckley, B., Nixon, S.W., Allen, B.T. 2004. Experimental studies of predation by bluefish *Pomatomus saltatrix* in varying densities of seagrass and macroalgae. *Marine Ecology Progress Series* **281**: 233-239.

Hauxwell, J., Cebrián, J., Furlong, C., Valiela, I. 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology* **82**: 1007-1022.

Hauxwell, J., Cebrián, J., Valiela, I. 2003. Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. *Marine Ecology Progress Series* **247**: 59-73.

Heck, K.L., Able, K.W., Fahay, M.P., Roman, C.T. 1989. Fishes and decapod crustaceans of Cape Cod eelgrass meadows: species composition, abundance patterns and comparison with unvegetated substrates. *Estuaries* **12**: 59-65.

Heck, K.L. Jr., Orth R.J. 2006. Predation in seagrass beds. p. 537-550 in A.W.D. Larkum et al. eds. Seagrasses: Biology, Ecology, and Conservation, Springer Publishing, Dordrecht, Netherlands. 691pp.

Heck, K.L. Jr., Thoman, T.A. 1981. Experiments on predator-prey interactions in vegetated habitats. *Journal of Experimental Marine Biology and Ecology* **53**: 125-134.

Hughes, J.E., Deegan, L.A., Weaver, M.J., Costa, J.E. 2002a. Regional application of an index of estuarine biotic integrity based on fish communities. *Estuaries* **25**: 250-263.

Hughes, J., Deegan, L.A., Wyda, J.C., Weaver, M.J., Wright, A. 2002b. The effects of eelgrass habitat loss on estuarine fish communities of southern New England. *Estuaries* **25**: 235-249.

Jaschinski, S., Sommer, U. 2008. Top-down and bottom-up control in an eelgrass-epiphyte system. *Oikos* **117**: 754-762.

Jenkins, G.P., May, H.M.A., Wheatley, M.J., Holloway, M.G. 1997. Comparison of fish assemblages associated with seagrass and unvegetated habitats of Port Phillip Bay and Corner Inlet, Victoria, Australia, with emphasis on commercial species. *Estuarine, Coastal and Shelf Science* **44**: 569-588.

Kingsford, M.J. 1995. Drift algae: a contribution to near-shore habitat complexity in the pelagic environment and an attractant for fish. *Marine Ecology Progress Series* **116**: 297-301.

Lazzari, M.A. 2002. Epibenthic fishes and decapod crustaceans in northern estuaries: a comparison of vegetated and unvegetated habitats in Maine. *Estuaries* **25**: 1210-1218.

Lazzari, M.A., Tupper, B. 2002. Importance of shallow water habitats for demersal fishes and decapod crustaceans in Penobscot Bay, Maine. *Environmental Biology of Fishes* **63**: 57-66.

Mattila, J. Chaplin, G., Eilers R., Heck, K.L., O'Neal J.P., Valentine J.F. 1999. Spatial and diurnal distribution of invertebrate and fish fauna of a *Zostera marina* bed and nearby unvegetated sediments in Damariscotta River, Maine (USA) *Journal of Sea Research* **41**: 321-332.

Meng, L., Cicchetti, G., Chintala, M. 2004. Nekton habitat quality at shallow water sites in two Rhode Island coastal systems. *Estuaries* **27**: 740-751.

McGlathery, K.J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *Journal of Phycology* **37**: 453-456.

Neckles, H.A., Wetzel, R.L., Orth, R.J. 1993. Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. *Oecologia* **93**: 285-295.

Nelson, W.P. 1979. Experimental studies of selective predation on amphipods: consequences for amphipod distribution and abundance. *Journal of Experimental Marine Biology and Ecology* **38**: 225-245.

Orth, R.J., Heck, K.L. 1980. Structural components of eelgrass (*Zostera marina*) meadows in the lower Chesapeake Bay- Fishes. *Estuaries* **3**: 278-288.

Orth, R.J., Heck, K.L., van Montfrans, J. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries* **7**: 278-286.

Orth, R.J., Moore, K.A. 1983. Chesapeake Bay: an unprecedented decline in submerged aquatic vegetation. *Science* **222**: 51-53.

Orth, R.J., van Montfrans, J. 1982. Predator-prey interactions in a *Zostera marina* ecosystem in the lower Chesapeake Bay, Virginia. p. 81-94 in R.J. Orth and J. van Montfrans eds. Interactions of Resident Consumers in a Temperate Estuarine Seagrass Community: Vaucluse Shores, Virginia, USA. 232 pp.

Peralta, G., Pérez-Lloréns, J.L., Hernández, I., Vergara, J.J. 2002. Effects of light availability on growth, architecture and nutrient content of the seagrass *Zostera noltii* Hornem. *Journal of Experimental Marine Biology and Ecology* **269**: 9-26.

Pihl, L., Baden, S., Kautsky, N., Ronnback, P., Soderqvist, T., Troell, M., Wennhage, H. 2006. Shift in fish assemblage structure due to loss of seagrass *Zostera marina* habitats in Sweden. *Estuarine, Coastal and Shelf Science* **67**: 123-132.

Schein, A. 2009. The estuarine fish community and food web structure in areas of sea lettuce (*Ulva lactuca*) and eelgrass (*Zostera marina*) within the Stanley River estuary Prince Edward Island. M.Sc. Thesis. University of Prince Edward Island, Charlottetown, PEI.

Short, F.T., Burdick, D.M., Wolf, J.S., Jones, G.E. 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina. Limnology and Oceanography* **40**: 740-749.

Sogard, S.M., Able, K.W. 1991. A comparison of eelgrass, sea lettuce macroalgae, and marsh creeks as habitats for Epibenthic fishes and decapods. *Estuarine and Coastal Shelf Science* **33**: 501-519.

Sogard, S.M., Powell, G.V.N., Holmquist, J.G. 1987. Epibenthic fish communities on Florida Bay banks: relations with physical parameters and seagrass cover. *Marine Ecology Progress Series* **40**: 25-39.

Szedlmayer, S.T., Able, K.W.1996. Patterns of seasonal availability and habitat use by fishes and decapod crustaceans in a Southern New Jersey estuary. *Estuaries* **19**: 697-709.

Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Costa, J., Peckol, P., DeMeo-Anderson, B., D'Avanzo, C., Babione, M., Sham, C.H., Brawley, J., Lajtha, K. 1992. Couplings of watersheds and coastal waters: sources and consequences of nutrient enrichment of Waquoit Bay, Massachusetts. *Estuaries* **15**: 433-457.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* **42**: 1105-1118.

Weldon, J., Garbary, D., Courtenay, S., Ritchie, W., Godin, C., Thériault, M-H., Boudreau, M., Lapenna, A. 2005. The community aquatic monitoring project (CAMP) for measuring marine environmental health in coastal waters of the southern Gulf of St. Lawrence: 2004 overview. *Canadian Technical Report of Fisheries and Aquatic Science* **2624**: viii + 53 pp.

Wyda, J.C., Deegan, L.A., Hughes, J.E., Weaver, M.J. 2002. The response of fishes to submerged aquatic vegetation complexity in two ecoregions of the Mid-Atlantic Bight: Buzzards Bay and Chesapeake Bay. *Estuaries* **25**:86-100.

CHAPTER 3: DIEL AND MONTHLY VARIATION IN THE EELGRASS (*ZOSTERA MARINA*) FISH COMMUNITY ASSEMBLAGE IN THE MIDGELL RIVER ESTUARY, PRINCE EDWARD ISLAND

3.1 Abstract

Eelgrass beds provide important habitat for many aquatic organisms including fishes. However, current information concerning fish utilization of eelgrass habitat has been largely restricted to day sampling efforts. Diel changes in the eelgrass fish community were investigated in the Midgell River estuary, Prince Edward Island during the summer season. Beach seines were conducted over 24 h periods twice monthly from June to August 2008. Significantly more individuals occurred in eelgrass habitats throughout the night hours (736.42 ± 101.87) than during the day (495.67 ± 36.26). Atlantic silverside (*Menidia menidia*) young-of-the-year (YOY), mummichog (*Fundulus heteroclitus*), and American eel (*Anguilla rostrata*) were captured significantly more often at night, while ninespine stickleback (*Pungitius pungitius*) were captured more frequently during the day. Diel variation differed between months; however, within a given month day catches were generally dominated by *Gasterosteus* spp. YOY, ninespine stickleback, threespine stickleback (*Gasterosteus aculeatus*), and northern pipefish YOY (*Syngnathus fuscus*). Night catches generally had higher numbers of mummichog and Atlantic silverside YOY. Temporal variation was driven by the emergence of YOY fish species and differed most between the beginning and end of the sampling season.

3.2 Introduction

Eelgrass provides a complex habitat within the estuarine environment and is known to support a diverse and abundant ichthyofauna. The structural complexity created by eelgrass beds provides increased food resources and lessens the risk of predation, allowing invertebrates and fish, especially juveniles, to thrive (Orth et al. 1984; Heck et al. 1989; Deegan et al. 1997). Generally, there is a positive correlation between eelgrass complexity within an estuary and fish abundance. This is particularly noticeable when sites containing eelgrass beds are compared to those sampled over bare substrate (e.g. Heck et al. 1989; Mattila et al. 1999; Hughes et al. 2002b). Hughes et al. (2002b) compared habitats of high, low and zero eelgrass complexity and found that although the high complexity habitat had the greatest fish abundances and species richness, the low complexity habitat provided attributes to the fish community that the zero complexity regions could not. The relationship between eelgrass abundance and fish populations has in recent times been confirmed in other studies, especially in areas affected by coastal eutrophication (see Hughes et al. 2002a and Wyda et al. 2002).

A weakness of many studies that evaluate eelgrass-associated fish communities is the tendency to restrict sampling to daylight hours. It has been shown that temporally-limited investigations tend to underestimate species abundances and richness (Stoner 1991; Rountree and Able 1993). A number of investigators have noted increased fish numbers at night (e.g. Lubbers et al. 1990, Rountree and Able 1993; Methven et al. 2001 among others). Such diel patterns may be the result of foraging behaviour, predator avoidance, interspecific competition or a combination of these factors (Rountree and Able 1993; Mattila et al. 1999). Additionally, diel environmental variability such as differences in dissolved oxygen concentration or temperature may further affect the diel activity of estuarine fishes (Rountree and Able 1993). Therefore conclusions drawn from studies employing only diurnal sampling may inadequately establish the importance of seagrass habitat to a variety of fish species (Gray et al. 1998). The addition of nocturnal sampling provides a more complete understanding of seagrass habitat utilization by various estuarine fish species.

The purpose of this investigation was to document the diel patterns of eelgrass habitat use by an estuarine fish community. While many studies have looked at the eelgrass-associated fish communities (Orth and Heck 1980; Heck et al. 1989; Deegan et al. 1997; Hughes et al. 2002ab; Lazarri 2002; Wyda et al. 2002 among others) relatively few have looked specifically at diel patterns (see Gray et al. 1998; Hagan and Able 2008). The above studies have taken place in south eastern Australia and along the eastern USA respectively; only one known study has looked at diel variability in estuarine fish communities in the Southern Gulf of St. Lawrence and involved passive sampling methods (see Joseph et al. 2006) which may have underestimated diel changes in the fish community. This study (1) documents diel abundance patterns in eelgrass habitat use by individual fish species and the fish community and (2) examines monthly variation (between June and August) in the fish community.

3.3 Methods

3.3.1 Study location

The study location was at the mouth of the Midgell River estuary where it joins St. Peter's Bay on the North shore of Prince Edward Island (Figure 3.1). The study site was on the northeast side of the estuary in an extensive area of eelgrass (*Zostera marina*). The eelgrass bed extended at least 75 m from shore and was sampled approximately 15 m from shore in the present study. The bed itself was situated on a gentle slope and therefore could be sampled throughout a 24 h period despite the change in tides. The maximal range of the semi-diurnal tidal cycle in the Midgell River estuary is 0.0 m to 0.9 m during spring tides; during neap tide events tides range from 0.3 m to 0.8 m.

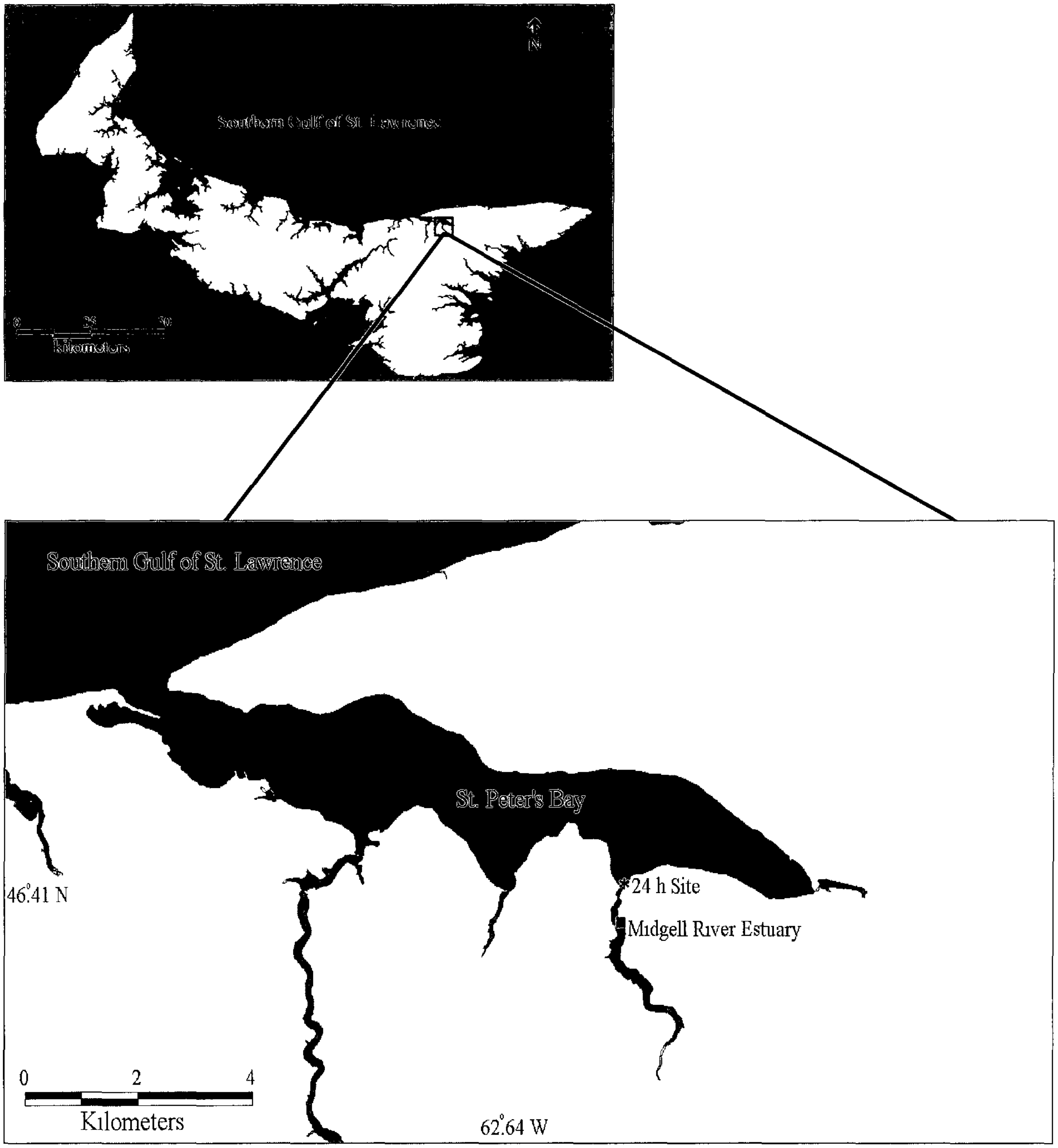

Figure 3.1. Diel sampling location in the Midgell River estuary, which flows into St. Peter's Bay on the north side of Prince Edward Island.

3.3.2 Fish sampling

Fish communities were sampled over a 24 h period six times between June 22nd and August 24th, 2008 (at approximately two week intervals and during neap tide events to better control for large fluctuations in water depth) to determine daily patterns of eelgrass use by different fish species. Every four hours starting at 10:00 (with the exception of June 22nd when seining started at 18:00) two consecutive seine hauls were completed (in non-overlapping areas less than 5 m apart) in the eelgrass habitat. Fish were captured using the beach seine and pentagonal sampling methods as outlined in Chapter 2, although in this case the net was brought to shore before being emptied into the large container. Fish were handled in the same manner as previously described.

3.3.3 Environmental data

Water temperature, salinity, dissolved oxygen, pH, and depth were measured as described in Chapter 2.

3.3.4. Data analysis

Repeated measures analysis of variance (ANOVA) was used to test for diel differences in total abundance, abundance of individual fish species, and species diversity throughout the sampling period. One way ANOVA was used to test for diel differences in water parameters between each of the six diel samples. ANOVAs were performed using the statistical procedure described in Chapter 2. Correlation analysis was used to determine if there were differences between water depth and the various measures of community structure (abundance, richness, diversity). Multivariate analyses were used to describe temporal and diel differences in the fish community as a whole. Both diel and monthly analyses were performed with (a) both adults and young-of-the-year (YOY) and with (b) YOY excluded (as YOY often behave differently) using ANOSIM and SIMPER analyses (Chapter 2).

3.4 Results

3.4.1 Environmental data

Most water parameters did not differ greatly within any of the diel sampling periods. There were no significant differences in day (6:00-20:00 h) or night (22:00-4:00 h) water temperature, dissolved oxygen concentration, pH, or salinity in any of the six 24 h samples. There were, however, large fluctuations in dissolved oxygen concentrations throughout the diel period on some sampling occasions. Salinity did not usually differ more than 3 ppt, and usually < 1 ppt, with the exception of August 6th which followed a heavy rainfall event and salinity fluctuated from 9 ppt – 22 ppt. Major fluctuations in water depth were not observed. The largest change in mean water depth in one 24 h period was 53.8 cm which was seen on August 24th. Generally, the difference in mean water depth throughout any given 24 h sample was < 40 cm. Between sampling intervals (i.e. 4 h periods) water depth did not typically fluctuate more than 10 cm. Significant differences in water depth between day and night samples were found for the June 27th, July 11th, and August 24th samples (Table 3.1); however, differences were not major, ranging from 11 cm to 35 cm (Figure 3.2). High or low tides were not restricted to either day or night samples.

3.4.2 Fish community

After 72 seine hauls spanning 144 hours, a total of 47,044 fish from 12 different species were captured (Appendix B.1 - B.6). When adult and young-of-the-year (YOY)

Table 3.1. Comparison of the mean day (6:00-20:00 h) and night (22:00-4:00 h) water parameters in all six sampling periods (June 22nd- August 24th, 2008, n = 8 for day samples and n = 4 at night during each period).

Environmental character	Day (± SE)	Night (± SE)
June 22nd		
Water temperature (°C)	20.56 (± 0.60)	20.07 (± 0.68)
Salinity (ppt)	24.68 (± 0.08)	24.58 (± 0.11)
Dissolved oxygen (mg/L)	11.98 (± 1.34)	9.82 (± 0.83)
pH	8.50 (± 0.11)	8.49 (± 0.13)
Avg. water depth (cm)	65.58 (± 7.35)	51.05 (± 3.45)
June 27th		
Water temperature (°C)	19.54 (± 0.44)	18.85 (± 0.19)
Salinity (ppt)	22.89 (± 0.33)	22.06 (± 0.48)
Dissolved oxygen (mg/L)	9.21 (± 0.58)	7.35 (± 0.35)
pH	8.31 (± 0.05)	8.26 (± 0.05)
Avg. water depth (cm)	72.25 (± 2.58)	84.95 (± 4.37)*
July 11th		
Water temperature (°C)	23.89 (± 0.45)	23.66 (± 0.31)
Salinity (ppt)	22.79 (± 0.22)	23.52 (± 0.18)
Dissolved oxygen (mg/L)	7.91 (± 0.91)	5.69 (± 0.27)
pH	8.35 (± 0.08)	8.26 (± 0.07)
Avg. water depth (cm)	66.18 (± 2.31)	79.25 (± 2.77)*
July 23rd		
Water temperature (°C)	21.38 (± 0.42)	21.46 (± 0.25)
Salinity (ppt)	23.23 (± 0.15)	24.75 (± 0.65)
Dissolved oxygen (mg/L)	10.71 (± 1.44)	8.27 (± 1.80)
pH	8.28 (± 0.11)	8.16 (± 0.15)
Avg. water depth (cm)	55.18 (± 5.93)	50.15 (± 3.59)
August 6th		
Water temperature (°C)	19.51 (± 0.35)	19.07 (± 0.16)
Salinity (ppt)	14.86 (± 1.67)	18.26 (± 3.10)
Dissolved oxygen (mg/L)	10.57 (± 1.51)	9.68 (± 1.38)
pH	7.68 (± 0.15)	7.66 (± 0.13)
Avg. water depth (cm)	63.53 (± 3.83)	73.50 (± 2.10)
August 24th		
Water temperature (°C)	22.79 (± 0.63)	22.41 (± 0.26)
Salinity (ppt)	18.78 (± 0.57)	19.43 (± 0.33)
Dissolved oxygen (mg/L)	13.18 (± 1.41)	12.29 (± 0.89)
pH	8.06 (± 0.12)	8.19 (± 0.06)
Avg. water depth (cm)	48.95 (± 4.69)	84.65 (± 5.27)*

Differences between diel periods were tested with a one-way analysis of variance (ANOVA) and significant results are indicated. *$p<0.05$.

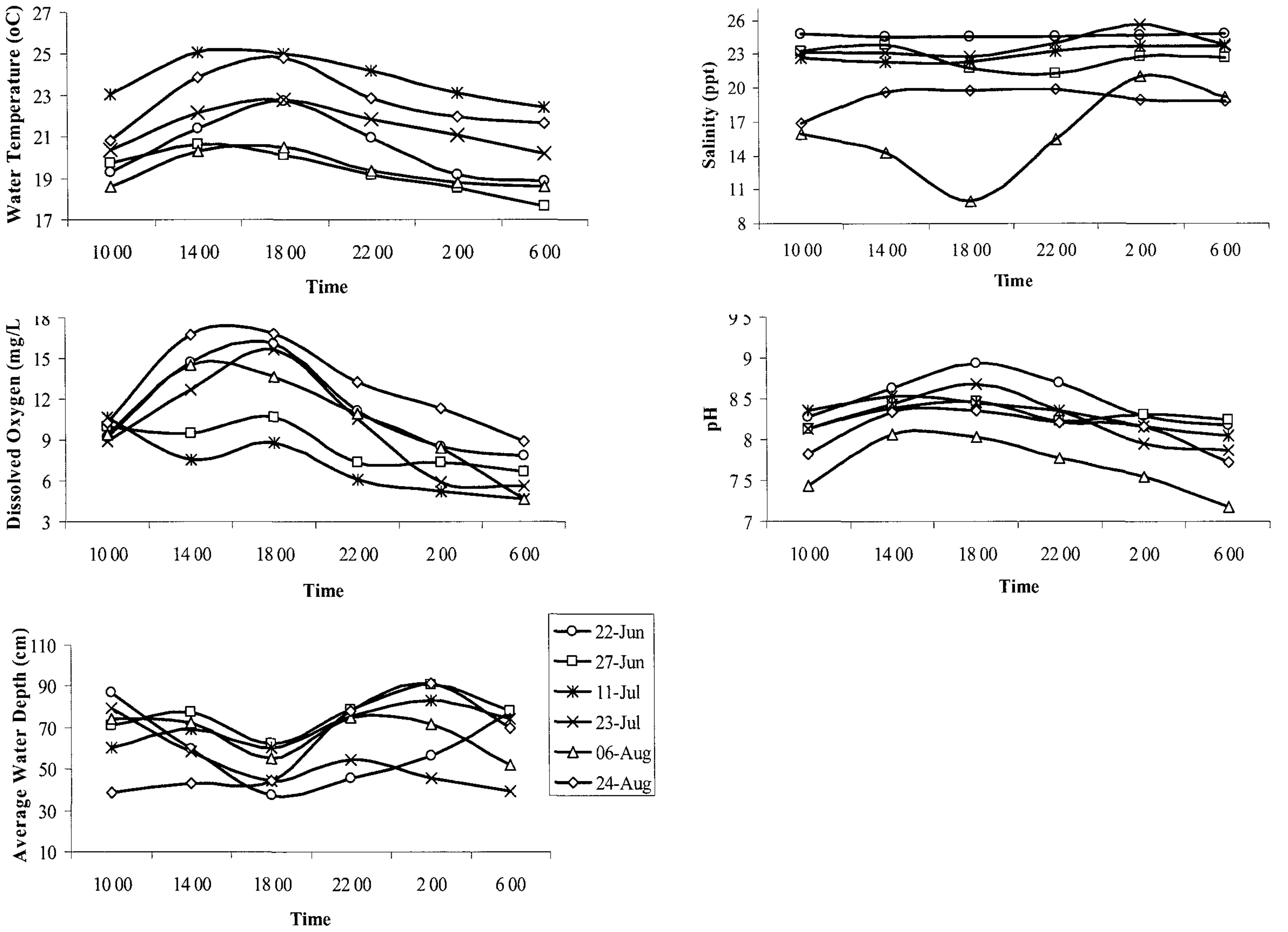

Figure 3.2. Mean water temperature (^{o}C), salinity (ppt), dissolved oxygen (mg/L), pH, and water depth (cm) during 24 h sampling in the Midgell River estuary during each time interval from June 22nd to August 24th, 2008. N = 2 seines per period.

fishes were analyzed together, six species comprised over 98% of the total catch: fourspine stickleback (*Apeltes quadracus*), mummichog (*Fundulus heteroclitus*), Atlantic silverside (*Menidia menidia*), threespine stickleback (*Gasterosteus aculeatus*), northern pipefish (*Syngnathus fuscus*), and ninespine stickleback (*Pungitius pungitius*). When YOY were excluded from the analysis, Atlantic silverside contributed less than 1% to the community structure; however the other five species still made up slightly over 97%. If the next most abundant species, blackspotted stickleback (*Gasterosteus wheatlandi*), are included these six species combined made up 99% of the community.

3.4.3 Diel differences in the fish community composition

No species was captured exclusively during the day or night (Table 3.2). Significantly more individuals occurred in eelgrass habitats throughout the night hours (736.42 ± 101.87) than during the day (495.67 ± 36.26) (Repeated measures (RM) ANOVA, $F_{6,5} = 8.30$, $p = 0.017$). The least number of fish were captured during dawn (6:00) samples (Table 3.3). Several species showed significant diel abundance patterns. Adult mummichog, silverside YOY and American eel were captured in significantly higher numbers during the night (RM ANOVA, $F_{6,5} = 99.473$, $p < 0.001$; $F_{4,7} = 42.665$, $p < 0.001$; $F_{6,5} = 5.733$, $p = 0.027$ respectively). Conversely, adult ninespine stickleback were significantly more prevalent during day samples (RM ANOVA, $F_{6,5} = 18.555$, $p = 0.003$) (Figure 3.3). Although variation in diel abundance was observed for other species, none of the differences were significant. The species composition of the eelgrass habitat during both day and night were comparable; therefore, the diel changes were not the result of different species using the habitat at different times of day, although species diversity was significantly higher at night (RM ANOVA, $F_{6,5} = 24.008$, $p = 0.002$). Water depth,

Table 3.2. Average abundance per seine haul of each fish species captured during the day or night throughout the diel sampling period (n = 48 for day samples and n = 24 for night samples).

Species: Common name (Scientific name)	Day (± SE)	Night (± SE)
American eel (*Anguilla rostrata*)	0.06 (± 0.04)	0.25 (± 0.11)*
Atlantic silverside (*Menidia menidia*)	0.33 (± 0.18)	0.92 (± 0.47)
Atlantic silverside YOY[1]	26.77 (± 8.13)	182.42 (± 37.47)*
Atlantic tomcod YOY (*Microgadus tomcod)*	0.08 (± 0.05)	0.08 (± 0.06)
Blackspotted stickleback (*Gasterosteus wheatlandi*)	7.17 (± 0.38)	4.96 (± 1.39)
Cunner (*Tautogolabrus adspersus)*	0.17 (± 0.05)	0.04 (± 0.04)
Fourspine stickleback (*Apeltes quadracus*)	166.58 (± 13.47)	171.04 (± 18.99)
Fourspine YOY	143.77 (± 21.02)	129.54 (± 25.55)
Gaspereau YOY (*Alosa* spp.)	0.29 (± 0.29)	0.38 (± 0.25)
Gasterosteus spp. YOY[2]	38.75 (± 11.53)	32.71 (± 10.45)
Mummichog (*Fundulus heteroclitus*)	17.65 (± 1.80)	73.50 (± 7.45)*
Mummichog YOY	58.25 (± 11.17)	114.71 (± 33.50)
Ninespine stickleback (*Pungitius pungitius*)	9.31 (± 1.01)	5.71 (± 0.84)*
Ninespine YOY	1.27 (± 1.01)	0.54 (± 0.35)
Northern pipefish (*Syngnathus fuscus*)	7.17 (± 1.15)	6.33 (± 1.36)

Table 3.2 continued

Northern pipefish YOY	5.63 (± 1.12)	4.29 (± 1.52)
Winter flounder (juv) (*Pseudopleuronectes americanus*)	0.02 (± 0.02)	0.04 (± 0.04)
Winter flounder YOY	0.02 (± 0.02)	0.00 (± 0)
Total	495.67 (± 36.26)	736.42 (± 101.87)

[1]YOY designates young-of-the-year fishes.
[2]Threespine and blackspotted stickleback YOY are difficult to distinguish as very small individuals therefore all young of this type are designated *Gasterosteus* spp. YOY.
Differences between diel abundances were tested with a repeated measures analysis of variance (ANOVA) and significant results are indicated. *p<0.05.

Table 3.3. Average abundance (no. /100 m^2) of fish per seine haul during each of the time intervals sampled (n = 48 for day samples and n = 24 for night samples)

	Day			Night		Day
Time Interval	10:00	14:00	18:00	22:00	2:00	6:00
Average Abundance (± SE)	513.92 (± 79.96)	560.67 (± 49.17)	488.50 (± 44.59)	782.33 (± 103.52)	689.92 (± 109.27)	418.42 (± 34.31)

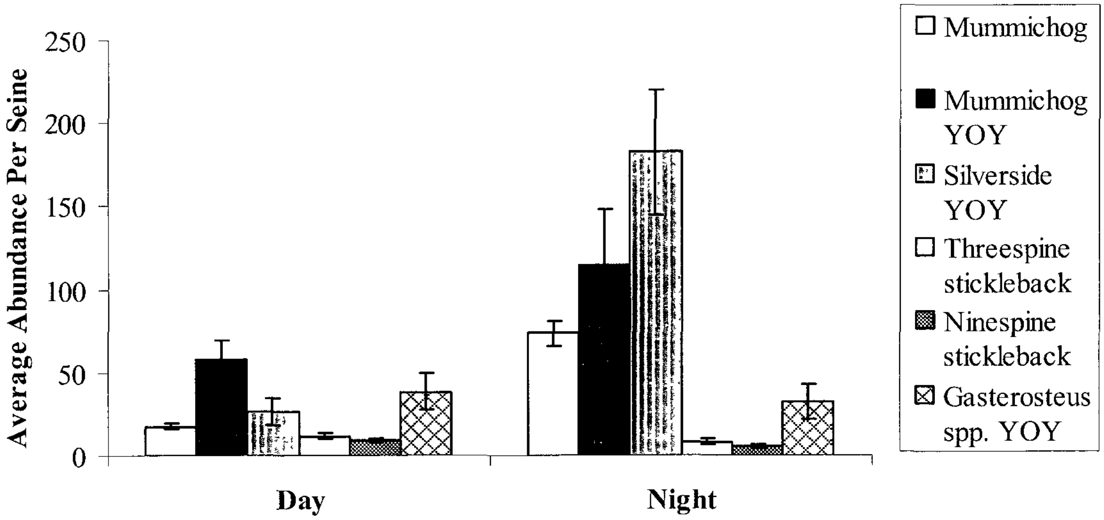

Figure 3.3. Average abundance per beach seine haul of the fish species contributing most to the disparity in the fish assemblage during the day and at night (n = 48 for day samples and n = 24 at night). Error bars are standard error.

the only environmental variable to differ between day and night samples, was not significantly correlated with any measure of community structure (abundance, richness, diversity).

3.4.4 Diel and monthly differences in the fish community

The fish community differed significantly between June, July, and August (two-way ANOSIM, Global R = 0.859, p = 0.001) although less so during the diel periods (two-way ANOSIM, Global R = 0.329, p = 0.001) (Table 3.4). Temporal variation among months was examined with both adults and YOY and when YOY were excluded (Figure 3.4). Differences in the entire fish community were primarily due to the higher numbers of northern pipefish and blackspotted, threespine and fourspine stickleback in June, *Gasterosteus* spp. YOY in July, fourspine stickleback YOY and silverside YOY in July and August and mummichog YOY in August (SIMPER analysis, Figure 3.5A, Appendix B.7). If YOY are excluded from the analysis the temporal difference is not as great; however the diel difference in the fish assemblage increases slightly (Global R = 0.595 and 0.349 respectively) (Table 3.4). Differences in the adult fish assemblage are the result of more northern pipefish and blackspotted, threespine, and ninespine stickleback in June, and more fourspine stickleback and mummichog in July and August (SIMPER analysis, Figure 3.5B, Appendix B.8).

Given the large temporal variation, diel differences in the fish community were analyzed (with adults and YOY and when YOY are excluded [Figure 3.6]) by separate one-way ANOSIM tests within each month. Diel differences, although very similar, were found to be higher in August (Global R = 0.476, p = 0.001), were slightly lower in June (Global R = 0.470, p = 0.001) and differed least in July (Global R = 0.460,

Table 3.4. Summary of two-way ANOSIM test results for the temporal variation in the fish community between months and between day and night with (A) adults and YOY and (B) YOY excluded.

(A)

Pairwise groups	**Global R value***	**Significance level**
Months combined	0.859	0.001
June vs. July	0.937	0.001
June vs. August	1.000	0.001
July vs. August	0.566	0.001
Day vs. Night	0.329	0.001

(B)

Pairwise groups	**Global R value**	**Significance level**
Months combined	0.595	0.001
June vs. July	0.520	0.001
June vs. August	0.808	0.001
July vs. August	0.523	0.001
Day vs. Night	0.349	0.001

*Global R 1.00-0.750 = well separated communities, 0.750-0.5 = some overlap in community structure but remaining different, 0.5-0.250 = overlap, still somewhat different, 0.250-0 = lots of overlap, little difference (Clarke and Warwick 2001).

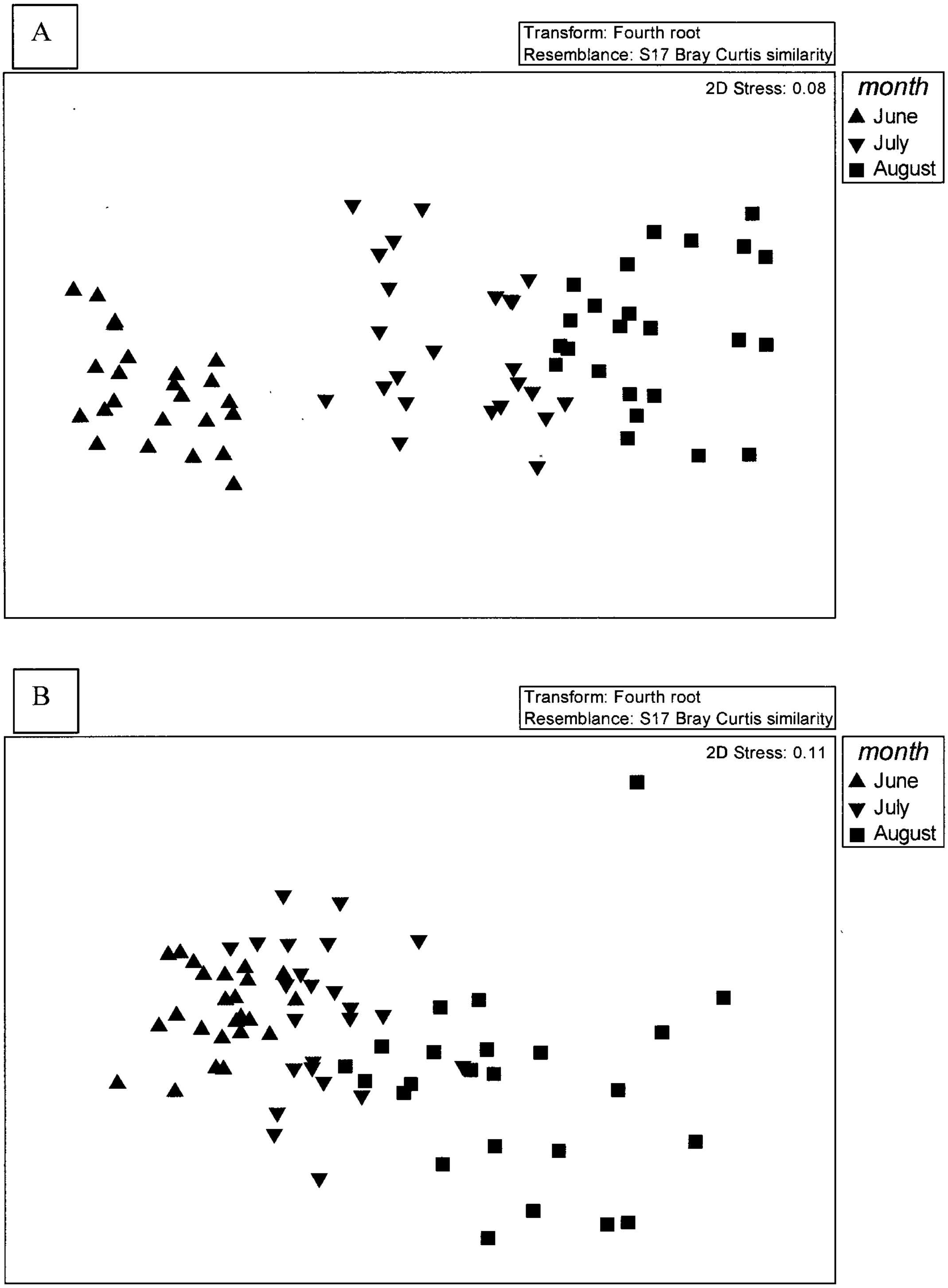

Figure 3.4. Non-metric multi-dimensional scaling (nMDS) plot illustrating temporal variation in the fish community throughout the three sampling months with A) adults and young-of-the-year (YOY) and B) YOY excluded. Each point represents one beach seine (n = 24 in each month).

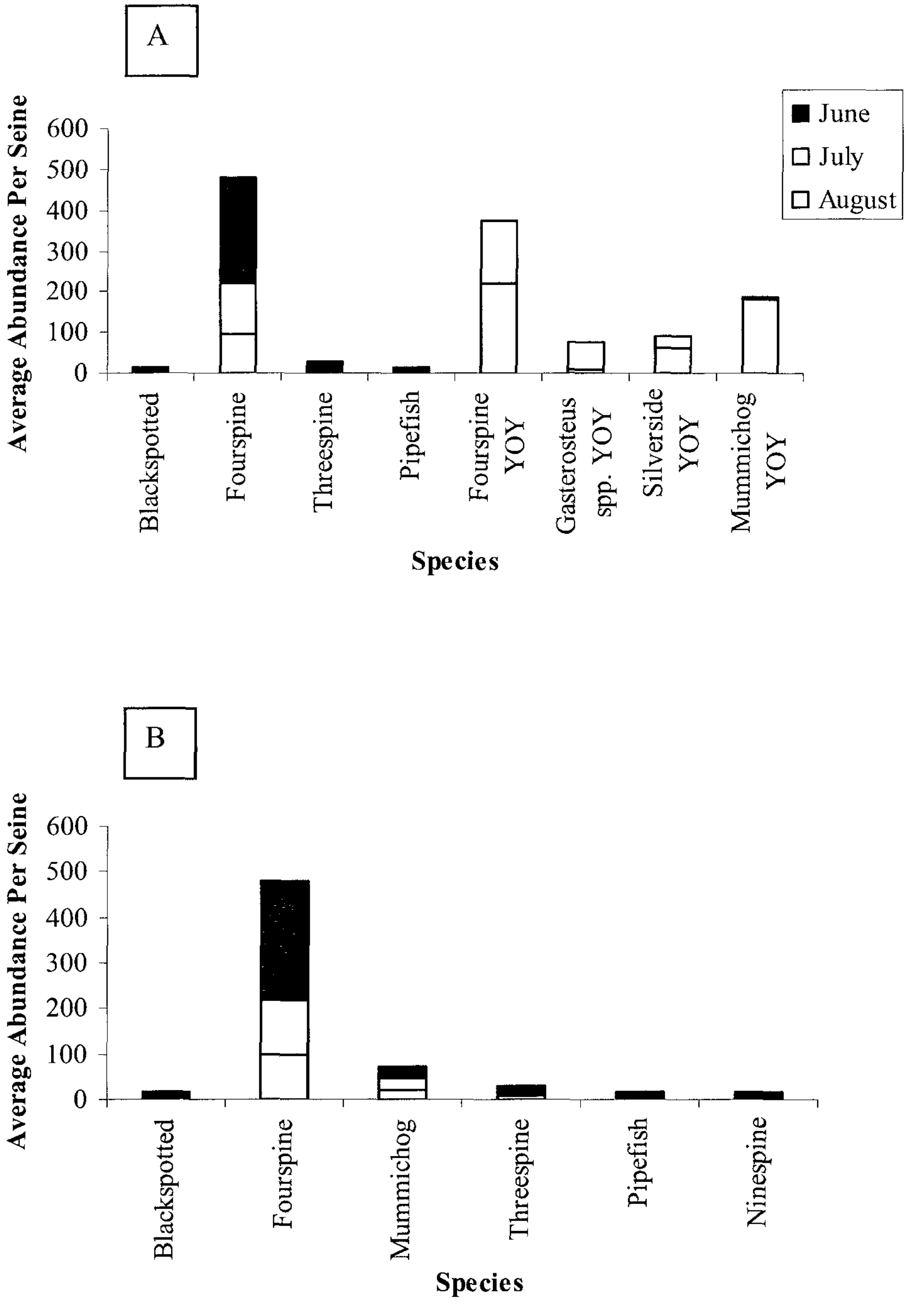

Figure 3.5. Average abundance (back transformed from fourth root data used in the analysis) per beach seine haul of the fish species contributing most to the monthly variation in the fish community throughout the sampling season by means of a SIMPER analysis with A) adults and young-of-the-year (YOY) and B) YOY excluded.
Note: threespine, fourspine, ninespine, and blackspotted are all stickleback species.

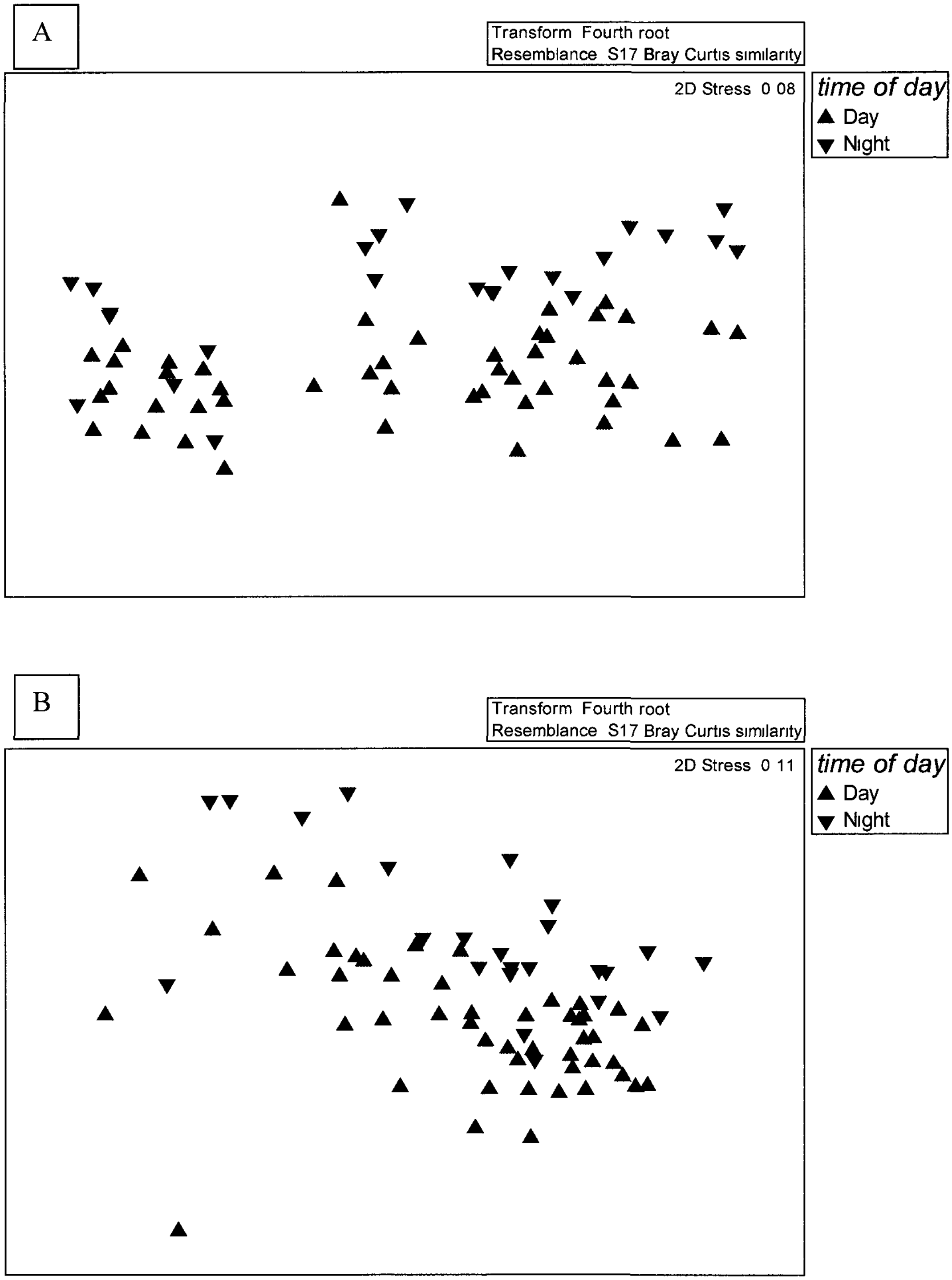

Figure 3.6. Non-metric multi-dimensional scaling (nMDS) plot illustrating day and night variation in the fish community throughout the three sampling months with A) adults and young-of-the-year (YOY) and B) YOY excluded. Each point represents one beach seine (n = 48 during the day and n = 24 at night).

p = 0.001). During day seines in June there were higher numbers of fourspine YOY, cunner, and *Gasterosteus* spp. YOY. During night seines in June there were more Atlantic silverside, American eel, and mummichog. In July, day catches had more mummichog YOY, northern pipefish YOY, *Gasterosteus* spp. YOY and blackspotted and ninespine stickleback, while night catches showed higher numbers of Atlantic silverside YOY and mummichog. Day catches in August had more *Gasterosteus* spp. YOY, northern pipefish YOY, and adult and YOY ninespine stickleback, while night catches once again were dominated by Atlantic silverside YOY and mummichog (SIMPER analysis, Table 3.5).

Excluding YOY from the analysis, diel differences in the adult fish community were greatest in June (Global R = 0.463, p = 0.001), while differing to a lesser extent in July and August (Global R = 0.393 and 0.190, respectively, p = 0.001 for both). Day seines in June had more cunner and fourspine, threespine and ninespine stickleback while night catches had higher numbers of Atlantic silverside, mummichog, and American eel. In July, day catches had more blackspotted and ninespine stickleback, while night catches showed higher numbers of mummichog and northern pipefish. In August, day catches were dominated by ninespine and threespine stickleback and northern pipefish, while night seines continued to show higher numbers of mummichog (SIMPER analysis, Table 3.6).

Table 3.5. SIMPER results of day and night differences in the fish community within each month (adults and YOY) showing species contributing more than 4% to the dissimilarity in the community. Abundances are back-transformed from the fourth root data used in the analysis.

Month	**Average dissimilarity**	**Species contributing**	**Average abundance/ 100 m^2 during the day**	**Average abundance/ 100 m^2 at night**	**Contribution to dissimilarity (%)**	**Cumulative contribution to dissimilarity (%)**
June	17.65 %	*Gasterosteus* YOY	1.31	0.04	15.79	15.79
		Mummichog	18.72	53.14	13.39	29.18
		Silverside	0.0007	0.30	12.4	41.58
		Fourspine YOY	0.05	0.005	9.12	50.7
		Cunner	0.05	0.0002	8.07	58.76
		American eel	0.00002	0.015	6.18	64.95
		Fourspine	274.40	240.98	6.09	71.03
		Atlantic tomcod YOY	0.002	0.002	5.78	76.81
		Pipefish	12.23	8.96	5.7	82.51
		Blackspotted	17.66	11.71	5.11	87.62
July	22.05%	Silverside YOY	7.23	210.72	21.46	21.46
		Mummichog YOY	9.60	1.75	13.14	34.61
		Mummichog	9.82	91.17	12.71	47.32
		Pipefish yoy	2.01	0.33	9.49	56.81
		Gasterosteus YOY	65.05	74.71	6.91	63.71
		Fourspine YOY	157.04	133.63	6.01	69.72
		Blackspotted	1.94	0.50	5.74	75.45
		Pipefish	4.93	5.92	4.45	79.9
		Ninespine	9.82	4.80	4.17	84.08
August	22.54%	Silverside YOY	22.17	263.77	18.97	18.97

Table 3.5 continued

	Gasterosteus YOY	10.04	3.42	8.78	27.75
	Mummichog	13.03	48.58	8.62	36.37
	Pipefish YOY	4.93	2.86	8.59	44.96
	Mummichog YOY	135.21	320.16	8.54	53.5
	Ninespine YOY	0.72	0.15	7.92	61.42
	Ninespine	2.22	0.60	7.52	68.94
	Threespine	0.81	0.02	7.42	76.36
	Gaspereau spp. YOY	0.0002	0.11	6.16	82.53
	Pipefish	0.05	0.03	5.34	87.86
	Fourspine YOY	222.00	226.63	5.27	93.14

Note: threespine, fourspine, ninespine, and blackspotted are all stickleback species, which is true for tables 3.5-3.6 inclusively.

Table 3.6. SIMPER results of day and night differences in the fish community within each month (YOY excluded from analysis) showing species contributing more than 4% to the dissimilarity in the community. Abundances are back-transformed from the fourth root data used in the analysis.

Month	**Average dissimilarity**	**Species contributing**	**Average abundance/ 100 m^2 during the day**	**Average abundance/ 100 m^2 at night**	**Contribution to dissimilarity (%)**	**Cumulative contribution to dissimilarity (%)**
June	13.38%	Mummichog	19.82	54.74	19.53	19.53
		Silverside	0.0005	0.48	19.33	38.86
		Cunner	0.04	0.0003	11.02	49.88
		American eel	0.00001	0.03	9.57	59.45
		Fourspine	271.71	240.98	9.26	68.71
		Pipefish	11.71	9.38	7.99	76.7
		Blackspotted	16.32	12.76	6.57	83.27
		Threespine	24.73	17.66	6.45	89.72
		Ninespine	11.22	7.78	6.39	96.11
July	17.74%	Mummichog	9.82	91.16	31.39	31.39
		Blackspotted	1.94	0.50	14.07	45.46
		Pipefish	4.93	5.92	10.96	56.41
		Ninespine	9.82	4.80	10.04	66.46
		Threespine	6.39	6.23	8.98	75.44
		Fourspine	122.96	125.94	8.98	84.42
		Silverside	0.004	0.0003	7.52	91.94
August	23.78%	Mummichog	13.03	48.58	25.11	25.11
		Ninespine	2.22	0.60	21.70	46.81
		Threespine	0.81	0.02	20.83	67.64
		Pipefish	0.05	0.03	14.81	82.45
		Fourspine	87.68	115.74	13.16	95.61

3.5 Discussion

This study examined diel and monthly variation in the nearshore fish community living in an eelgrass habitat in the Midgell River estuary, Prince Edward Island. Fish assemblages in the area were similar to those reported in other studies in the southern Gulf of St. Lawrence (e.g. Joseph et al. 2006; Thériault 2006, Weldon et al. 2008) and to a lesser extent those from the northeastern United States (e.g Orth and Heck 1980; Heck et al. 1989; Lubbers et al. 1990; Mattila et al. 1999; Lazzari 2002 among others). Typically, a greater number of species were captured in studies conducted further south, which could represent a latitudinal gradient in species richness. Differences in sampling gear among these studies could also be contributing to the variation in number of species captured. In the present study an active sampling method, beach seining, was used which is a common technique for sampling nearshore estuarine habitats and therefore deemed appropriate for sampling the eelgrass ichthyofauna.

Throughout the six diel periods, the fish assemblages showed prominent differences. As noted by Gray et al. (1998), nocturnal sampling efforts allow the recognition of habitat utilization by certain fish species that would not be otherwise observed by day sampling. Had sampling been restricted to a diurnal time scale stickleback species would have incorrectly reported as the primary inhabitants of eelgrass habitat, as Atlantic silverside (*M. menidia*) and mummichog (*F. heteroclitus*) were found in lower numbers during the day. In particular, the abundance of Atlantic silversides would have been seriously underestimated. No species was caught exclusively during diurnal or nocturnal samples. This contradicts results by Hagan and Able (2008) who captured threespine stickleback (*G. aculeatus*), Atlantic menhaden (*Brevoortia tyrannus*) and silver anchovy (*Engraulis eurystole*) solely during night samples. Further, Joseph et al. (2006) captured American eel (*A. rostrata*) exclusively during night catches. It remains apparent that to properly understand habitat usage by various fish species, sampling must be completed during both diurnal and nocturnal periods (Gray et al. 1998; Rountree and Able 1993).

Increased numbers of fish captured at night is not uncommon. Hagan and Able (2008) found that total numbers of fish captured in the Great Bay estuary of southern New Jersey were higher during the night throughout the majority of the sampling season which spanned over one year. Joseph et al. (2006), while studying in the Kouchibouguac estuary in New Brunswick, Canada, also captured a greater number of individuals at night. Similar results were observed by Robertson (1980) (southeastern Australia), Lubbers et al. (1990) (Chesapeake Bay), and Methven et al. (2001) (Trinity Bay, Newfoundland). Lubbers et al. (1990) found highest and lowest fish abundances occurred around midnight and dawn, respectively. Findings were similar in this study as the most fish were usually captured in the 22:00 h seines and lowest abundances always occurred in the 6:00 h samples, which could be related to differences in environmental parameters (i.e. oxygen, see below).

Higher numbers of fish in night samples were due to the increased numbers of adult mummichog and Atlantic silverside YOY; American eel was also captured more frequently at night, but in low numbers. High nocturnal abundances of Atlantic silverside YOY appears to be consistent with findings of past studies. Rountree and Able (1993) found that the mean abundance of Atlantic silversides captured in subtidal seine samples during the day was 536 (± 249 SE), while at night abundance was 3087 (± 1146). In this study we report that the mean day and night abundance of Atlantic silverside YOY is 26.7

(± 8.13) and 182.4 (± 37.47) respectively. Although total numbers were notably lower in this study (possibly due to differences in seine size) the variation between day and night was consistent. Lubbers et al. (1990) and Hagan and Able (2008) documented higher numbers of Atlantic silverside during night samples as well. However, Hagan and Able (2008) found numbers of Atlantic silverside YOY were not consistently higher at night; during the late summer, day catches tended to be dominated by Atlantic silverside YOY, a phenomenon not observed in the current study. Higher numbers of mummichog at night was not as commonly noted. Although Rountree and Able (1993) did sample more mummichog at night, their results were not significant. In contrast, Heck et al. (1989) found that the numbers of mummichog captured in eelgrass habitats was substantially higher during day samples.

Daily patterns of habitat use may be strongly related to changes in behaviour due to foraging opportunities and predator avoidance. Robertson and Howard (1978) found that silver fish (*Atherinasoma presbyteroides*) and yellow-eye mullet (*Aldrichetta forsteri*), which are visual planktivores, fed in eelgrass beds during the day when calanoid copepods, a major prey item, were easier to locate and thus both fish species were more prevalent during day catches. Further, Robertson (1980) found that common weedfish (*Clinus perspicillatus*) and south Australian cobbler (*Gymnapistes marmoratus*) were captured more frequently in eelgrass at night when prey species such as amphipods and spider crabs (*Halicarcinus* spp.) were more active. Ninespine stickleback (*P. pungitius*) are known to be visual predators that feed on calanoid copepods, isopods, and amphipods (Antholz et al. 1991), which use eelgrass blades as habitat (Deegan 2002), and therefore their dominance in day catches during this study may be related to feeding activity. Interestingly, threespine stickleback are also diurnal feeders (Beukema 1968); they were also captured more frequently during the day in our study, as well as those conducted by Heck et al. (1989) and Joseph et al. (2006). Conversely, Hagan and Able (2008) captured them exclusively at night and stated that this could have been the effect of gear selectivity or avoidance.

Diel patterns in mummichog abundance may also be the result of foraging activities; however, mummichogs are known to be visual day feeders (Weisberg et al. 1981; Clark et al. 2003). Weisberg et al. (1981) found that mummichog feeding peaks occurred during day hours and tended to correspond to high tides. Clark et al. (2003) sampled significantly more mummichog in nearshore shallow waters during the day than during night hours, which corresponded to the higher abundance of grass shrimp (*Palaemonetes pugio*) (a preferred food item) during the day. Although these findings are not consistent with other studies, higher numbers of mummichogs sampled at night during the present study may also be related to food availability. Schein (2009) found that mummichog diet in the Stanley River estuary, Prince Edward Island was composed primarily of gammarid amphipods. The numbers of small invertebrates such as amphipods increased substantially during night seine samples (pers. obs.); small invertebrates tend to inhabit seagrass beds since they act as a refuge from water currents (Pihl et al. 2006). The increase in mummichog abundance during night catches may therefore be a response to habitat-mediated prey activity and availability.

In contrast, the high nocturnal abundance of Atlantic silverside YOY is more likely the result of predator avoidance. Rountree and Able (1993) who studied diel fish assemblage differences in marsh creeks, suggested that Atlantic silverside YOY move into shallower areas at night due to higher abundances of predators (Northern barracuda, *S. borealis* and bluefish, *P. saltatrix*) moving inshore from deeper waters at night. Mattila et al. (1999) also suggested that higher numbers of some fish at night may be the result of nearshore movement to avoid predation by larger fish species. It is possible that this was the driving force for Atlantic silverside YOY movement in our study, as larger predatory species (e.g. Atlantic tomcod, *Microgadus tomcod*) could be moving inshore from deeper waters during night hours.

In addition to foraging activity and predator avoidance, differential abundance patterns could be a response to changes in water parameters. In particular, low total abundances in 6:00 h seines may have been the result of lower dissolved oxygen levels. This response was particularly noticeable on July 11th, and Aug 6th when morning dissolved oxygen levels dropped below 4 mg/L. Wannamaker and Rice (2000) conducted experimental trials on a variety of fish species' responses to changes in dissolved oxygen concentrations. They concluded that spot (*Leiostomus xanthurus*), pinfish (*Lagodon rhomboids*), white mullet (*Mugil curema*), and mummichog (*F. heteroclitus*), detected and behaviourally responded to low water oxygen concentrations. The most typical response was to avoid the areas of lowest oxygen concentrations. Phil et al. (1991) also concluded that fish generally move out of areas in which low oxygen concentrations occurred. Fluctuations in water depth have also been shown to alter fish abundances (Rozas 1995). Although water levels were typically higher at night, depth changes were minor and are not believed to be the driving factor which influenced fish movement patterns in this study; as the abundance pattern remained the same despite the change in tides. Predation pressures and foraging opportunities likely lead to the nocturnal abundance patterns observed (as discussed above); however the influence of water parameters, such as oxygen, given the large fluctuation between dusk and dawn, may also be important.

Monthly differences in the eelgrass fish community were largely due to the emergence of young-of-the-year (YOY) species as the summer progressed, a common trend in estuarine studies. Eelgrass beds are noted as key nursery and spawning areas (Deegan et al. 1997; Heck et al. 2003), this was particularly noticeable in our study as adult fish dominated the population in June, while juveniles dominated in July and August. When YOY were excluded from the analysis temporal patterns for all adults followed a clear pattern, numbers were highest in June and consistently decreased throughout subsequent sampling months. Hagan and Able (2003) found that the estuarine fish assemblage differed temporally with larval fishes contributing heavily to seasonal differences. Lazzari et al. (1999) also found that increased recruitment, corresponding to higher water temperatures during summer catches, resulted in a marked increase in species richness and abundance. Although this study focused on a relatively short time period, it included both the pre- and post-recruitment periods and changes in the fish assemblages were clearly noted during the sampling period. It is not believed that spawning activity played a role in dictating diel changes, as the patterns remained consistent prior to and following the emergence of larval fishes.

In summary, it was apparent that restricting sampling to daylight hours would have underestimated the abundance of fish species and given an unrepresentative view of the entire fish assemblage in eelgrass beds. However, it was interesting to note the similarity in the entire fish assemblage among diel sampling periods. Differences in the diel fish community were likely strongly behaviour-mediated and related to patterns of food abundance and predator-prey interactions with variability in certain environmental parameters playing a secondary role. Diel sampling is essential in understanding changes in abundance patterns and seasonal abundance (Heck et al. 1989; Rountree and Able, 1993; Hagan and Able 2008; this study), as well as species interactions (i.e. predator-prey) (Rountree and Able 1993; Mattila et al. 1999). This study shows the benefits of incorporating both diurnal and nocturnal sampling efforts into any sampling regime that examines estuarine fish assemblages and associated habitat use by various fish species.

3.6 Literature Cited

Antholz, B., Meyer-Antholz, W., Zander, C.D. 1991. Feeding activities of two euryhaline small-sized fish in a western Baltic brackish fjord. *Helgoländer Meeresunters* **45**: 287-300.

Beukema, J. 1968. Predation by the three spined stickleback (*Gasterosteus aculeatus* L.): the influence of hunger and experience. *Behaviour* **31**: 1- 126.

Clark, K.L., Ruiz, G.M., Hines, A.H. 2003. Diel variation in predator abundance, predation risk and prey distribution in shallow-water estuarine habitats. *Journal of Experimental Marine Biology and Ecology* **287**: 37-55.

Clarke, K., R., Warwick, R., M. 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E: Plymouth, UK.

Deegan, L.A. 2002. Lessons learned: the effects of nutrient enrichment on the support of nekton by seagrass and salt marsh ecosystems. *Estuaries* **25**: 727-742.

Deegan, L.A., Finn, J.T., Ayvazian, S.G., Ryder-Kieffer, C.A., Buonaccorsi, J. 1997. Development and validation of an estuarine biotic integrity index. *Estuaries* **20**: 601-617.

Gray, C.A., Chick, R.C., McElligott, D.J. 1998. Diel changes in assemblages of fishes associated with shallow seagrass and bare sand. *Estuarine, Coastal and Shelf Science* **46**: 849-859.

Hagan, S.M. Able, K.W. 2003. Seasonal changes of the pelagic fish assemblage in a temperate estuary. *Estuarine, Coastal and Shelf Science* **56**: 15-29.

Hagan, S.M. Able, K.W. 2008. Diel variation in the pelagic fish assemblage in a temperate estuary. *Estuaries and Coasts* **31**: 33-42.

Heck, K.L., Able, K.W., Fahay, M.P., Roman, C.T. 1989. Fishes and decapod crustaceans of Cape Cod eelgrass meadows: species composition, abundance patterns and comparison with unvegetated substrates. *Estuaries* **12**: 59-65.

Heck, K.L., Hays, G., Orth, R.J. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress Series* **253**: 123-136.

Hughes, J.E., Deegan, L.A., Weaver, M.J., Costa, J.E. 2002a. Regional application of an index of estuarine biotic integrity based on fish communities. *Estuaries* **25**: 250-263.

Hughes, J., Deegan, L.A., Wyda, J.C., Weaver, M.J., Wright, A. 2002b. The effects of eelgrass habitat loss on estuarine fish communities of southern New England. *Estuaries* **25**: 235-249.

Joseph, V., Locke A., Godin J.J. 2006. Spatial distribution of fishes and decapods in eelgrass (*Zostera marina* L.) and sandy habitats of a New Brunswick estuary, eastern Canada. *Aquatic Ecology* **40**: 111-123.

Lazzari, M.A. 2002. Epibenthic fishes and decapod crustaceans in northern estuaries: a comparison of vegetated and unvegetated habitats in Maine. *Estuaries* **25**: 1210-1218.

Lazzari, M.A., Sherman, S., Brown, C.S., King, J., Joule, B.J., Chenoweth, S.B., Langton, R.W. 1999. Seasonal and annual variations in abundance and species composition of two nearshore fish communities in Maine. *Estuaries* **22**: 636-647.

Lubbers, L., Boynton, W.R., Kemp, W.M. 1990. Variations in structure of estuarine fish communities in relation to abundance of submersed vascular plants. *Marine Ecology Progress Series* **65**: 1-14.

Mattila, J. Chaplin, G., Eilers R., Heck, K.L., O'Neal J.P., Valentine J.F. 1999. Spatial and diurnal distribution of invertebrate and fish fauna of a *Zostera marina* bed and nearby unvegetated sediments in Damariscotta River, Maine (USA). *Journal of Sea Research* **41**: 321-332.

Methven, D.A., Haedrich, R.L., Rose, G.A. 2001. The fish assemblage of a Newfoundland estuary: diel, monthly and annual variation. *Estuarine, Coastal and Shelf Science* **52**: 669-687.

Nemerson, D.M., Able, K.W. 2004. Spatial patterns in diet and distribution of juveniles of four fish species in Delaware Bay marsh creeks: factors influencing fish abundance. *Marine Ecology Progress Series* **276**: 249-262.

Orth, R.J., Heck, K.L. 1980. Structural components of eelgrass (*Zostera marina*) meadows in the lower Chesapeake Bay- Fishes. *Estuaries* **3**: 278-288.

Orth, R.J., Heck, K.L., Van Montfrans, J. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries* **7**: 278-286.

Pihl, L.S., Baden, S.P., Diaz, R.J. 1991. Effects of periodic hypoxia on distribution of demersal fish and crustaceans. *Marine Biology* **108**: 349-360.

Pihl, L., Baden, S., Kautsky, N., Ronnback, P., Soderqvist, T., Troell, M., Wennhage, H. 2006. Shift in fish assemblage structure due to loss of seagrass *Zostera marina* habitats in Sweden. *Estuarine, Coastal and Shelf Science* **67**: 123-132.

Rountree, R.A., Able, K.W. 1993. Diel variation in decapod crustacean and fish assemblages in New Jersey polyhaline marsh creeks. *Estuarine, Coastal and Shelf Science* **37**: 181-201.

Robertson, A.I. 1980. The structure and organization of an eelgrass fish fauna. *Oecologia* **47**: 76-82.

Robertson, A.I. Howard, R.K., 1978. Diel trophic interactions between vertically-migrating zooplankton and their fish predators in an eelgrass community. *Marine Biology* **48**: 207-213.

Rozas, L.P. 1995. Hydroperiod and its influence on nekton use of the salt marsh: a pulsing ecosystem. *Estuaries* **18**: 579–590.

Schein, A. 2009. The estuarine fish community and food web structure in areas of sea lettuce (*Ulva lactuca*) and eelgrass (*Zostera marina*) within the Stanley River estuary Prince Edward Island. M.Sc. Thesis. University of Prince Edward Island, Charlottetown, PEI.

Stoner, A.W. 1991. Diel variation in the catch of fishes and penaeid shrimps in a tropical estuary. *Estuarine, Coastal and Shelf Science* **33**: 57-69.

Thériault, M-H. 2006. Les effets des effluents d'usines de transformation des produits marins sur la communauté de poissons côtiers et sur deux espèces sentinelles dans le sud du golfe du Saint Laurent. M.Sc. Thesis. Université de Moncton, Moncton, NB.

Wannamaker, C.M., Rice, J.A. 2000. Effects of hypoxia on movements and behaviour of selected estuarine organisms from the southeastern United States. *Journal of Experimental Marine Biology Ecology* **249**: 145-163.

Weisberg, S.B., Whalen, R., Lotrich, V.A. 1981. Tidal and diurnal influence on food consumption of a salt marsh killifish *Fundulus heteroclitus*. *Marine Biology* **61**: 243-246.

Weldon, J., Courtenay, S. and Garbary, D. 2008. The Community Aquatic Monitoring Program (CAMP) for measuring Marine Environmental Health in Coastal Waters of the southern Gulf of St. Lawrence: 2007 Overview. *Canadian Technical Report of Fisheries and Aquatic Science* 2825: viii + 75 p.

Wyda, J.C., Deegan, L.A., Hughes, J.E., Weaver, M.J. 2002. The response of fishes to submerged aquatic vegetation complexity in two ecoregions of the Mid-Atlantic Bight: Buzzards Bay and Chesapeake Bay. *Estuaries* **25**: 86-100.

CHAPTER 4: INVESTIGATING FISH COMMUNITY RESPONSES TO VARIABILITY IN EELGRASS (*ZOSTERA MARINA*) HABITAT CONDITION IN EIGHT PRINCE EDWARD ISLAND ESTUARIES

4.1 Abstract

Seagrasses increase habitat complexity within estuaries and play a central role in supporting fish assemblages. However, anthropogenic activities within watersheds are prompting eutrophication of estuaries and altering estuarine seagrass habitat structure. The fish community and associated eelgrass habitat in eight estuaries in Prince Edward Island (PEI) were sampled to determine how the structure of the community differed depending on the characteristics of the eelgrass (*Zostera marina*) habitat. Beach seines were conducted at ten sites in each of the eight estuaries in June and August 2009. The sites were chosen based on variability in eelgrass condition (shoot density, average canopy height, percent cover, above-ground biomass, and epiphyte biomass) within and between estuaries. The adult fish community in both June and August was influenced most by variability in water temperature, salinity and dissolved oxygen, while in August young-of-the-year (YOY) responded to water quality measures as well as eelgrass complexity. Individual species responses to eelgrass measures showed that numbers of *Gasterosteus* spp. YOY, winter flounder YOY, cunner YOY, and northern pipefish YOY were correlated with higher eelgrass shoot density, while mummichog numbers were negatively correlated with higher eelgrass complexity. These results confirm the nursery role of eelgrass within the estuarine environment.

4.2 Introduction

Seagrass beds are an important aspect of estuarine ecosystems, and serve as key habitats for numerous fish and invertebrate species (Orth and Heck 1980, Orth et al. 1984, Heck et al. 1989, Deegan et al. 1997, Hughes et al. 2002, Wyda et al. 2002). Seagrasses such as eelgrass (*Zostera marina*) provide smaller fish protection from predators (Orth and Heck 1980) while at the same time harbouring considerable quantities of food (Orth et al. 1984; Lubbers et al. 1990). For these reasons, many fish utilize eelgrass beds as nursery habitat (Deegan et al. 1997; Gotceitas et al. 1997; Heck et al. 2003). However nutrient enrichment, particularly nitrogen loading as the result of anthropogenic activities within watersheds, is resulting in seagrass decline (Hauxwell et al. 2001; Deegan 2002). Most notably, nutrient additions are resulting in enhanced growth of macro and epiphytic algae (Borum and Wium-Anderson 1980; Borum 1985; Valiela et al. 1997; Hauxwell et al. 2001) that impose negative impacts on seagrass by competing for light and space.

Light limitations caused by macroalgae (e.g. sea lettuce, *Ulva lactuca*) increase shoot mortality, limit growth, and decrease shoot density of eelgrass (Duarte 1995; Short et al. 1995, Hauxwell et al. 2001; Deegan et al. 2002; Hauxwell et al. 2003, this study - see Chapter 2). Further, insufficient light can reduce recruitment and prevent seedling growth (Hauxwell et al. 2001). Increased epiphyte loads on eelgrass blades decrease photosynthetic ability and seagrass biomass by decreasing blade growth rates and accelerating blade loss (Neckles et al. 1993; Williams and Ruckelshaus 1993; Philippart 1995). Changes in the physical complexity of the seagrass habitat as a result of increased shoot mortality and blade loss may have important implications with respect to fish species abundance and richness as these may reduce habitat complexity (Orth and Heck 1980; Deegan et al. 1997; Wyda et al. 2002).

Within the estuarine environment, blades of seagrass plants provide a complex habitat that allows for more faunal associations (Bell and Westoby 1986). Although the physical characters (i.e. shoot density, height, cover) vary between seagrass beds, previous examinations of seagrass bed habitat have shown that they support higher numbers of fish species as well as higher numbers of individuals, especially when compared to bare areas (Orth and Heck 1980; Heck et al. 1989; Lazzari et al. 2003). Heck et al. (1989) found that the mean abundance for fish in eelgrass beds was eight times higher than that for a sandy substrate. Hughes et al. (2002) found that estuarine habitats with high eelgrass shoot density and thick canopy cover have significantly higher species richness when compared to habitats with intermediate to low eelgrass density although areas that support even sparse eelgrass beds have more diverse fish communities than areas with no vegetation.

The significance of eelgrass habitats to fish communities within the estuarine and coastal environment is often assessed based on comparisons with sandy substrates (see Heck et al. 1989; Mattila et al. 1999; Lazzari 2002; Joseph et al. 2006 among others). A few studies have looked at seagrass complexity on a narrower scale, categorizing regions of varying eelgrass density into zero, low, and high complexity and examining fish community differences (see Hughes et al. 2002; Wyda et al. 2002). While the relationship between dense eelgrass habitats and abundant and species rich fish communities have been established, little information is available concerning the relationship between fish communities and eelgrass bed condition on a finer scale.

On Prince Edward Island (PEI), Canada, eutrophication of estuaries is resulting in dramatic shifts in estuarine structure. The most noticeable response is the proliferation of sea lettuce and a corresponding decline in the growth and abundance of eelgrass. Changes in vegetation structure have profound implications with respect to the ecological functioning and value of these regions. This study examined the responses of the near-shore fish community (abundance, species richness, diversity) to variation in eelgrass habitat condition. The eelgrass habitat was assessed based on five chosen metrics: shoot density, percent cover, above-ground biomass, canopy height, and epiphyte biomass.

4.3 Methods

4.3.1 Study area

The study sites were Kildare River, Freeland Creek, Brooks River, Bideford River, Stanley River, Wheatley River, Tracadie Bay, and Midgell River estuaries located on the north shore of Prince Edward Island (Figure 4.1). Kildare, Freeland, Stanley, and Midgell River estuaries were chosen based on previous knowledge of eelgrass presence within the estuary. The remaining four were chosen during this study based on the presence of eelgrass and location, such that all eight estuaries spanned much of the north shore of PEI. Prior to sampling, the eelgrass habitat in each estuary was visually assessed for differences in density, canopy height, and percent cover. Based on these differences, ten sampling sites were chosen to provide variability in eelgrass habitat within the estuary (i.e. 80 study sites in total). Each site was sampled in June (7-28) and August (3-22), 2009. Distances between sites depended largely on the size of the estuary. The approximate area of the estuary, area within each estuary in which sampling occurred, and the average distance between sites within estuaries were estimated using MapInfo software. The largest distances between sites occurred in Tracadie, while sites were closest together in Midgell (Table 4.1). Locations of the study sites within each estuary are provided in Appendix C.

4.3.2 Fish sampling

At each site, fish were captured using a beach seine and the pentagonal sampling method outlined in the Chapter 2 (as with the diel sampling, the seine net was brought to shore before emptying the catch). In August, fish were separated into adults and young-of-the-year (YOY). YOY were classified by the following standards: mummichog ≤ 3 cm,

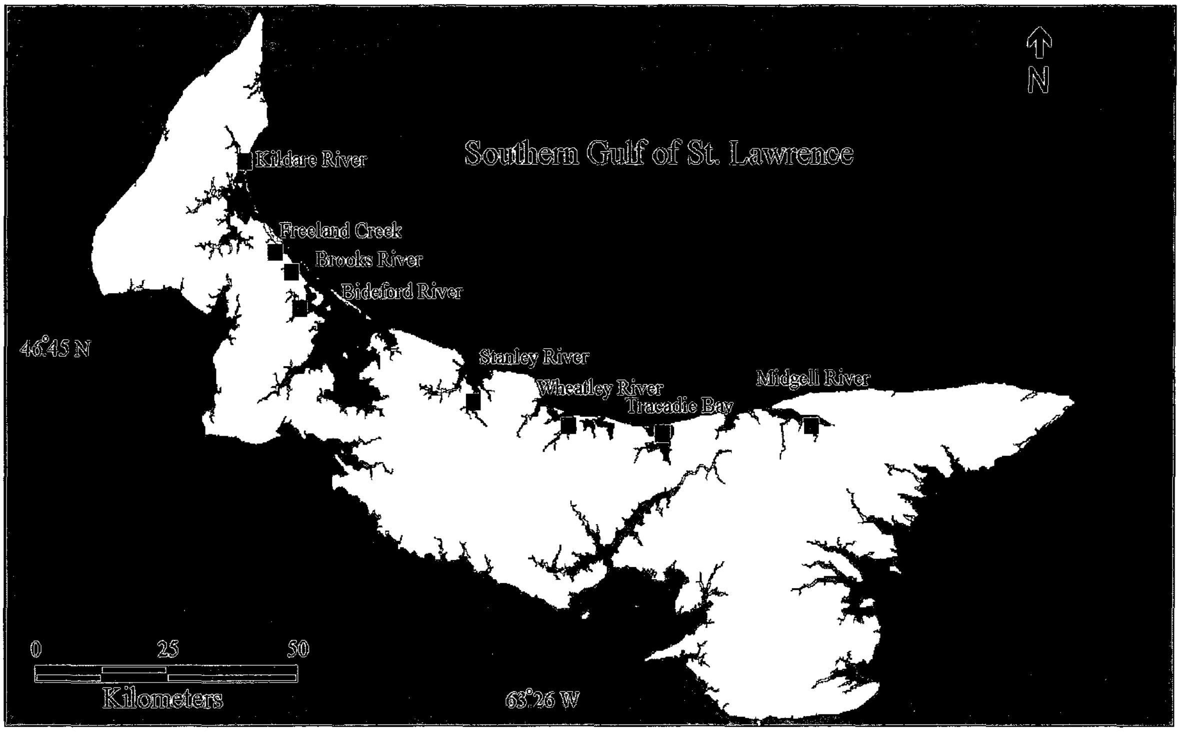

Figure 4.1. Location of the eight estuaries sampled on Prince Edward Island.

Table 4.1. Total area, sampling area, and average distance between sites within each estuary of the eight estuaries sampled in June and August 2009.

Estuary	Total area of entire estuary (km^2) (estimated)	Area within estuary in which sampling occurred (km^2)	Avg. distance between seining sites (km)
Midgell River	0.09	0.05	0.02
Tracadie Bay	18.46	9.11	1.12
Wheatley River	11.86	5.87	0.96
Stanley River	2.55	1.09	0.51
Bideford River	1.63	0.64	0.31
Brooks River	0.95	0.45	0.32
Freeland Creek	0.42	0.25	0.19
Kildare River	3.64	3.15	0.29

Note: areas estimated using MapInfo GIS software.

Atlantic silverside ≤ 8 cm, flounder ≤ 3 cm, stickleback species ≤ 1.5 cm (Weldon et al. 2005).

4.3.3 Habitat descriptions

In the middle of the sampling area water temperature (°C), dissolved oxygen concentration (mg/L), and salinity (ppt) were determined (see Chapter 2). Eelgrass percent cover was determined by walking along three transects within the pentagonal area. One transect was 1 m inside the inshore stakes, one in the middle of the pentagon and one 1 m inside the outer stakes. Using a 0.5 m x 0.5 m viewing frame the percent cover of the eelgrass bed canopy was estimated (within 10%) every meter along each of the three transects. Eelgrass samples were collected using a 10 cm diameter core. Six cores were collected from each sample area in a semi-random manner (one core sample from around each stake and in the middle of the sampling area) to obtain samples that best represented the entire eelgrass bed habitat. Samples were placed in a Ziploc® bag, stored in a cooler on ice and subsequently frozen at -20 °C until processed.

Shoot density, average canopy height, above ground biomass, and epiphyte biomass of each sample were determined. Shoot density of the samples (6 x 10 cm cores) was determined by counting the number of shoots attached to a root system. Average canopy height of the eelgrass was chosen to be the mean length of the longest blade of each eelgrass shoot within the sample (Sogard et al. 1987). Epiphytes were scraped off the eelgrass blades using the edge of a glass microscope slide into distilled water and filtered using vacuum filtration onto pre-combusted and pre-weighed glass fiber filters (Whatman Type GF/A, 4.7cm). Epiphyte biomass was then determined by drying samples at 60 °C for 48 h (Neckles et al. 1993). Above-ground biomass of the eelgrass blades was determined in the same manner as epiphytes (Neckles et al. 1993; Jaschinski and Sommer 2008). Together, the eelgrass/epiphyte biomass, shoot density, average canopy height and percent cover were used to assess eelgrass condition.

4.3.4 Sediment analysis

At the end of each sample in June, sediment was collected from within the sampling area using the same core as for the eelgrass. The top 10 cm of sediment was collected, placed in a Ziploc® bag and frozen at -20 °C until analysis. Samples were analyzed for moisture content, organic content and grain size proportions. Moisture content was determined by decrease in original mass by drying at 60 °C for 24 h. Organic content was determined by loss upon combustion at 550 °C for 3 h. Grain size distribution was established by shaking a portion of the sample through 1 mm (sand) and 63 μm (silt) sieves for approximately 10 min and weighing each size class (Finley 2008).

4.3.5 Data analysis

One-way analysis of variance (ANOVA) was used to test for differences in water parameters and eelgrass characteristics between estuaries within each month to demonstrate variability in habitat characteristics. Tukey's post-hoc tests were used when significant differences were found with an ANOVA for comparisons between estuaries. Principle components analysis was used to graphically show the relationships between eelgrass and water parameters in both June and August.

Multivariate analyses (PRIMER®, v.6.1.6 (PRIMER-E Ltd, 2006, Plymouth, UK) were used to examine monthly differences in the fish community. Fourth root transformations were performed on the fish community data to down weigh the effects of dominant species, allowing the effects of intermediate and rarer species to be more influential at each site (Clarke and Warwick 2001). To examine temporal patterns, analyses were completed with adults only in June and with a) adults and young-of-the-year (YOY) and b) adults only in August. ANOSIM and SIMPER analyses were used to test for significant differences in fish community composition and see which species were contributing most to these differences (Chapter 2).

Fish community structure (abundance/100m^2 of each species, species richness and species diversity) in relation to the various characteristics of eelgrass and water parameters were analyzed using Spearman rank correlations (as various transformations failed to normalize the data). Correlations between water parameters were first assessed and if two variables were highly related ($r > 0.8$) one of the two was dropped from the analysis to reduce the number of variables in the data set. Spearman rank correlation analyses were completed with Statistica® v.8 software (StatSoft, Inc, 2007). Correlations between the fish community measures and water parameters/eelgrass characters were performed with adults in a) June and b) August and c) adults and YOY in August. Spearman rank correlations of abundances of individual species were used to examine the relationship between fish density and eelgrass characters in August to see which species were most influenced by the eelgrass.

4.4 Results

4.4.1 Water parameter and eelgrass variability

There were significant differences in environmental parameters among estuaries in both sampling months. All data are presented in Tables 4.2 and 4.3 for June and August respectively. Between each estuary, variability in eelgrass characters was found. In general, all measured eelgrass characteristics (shoot density, canopy height, percent cover, above-ground biomass, and epiphyte biomass) within each estuary increased from June to August (Tables 4.4 - 4.5). The lone exceptions to this were Stanley and Bideford in which shoot density and percent cover decreased in August.

Principal components analysis revealed that June shoot density (0.87), percent cover (0.86), and above-ground biomass (0.92) were the most discriminating eelgrass variables along axis 1 and were positively associated with salinity, dissolved oxygen (DO), and temperature (Figure 4.2). Canopy (0.74) and epiphyte biomass (0.73) were most discriminating on axis 2 and positively related to temperature and DO. August results showed that shoot density (-0.89), percent cover (-0.90), and above-ground biomass (-0.72) were the most discriminating variables on the first axis and were positively associated with salinity and negatively correlated with temperature and DO (Figure 4.3). Canopy was most important on axis 2 (-0.86) and negatively related to salinity. As environmental and eelgrass variables are interrelated, both are used to determine the influence on the fish community.

4.4.2 Fish community assemblage and temporal patterns

A total of 54,753 individuals of 16 different species were captured during the two sampling periods (Tables C.1 – C.2, Appendix C). Within any given estuary, the fish species composition was dominated by six species: fourspine stickleback (*Apeltes quadracus*), threespine stickleback (*Gasterosteus aculeatus*), blackspotted stickleback (*Gasterosteus wheatlandi*), ninespine stickleback (*Pungitius pungitius*), mummichog (*Fundulus heteroclitus*) and Atlantic silverside (*Menidia menidia*). Multivariate results

Table 4.2. Mean and range of all water parameters in each of the eight estuaries in June 2009. N = 10 sites in each estuary. Significant differences between estuaries for each variable are indicated by different superscripts (Tukey's $p < 0.05$).

	Temperature (°C)		Salinity (ppt)		Dissolved Oxygen (mg/L)		Avg. Water Depth (cm)	
Estuary	Mean (± SE)	Range	Mean (± SE)	Range	Mean (± SE)	Range	Mean (± SE)	Range
Midgell	20.43 (0.47)^{AC}	17.43, 22.32	8.30 (3.91)^{A}	0.36, 26.59	10.99 (0.47)^{AC}	9.52, 13.41	67.54 (3.10)^{A}	57.4, 81.8
Tracadie	19.97 (0.47)^{AC}	17.93, 22.94	25.51 (0.76)^{BC}	19.64, 27.47	11.06 (0.49)^{AC}	8.12, 13.06	64.60 (2.74)^{A}	50.2, 78.0
Wheatley	20.82 (0.57)^{AC}	17.33, 23.50	25.63 (0.79)^{BC}	19.88, 27.82	15.55 (0.90)^{B}	10.80, 21.48	54.82 (4.75)^{A}	30.0, 82.0
Stanley	20.08 (0.39)^{AC}	17.94, 22.22	18.42 (1.05)^{C}	14.34, 24.71	15.25 (0.51)^{B}	12.18, 17.62	69.44 (3.56)^{A}	55.0, 95.4
Bideford	19.39 (0.42)^{AC}	16.54, 21.02	18.87 (2.88)^{C}	4.03, 27.00	11.03 (0.49)^{AC}	9.32, 11.77	56.76 (4.03)^{A}	40.2, 83.2
Brooks	15.55 (0.20)^{B}	14.45, 16.57	27.92 (0.29)^{B}	26.57, 28.81	9.13 (0.18)^{A}	8.44, 10.03	62.04 (3.90)^{A}	38.8, 77.8
Freeland	18.90 (0.77)^{A}	14.66, 21.58	27.85 (0.18)^{B}	26.88, 28.98	11.07 (0.23)^{AC}	10.07, 12.29	56.90 (5.33)^{A}	29.4, 84.8
Kildare	21.33 (0.76)^{C}	18.52, 24.80	25.94 (0.30)^{BC}	23.87, 27.33	11.67 (0.37)^{C}	9.74, 13.12	70.32 (2.52)^{A}	50.6, 78.8

Table 4.3. Mean and range of all water parameters in each of the eight estuaries in August 2009. N = 10 sites in each estuary. Significant differences between estuaries for each variable are indicated by different superscripts (Tukey's $p < 0.05$).

	Temperature (°C)		Salinity (ppt)		Dissolved Oxygen (mg/L)		Avg. Water Depth (cm)	
Estuary	Mean (± SE)	Range	Mean (± SE)	Range	Mean (± SE)	Range	Mean (± SE)	Range
Midgell	24.50 (0.58)^{A}	22.24, 27.07	16.59 (1.41)^{A}	11.29, 21.71	12.03 (0.51)^{AC}	9.27, 14.19	67.12 (3.14)^{A}	53.8, 82.0
Tracadie	21.41 (0.69)^{B}	19.31, 25.84	26.01 (0.28)^{B}	24.82, 27.70	9.93 (0.73)^{A}	7.80, 12.97	74.94 (4.00)^{A}	52.6, 96.4
Wheatley	22.88 (1.06)^{AB}	18.23, 27.13	25.91 (0.36)^{B}	23.94, 27.32	10.79 (0.85)^{ABC}	8.26, 13.58	76.32 (8.07)^{A}	17.8, 99.8
Stanley	25.17 (0.50)^{A}	23.28, 27.42	21.33 (0.41)^{C}	19.63, 23.11	13.67 (0.81)^{C}	9.84, 16.08	63.32 (3.75)^{A}	49.2, 82.8
Bideford	24.31 (0.22)^{A}	23.11, 25.19	25.57 (0.28)^{B}	23.47, 26.25	9.39 (0.72)^{A}	6.15, 13.17	73.30 (3.27)^{A}	58.0, 95.6
Brooks	25.37 (0.52)^{A}	23.22, 27.50	25.54 (0.57)^{B}	22.45, 27.64	11.22 (0.66)^{ABC}	8.03, 15.93	61.80 (4.63)^{A}	35.0, 82.8
Freeland	23.34 (0.57)^{AB}	19.05, 25.28	26.54 (0.25)^{B}	25.53, 28.06	9.74 (0.58)^{A}	6.59, 12.73	68.08 (3.86)^{A}	52.0, 88.4
Kildare	24.70 (0.21)^{A}	23.78, 25.95	25.09 (0.37)^{B}	23.31, 26.58	8.49 (0.85)^{B}	3.34, 12.17	76.02 (5.58)^{A}	47.8, 102.4

Table 4.4. Mean eelgrass variability in each of the eight estuaries in June 2009. N = 10 sites in each estuary. Significant differences between estuaries for each eelgrass character are indicated by different superscripts (Tukey's $p < 0.05$).

	Shoot Density (per six cores)	Canopy Height (cm)	Percent Cover	Above-ground Biomass (g dry wgt)	Epiphyte Biomass (g dry wgt)
Estuary	Mean (± SE)	Mean (± SE)	Mean (± SE)	Mean (± SE)	Mean (± SE)
Midgell	22.8 (4.77)^{A}	26.36 (2.57)^{ABC}	18.11 (4.47)^{A}	3.01 (0.87)^{A}	0.01 (0.003)^{A}
Tracadie	69.1 (4.32)^{B}	27.72 (3.19)^{ABC}	72.4 (5.68)^{B}	9.42 (1.05)^{BC}	0.02 (0.012)^{A}
Wheatley	66.4 (6.78)^{BC}	34.83 (2.84)^{A}	67.92 (6.59)^{BC}	11.2 (0.73)^{B}	0.02 (0.007)^{A}
Stanley	29.8 (3.65)^{A}	28.42 (2.47)^{ABC}	29.76 (7.6)^{A}	6.56 (0.79)^{AC}	0.06 (0.027)^{A}
Bideford	54.9 (4.97)^{C}	22.16 (1.42)^{BC}	60.61 (4.65)^{BC}	5.34 (0.57)^{AD}	0.02 (0.008)^{A}
Brooks	57.8 (7.75)^{BC}	20.78 (1.97)^{B}	57.33 (6.87)^{BC}	5.84 (0.59)^{AE}	0.02 (0.01)^{A}
Freeland	40.9 (3.60)^{AC}	21.53 (1.53)^{B}	42.69 (5.59)^{AC}	4.15 (0.43)^{A}	0.01 (0.002)^{A}
Kildare	69 (9.47)^{BC}	32.14 (3.02)^{AC}	52.01 (8.21)^{BC}	8.44 (1.16)^{BCDE}	0.03 (0.01)^{A}

Table 4.5. Mean eelgrass variability in each of the eight estuaries in August 2009. N = 10 sites in each estuary. Significant differences between estuaries for each eelgrass character are indicated by different superscripts ($p < 0.05$).

	Shoot Density (per six cores)	Canopy Height (cm)	Percent Cover	Above-ground Biomass (g dry wgt)	Epiphyte Biomass (g dry wgt)
Estuary	Mean (± SE)	Mean (± SE)	Mean (± SE)	Mean (± SE)	Mean (± SE)
Midgell	29.7 (4.78)^{A}	67.62 (5.23)^{A}	33.57 (7.93)^{AC}	8.82 (0.94)^{AC}	0.73 (0.44)^{AB}
Tracadie	84.9 (5.46)^{BC}	39.2 (5.12)^{B}	76.64 (4.96)^{BD}	13.08 (1.25)^{BC}	0.11 (0.03)^{A}
Wheatley	76.0 (8.18)^{BCD}	46.79 (3.31)^{B}	76.97 (4.21)^{BD}	14.66 (0.99)^{B}	0.16 (0.04)^{AB}
Stanley	22.5 (3.10)^{A}	49.65 (3.25)^{B}	24.86 (5.99)^{C}	7.58 (1.10)^{A}	0.51 (0.36)^{AB}
Bideford	42.0 (2.02)^{AD}	44.17 (3.40)^{B}	55.07 (5.39)^{AD}	7.92 (1.09)^{A}	0.87 (0.47)^{B}
Brooks	79.2 (7.93)^{B}	40.1 (3.39)^{B}	68.04 (5.96)^{BD}	11.41 (0.73)^{ABC}	0.14 (0.04)^{AB}
Freeland	46.6 (6.20)^{AC}	43.3 (3.40)^{B}	64.62 (7.62)^{BD}	10.26 (1.14)^{ABC}	0.07 (0.04)^{A}
Kildare	73.9 (6.88)^{B}	46.85 (3.62)^{B}	66.96 (6.91)^{BD}	11.33 (0.98)^{ABC}	0.08 (0.03)^{A}

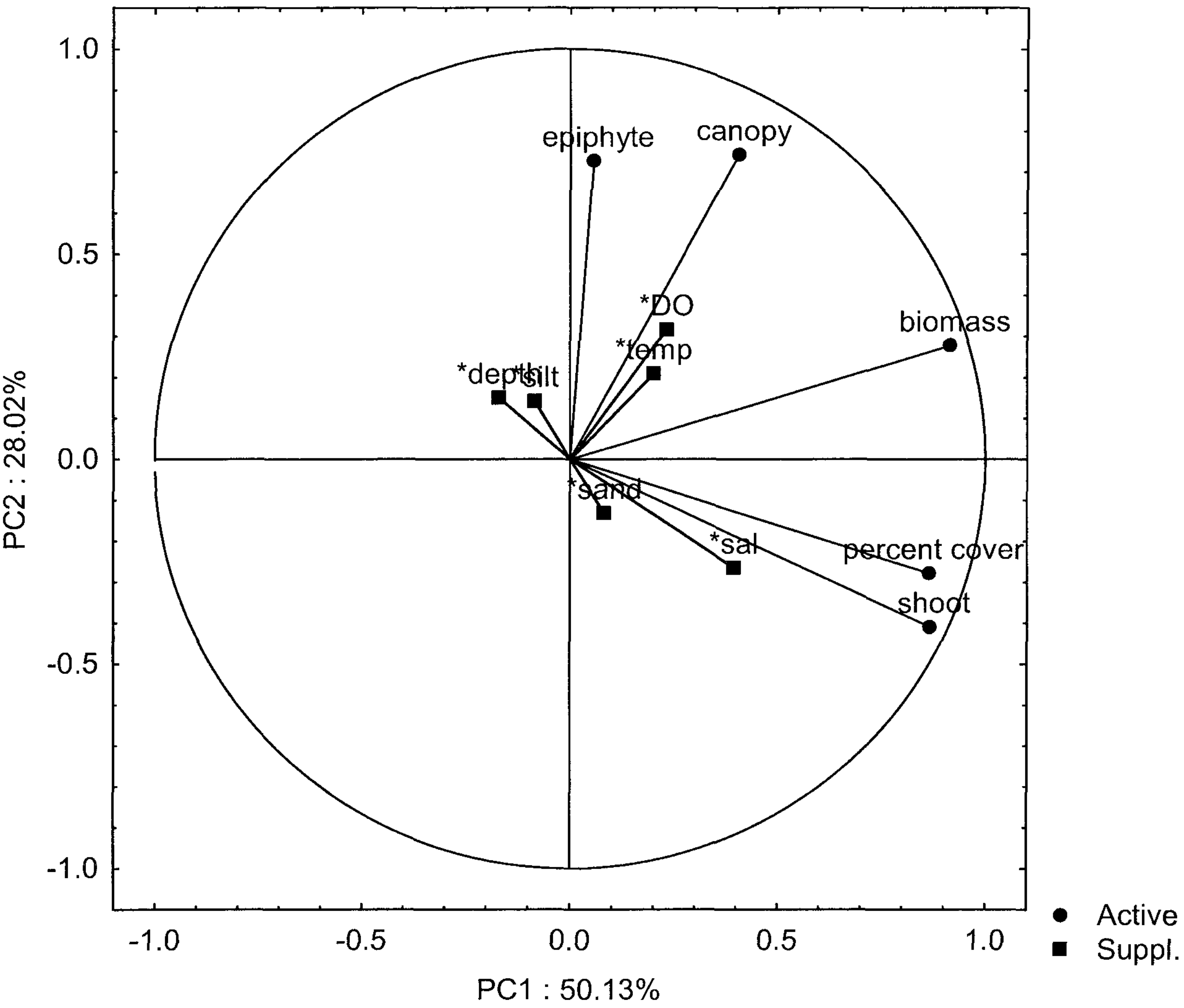

Figure 4.2. PCA of the eelgrass variables with water parameters shown as supplementary variables in June 2009.

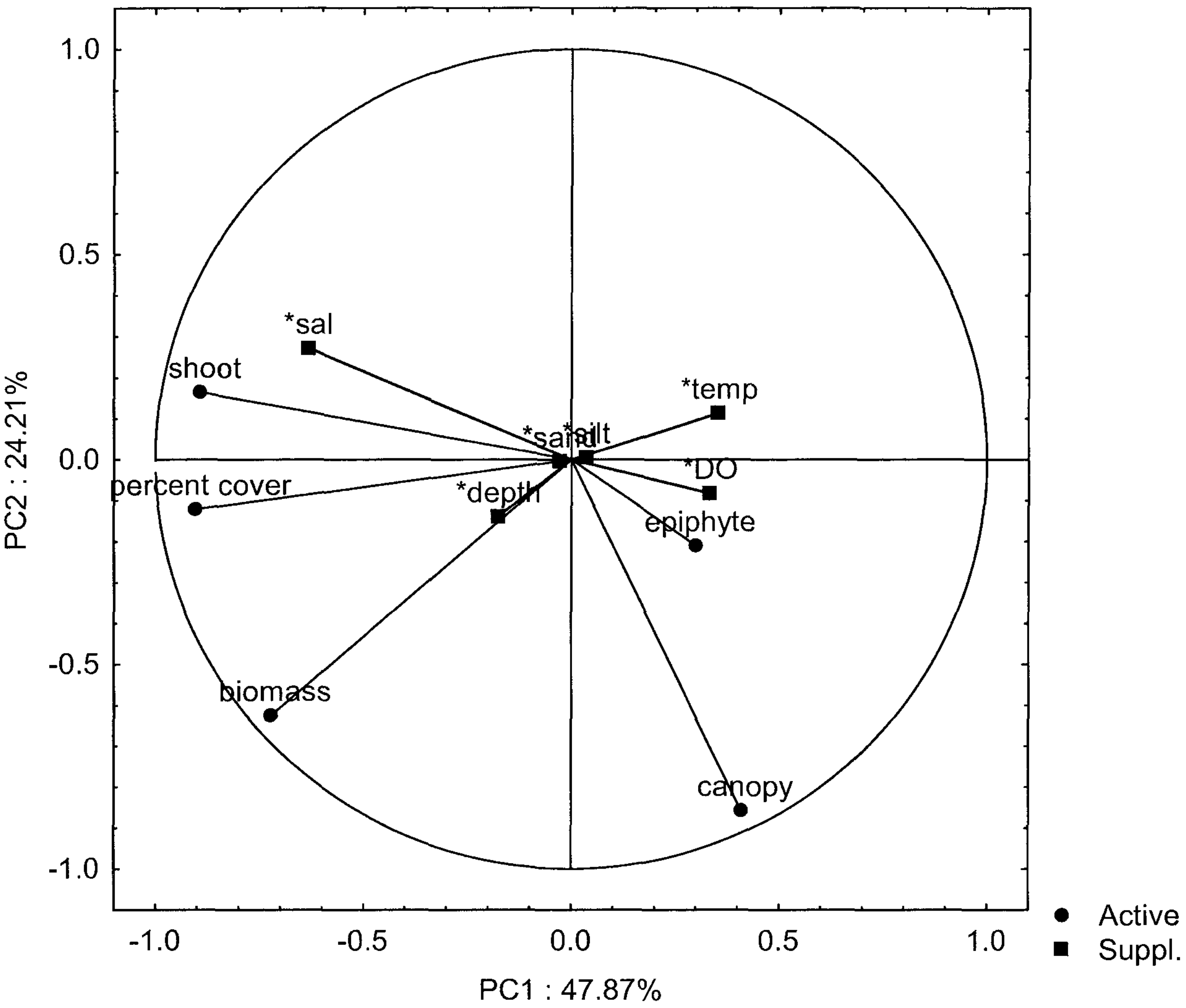

Figure 4.3. PCA of the eelgrass variables with water parameters shown as supplementary variables in August 2009.

using one-way ANOSIM showed that there was a significant difference between months (Global R = 0.917, p = 0.001) in the entire fish assemblage. Much of this difference was due to young-of-the-year (YOY) in August; when YOY were excluded from the analysis, communities were more similar (Global R = 0.670, p = 0.001) (Figure 4.4). SIMPER results show that there are more threespine and blackspotted stickleback and mummichog in June and more fourspine stickleback YOY, *Gasterosteus* spp. YOY, mummichog YOY, Atlantic silverside YOY, northern pipefish YOY, and ninespine stickleback in August (Table 4.6A). Excluding YOY, SIMPER analysis showed that there were more blackspotted, threespine and fourspine stickleback, mummichog, cunner and, northern pipefish in June and higher numbers of ninespine stickleback in August (Table 4.6B). Therefore communities were analyzed as separate entities in June and August, as the response to eelgrass and water parameters may differ temporally as the variables themselves change.

4.4.3 Fish community structure in relation to water parameters and eelgrass characteristics

Spearman rank correlation analysis between the various water and eelgrass characters in all eight estuaries (analyzed together to represent a continuum of eelgrass habitat and water parameter differences) and measures of the fish community (abundance, richness, diversity) showed that eelgrass was not important in structuring the adult community in June or August (Table 4.7). The adult fish community in June was more abundant in areas of higher water temperature and lower salinity, while community diversity was influenced by salinity and dissolved oxygen. In August, species richness in the adult community was higher in areas of lower water temperature and higher water depth; however, when the August community included YOY, abundance and diversity

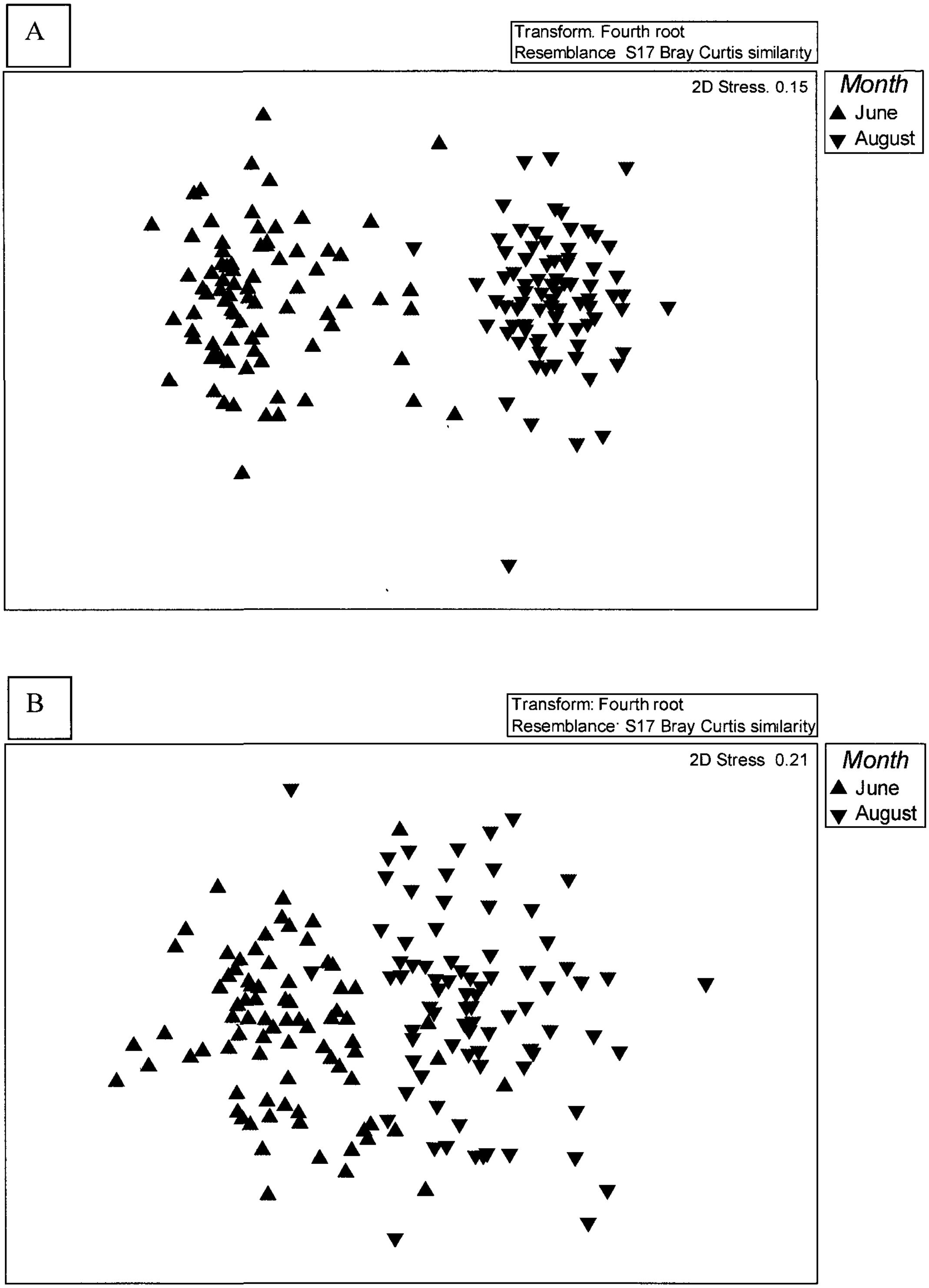

Figure 4.4. Non-metric multi-dimensional scaling (nMDS) plot illustrating temporal variation in the fish community throughout the two sampling months with A) adults and young-of-the-year (YOY) and B) YOY excluded. Each point represents one beach seine, n = 80 in each month.

Table 4.6. SIMPER results of temporal differences in the fish community based on the two sampling periods showing species contributing more than 4% to the dissimilarity in the community with A) adults and young-of-the-year (YOY) combined and B) YOY excluded. Abundances are back-transformed from the fourth root data used in the analysis.

Temporal Comparison: June vs. August	**Average dissimilarity**	**Species contributing**	**Average abundance/ 100 m^2 June**	**Average abundance/ 100 m^2 August**	**Contribution to dissimilarity (%)**
A)	56.49%	Fourspine YOY*	0.02	44.31	10.9
		Gasterosteus YOY**	0.17	42.28	9.84
		Mummichog YOY	0.00	14.76	9.39
		Silverside YOY	0.00	9.38	8.33
		Blackspotted	11.46	0.00	8.14
		Pipefish YOY	0.00	4.42	7.00
		Threespine	41.62	1.81	6.86
		Ninespine	3.13	9.17	5.72
		Mummichog	10.50	6.89	5.28
		Ninespine YOY	0.01	0.60	4.44
		Fourspine	228.98	132.07	4.28
		Cunner	0.55	0.00	4.09
		Pipefish	0.50	0.00	4.05
B)	40.33%	Blackspotted	11.46	0.00	17.67
		Threespine	41.62	1.81	15.07
		Ninespine	3.13	9.17	12.68
		Mummichog	10.50	6.89	11.62
		Fourspine	228.98	132.07	9.36
		Cunner	0.55	0.00	8.80
		Pipefish	0.50	0.00	8.79
		Silverside	0.17	0.00	6.77

* YOY designates young-of-the-year fishes

**Threespine and blackspotted stickleback YOY are difficult to distinguish as very small individuals therefore all young of this type are designated *Gasterosteus* YOY.

Note: threespine, fourspine, ninespine, and blackspotted are all stickleback species.

Table 4.7. Spearman rank correlation analysis of the various measures of fish community structure A) abundance, B) richness, and C) diversity of the adult community in June and August as well as the entire community in August against the various measures of water parameters and eelgrass habitat condition. Significant correlations ($p < 0.05$) are shown in bold.

A)	June Adults	August Adults	August Adults and YOY
Water parameter/eelgrass variable	r	r	R
Water temperature	**0.25**	-0.03	-0.02
Salinity	**-0.29**	0.03	-0.02
Dissolved oxygen	-0.05	-0.12	-0.14
Water depth	0.01	0.14	0.19
Sediment sand content	-0.19	-0.16	-0.20
Eelgrass shoot density	-0.22	-0.02	**0.29**
Avg. canopy height	0.17	0.12	-0.09
Above-ground biomass	-0.09	-0.04	0.01
Epiphyte biomass	0.17	0.14	0.14

B)	June Adults	August Adults	August Adults and YOY
Water parameter/eelgrass variable	r	r	R
Water temperature	0.19	**-0.27**	**-0.29**
Salinity	-0.16	0.09	**0.28**
Dissolved oxygen	0.18	-0.17	**-0.31**
Water depth	0.07	**0.28**	**0.46**
Sediment sand content	0.15	-0.01	0.15
Eelgrass shoot density	0.03	0.19	**0.33**
Avg. canopy height	-0.01	-0.11	-0.06

Table 4.7 continued

Above-ground biomass	0.08	0.11	**0.26**
Epiphyte biomass	0.18	-0.12	-0.02

C)	June Adults	August Adults	August Adults and YOY
Water parameter/eelgrass variable	r	r	R
Water temperature	-0.04	-0.01	0.07
Salinity	**-0.35**	-0.19	-0.21
Dissolved oxygen	**-0.26**	0.14	0.14
Water depth	-0.01	-0.14	-0.17
Sediment sand content	-0.02	**0.29**	-0.03
Eelgrass shoot density	-0.20	-0.20	**0.25**
Avg. canopy height	-0.03	0.21	0.09
Above-ground biomass	-0.13	-0.02	0.21
Epiphyte biomass	0.18	-0.19	-0.12

were positively correlated with shoot density, while richness was positively correlated with shoot density and above-ground biomass as well as salinity and water depth (Table 4.7 page 106).

Spearman rank correlations of the abundance of individual species in the August community and various eelgrass characteristics revealed that a number of YOY species were almost exclusively related to several eelgrass characters. *Gasterosteus* spp. YOY, winter flounder YOY, and cunner YOY were positively correlated to eelgrass shoot density and above-ground biomass. Northern pipefish YOY were positively correlated to eelgrass shoot density. In contrast, mummichog YOY were negatively correlated to eelgrass shoot density and above-ground biomass, but positively related to epiphyte biomass, while ninespine YOY were also negatively correlated to shoot density (Table 4.8).

Table 4.8. Spearman rank correlation analysis of the various fish species in August against the various measures of eelgrass habitat condition. Significant correlations ($p < 0.05$) are shown in bold.

Eelgrass character	Eelgrass shoot density	Avg. canopy height	Above-ground biomass	Epiphyte biomass
Fish species	r	r	r	r
Atlantic silverside (*Menidia menidia*)	0.20	0.03	0.12	-0.11
Atlantic silverside YOY	-0.14	0.02	-0.15	0.10
Blackspotted stickleback (*Gasterosteus wheatlandi*)	0.16	-0.21	0.04	-0.19
Cunner (*Tautogolabrus adspersus*)	-0.01	-0.05	-0.03	0.08
Cunner YOY	**0.36**	-0.04	**0.23**	-0.14
Fourspine stickleback (*Apeltes quadracus*)	0.08	0.04	0.06	0.16
Fourspine YOY	0.02	0.08	0.03	0.13
Gasterosteus spp. YOY	0.10	**0.29**	**0.36**	0.18
Mummichog (*Fundulus heteroclitus*)	**-0.22**	**0.23**	-0.13	0.07
Mummichog YOY	**-0.36**	0.18	**-0.38**	**0.29**
Ninespine stickleback (*Pungitius pungitius*)	-0.17	**0.42**	0.08	0.19
Ninespine YOY	**-0.34**	**0.30**	-0.21	0.15

Table 4.8 continued

Northern pipefish (*Syngnathus fuscus*)	0.09	-0.19	0.04	0.06
Northern pipefish YOY	**0.29**	-0.02	-0.01	-0.12
Threespine stickleback (*Gasterosteus aculeatus*)	-0.03	-0.06	-0.02	0.03
Winter flounder (juv) (*Pseudopleuonectes americanus*)	**0.34**	-0.17	**0.22**	-0.05
Winter flounder YOY	**0.35**	-0.19	**0.26**	-0.04

4.5 Discussion

This study examined how the fish community responded to varying characteristics of eelgrass habitat within the estuarine environment. Degradation of seagrass habitat has been shown to negatively affect the fish community in other estuaries. Hughes et al. (2002) and Wyda et al. (2002) found higher abundance and species richness in fish assemblages sampled in areas of higher eelgrass complexity, compared to areas of lower complexity. This suggests that changes in eelgrass habitat structure have notable impacts on a variety of fish species. The results of this study suggest that the adult fish community responds primarily to measures of water quality while young-of-the-year (YOY) fishes are influenced more by eelgrass habitat complexity.

4.5.1 Water parameters and eelgrass variability

Sites in this study were selected based on previous knowledge of eelgrass habitat and visual assessment of estuaries prior to the sampling season, to reflect the variability both within and between estuaries allowing us to examine how fish were responding to differences in eelgrass habitat. Generally, Midgell, Stanley, and Freeland had lower measures of eelgrass shoot density, percent cover and above-ground biomass within the estuaries compared to the other five. Midgell was the most different system compared to the others, as the inner sites sampled contained very little eelgrass, most likely due to low salinity levels. Eelgrass tends to perform optimally at salinities above 15 ppt (Nejrup and Pedersen 2008). Thom et al. (2003) found a linear relationship between eelgrass shoot density and water salinity, highest shoot densities occurred in the most saline habitats. Shoot densities and associated percent cover measurements in the current study were also related to salinity. Oxygen can also be important in eelgrass maintenance. Greve et al. (2003) found that low oxygen levels can be physiologically stressful on eelgrass plants increasing the rate of blade and shoot mortality. Shoot density in the present study was negatively associated with oxygen levels in August when water oxygen levels were lowest confirming that decreased dissolved oxygen levels can impede eelgrass growth. Together, this demonstrates the relationship between water quality parameters and eelgrass condition.

4.5.2 Fish community composition and temporal patterns

The fish community composition of all eight estuaries in both months was dominated by fourspine, threespine, ninespine, and blackspotted stickleback, mummichog, and Atlantic silverside. All fish species captured in this study were also sampled in other studies within the southern Gulf of St. Lawrence (e.g. Joseph et al. 2006, Thériault 2006, Weldon et al. 2007-2009), suggesting that the fish assemblages in PEI estuaries are characteristic of assemblages found in this region. Dominance by certain species, as seen is this study is common in this area and elsewhere (e.g. Cabral et al. 2001; Akin et al. 2005; Elliott et al. 2007). In addition, given the proximate locations of the estuaries studied in this investigation, it is not surprising that the fish species comprising the community were similar. Most differences in species richness that did occur were the result of occasional and rare species such as cunner (adults and YOY) (*Tautogolabrus adspersus*), grubby YOY (*Myoxocephalus aenaeus*), Atlantic tomcod YOY (*Microgadus tomcod*), and juvenile white hake (*Urophycis tenuis*).

In June, the fish community was dominated by adults, and in August, YOY occurred in all samples. Seasonal changes in the estuarine fish community composition have been well documented, and are primarily the result of the emergence of YOY fishes (Lazzari et al. 1999; Hagan and Able 2003). Lazarri et al. (1999) found that during the spring and summer, abundance and species richness increased in an estuary in Maine, as a result of the appearance of 0+ aged fishes. Sampling occurred in June and August in this study to explore how adults and YOY of various fish species were interacting with eelgrass. Generally eelgrass was more important for YOY fishes than adults (see below).

4.5.3 Fish community composition in response to variability in water parameters and eelgrass

The nursery role of eelgrass habitat was shown by measures of abundance, richness and diversity of fishes being significantly correlated with more complex (higher shoot density) eelgrass habitat. In general, fish tend to associate with eelgrass since it offers a refuge from predation (the blades of eelgrass decrease the visual ability of predators) (Orth and Heck 1980; Orth et al. 1984), and provides a rich food supply (Orth et al. 1984; Lubbers et al. 1990), as preferred food items such as isopods and amphipods live on eelgrass blades (Deegan 2002). For these reasons eelgrass acts as a nursery for juvenile fishes (Deegan et al. 1997; Gotceitas et al. 1997; Heck et al. 2003). Many studies have shown the value of eelgrass habitat to a variety of juvenile fish species (Deegan et al. 1997; Gotceitas et al. 1997; Heck et al. 2003; Joseph et al. 2006; Lazzari and Stone 2006, among others). Joseph et al. (2006) sampled juvenile white hake exclusively in eelgrass habitats and juvenile cunner more frequently in eelgrass habitats. Lazzari and Stone (2006) found that juvenile Atlantic tomcod, white hake, and winter flounder were all sampled more frequently in eelgrass than unvegetated substrates. In the present study YOY cunner, winter flounder (*Pseusopleuronectes americanus*), northern pipefish (*Syngnathus fuscus*), and *Gasterosteus* spp. YOY were all positively correlated with higher eelgrass shoot density and above-ground biomass confirming that a variety of YOY fishes prefer eelgrass habitat. Interestingly, Deegan et al. (1997) found that the nursery function of eelgrass beds in Waquoit and Buttermilk Bays (Massachusetts) was significantly decreased in areas of low habitat complexity (< 100 shoots m^{-2}). The findings of the present study support the idea that eelgrass is a suitable and necessary nursery habitat, showing that higher eelgrass shoot density supports a higher abundance and more specious community of juveniles.

In contrast to a number of studies (e.g. Heck et al. 1989; Hughes et al. 2002), the current study found that mummichog occurred more frequently in lower complexity habitats. Generally mummichog were associated with lower shoot density and higher epiphyte biomass, all of which are associated with estuarine eutrophication (Neckles et al. 1993; Duarte 1995; Valiela et al. 1997). However, these findings are consistent with other studies of estuarine fish communities on Prince Edward Island. Schein (2009) found that mummichog were captured in higher numbers in areas where macroalgal (*Ulva lactuca*) biomass was greatest compared to areas with higher amounts of eelgrass, stating that this could have been a response to higher prey availability or depict the tolerance of mummichog to varying environmental conditions. Finley also (2008) found significantly higher numbers of mummichog associated with habitats where eelgrass had been replaced by macroalgae.

Although eelgrass complexity played a role in structuring the entire fish community in August, specific water parameters were of higher importance to adult fish in June and August. Two parameters best known to influence fish community composition in estuaries are temperature (Thiel et al. 1995; Marshall and Elliot 1998;

Araújo et al. 1999) and salinity (Maes et al. 2004; Selleslagh and often significantly correlated with the various measures of fish community structure.

Salinity is largely influential in fish community organization (Maes et al. 2004; Selleslagh and Amara 2008; Selleslagh et al. 2009). Selleslagh and Amara (2008) examined the fish community composition in the Canche estuary (France) and found that salinity was among the top three variables influencing the variation in the fish community. Marshall and Elliot (1998) found that salinity affected abundance, richness and biomass of the fish community. In the current study salinity was negatively correlated to abundance and diversity in June.

Differences in dissolved oxygen concentrations (DO) could also influence the fish community (Wannamaker and Rice 2000; Stevens et al. 2006). DO levels were found to range from 8.12 to 21.48 mg/L in all the estuaries in June and 3.34 to 16.08 mg/L in August in this study. All of the values were above hypoxic (< 2 mg/L) or anoxic (< 0.2 mg/L) levels (Rosenberg and Loo 1988), although the lower values found in August could have contributed to changes in community structure. Further, DO was measured during the day and has been shown to fluctuate throughout diel periods (Chapter 3) which could potentially impact the fish community. Part of this response may have been manifested by the negative correlations between DO and diversity in June and species richness in August.

Water temperature was also shown to be an important determinant of fish community structure in this study and was important on both a spatial and temporal scale. In June there was considerable spatial variability in temperature within estuaries, and temporally throughout the three sampling weeks, while in August temperature was high but not as variable. Thiel et al. (1995) found that water temperature was the best predictor of total abundance, especially on a seasonal basis. This was apparent in the current study, as temperature was positively correlated with abundance in June. Araújo et al. (1999) found that temperature was the best predictor of abundances of certain species such as dace (*Leuciscus leuciscus*), perch (*Perca fluviatilis*), and flounder (*Platichthys flescus*). In the present study species richness was negatively correlated with temperature in August suggesting that some species avoided the warmer nearshore temperatures. Temperature affects the seasonality and variability of migration, spawning and recruitment patterns which influence fish community structure within an estuary (Wheeler 1969).

The physical features of the habitat can be highly dependent on one another making it difficult to determine, with precision, which environmental features are structuring the fish community (Akin et al. 2005). Even though measures of the adult community in this study were correlated only to specific water parameters and not to hiogher measures of eelgrass shoot density or above-ground biomass, this relationship may reflect an undisclosed relationship with eelgrass as some environmental parameters and eelgrass characters were related. Therefore, it is possible that only sites which differ greatly in habitat structure i.e. low vs. high complexity can be used to see an effect of eelgrass habitat complexity on the fish community. The fact that eelgrass is present may provide adequate resources for fishes, leaving water parameters as the driving force in determining fish community structure. Despite measuring a suite of physical habitat characteristics, accounting for variation in species distributions is difficult (Martino and Able 2003; Selleslagh and Amara 2008).

To conclude, this study showed the fish community structure within the estuarine environment is influenced by a combination of environmental and eelgrass variables. Water parameters such as salinity and temperature may be the major determinants influencing habitat associations and community structure; however, it was apparent that YOY fish species were associated with areas of higher eelgrass shoot density and above-ground biomass confirming the importance of the nursery function of this habitat. This is important in light of current anthropogenic impacts on the estuarine environment. Eutrophication results in the replacement of high complexity eelgrass habitat with that of low complexity containing a few shoots or which becomes dominated by macroalgae (Hughes et al. 2002, Wyda et al. 2002). As estuaries become increasingly eutrophic and eelgrass habitat is lost, it is likely that the fish community in PEI estuaries will become less diverse as the capacity of the environment to support various YOY species decreases.

4.6 Literature Cited

Akin, S., Buhan, E., Winemiller, K.O., Yilmaz, H. 2005. Fish assemblage structure of Koycegiz Lagoon-Estuary, Turkey: spatial and temporal distribution patterns in relation to environmental variation. *Estuarine, Coastal and Shelf Science* **64**: 671-684.

Araújo, F.G., Bailey, R.G., Williams, W.P. 1999. Spatial and temporal variability in fish populations in the upper Thames estuary. *Journal of Fish Biology* **55**: 836-853.

Bell, J. D., Westoby, M. 1986. Importance of local changes in leaf height and density to fish and decapods associated with seagrasses. *Journal of Experimental Marine Biology and Ecology* **104**: 249-274.

Borum, J. 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. *Marine Biology* **87**: 211-218.

Borum, J., Wium-Andersen, S. 1980. Biomass and production of epiphytes on eelgrass (*Zostera marina*) in the Øresund, Denmark. *Ophelia* **(Suppl 1)**: 57-64.

Cabral, H.N., Costa, M.J., Salgado, J.P. 2001. Does the Tagus estuary fish community reflect environmental changes? *Climate Research* **18**: 119-126.

Deegan, L.A. 2002. Lessons learned: the effects of nutrient enrichment on the support of nekton by seagrass and salt marsh ecosystems. *Estuaries* **25**: 727-742.

Deegan, L.A., Finn, J.T., Ayvazian, S.G., Ryder-Kieffer, C.A., Buonaccorsi, J. 1997. Development and validation of an estuarine biotic integrity index. *Estuaries* **20**: 601-617.

Deegan, L.A., Wright, A., Ayvazian, S.G., Finn, J.T., Golden, H., Rand-Merson, R., Harrison, J. 2002. Nitrogen loading alters seagrass ecosystem structure and support of higher trophic levels. *Aquatic Conservation: Marine and Freshwater Ecosystems* **12**: 193-212.

Duarte, C. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* **41**: 87-112.

Elliott, M., Whitfield, A.K., Potter, I.C., Blaber, S.J.M., Cyrus, D.P., Nordlie, F.G., Harrison, T.D. 2007. The guild approach to categorizing estuarine fish Assemblages:a global review. *Fish and Fisheries* **8**: 241-268.

Finley, M.A. 2008. Investigating the suitability of northern mummichog (*Fundulus heteroclitus macrolepidotus)* for the assessment of cumulative and non-point source pollution in Prince Edward Island estuaries. MSc Thesis. University of Prince Edward Island, Charlottetown, PEI.

Gotceitas, V., Fraser, S., Brown, J.A. 1997. Use of eelgrass beds (*Zostera marina*) by juvenile Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Science* **54**: 1306-1319.

Greve, T.M., Borum, J., Pedersen, O. 2003. Meristematic oxygen variability in eelgrass (*Zostera marina*). *Limnology and Oceanography* **48**: 210-216.

Hagan, S.M. Able, K.W. 2003. Seasonal changes of the pelagic fish assemblage in a temperate estuary. *Estuarine, Coastal and Shelf Science* **56**: 15-29.

Hauxwell, J., Cebrián, J., Furlong, C., Valiela, I. 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology* **82**: 1007-1022.

Heck, K.L., Able, K.W., Fahay, M.P., Roman, C.T. 1989. Fishes and decapod crustaceans of Cape Cod eelgrass meadows: species composition, abundance patterns and comparison with unvegetated substrates. *Estuaries* **12**: 59-65.

Heck, K.L., Hays, G., Orth, R.J. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress Series* **253**: 123-136.

Hughes, J., Deegan, L.A., Wyda, J.C., Weaver, M.J., Wright, A. 2002. The effects of eelgrass habitat loss on estuarine fish communities of southern New England. *Estuaries* **25**: 235-249.

Jaschinski, S., Sommer, U. 2008. Top-down and bottom-up control in an eelgrass-epiphyte system. *Oikos* **117**: 754-762.

Joseph, V., Locke A., Godin J.J. 2006. Spatial distribution of fishes and decapods in eelgrass (*Zostera marina* L.) and sandy habitats of a New Brunswick estuary, eastern Canada. *Aquatic Ecology* **40**: 111-123.

Lazzari, M.A. 2002. Epibenthic fishes and decapod crustaceans in northern estuaries: a comparison of vegetated and unvegetated habitats in Maine. *Estuaries* **25**: 1210-1218.

Lazzari, M.A., Sherman, S., Brown, C.S., King, J., Joule, B.J., Chenoweth, S.B., Langton, R.W. 1999. Seasonal and annual variations in abundance and species composition of two nearshore fish communities in Maine. *Estuaries* **22**: 636–647.

Lazzari, M.A., Sherman, S., Kanwit, J.K. 2003. Nursery use of shallow habitats by epibenthic fishes in Maine nearshore waters. *Estuarine, Coastal and Shelf Science* **56**: 73-84.

Lazzari, M.A., Stone, B.Z. 2006. Use of submerged aquatic vegetation as habitat by young-of-the-year epibenthic fishes in shallow Maine nearshore waters.

Estuarine, Coastal and Shelf Science **69**: 591-606

Lubbers, L., Boynton, W.R., Kemp, W.M. 1990. Variations in structure of estuarine fish communities in relation to abundance of submersed vascular plants. *Marine Ecology Progress Series* **65**: 1-14.

Maes, J, van Damme, S, Meire, P., Ollevier, F. 2004. Statistical modeling of seasonal and environmental influences on the population dynamics of an estuarine fish community. *Marine Biology* **145**: 1033-1042.

Marshall, S., Elliott, M. 1998. Environmental influences on the fish assemblages of the Humber Estuary, U.K. *Estuarine, Coastal and Shelf Science* **46**: 175-184.

Martino, E.J., Able, K.W., 2003. Fish assemblages across the marine to low salinity transition zone of a temperate estuary. *Estuarine, Coastal and Shelf Science* **56**: 969-987.

Mattila, J. Chaplin, G., Eilers R., Heck, K.L., O'Neal J.P., Valentine J.F. 1999. Spatial and diurnal distribution of invertebrate and fish fauna of a *Zostera marina* bed and nearby unvegetated sediments in Damariscotta River, Maine (USA). *Journal of Sea Research* **41**: 321-332.

Neckles, H.A., Wetzel, R.L., Orth, R.J. 1993. Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. *Oecologia* **93**: 285-295.

Nejrup, L.B., Pedersen, M.F. 2008. Effects of salinity and water temperature on the ecological performance of *Zostera marina. Aquatic Botany* **88**: 239-246.

Orth, R.J., Heck, K.L. 1980. Structural components of eelgrass (*Zostera marina*) meadows in the lower Chesapeake Bay- Fishes. *Estuaries* **3**: 278-288.

Orth, R.J., Heck, K.L., Van Montfrans, J. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries* **7**: 278-286.

Philippart, CJ.M. 1995. Effects of shading on growth, biomass and population maintenance of the intertidal seagrass *Zostera noltii* Hornem. in the Dutch Wadden Sea. *Journal of Experimental Marine Biology and Ecology* **188**: 199-213.

Rosenberg, R., Loo, L.-O. 1988. Marine eutrophication induced oxygen deficiency: effects on soft bottom fauna, Western Sweden. *Ophelia* **29**: 213-225.

Schein, A. 2009. The estuarine fish community and food web structure in areas of sea lettuce (*Ulva lactuca*) and eelgrass (*Zostera marina*) within the Stanley River estuary Prince Edward Island. M.Sc. Thesis. University of Prince Edward Island, Charlottetown, PEI.

Selleslagh, J., Amara, R. 2008. Environmental factors structuring fish composition and assemblages in a small macrotidal estuary (eastern English Channel). *Estuarine, Coastal and Shelf Science* **79**: 507-517.

Selleslagh, J., Amara, R., Laffargue, P., Lesourd, S., Lepage, M. 2009. Fish composition and assemblage structure in three Eastern English Channel macrotidal estuaries: A comparison with other French estuaries. *Estuarine, Coastal and Shelf Science* **81:** 149-159.

Short, F.T., Burdick, D.M., Wolf, J.S., Jones, G.E. 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina. Limnology and Oceanography* **40**: 740-749.

Sogard, S.M., Powell, G.V.N., Holmquist, J.G. 1987. Epibenthic fish communities on Florida Bay banks: relations with physical parameters and seagrass cover. *Marine Ecology Progress Series* **40**: 25-39.

Stevens, P.W., Blewett, D.A., Casey, J.P. 2006. Short term effects of a low dissolved oxygen event on estuarine fish assemblages following the passage of hurricane Charley. *Estuaries and Coasts* **29**: 997-1003.

Thériault, M-H. 2006. Les effets des effluents d'usines de transformation des produits marins sur la communauté de poissons côtiers et sur deux espèces sentinelles dans le sud du golfe du Saint Laurent. M.Sc. Thesis. Université de Moncton, Moncton, NB.

Thiel, R., Sepúlveda, R., Kafemann, R., Nellen, W. 1995. Environmental factors as forces structuring the fish community of the Elbe Estuary. *Journal of Fish Biology* **46**: 47-69.

Thom, R.M., Borde, A.B., Rumrill, S., Woodruff, D.L., Williams, G.D., Southard, J.A., Sargeant, S.L. 2003. Factors influencing spatial and annual variability in eelgrass (Zostera marina L.) meadows in Willapa Bay, Washington, and Coos Bay, Oregon, estuaries. *Estuaries* **26**: 1117-1129.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* **42**: 1105-1118.

Wannamaker, C.M., Rice, J.A. 2000. Effects of hypoxia on movements and behaviour of selected estuarine organisms from the southeastern United States. *Journal of Experimental Marine Biology and Ecology* **249**: 145-163.

Weldon, J., Courtenay, S. and Garbary, D. 2007. The Community Aquatic Monitoring

Program (CAMP) for measuring Marine Environmental Health in Coastal Waters of the southern Gulf of St. Lawrence: 2005 Overview. *Canadian Technical Report of Fisheries and Aquatic Science* **2708**: viii + 47 p.

Weldon, J., Courtenay, S. and Garbary, D. 2008. The community aquatic monitoring program (CAMP) for measuring marine environmental health in coastal waters of the southern Gulf of St. Lawrence: 2007 Overview. *Canadian Technical Report of Fisheries and Aquatic Science* **2825**: viii + 75 p.

Weldon, J., Garbary, D., Courtenay, S., Ritchie, W., Godin, C., Thériault, M-H., Boudreau, M., Lapenna, A. 2005. The community aquatic monitoring project (CAMP) for measuring marine environmental health in coastal waters of the southern Gulf of St. Lawrence: 2004 overview. *Canadian Technical Report of Fisheries and Aquatic Science* **2624**: viii + 53 pp.

Wheeler, A. 1969. Fish-life and pollution in the lower Thames: a review and preliminary report. *Biological Conservation* **2**: 25-30.

Williams, S.L., Ruckelshaus, M.H. 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. *Ecology* **74**: 904-918.

Wyda, J.C., Deegan, L.A., Hughes, J.E., Weaver, M.J. 2002. The response of fishes to submerged aquatic vegetation complexity in two ecoregions of the Mid-Atlantic Bight: Buzzards Bay and Chesapeake Bay. *Estuaries* **25**:86-100.

CHAPTER 5: DETERMINING THE IMPACTS OF EELGRASS (*ZOSTERA MARINA*) HABITAT DEGRADATION ON NORTHERN PIPEFISH (*SYNGNATHUS FUSCUS*) POPULATIONS

5.1 Abstract

Northern pipefish, *Syngnathus fuscus*, are common members of the eelgrass- (*Zostera marina*) associated ichthyofauna; however, relatively little information is available concerning their life history characteristics. This is particularly important given that eelgrass habitat in many areas is declining, primarily in response to eutrophication. The focus of this study was to document the population and reproductive characteristics of *S. fuscus* and, in addition, determine whether eelgrass habitat loss was affecting *S. fuscus* populations. *S. fuscus* were collected from the Stanley River estuary, which is currently experiencing eelgrass habitat decline and replacement by macroalgae (*Ulva lactuca*). Sampling occurred in ten day intervals from May to October, 2009 in an eelgrass and sea lettuce-dominated habitat within the estuary. Adults dominated the population until mid-July when young-of-the-year (YOY) became increasingly abundant and after which dominated. Growth rates of adult and YOY *S. fuscus* were estimated to be 1.0 mm d^{-1} and 1.9 mm d^{-1} respectively. Sex ratios were strongly male-biased in the sites sampled. Sexually mature females ranged from 146 to 208 mm TL, while males ranged from 116 to 180 mm TL. All pipefish (males, females, YOY) were significantly more abundant in the eelgrass habitat. Male *S. fuscus* living in the sea lettuce-dominated habitat did not show significantly lower brood pouch somatic indices (BPSI). However, males sampled from the sea lettuce habitat did have significantly lower brood sizes (number of embryos), although this was related to their smaller size. Female gonadosomatic indices (GSI) did not differ between the habitats. This study demonstrated the association of pipefish to eelgrass habitat and it described baseline information on reproductive status between the habitats, which may prove important as eelgrass habitat continues to deteriorate.

5.2 Introduction

Syngnathus fuscus are widely distributed, ranging from the Southern Gulf of St. Lawrence surrounding Atlantic Canada down the eastern seaboard of the United States to Jupiter Inlet, Florida (Dawson 1982). Northern pipefish are common members of eelgrass ichthyofauna (Orth and Heck 1980; Heck et al. 1989; Hughes et al. 2002; Wyda et al. 2002) and are known for their unique reproductive strategies (Berglund et al. 1986; Berglund and Rosenqvist 1990; Vincent et al. 1995; Campbell and Able 1998). However, comprehensive details concerning the life history and population characteristics of northern pipefish are largely lacking.

A considerable amount of interest in pipefish biology stems from their distinctive mode of reproduction. *S. fuscus*, along with other Syngnathid species, exhibit sex role reversal: females deposit eggs into the male's ventral brood pouch where the male fertilizes and gestates them until independent young are released (Vincent et al. 1992; Vincent et al. 1995). Females typically compete for males and therefore have features making them more attractive to males (Berglund and Rosenqvist 1990; Vincent et al. 1994). *S. fuscus* females are larger than male counterparts (Ripley and Foran 2006) and show bright yellow ventral coloration during the reproductive period. Females display to males by rising slightly above eelgrass beds and moving up and down to improve visibility (Vincent et al. 1994). When a male chooses a female he will accept eggs from that individual. *S. fuscus* are believed to acquire eggs from a single female. Campbell and Able (1998) found all embryos within a male's brood pouch to be at the same developmental stage. However, copulations with multiple females have been noted in other *Syngnathus* species (*S. typhle*, Berglund et al. 1986; Vincent et al. 1994; *S. floridae*, Jones and Avise 1997; *S. schlegeli* Watanabe and Watanabe 2001).

During gestation, the embryos are protected within an extensive brood pouch. Bilateral skin folds that enclose the embryos of *S. fuscus* are considered to be one of the highest forms of brood pouch development among the Syngnathids (Wilson et al. 2001). Embryos in the brood pouch are provided protection and gas exchange while fluids within the pouch are maintained similar to that of the male to decrease osmotic stress (Berglund et al. 1986; Vincent et al. 1994; Wantanabe et al. 1999; Foster and Vincent 2004). The duration of the gestation period is unknown for *S. fuscus*. However, it ranges from two to six weeks in other pipefish species (Vincent et al. 1994; Watanabe and Watanabe 2001). While other pipefish (i.e. *S. typhle*) are known to gestate multiple broods during the reproductive season (Vincent et al. 1994), the number of broods *S. fuscus* is capable of rearing each season is unknown (Campbell and Able 1998).

Despite a general lack of information on *S. fuscus* reproductive biology, habitat requirements have been well documented. *S. fuscus* are known for their close association with seagrasses and an abundant member of the eelgrass ichthyofauna in a number of estuaries along the eastern coast of the USA (Orth and Heck 1980, Wyda et al. 2002). Studies carried out in Chesapeake Bay, USA, found that *S. fuscus* were the second most abundant species surveyed in eelgrass beds (Orth and Heck 1980). Wyda et al. (2002) also noted that *S. fuscus* were a common species captured during trawl surveys in eelgrass, present in more than 10% of all hauls. Heck et al. (1989) found that *S. fuscus* was one of six species that together comprised 98% of the total fish catch in Cape Cod estuaries.

Seagrass beds are habitats that are characterized by a high degree of structural complexity (Curtis and Vincent 2005) and there is a tendency for higher abundances of pipefish in areas of higher eelgrass complexity (i.e. shoot density) (e.g. Hughes et al.

2002, Wyda et al. 2002). Hughes et al. (2002) stated that *S. fuscus*, which were found in higher numbers in high density eelgrass habitats, were strongly affected by the disappearance of eelgrass in Waquoit and Buttermilk Bays, Massachusetts. Further, the authors found that eelgrass-dependent species did not typically seek refuge in alternative habitats such as macroalgae when eelgrass disappeared. These results suggest a strong dependence of this species on eelgrass habitats within the estuarine environment.

Eelgrass habitat in the Atlantic region has been in decline in some areas over the past decade (see Hanson 2004). Anthropogenic activities within watersheds have resulted in excessive algal growth, hypoxic and anoxic water conditions and loss of seagrass habitat (Deegan et al. 2002; Hughes et al. 2002; Bricker et al. 2008). Of particular concern is the increase in macroalgal growth. Although macroalgae (e.g. *Ulva lactuca*) are a natural component of the estuarine ecosystem, their growth is usually restricted by a limited concentration of nutrients. Under eutrophic conditions, macroalgal biomass can quickly surpass that of the eelgrass (Deegan et al. 2002). One alga of particular concern in this region is sea lettuce (*Ulva lactuca* L.). *Ulva* readily uses excess nutrients in a system and dominates estuaries by forming large free floating mats which severely impact eelgrass beds (Hauxwell et al. 2001; this study, Chapter 2). Deegan et al. (2002) noted that the removal of macroalgae from a contained study site resulted in increased biomass of eelgrass plants. They concluded that macroalgae impedes eelgrass growth by interception of light and competition for space, resulting in the subsequent shading and smothering of eelgrass beds and ultimately their removal from the system.

Continued loss and degradation of necessary habitat could threaten *S. fuscus* populations. Although various life history traits, feeding ecology and seasonal migration have been at least partially described (Ryer and Orth 1987; Lazarri and Able 1990;

Campbell and Able 1998), these studies were completed in Chesapeake Bay and Great Bay-Little Egg Harbour estuaries in the United States. Notably, life history traits were based on assembled data from a variety of locations, spanning a number of sampling seasons and using a number of different sampling methods. No known studies have looked at population parameters in the Southern Gulf of St. Lawrence. Given that this is the northern limit of their range, it is possible that differences in life history characteristics coupled with habitat loss may make populations vulnerable. Habitat degradation necessitates extensive knowledge of their life history and habitat selection for monitoring and possibly conservation purposes (Ripley and Foran 2006). The purpose of this project was to gather baseline population parameters (population structure, adult sex ratios, growth rates) of *S. fuscus* populations in Prince Edward Island estuaries and to examine population and reproductive differences in pipefish living in an eelgrass- and sea lettuce-dominated habitat.

5.3 Methods

5.3.1 Study area

Sampling took place in the Stanley River estuary, Prince Edward Island. This represents a unique estuarine habitat with three upper tributaries- Trout River, Founds Mills, and Granville Creek- which collectively drain into the Stanley River estuary and New London Bay on the north shore of PEI. The three upper tributaries support large blooms of sea lettuce during the summer which may account for the increasing amount found in Stanley River. Within Stanley River itself there remain some extensive eelgrass beds. The sea lettuce habitat was located upstream where Trout River joins the Stanley River estuary, while the eelgrass habitat was located approximately 700 m downstream in the Stanley River estuary (Figure 5.1). In addition, pipefish were sampled less extensively in seven other PEI estuaries (Figure 5.2) for information concerning variability in population structure, sex ratios, lengths, weights, and timing of reproductive events.

5.3.2 Fish sampling

Fish were collected and sampled within 4 h of low tide during daylight hours (10:00 – 19:00) from the Stanley River estuary in ten day intervals from May 8th, 2009-October 24th, 2009. Five seines (separated by no more than 3 m and 30 min) in the sea lettuce and eelgrass dominated habitat (in May only the sea lettuce site was sampled) were carried out consecutively, covering an area of approximately 400 m^2. Fish were captured using a beach seine and pentagonal sampling technique (see Chapter 2, with the exception that the seine net was pulled to shore before emptying the catch into the sorting container). All adult pipefish captured were counted, sexed, weighed, and measured (total length (mm), TL). Ten adults (five males and five females, if captured) were retained from each of the habitats to assess reproductive condition. Due to their small size, young-of-the-year (YOY) pipefish were weighed en mass although lengths were determined for each individual. All other fish were released. Retained fish were placed in large Ziploc® bags filled with water from the estuary, placed in a cooler on ice and transported to the laboratory.

In the lab, adult pipefish were euthanized (Tricaine methanesulfonate salt) weighed and measured. Liver weight (both sexes) and gonad weight (females, as the testes of males were minute) were obtained so liver somatic indices (LSI) (liver weight/eviscerated body weight *100) and gonadosomatic indices (GSI) (gonad weight/eviscerated body weight *100) could be determined. Body condition of

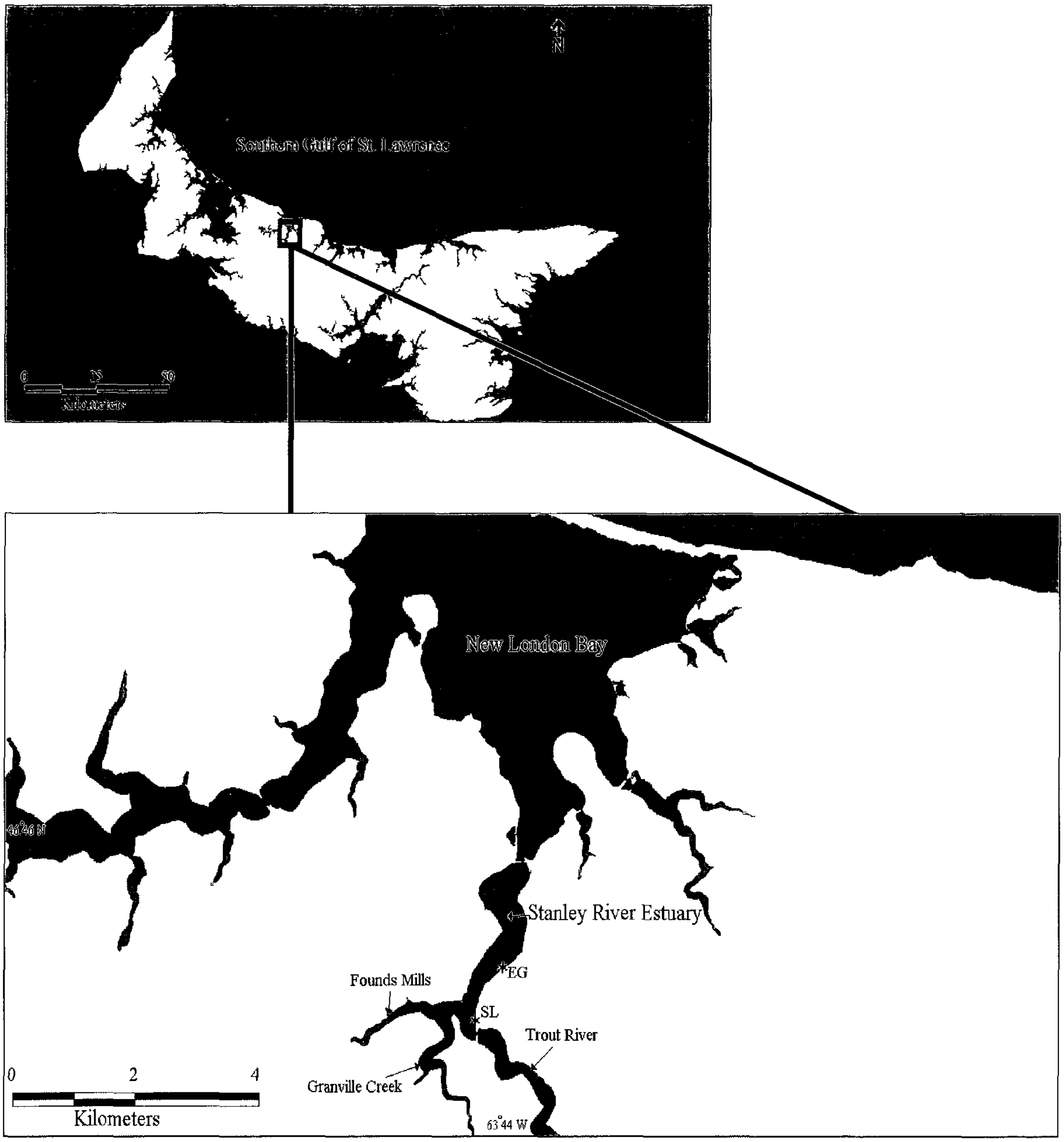

Figure 5.1. Location of the Stanley River Estuary on the north shore of Prince Edward Island. Sea lettuce (SL) and eelgrass (EG) sampling locations are marked within the Stanley River Estuary.

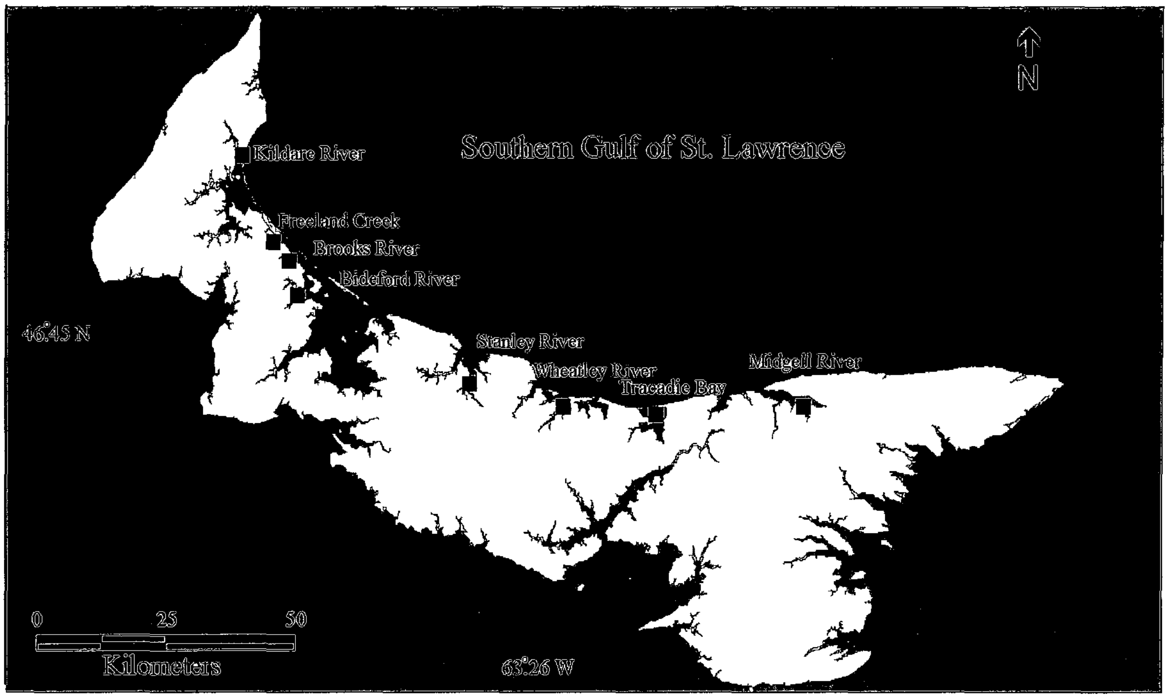

Figure 5.2. Location of all eight estuaries sampled including Stanley River.

males and females was also determined (females: eviscerated body weight/length^3, males: eviscerated frozen body weight (without brood)/length^3). Male pipefish were individually labelled and frozen so that brood contents could be removed non-destructively later. Although an attempt was made to remove the broods from fresh material, eggs/embryos in the pouch were too easily damaged. When pipefish were thawed, brood pouch contents were removed and weighed, and eviscerated frozen body weight was determined. Brood sizes (number of embryos) and brood pouch somatic indices (BPSI) (entire brood pouch weight/eviscerated frozen body weight (without brood) *100) were also determined (modified from Bolland and Boettcher 2005). Brood stages of embryos were noted and consisted of egg, eyed egg (development started with visible eyespots), and mature (ready to hatch, with little to no yolk sac remaining) (modified from Bolland and Boettcher 2005).

In seven other PEI estuaries, pipefish were recorded (sex, weight, length, and developmental stage in males (as described above), if applicable), from each of ten seines, and ten (five males, five females) were retained from the estuary in both June and August (if captured) to look at population structure, sex ratios, lengths, weights, and reproductive condition. In 2008, up to ten adult pipefish were collected from each of eight sites from the Midgell and Stanley river estuaries to examine differences in weight, length, and reproductive condition.

5.3.3 Environmental data

Water temperature, salinity, and dissolved oxygen were measured as described in Chapter 2. On July 9th, the extent of eelgrass coverage in both sampling sites was determined using a 0.5 m x 0.5 m viewing frame. Three transects were sampled along the outer, inner and middle portions of the entire sampling area which covered approximately 80 m. Approximately every meter the percent cover of eelgrass in the area was visually assessed to the nearest 10%.

5.3.4 Data analysis

Abundance of *S. fuscus* was determined as catch per unit effort from each sampling period. Monthly modal progression length frequency histograms (10 mm increments) were used to show abundances of adults and YOY and to estimate growth rates of adults and YOY. Deviations from 1:1 sex ratios of adults were tested using a Chi-Squared test for individuals captured during each sampling period in June and July. Normality and homogeneity of variance were evaluated using probability plots and Levene's test, respectively. If necessary, data were log transformed to satisfy the assumptions of parametric statistics. Regression analysis was used to look at the relationship between male length and brood size.

One-way analysis of variance (ANOVA) tests were used to explore differences in adult and YOY (young-of-the-year) abundances between the eelgrass and sea lettuce habitats. Two-way ANOVA tests were used to explore differences in pipefish length (factors: habitat and sex). Analysis of covariance (ANCOVA) was used to explore differences in brood pouch somatic indices (BPSI), brood sizes, and gonadosomatic index (GSI) between the two habitats. For BPSI and brood sizes the first two sampling dates (June 9th and June 18th) were excluded from the analysis which reduced temporal variability and corresponded to peaks in reproductive activity to best detect potential differences between sites. For GSI, the June 9th sampling date was dropped as the sample size was too low. The same sampling periods mentioned above were used to examine differences in body condition for males and females. Liver somatic index (LSI) showed inconsistent trends and was not analyzed statistically. Weight was used as a covariate in determining differences between BPSI and GSI, while length was used as a covariate in determining differences in brood sizes and condition. All data were log transformed and tested for normality, alpha was set at $p = 0.05$.

5.4 Results

5.4.1 Stanley River

5.4.1.1 Environmental data

Temperature showed seasonal fluctuations increasing from June to August and then decreased into October. Mean monthly temperature within the estuary from June to October was 19.36 ± 0.3 °C, 21.60 ± 0.17 °C, 22.75 ± 0.44 °C, 20.32 ± 0.71 °C, and 12.23 ± 0.80 °C respectively. Salinity ranged from 21-24 ppt in all samples with the exception of October 24th when it was between 9.7 and 12.5 ppt. Dissolved oxygen concentrations in the water column were always above 7 mg/L. There were no significant differences in the abiotic characteristics of the eelgrass and sea lettuce sampling sites; mean temperature, salinity and dissolved oxygen within a given sampling period differed by no more than 2°C, 2.36 ppt, and 3.9 mg/L respectively.

5.4.1.2 Population characteristics and growth rates

Monthly length-frequency distributions suggest that *S. fuscus* captured during the sampling period represent two year classes (Figure 5.3). In June there appeared to be a single cohort ranging from 116 mm to 186 mm TL. In July when young-of-the-year (YOY) first appeared there were two distinct cohorts (30 mm to 106 mm and 148 mm to

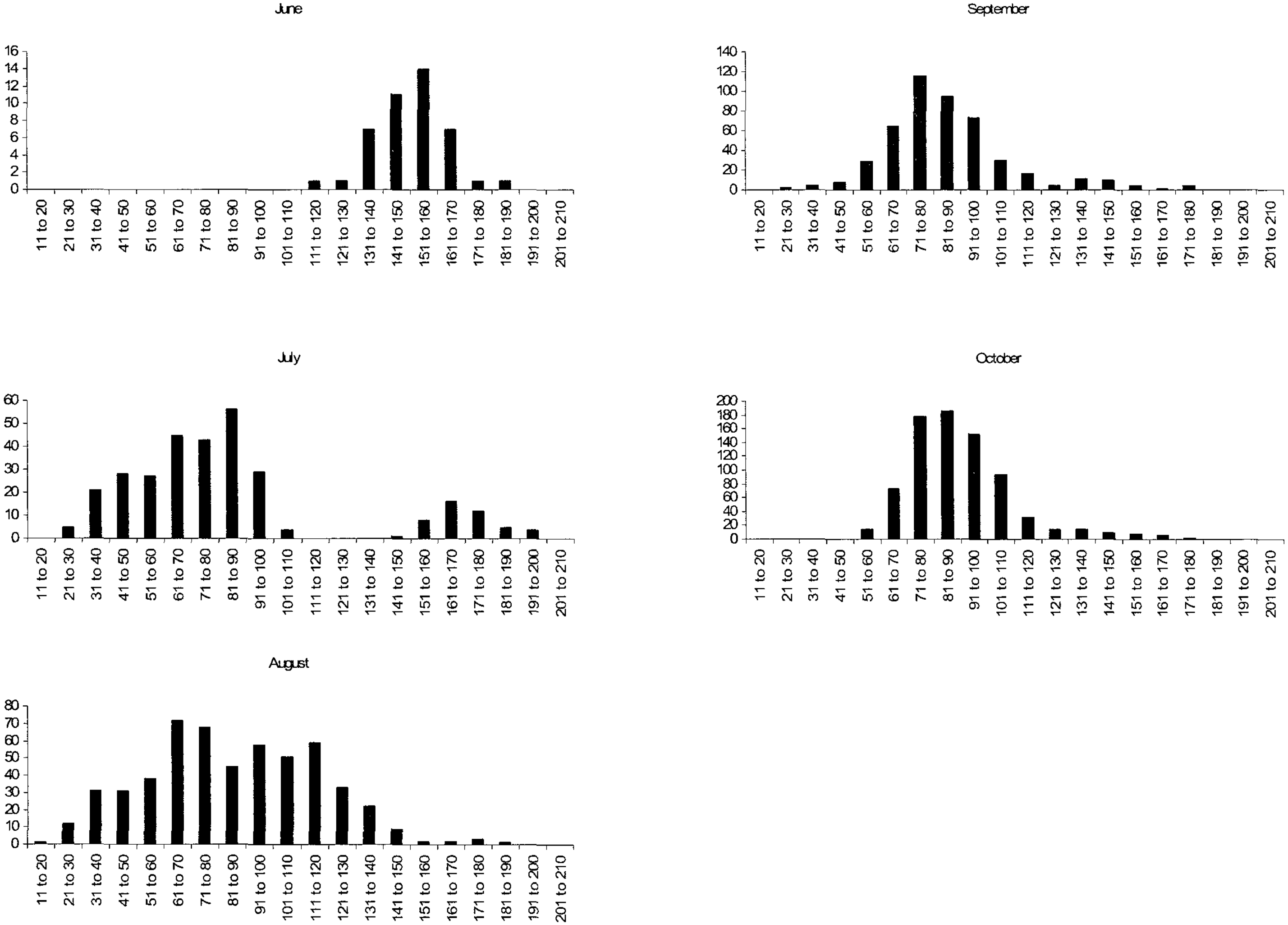

Figure 5.3. Monthly length-frequency distributions *S. fuscus* in the Stanley River estuary. N = 30 seine hauls per month from June – August and October and 20 seines in September. Note differences in y-axis in all months.

200 mm). In August, few adults were captured leaving YOY of varying lengths (17 mm to 151 mm). In September there was a noticeable increase in the number of smaller individuals (51 mm to 110 mm). By October there were no longer individuals less than 50 mm TL being captured and sizes ranged from (52 mm to 172 mm). Based on these data there were an estimated total of 289 adult and 2051 YOY *S. fuscus* captured in Stanley River. In May, 37 adults were captured from our sea lettuce site. Adults dominated the population until mid July, after which YOY became increasingly abundant and made up the bulk of the catch (Figure 5.4). The first YOY was captured on July 9th; from August 9th to the end of the sampling period only eight adults were captured.

Determining the growth rates for *S. fuscus* is somewhat subjective, especially for YOY given the large size range. Adult growth rate is more straightforward. By averaging the length of individuals in the first sample in June (June 9th) and the last sample in which a fair number of adults were captured (July 19th) the growth rates of both males and females were estimated at 1 mm d^{-1}(Figure 5.5). Female *S. fuscus* were consistently larger than their male counterparts (2-way ANOVA, $F_{1,85} = 11.380$, $p = 0.001$) (Figure 5.5). YOY growth was determined in the same manner and estimated to be 1.9 mm d^{-1}. A second method was also used for YOY growth rate estimation and involved examining length-frequency histograms for each sampling period (Figure 5.6). By following a cohort of YOY from July 9th- Aug 9th, YOY growth rate was estimated to be 2.2 mm d^{-1}.

5.4.1.3 Sex ratios and reproductive characteristics

There were consistently more adult males than females captured during each sampling period, ($\chi^2 = 23.645$, $p < 0.001$) with 2.8 males for every female (Figure 5.7). Brooding males and reproductively mature females were captured from early June until

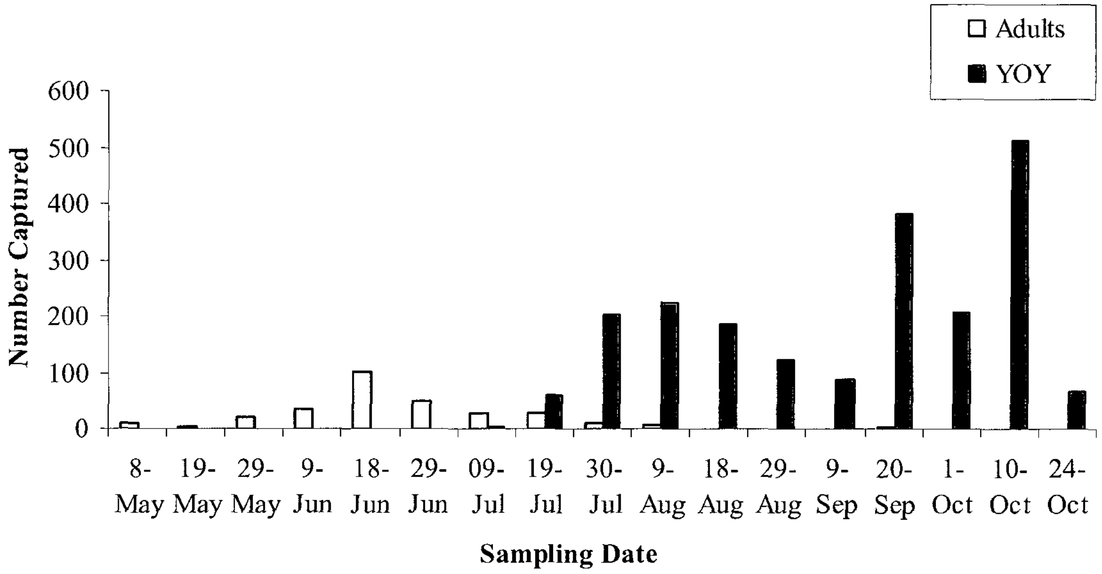

Figure 5.4. *Syngnathus fuscus* population structure in the Stanley River estuary. N = 10 seine hauls during each sampling date.

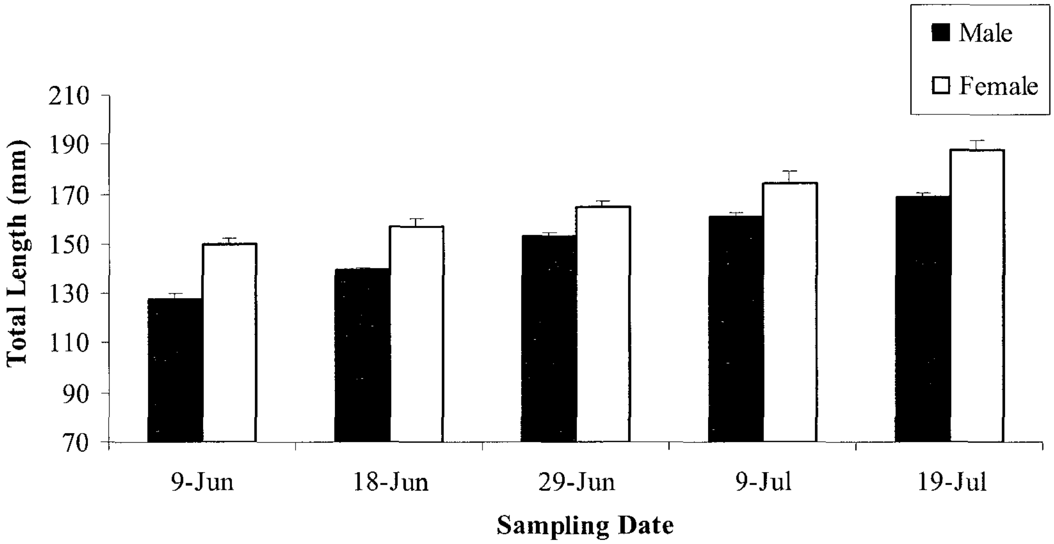

Figure 5.5. Growth rates and dimorphism of *S. fuscus* males and females in the Stanley River estuary. N = 10 seine hauls (1-11 fish per haul) during each sampling date. Error bars are standard error.

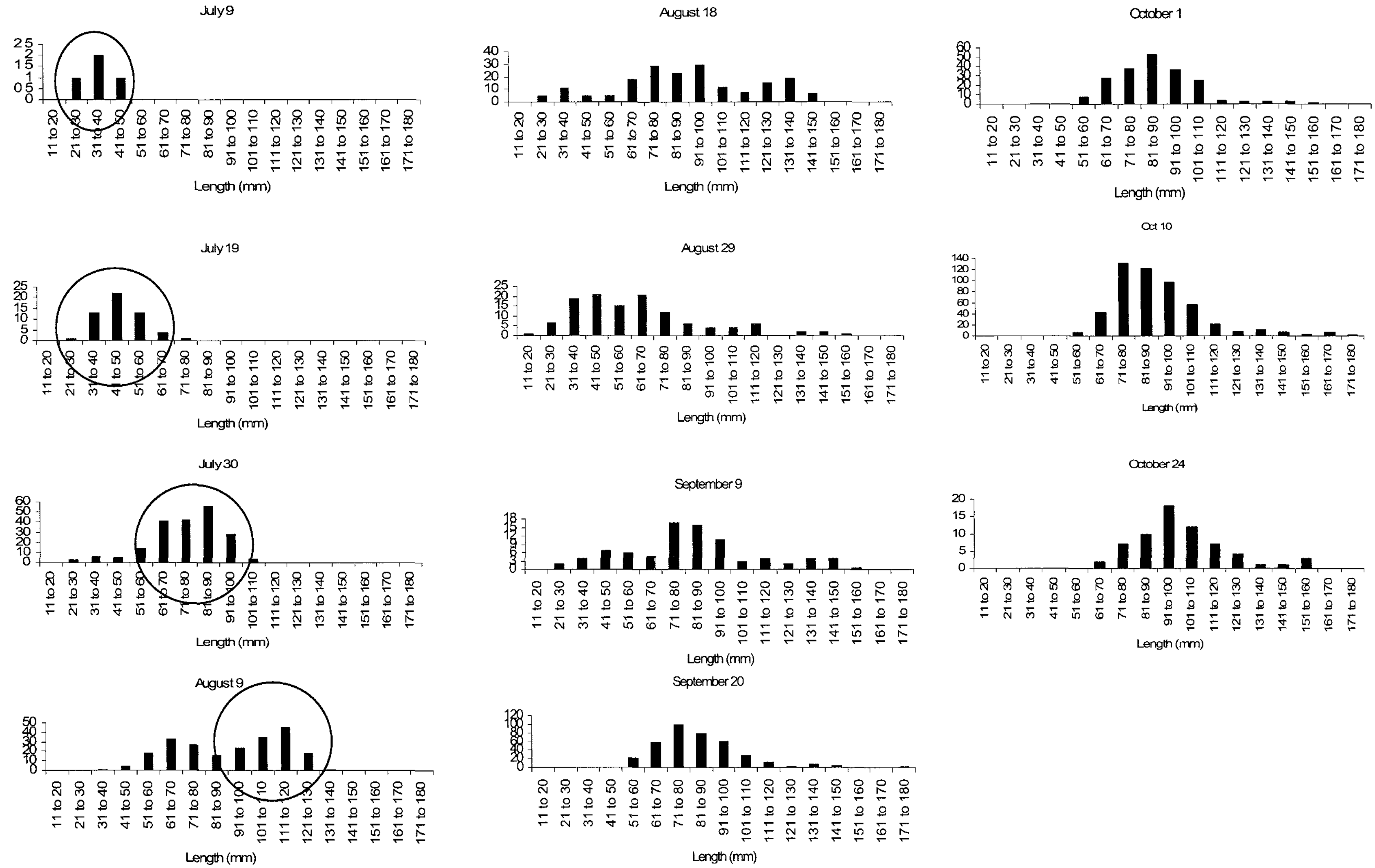

Figure 5.6. Modal progression length frequency histograms of YOY *S. fuscus* in the Stanley River estuary. N = 10 seine hauls during each sampling date. Note differences in y-axis.

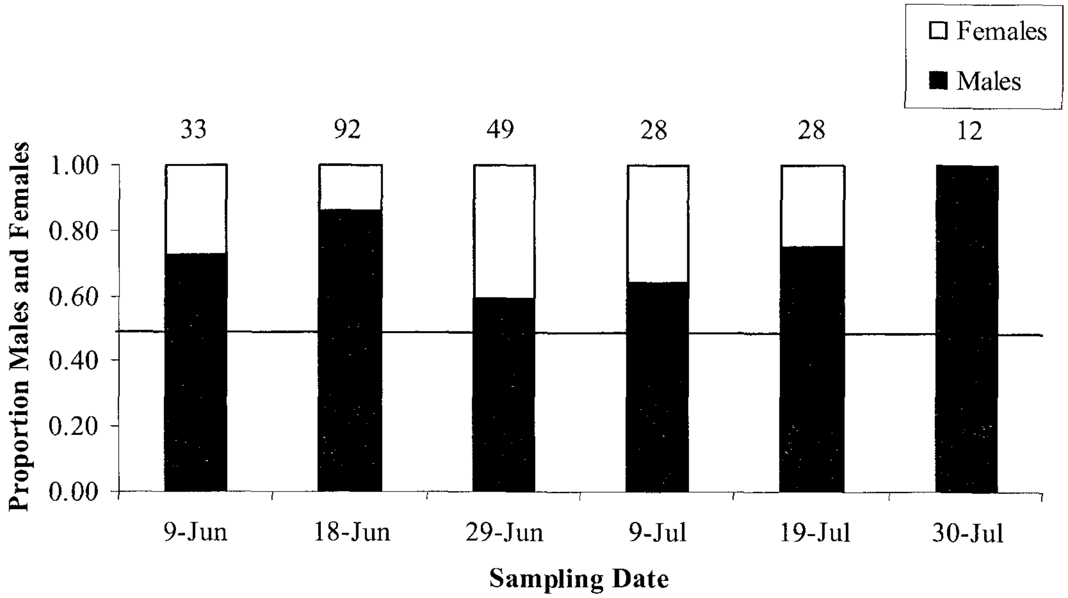

Figure 5.7. Sex ratios of adult male and female *S. fuscus* throughout the sampling period. Sample sizes are indicated above bars.

September indicating a spawning season that spanned at least four months. However, the majority of reproductively active individuals were captured in June and July. Female size ranged from 137 mm to 208 mm TL. The smallest sexually mature female was 146 mm as distinguished by the presence of well-developed ovaries with orange oocytes. Male size ranged from 98 mm to 180 mm. The smallest sexually mature male, holding fertilized eggs in a developed brood pouch, measured 116 mm. Brood sizes ranged from 38 to 385 embryos; there was a significant positive relationship between male length and brood size ($r^2 = 0.512$, $p < 0.001$, $n = 57$) (Figure 5.8). This corresponds to the size of the brood pouch of males increasing as males grow throughout the summer. Embryos within the brood pouch were identical in developmental stage with the exception of one male which had eyed embryos on the right side of the brood pouch and eggs on the left.

5.4.2 Differences between eelgrass and sea lettuce sites

Mean eelgrass cover in the sea lettuce habitat was estimated to be 14%, while cover in the eelgrass site was 64% based on an assessment on July 9th. The amount of sea lettuce did change throughout the season; however, this estimate is representative of the vegetation differences between the sites. The sea lettuce habitat consistently had a larger volume of sea lettuce and small quantities of eelgrass, while the eelgrass habitat had a visible expanse of eelgrass plants with small, variable quantities of sea lettuce floating through due to changes in tides or wind/wave action.

5.4.2.1 Population characteristics and growth rates

Adults and YOY were significantly more abundant in the eelgrass site than the sea lettuce site throughout the sampling period (ANOVA, $F_{1,58} = 64.403$, $p < 0.001$, and $F_{1,108}$

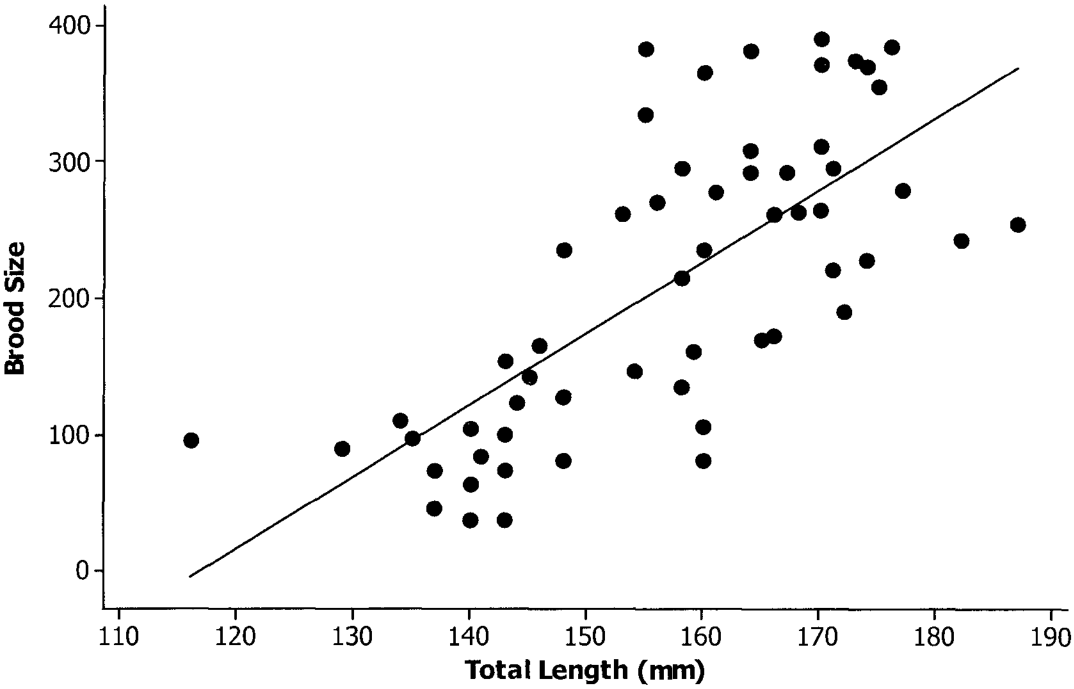

Figure 5.8. Regression analysis of the total length (mm) of *S. fuscus* males, in relation to brood sizes, sampled from June 9th to July 30th, n = 57.

= 5.752 p = 0.018, respectively) (Figure 5.9). If males and females are analyzed separately, the trend remains consistent; both sexes were more abundant in eelgrass. Males were significantly smaller in sea lettuce (2-way ANOVA, $F_{1,53} = 11.037$, p = 0.002). Female length did not differ significantly between the habitats (2-way ANOVA, $F_{1,27} = 3.832$, p = 0.061) (Figure 5.10). Growth rates of males in both habitats were approximately 0.95 mm d^{-1} (June 9th – July 19th) in sea lettuce and 1.05 mm d^{-1} in eelgrass. Female growth rate in sea lettuce was 0.87 mm d^{-1} and 0.95 mm d^{-1} in eelgrass. Growth rate of YOY pipefish in eelgrass was estimated at 1.90 mm d^{-1} (July 9th – Oct 10th) for the entire season, while in sea lettuce was about 1.87 mm d^{-1}.

5.4.2.2 Reproductive characteristics

Male *S. fuscus* showed no significant differences in reproductive characteristics between the eelgrass and sea lettuce habitats. Males in the sea lettuce had no significant difference in brood pouch weight, using body weight as a covariate when compared to those living in eelgrass (ANCOVA, $F_{1,36} = 2.961$, p = 0.093) (illustrated as brood pouch somatic index BPSI; Figure 5.11). However, both shared a common trend: BPSI increased dramatically during the month of June, and remained relatively constant in July (Figure 5.12). Brood sizes (number of embryos) were significantly different between the habitats. (ANOVA, $F_{1,56} = 7.779$, p = 0.007). Mean brood sizes in the sea lettuce and eelgrass habitats were 169 ± 17 and 235 ± 18 embryos respectively. However, when variability in length is controlled, there was no significant difference in brood size between the habitats (ANCOVA, $F_{1,36} = 0.593$, p = 0.446) (Figure 5.13). Female gonad weight with body weight as a covariate also did not differ between the two habitats (ANCOVA, $F_{1,25} = 0.173$, p = 0.681) (Figure 5.14). GSI of females living in both sea lettuce and eelgrass

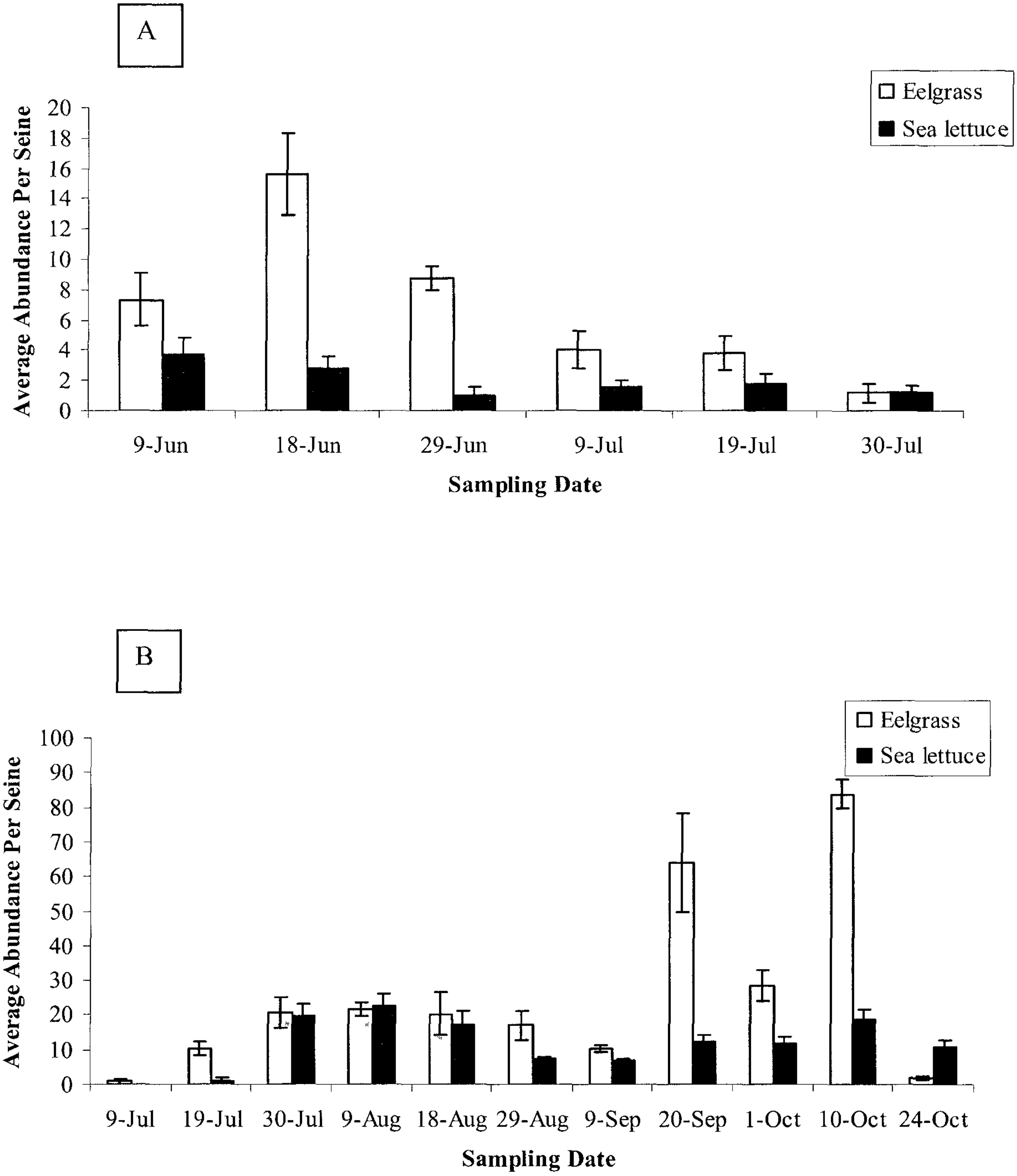

Figure 5.9. Average abundance of A) adult and B) YOY *S. fuscus* captured per beach seine haul in eelgrass and sea lettuce habitats throughout the sampling period. N = 5 seines in each habitat. Error bars are standard error.

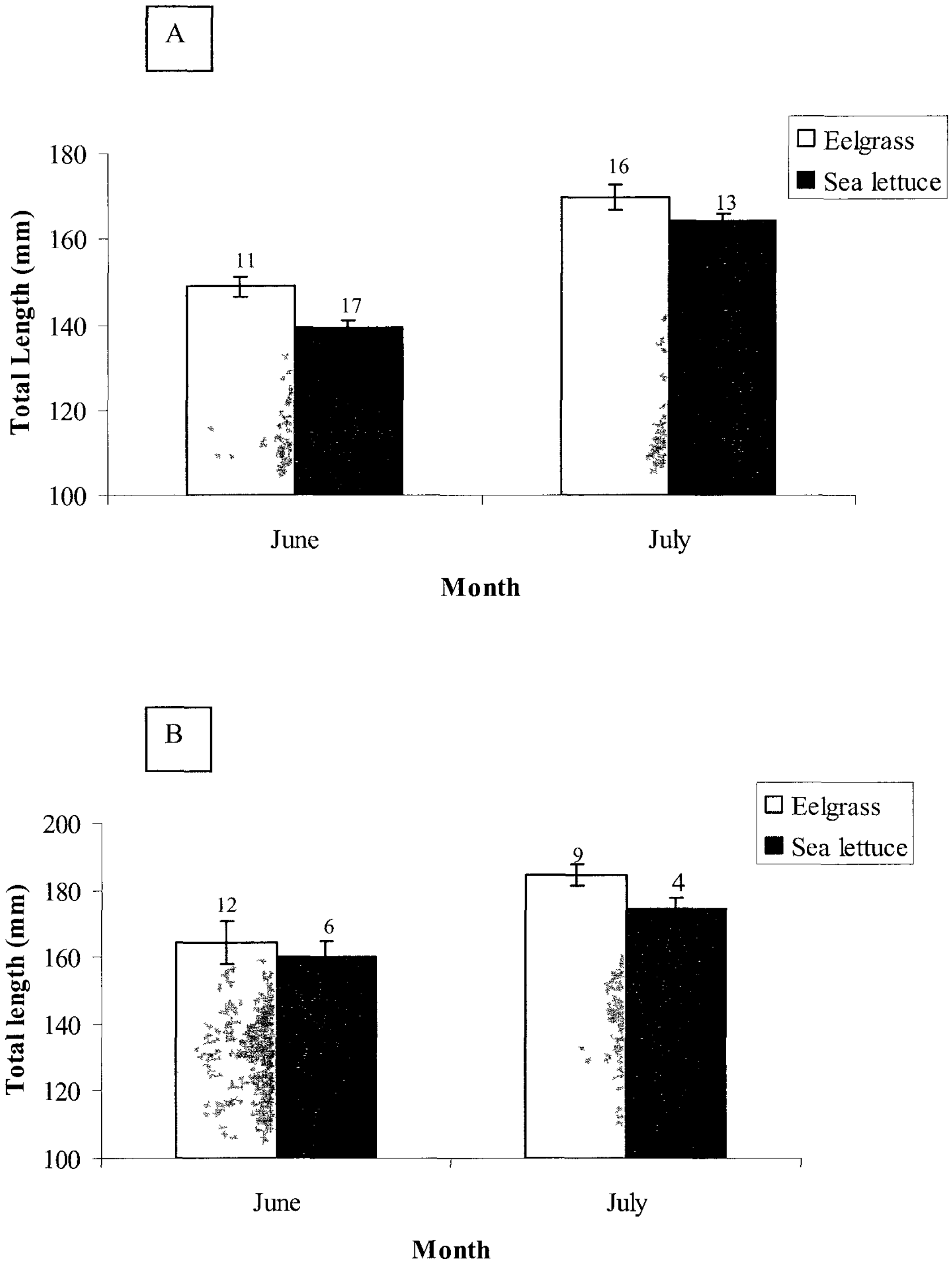

Figure 5 10 Mean total length (mm) of A) male and B) female *S fuscus* in the eelgrass and sea lettuce habitat during June and July Sample sizes are indicated above bars Error bars are standard error

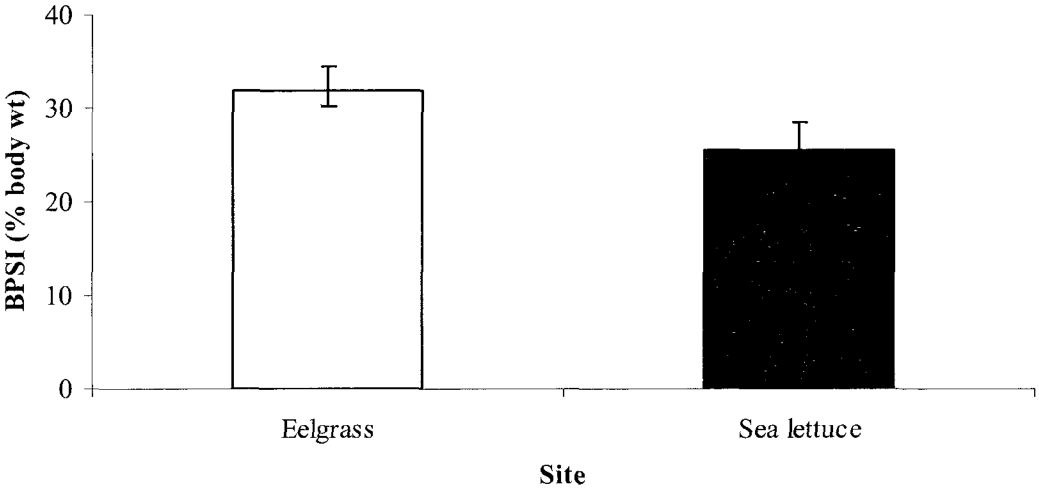

Figure 5.11. Mean brood pouch somatic index (BPSI) of male *S. fuscus* in the eelgrass and sea lettuce habitat between June 29th and July 30th, calculated using eviscerated frozen body weight as the covariate (n = 20 for eelgrass and 19 for sea lettuce). Error bars are standard error.

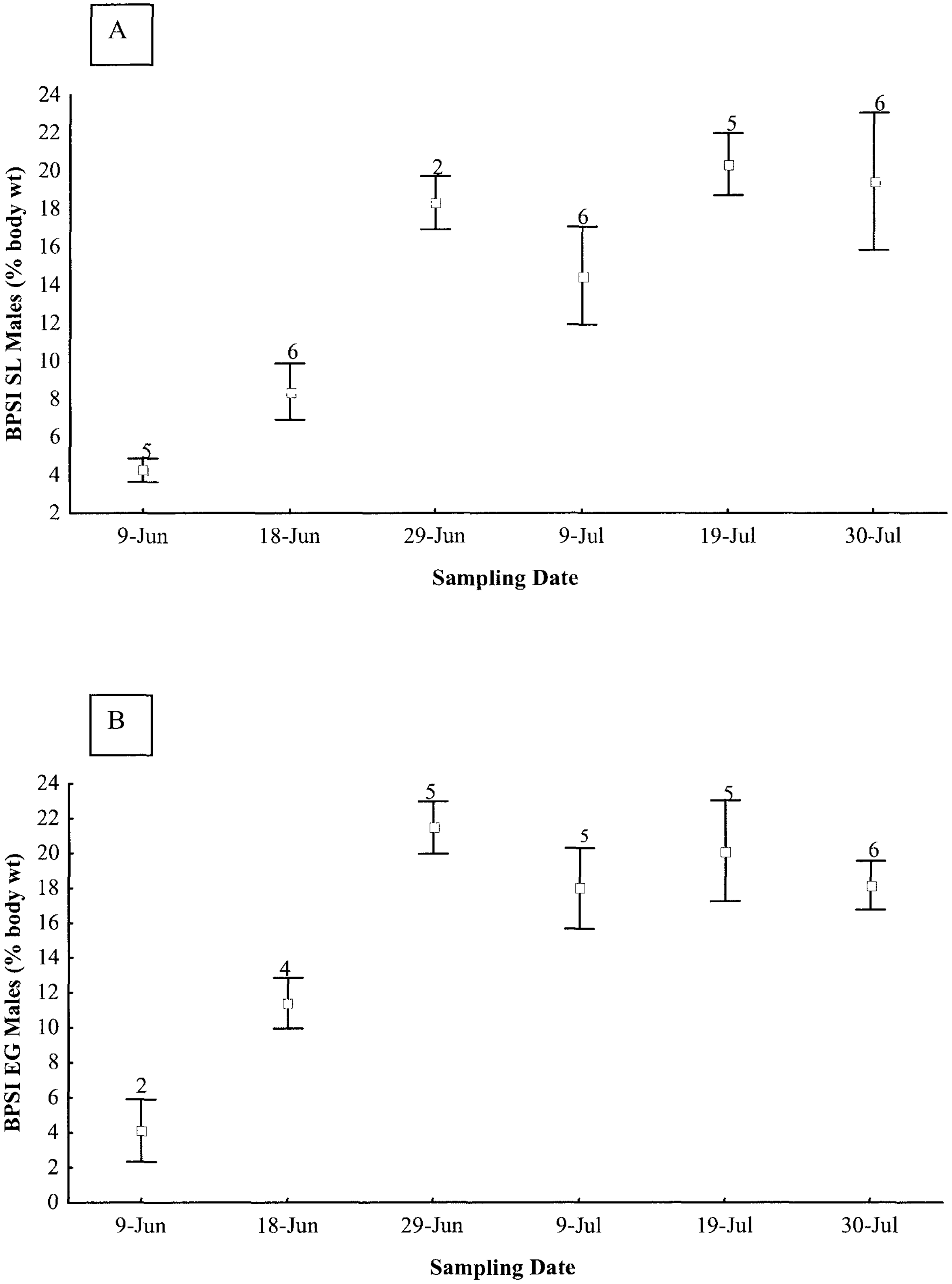

Figure 5.12. Mean brood pouch somatic index (BPSI) of A) males in the sea lettuce habitat and B) males in the eelgrass habitat throughout the sampling period. Sample sizes are indicated above bars. Error bars are standard error.

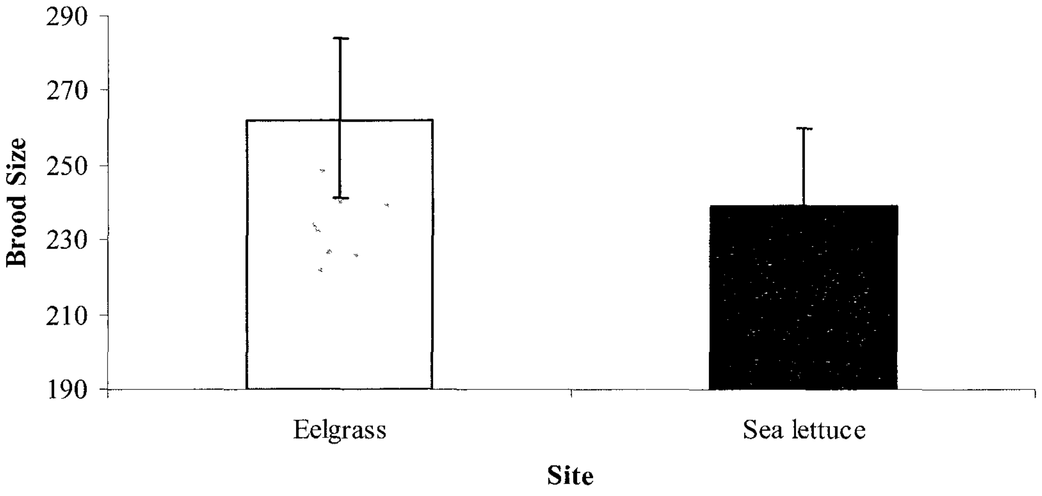

Figure 5.13. Mean brood size of *S. fuscus* males in the eelgrass and sea lettuce habitat between June 29th and July 30th, calculated using length as the covariate (n = 20 in eelgrass and 19 in sea lettuce). Error bars are standard error.

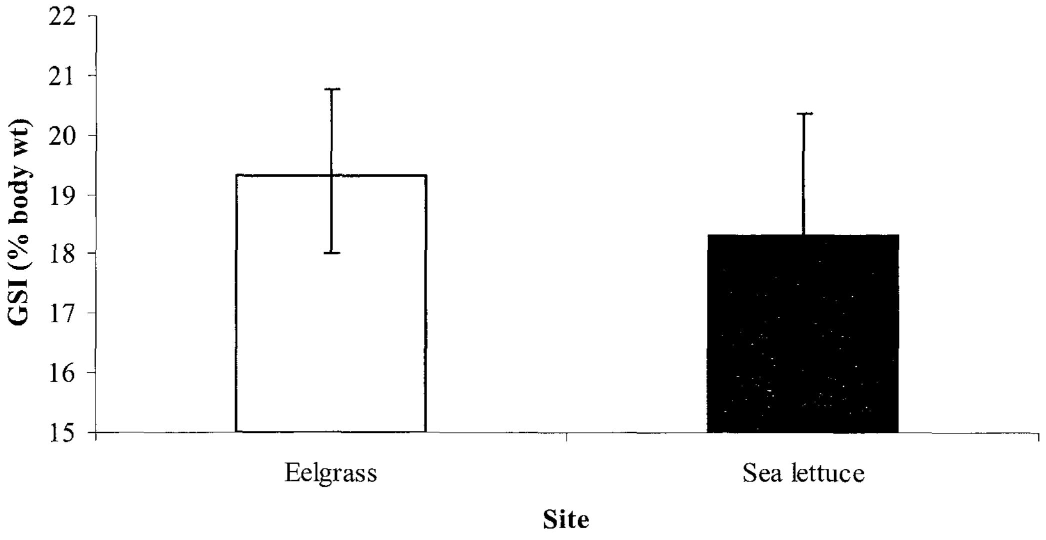

Figure 5.14. Mean gonadosmatic index (GSI) of female *S. fuscus* in the eelgrass and sea lettuce habitat between June 18th and July 19th,calculated using eviscerated body weight as the covariate (n = 19 in eelgrass and 9 in sea lettuce). Error bars are standard error.

increased through the month of June and subsequently declined in July (Figures 5.15). The peak in female GSI corresponded to a peak in male BPSI that occurred in late June. Trends for LSI for males and females in both habitats are shown in figures 5.16 and 5.17, no clear patterns were detected and therefore no statistical tests were preformed. Lastly, body condition of *S. fuscus* males and females are shown in figures 5.18 and 5.19 respectively. Condition of males did not differ significantly between the sea lettuce and eelgrass ($F_{1,36} = 0.811$, $p = 0.373$), while condition of females was significantly lower in the sea lettuce habitat (ANCOVA, $F_{1,25} = 4.144$, $p = 0.05$) (Figure 5.20).

5.4.3 Other estuaries

Tracadie had significantly more adult pipefish per seine haul than Kildare, Freeland, Brooks, Stanley, Wheatley, and Midgell (ANOVA, $F_{7,72} = 21.785$, $p < 0.001$, Tukey's $p < 0.05$), while significantly more YOY pipefish were captured per seine haul in Bideford than in Kildare, Freeland, Brooks, Stanley, Tracadie, and Midgell (ANOVA, $F_{7,72} = 29.490$, $p < 0.001$, Tukey's $p < 0.05$) (Figure 5.21). Males were significantly more abundant than females in June and August ($\chi^2 = 114.729$, $p < 0.001$ and $\chi^2 = 10.074$, $p = 0.002$ respectively). In June, Tracadie males were significantly smaller than Stanley males. Although Tracadie males also had lower brood sizes, BPSI was smallest in males in Midgell (Table 5.1). In August, males captured in Midgell and Tracadie were significantly smaller than males from Stanley. While Tracadie males had the smallest brood sizes and BPSI there were no significant differences between estuaries (Table 5.1). Comparing females in June, Bideford females were significantly larger. Although Stanley females had the highest GSI, there were no significant differences between estuaries (Table 5.2). In August there were no significant differences between any of the measured

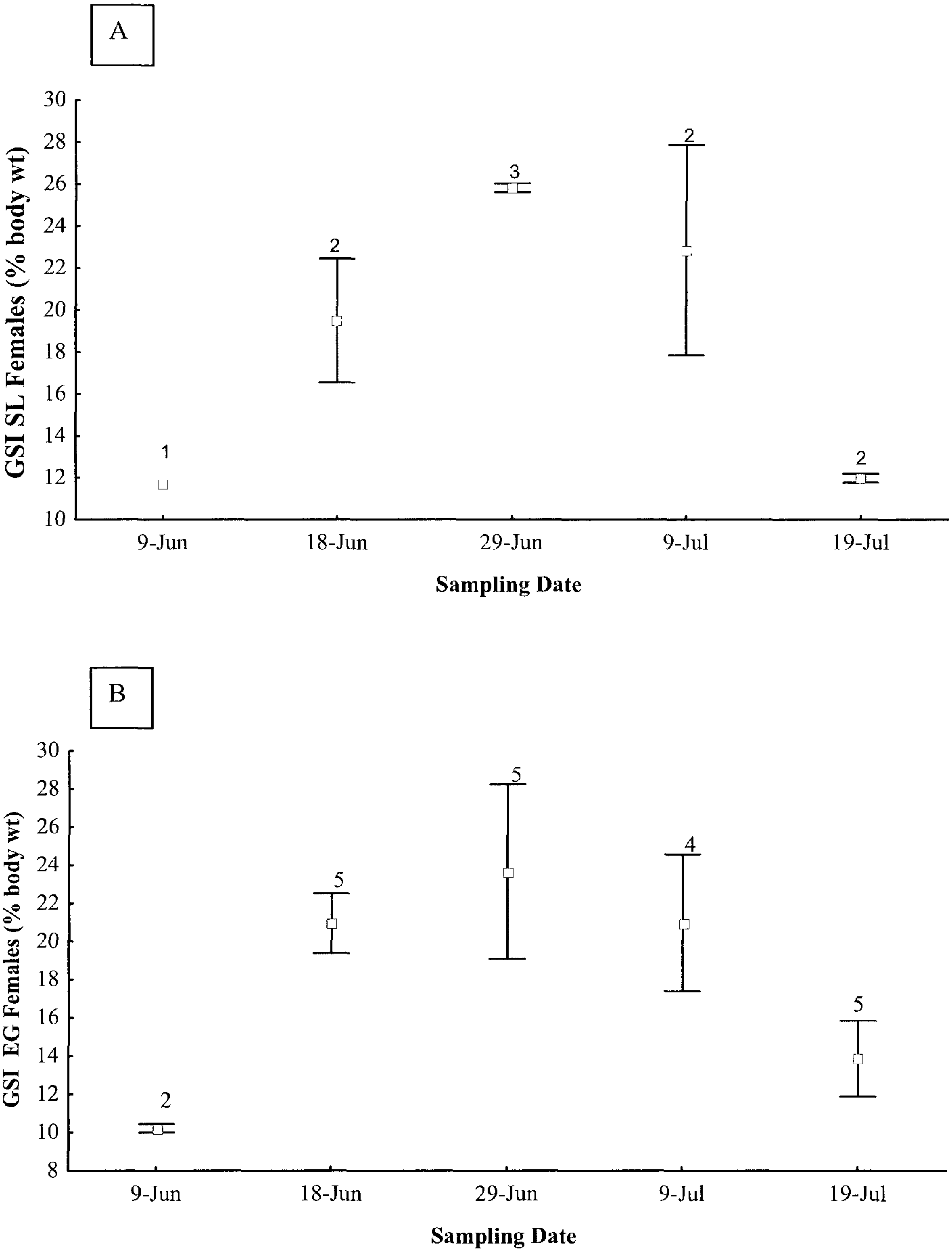

Figure 5.15. Mean gonadosomatic index (GSI) of A) females in the sea lettuce habitat and B) females in eelgrass throughout the sampling period. Sample sizes are indicated above bars. Error bars are standard error.

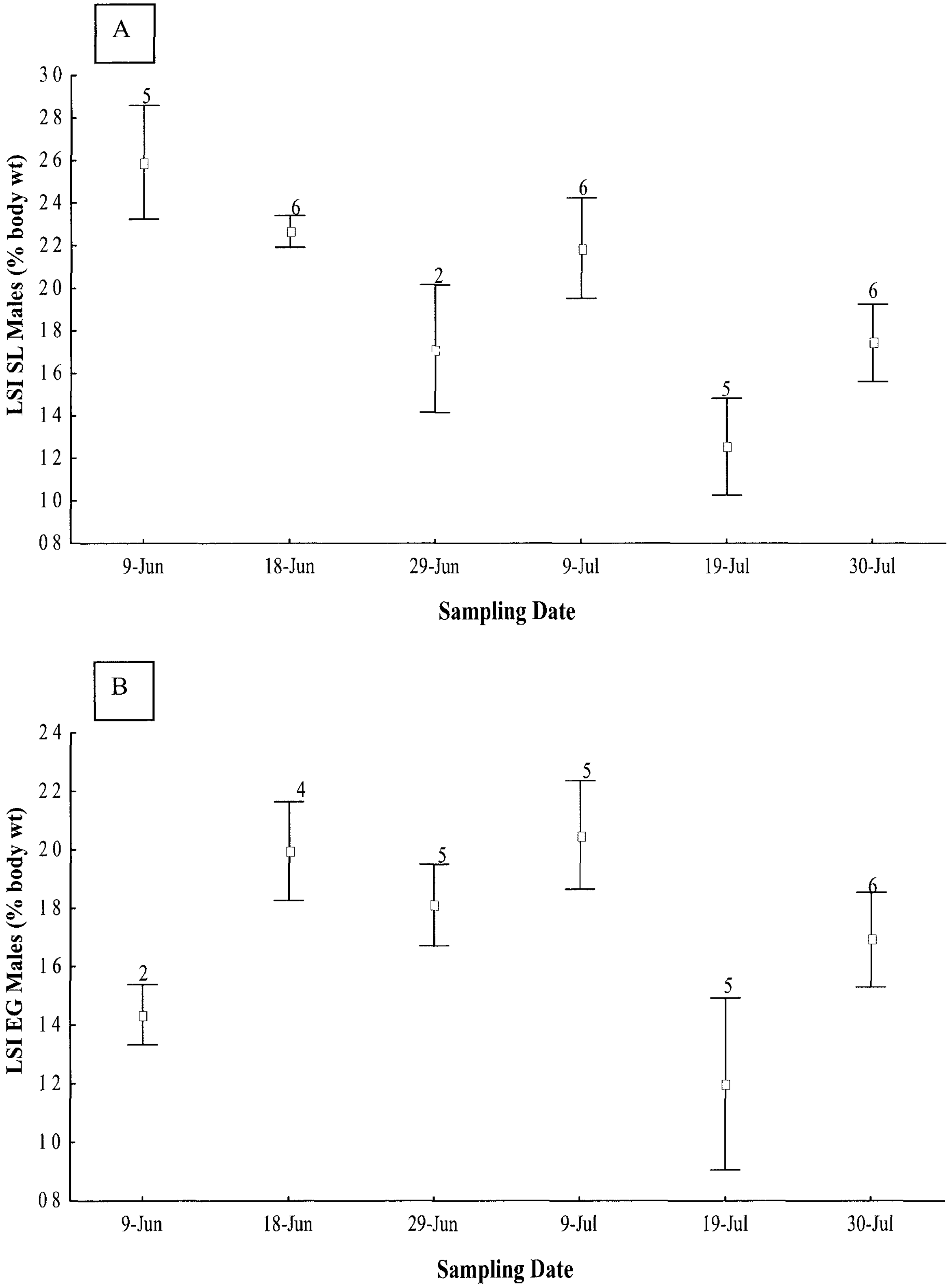

Figure 5. 16. Mean liver somatic index (LSI) of A) males in the sea lettuce habitat and B) males in eelgrass throughout the sampling period. Sample sizes are indicated above bars. Error bars are standard error.

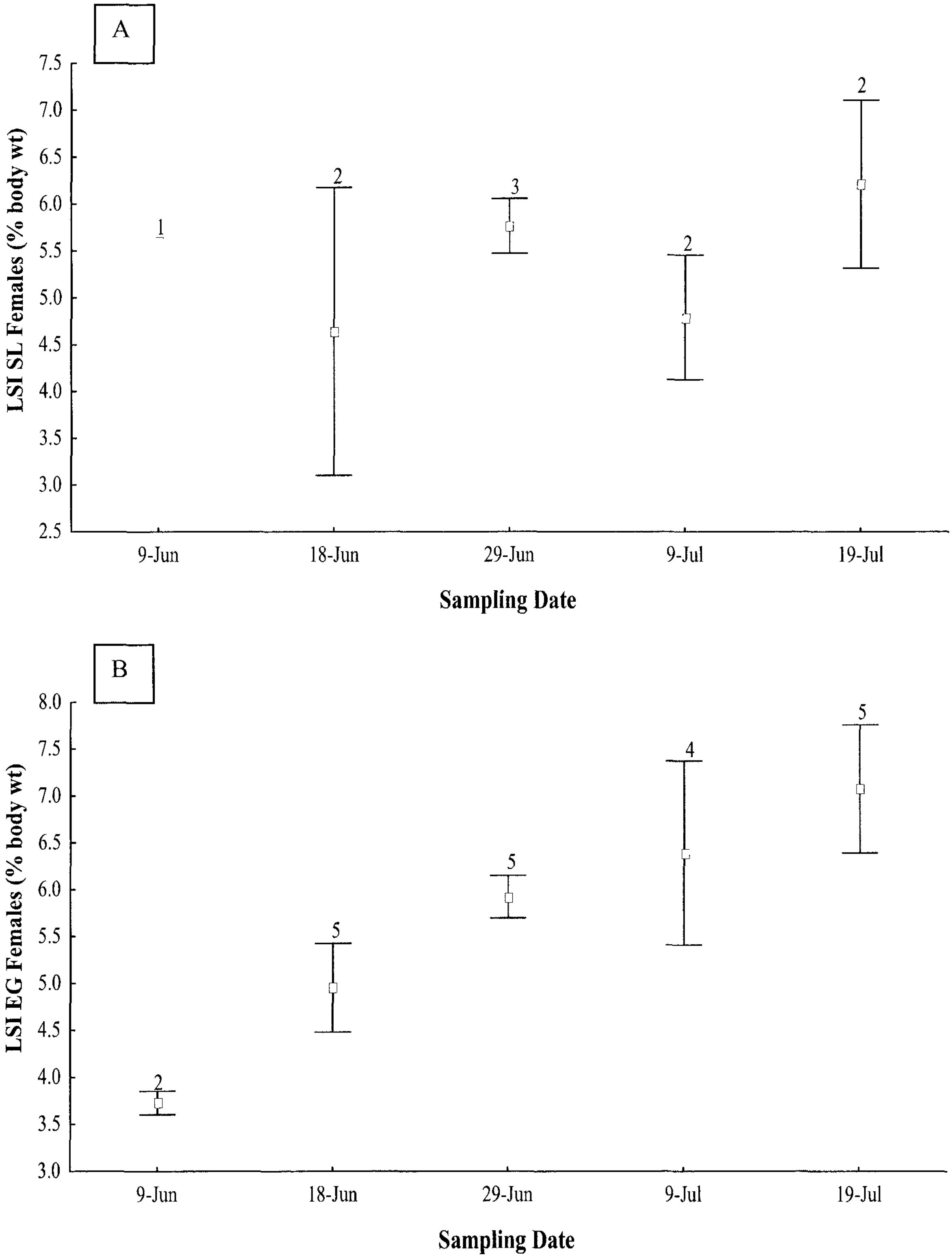

Figure 5.17. Mean liver somatic index (LSI) of A) females in the sea lettuce habitat and B) females in eelgrass throughout the sampling period. Sample sizes are indicated above bars. Error bars are standard error.

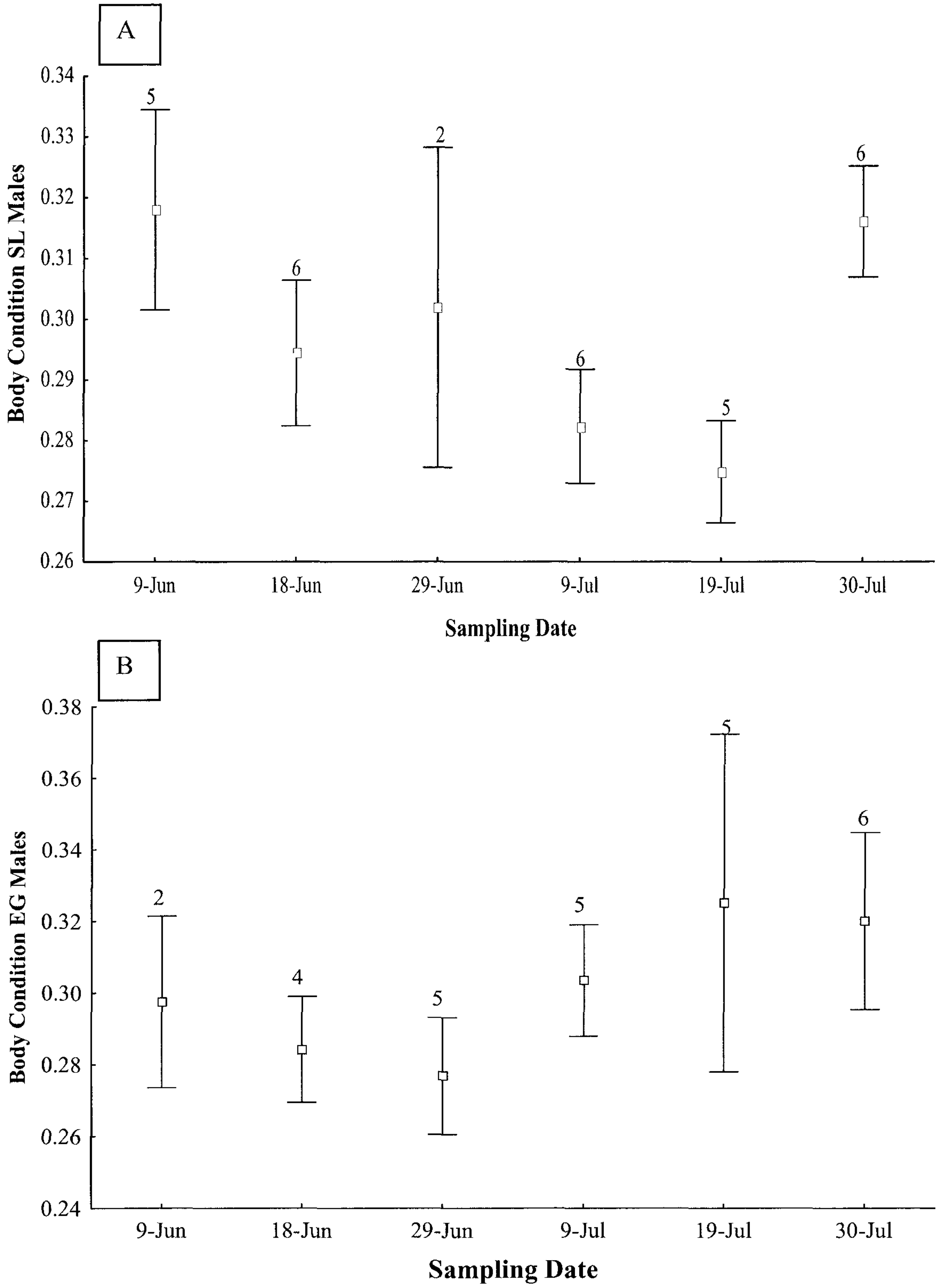

Figure 5.18. Mean body condition of A) males in the sea lettuce habitat and B) males in eelgrass throughout the sampling period. Sample sizes are indicated above bars. Error bars are standard error.

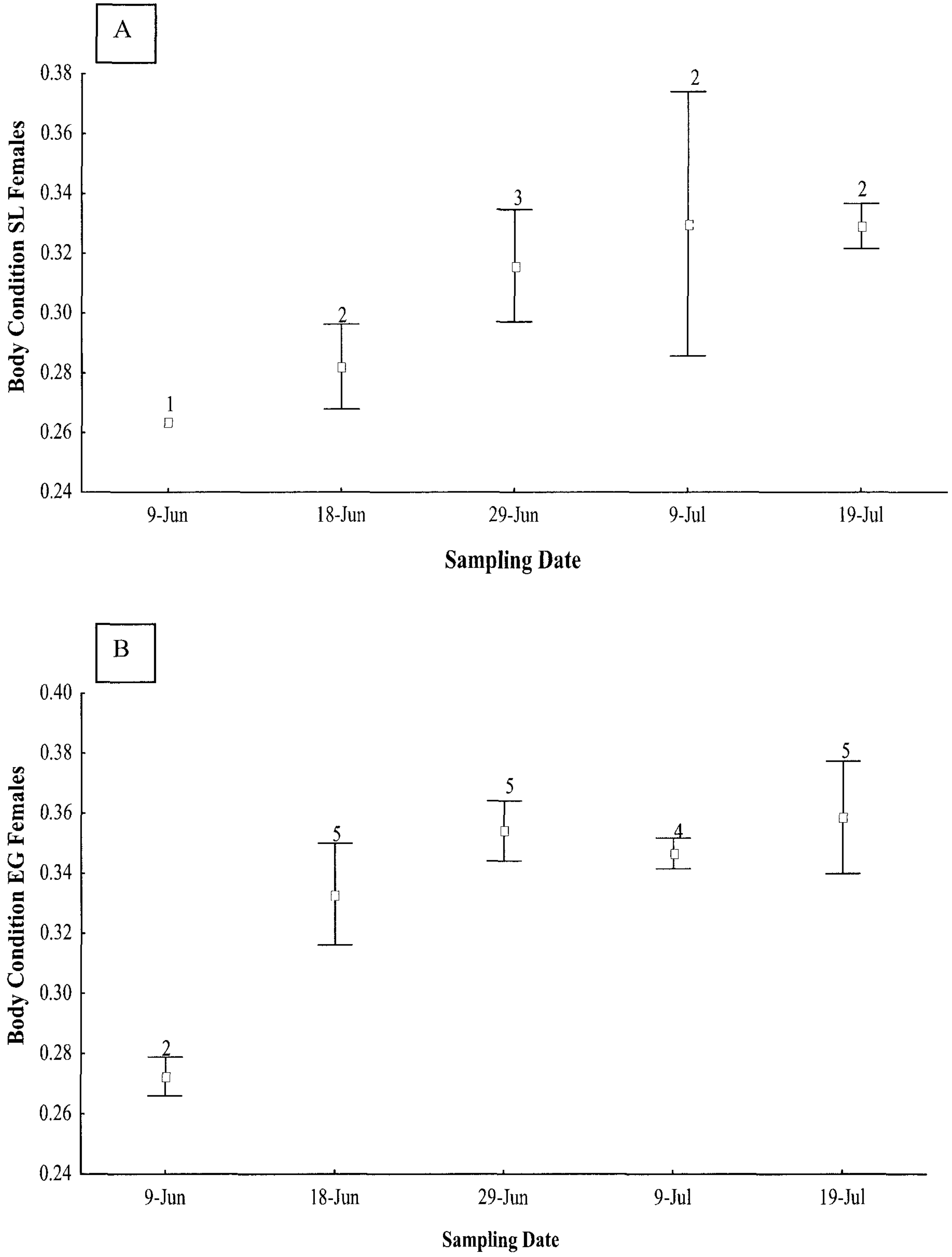

Figure 5.19. Mean body condition of A) females in the sea lettuce habitat and B) females in eelgrass throughout the sampling period. Sample sizes are indicated above bars. Error bars are standard error.

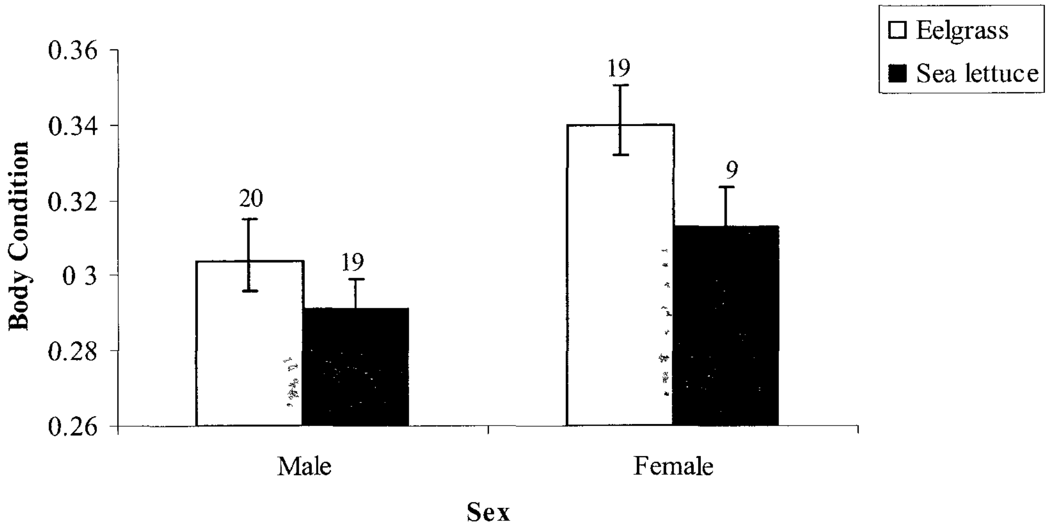

Figure 5.20. Mean body condition of male and female *S. fuscus* in the eelgrass and sea lettuce habitat calculated using length as the covariate. Sample sizes are indicated above bars. Error bars are standard error.

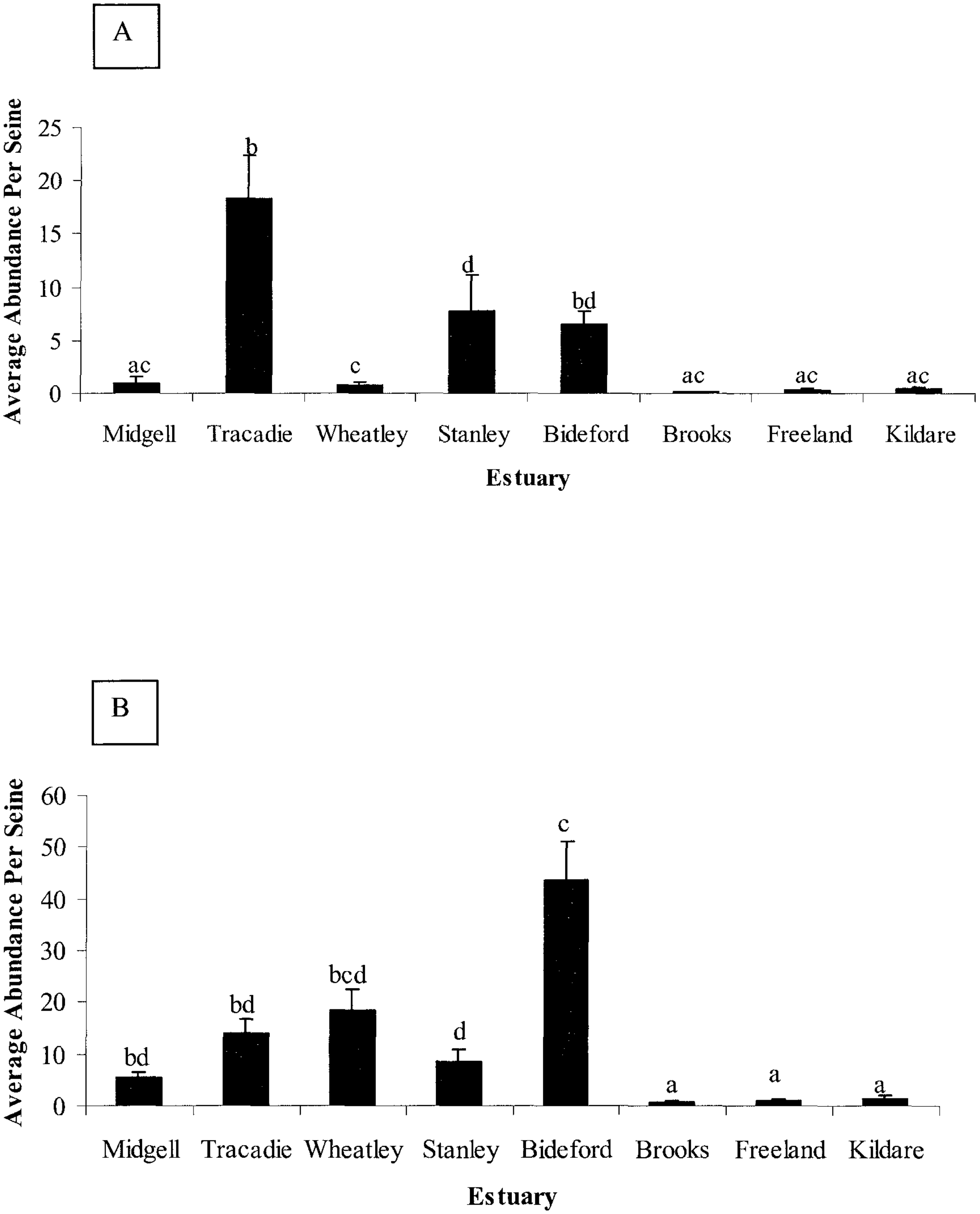

Figure 5.21. Average abundance per beach seine haul of A) adult *S. fuscus* captured in each estuary in June (n = 10 seine hauls in each estuary) and B) number of young-of-the-year (YOY) *S. fuscus* captured in each estuary in August (n = 10 seine hauls in each estuary). Bars sharing common letters are not significant. Error bars are standard error.

Table 5.1. Mean LSI, BPSI, brood size, weight, and length of *S. fuscus* males in June 2009 in Midgell, Tracadie, Stanley, and Bideford River estuaries and in August in Midgell, Tracadie, and Stanley River estuaries.

Estuary	Sample size	Sample date	LSI (± SE)	BPSI (± SE)	Brood size (± SE)	Weight (g) (± SE)	Length (mm) (± SE)
June							
Midgell	5	16 June	2.48 (± 0.19)	13.13 (± 1.91)	120.4 (± 9.90)	1.08 (± 0.05)	133.8 (± 3.4)
Tracadie	5	19 June	2.75 (± 0.29)	16.42 (± 5.35)	85.6 (± 14.1)	0.90 (± 0.04)	128.2 (± 2.8)
Stanley	5	18 June	2.00 (± 0.17)	13.76 (± 1.96)	120.4 (± 15.1)	1.23 (± 0.06)*	144.6 (± 3.1)*
Bideford	5	14 June	1.97 (±0.19)	16.96 (± 3.11)	117.8 (± 18.4)	1.12 (± 0.08)	138.6 (± 2.0)
August							
Midgell	5	15 Aug	1.37 (± 0.18)	32.85 (± 1.46)	318 (± 11.3)	2.15 (± 0.06)	158.0 (± 1.5)
Tracadie	5	16 Aug	1.75 (± 0.24)	24.90 (± 4.56)	281.4 (± 33.6)	2.08 (± 0.12)	163.0 (± 2.7)
Stanley	5	9 Aug	1.71 (± 0.23)	26.62 (± 3.84)	329 (± 32.1)	2.50 (± 0.13)*	171.8 (± 3.5)*

Within each month significant differences between estuaries for each variable are indicated ($p < 0.05$). Weight and length were tested using one-way analysis of variance (ANOVA) while LSI, BPSI, and brood size using analysis of covariance (ANCOVA, weight as the covariate).

Table 5.2. Mean GSI, LSI, weight, and length of *S. fuscus* females in June in Midgell, Tracadie, Bideford, and Stanley River estuaries and Midgell and Tracadie estuaries in August 2009.

Estuary	Sample size	Sample date	GSI (± SE)	LSI (± SE)	Weight (g) (± SE)	Length (mm) (± SE)
June						
Midgell	5	16 June	19.06 (± 2.41)	5.72 (± 0.81)	1.44 (±0.06)	146.8 (± 2.4)
Tracadie	5	19 June	14.98 (± 2.07)	7.65 (± 1.07)	1.53 (±0.09)	151.2 (± 1.8)
Stanley	5	18 June	20.95 (± 1.57)	4.95 (± 0.47)	1.80 (±0.07)	158.2 (± 2.2)
Bideford	4	14 June	15.54 (± 1.39)	7.90 (± 0.34)	2.60 (±0.15)*	172.8 (± 4.0)*
August						
Midgell	5	15 Aug	23.15 (± 2.93)	7.22 (± 0.83)	3.68 (±0.41)	187.6 (± 6.4)
Tracadie	2	16 Aug	11.69 (± 2.46)	5.86 (± 0.07)	2.53 (±0.02)	173.5 (± 0.5)

Within each month significant differences between estuaries for each variable are indicated ($p < 0.05$). Weight and length were tested using one-way analysis of variance (ANOVA) while LSI and GSI using analysis of covariance (ANCOVA, weight as the covariate).

variables between estuaries.

5.4.4 2008 results

S. fuscus were collected from Midgell and Stanley in June and July 2008. The sample size was larger since we attempted to take ten pipefish from each of eight sites that we were seining. In total 32 pipefish were collected from Midgell and 98 from Stanley throughout the two months; no females were captured in Midgell. Stanley had significantly more pipefish than Midgell throughout the sampling season (2-way ANOVA $F_{1,28} = 16.062$, $p < 0.01$) (Figure 5.22). When comparing the characteristics of the fish, Stanley males were significantly larger and longer, and had significantly larger brood sizes than Midgell. However, BPSI (brood pouch somatic indices) showed few differences between the estuaries. Lastly, the body condition of males was significantly higher in Stanley in June (Table 5.3).

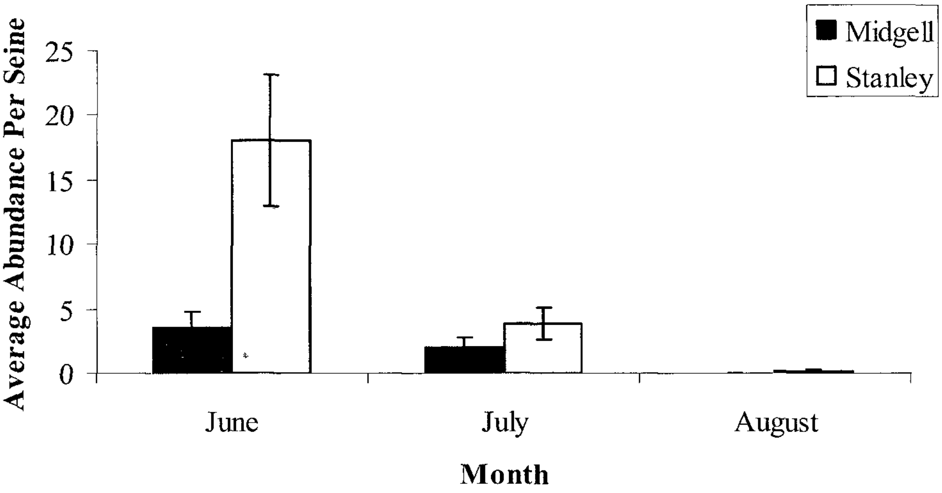

Figure 5.22. Average abundance of *S. fuscus* captured per seine haul in Midgell and Stanley River estuaries in 2008. N = 8 seine hauls in each month in each estuary. Error bars are standard error.

Table 5.3: Mean BPSI, brood size (males), GSI (females), weight, length, and body condition of *S. fuscus* in Midgell and Stanley River estuaries in June and July 2008. N/A= data not applicable for that variable.

Estuary	BPSI (± SE)	Brood size (± SE)	GSI (± SE)	Weight (g) (± SE)	Length (mm) (± SE)	Body Condition (± SE)
Males – June						
Midgell (n=22)	19.52 (± 0.72)	80.7 (± 6.2)	N/A	0.80 (± 0.03)	124.1 (± 0.18)	0.0419 (± 0.0007)
Stanley (n=42)	19.69 (± 0.48)	118.1 (± 7.1)	N/A	1.32 (± 0.03)*	137.6 (± 0.10)*	0.0504 (± .0006)*
Males – July						
Midgell (n=10)	22.56 (± 1.30)	225.4 (± 19.3)	N/A	1.86 (± 0.07)	15.46 (± 0.20)	0.0505 (± 0.002)
Stanley (n=23)	24.75 (± 0.86)	354.4 (± 16.2)	N/A	2.37 (± 0.07)*	16.93 (± 0.16)*	0.0494 (± 0.001)
Females						
Stanley-June (n=24)	N/A	N/A	3.09 (± 1.20)	1.89 (± 0.08)	155.4 (± 1.5)	N/A
Stanley-July (n=8)	N/A	N/A	13.00 (± 0.85)	3.4 (± 0.22)	184.3 (± 3.0)	N/A

Differences between *S. fuscus* in Midgell and Stanley were tested with a one-way analysis of variance (ANOVA) and significant results are indicated. *p<0.05.

5.5 Discussion

5.5.1 Population structure and growth rates

Modal progression length frequency histograms showed that adult growth rates of *S. fuscus* in this study were similar to those reported elsewhere. Ripley and Foran (2006) report a growth rate of 1.0 mm d^{-1} from May to September, while Campbell and Able (1998) found that *S. fuscus* grew at a rate of 1.2 mm d^{-1}. However, in both these studies young-of-the-year (YOY) fishes appeared earlier in the season (May-early June), and by late June the separation between adult and YOY size classes was not distinct; therefore the growth rate reported is an overall value. In the present study YOY fishes did not appear until July and size classes were more distinct, therefore we were able to report growth rates for both adult (1.0 mm d^{-1}) and YOY fishes (1.9 mm d^{-1}). However, Ripley and Foran (2006) did find that YOY fishes grew faster than adult conspecifics.

Population structure of *S. fuscus* was similar to that found by Campbell and Able (1998) in Chesapeake Bay, Virginia and Ripley and Foran (2006) in Chincoteague Bay, Virginia with larger individuals captured exclusively in the early spring and the population becoming YOY dominated as the season progressed. The current study differed in that YOY appeared later in the season, which is most likely a latitudinal response to temperature, as PEI estuaries do not typically warm up as quickly as estuaries further south. Among the Syngnathids water temperature strongly impacts both seasonal migration (Lazzari and Able 1990; Vincent et al. 1995) and reproduction (Campbell and Able 1998; Bolland and Boettcher 2005; Ripley and Foran 2006; Ahnesjö 2008). Based on this collection and that of Campbell and Able (1998) and Ripley and Foran (2006) it is believed that this species is best represented by two year classes, growing in the first season, and spawning in season two. It is possible, however, that some adult individuals may be two years old entering the spawning season. Otolith or some other aging procedure is needed to confirm the life span of this species. Although determination of age by otoliths was attempted in the present study, it proved unsuccessful as the otoliths are very small and difficult to locate.

Using modal progression length frequency to represent population structure and calculate growth rates can be subjective and is based upon selectivity of the sampling gear while further assuming that mortality in each cohort would be comparable (Campbell and Able 1998; Takahashi et al. 2003; Ripley and Foran 2006). In terms of sampling gear selectivity, Campbell and Able (1998) found that most of the smaller fishes sampled were captured in plankton nets, while sampling gear with mesh size (≥ 3 mm) captured individuals larger than 45 mm. Our seine net may not have captured all the smaller individuals in the sampling area, but consistently captured individuals that were between 20 and 35 mm (smallest 17 mm) which is similar to Bolland and Boettcher (2005) who captured individuals as small as 23 mm using a 5 mm mesh size and Ripley and Foran (2006) who captured individuals around 32 mm using a 4 mm mesh size. As noted by Ripley and Foran (2006) and observed in this study, the mixture of algae, eelgrass, and nekton intertwined in the seine net likely prevented escape and allowed capture of most of the smaller individuals. Lastly, mortality was most likely greater for smaller *S. fuscus*, which are at a higher risk for predation and cannibalism (as YOY are often found in the guts of adults, particularly females (pers. obs.). However, as noted by Campbell and Able (1998) and Ripley and Foran (2006) such events cannot be controlled in field studies. Therefore, the use of modal progression was appropriate to determine population structure and growth rates of *S. fuscus* over the time period investigated.

5.5.2 Sex ratios and reproductive characters

In the nearshore areas sampled in this study, the sex ratio of *S. fuscus* was biased towards males. Male biased sex ratios have been observed for *S. rostellatus* (Vincent et al. 1995), *S. schlegeli* (Watanabe and Watanabe 2001), and *N. lumbriciformis* (Lyons and Dunne 2003). Roelke and Sogard (1993) found that *S. fuscus* show sex-based habitat preferences in which gestating males remain in eelgrass habitat where they are protected and females would travel from seagrass beds and across bare areas, possibly searching for mates which may influence sex ratios. Interestingly, Ripley and Foran (2006) found that the sex ratio of *S. fuscus* within an eelgrass habitat of similar depth to that sampled in this study was female biased. If seining in their study had occurred in deeper waters or across an eelgrass/ bare substrate mixed habitat the sex ratio bias may have been reduced or disappeared. Females of other Syngnathid species are known to occur in deeper inshore waters (Vincent et al. 1995) and female *S. fuscus* are more likely to occur in unvegetated areas (Roelke and Sogard 1993) than are males. The sex ratio difference observed between this study and that of Ripley and Foran (2006) is intriguing and may reflect population differences and higher competition for mates among different study areas.

Brooding period and reproductive activity spanned a slightly shorter time period in the Stanley River estuary (June-September) when compared to studies conducted further south (i.e. Campbell and Able 1998 and Ripley and Foran 2006), which, as discussed above, is likely related to water temperature. The length of brooding males in this study (116 to 180 mm TL) was comparable to those found by Campbell and Able (1998) (119 to 222 mm) and Ripley and Foran (2006) (99 to 191 mm); however we found maximum male length to be slightly smaller. Female length was found to be significantly larger than males which is typical of *S. fuscus* (Ripley and Foran 2006) and other Syngnathids

(Berglund and Rosenqvist 1990; Vincent et al. 1995; Bolland and Boettcher 2005). Brood sizes were found to be substantially smaller in this study (38 to 385 embryos, mean 220) than those reported by Campbell and Able (1998) (45 to 1380 embryos), but comparable to other Syngnathid species e.g. *S. scovelli* (175 to 296 embryos) (Target 1984) and *S. biaculeatus* (60 to 200 embryos) (Takahashi et al. 2003). Unlike Campbell and Able (1998), we found that brood sizes were positively correlated with male length. This is most likely a consequence of male growth throughout the summer; as brood pouch length increased more embryos could be supported.

Contrary to Campbell and Able (1998), one brooding male pipefish encountered in this study had embryos at different developmental stages. The mating system of *S. fuscus* has not been documented (Roelke and Sogard 1993). A number of other Syngnathid species are monogamous (Berglund et al. 1989; Vincent et al. 1995; Takahashi et al. 2003; Foster and Vincent 2004), or more rarely polyandrous, as seen in *Nerophis ophidian* (Berglund et al. 1986) and *Syngnathus typhle* (Berglund and Rosenqvist 1990) were females hydrate enough eggs for more than one male. Polygnous systems have been found in *S. floridae* (Jones and Arvise 1997), and polygamous systems have also been described for *S. schlegeli* (Watanabe and Watanabe 2001), and *S. acus* and *S. rostellatus* (Vincent et al. 1995). Further research on *S. fuscus* reproductive biology is needed to substantiate the mating pattern of this species.

5.5.3 Differences between eelgrass and sea lettuce sites

5.5.3.1 Population characters and growth rates

All pipefish (males, females, and YOY) were captured in significantly higher numbers in eelgrass than in sea lettuce in this study which supports the idea that high eelgrass habitat complexity is important and necessary in maintaining pipefish populations. It is well known that seagrasses act as a refuge from predation, offer abundant food supplies and are considered nursery habitats for many species of fish (Orth et al. 1984; Lubbers et al. 1990; Heck et al. 2003). It was anticipated, therefore, that more pipefish would be found in the eelgrass-dominated habitat. Alterations in habitat complexity often have noticeable effects on fish assemblages (Hughes et al. 2002; Wyda et al. 2002). Wyda et al. (2002) found that S. *fuscus* were captured more frequently in areas of higher eelgrass density, and noted that fish abundance remained low in areas dominated by macroalgae; they therefore concluded that macroalgae does not provide a suitable alternative habitat. Hughes et al. (2002) studied fish responses to changes in eelgrass density over the long-term (one decade) and found that most species, including *S. fuscus,* which had originally been found in highest numbers in highest shoot density eelgrass habitats, were most affected by decreases in eelgrass density.

An interesting finding in this study was the significantly smaller size of males in the sea lettuce dominated habitat. Since eelgrass habitat in the area is declining, habitat use may have been dictated by density-dependent selection or interspecific competition (Pimm and Rosenzweig 1981; Rosenzweig 1991) which may have forced some of the smaller individuals into the sea lettuce habitat. Alternatively, Bell and Westoby (1986) proposed a "settle and stay" hypothesis which states that since the risk of predation outside of eelgrass habitat is high, individuals should remain in the seagrass bed in which they initially settle regardless of habitat quality relative to other habitat areas. If suboptimal habitats result in reduced growth, as discussed by Huey (1991), then males settling in sea lettuce dominated habitats would be smaller as witnessed in the present study. The difference in growth rates for males between the habitats was 0.1 mmd^{-1} which cumulatively approached a 1 cm difference throughout the sampling period. However, it is possible that density-dependent selection and/or interspecific competition may have contributed to size-specific differences between the habitats seen in this study.

5.5.3.2 Reproductive characteristics

In this study the BPSI (brood pouch somatic index) of male *S. fuscus* was not significantly reduced in the sea lettuce site, while brood sizes of males in the sea lettuce site were smaller than males living in eelgrass. Males living in sea lettuce had approximately 66 fewer embryos in their brood pouch when compared to males living in eelgrass. This reflects the size differences among males from both habitats, not necessarily a difference in the reproductive potential of males in sea lettuce to brood embryos. It is possible that smaller (or younger) males are being restricted to lower quality habitats, therefore the point that males in sea lettuce are supporting smaller broods is important to recognize.

As with male BPSI and relative brood sizes, female gonad size was not affected between the two sites. Interestingly, female body condition was found to be significantly lower in the sea lettuce site. Body condition is the relationship between eviscerated body mass and length, and is commonly used to measure energy storage in fish (Finley, 2008). It is possible that *S. fuscus* females living in sea lettuce were not able to store as much energy (possible lack of food resources), and/or had to use more energy (for reproduction or maintenance) making the body mass and therefore body condition significantly smaller.

5.5.4 Other estuaries and 2008 results

The main purpose of gathering data from the seven other estuaries was to examine potential variation in population and reproductive characteristics around PEI. Inter-estuary differences in temperature, salinity, substrate, vegetation abundance, or food availability often make comparisons between estuaries difficult to interpret (Schein 2009). Results from the seven estuaries show that although population structure (adult and YOY abundance) between estuaries are different, a male-biased sex ratio appears to be common, and there are notable differences in size and reproductive condition of *S. fuscus* among PEI estuaries. Possible reasons for this include variation in any or all of the habitat variables stated above. Reproductive characteristics (e.g. BPSI and brood sizes) varied among estuaries. Interestingly, there appeared to be a geographic range for *S. fuscus* on PEI, as few were captured in the three estuaries north west of Bideford River. This may be important given that PEI is situated within the northern limit of the range of *S. fuscus* (Dawson 1982). A study by Joseph et al. (2006) in eelgrass beds in the Kouchibouguac estuary, mid-eastern, New Brunswick did not capture a single *S. fuscus*. Likewise Thériault (2006) did not capture *S. fuscus* in Lamèque Bay, northern New Brunswick. In contrast, *S. fuscus* are present in small numbers around western Newfoundland (Hooper, D. pers. comm.). Continued and further studies on *S. fuscus* abundance in these and other PEI estuaries would be interesting to determine population dynamics on PEI.

Results from the 2008 season showed differences between the Midgell and Stanley River estuaries. Collection of *S. fuscus* in 2008 was by and large a precursor to the study in Stanley River in 2009. The Midgell River estuary had significantly less eelgrass, and consequently smaller *S. fuscus* individuals, with lower reproductive outputs than males living in the more abundant eelgrass habitats in the Stanley River estuary. Therefore it was decided to assess potential differences in reproductive characteristics and population structure between a favourable (eelgrass dominated) and unfavourable (sea lettuce dominated) habitat within the same estuary (Stanley River) thus controlling for excessive environmental variability.

This study provides information on basic population and life history characteristics of *S. fuscus* in the southern Gulf of St. Lawrence (the northern most range for this species), in addition to looking at the impacts of eelgrass habitat degradation on *S. fuscus* populations. These data will prove to be useful as baseline information for future studies on *S. fuscus* populations with definite predictions of continued habitat degradation on Prince Edward Island and elsewhere.

5.6 Literature cited

Ahnesjö, I. 2008. Behavioural temperature preference in a brooding male pipefish *Syngnathus typhle*. *Journal of Fish Biology* **73**: 1039-1045.

Bell, J. D., Westoby, M. 1986. Importance of local changes in leaf height and density to fish and decapods associated with seagrasses. *Journal of Experimental Marine Biology and Ecology* **104**: 249-274.

Berglund, A., Rosenqvist, G. 1990. Male limitation of female reproductive success in a pipefish: effects of body-size differences. *Behavioral Ecology and Sociobiology* **27**: 129-133.

Berglund, A., Rosenqvist, G., Svensson, I. 1986. Mate choice, fecundity and sexual dimorphism in two pipefish species (Syngnathidae). *Behavioral Ecology and Sociobiology* **19**: 301-307.

Berglund, A., Rosenqvist, G., Svensson, I. 1989. Reproductive success of females limited by males in two pipefish species. *The American Naturalist* **133**: 506-516.

Bolland, J., Boettcher, A. 2005. Population structure and reproductive characteristics of the gulf pipefish, *Syngnathus scovelli*, in Mobile Bay, Alabama. *Estuaries* **28**: 957-965.

Bricker, S.B. Longstaff, B., Dennison, W., Jones, A., Boicourt, K., Wicks, C., Woerner, J. 2008. Effects of nutrient enrichment in the nation's estuaries: a decade of change. *Harmful Algae* **8**: 21-32.

Campbell, B.C., Able, K.W. 1998. Life history characteristics of the northern pipefish, *Syngnathus fuscus*, in Southern New Jersey. *Estuaries* **21**: 470-475.

Curtis, J.M.R., Vincent, A.C.J. 2005. Distribution of sympatric seahorse species along a gradient of habitat complexity in a seagrass-dominated community. *Marine Ecology Progress Series* **291**: 81-91.

Dawson, C.E. 1982. Doryrhamhinae and Syngnathidae, p.1-172. In J.S Böhlke eds. Fishes of the Western North Atlantic, Sears Foundation for Marine Research, Yale University, New Haven, Connecticut.

Deegan, L.A., Wright, A., Ayvazian, S.G., Finn, J.T., Golden, H., Rand-Merson, R., Harrison, J. 2002. Nitrogen loading alters seagrass ecosystem structure and support of higher trophic levels. *Aquatic Conservation: Marine and Freshwater Ecosystems* **12**: 193-212.

Finley, M.A. 2008. Investigating the suitability of northern mummichog (*Fundulus heteroclitus macrolepidotus)* for the assessment of cumulative and non-point source pollution in Prince Edward Island estuaries. M.Sc. Thesis. University of Prince Edward Island, Charlottetown, PEI.

Foster, S.J., Vincent, A.C.J. 2004. Life history and ecology of seahorses: implications for conservation and management. *Journal of Fish Biology* **65**: 1-61.

Hanson, A.R. (ed) 2004. Status and conservation of eelgrass (*Zostera marina*) in Eastern Canada. Technical Report Series No.412. Canadian Wildlife Service, Atlantic Region. 40pp.

Hauxwell, J., Cebrián, J., Furlong, C., Valiela, I. 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology* **82**: 1007-1022.

Heck, K.L., Able, K.W., Fahay, M.P., Roman, C.T. 1989. Fishes and decapod crustaceans of Cape Cod eelgrass meadows: species composition, abundance patterns and comparison with unvegetated substrates. *Estuaries* **12**: 59-65.

Heck, K.L., Hays, G., Orth, R.J. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress Series* **253**: 123-136.

Huey, R.B. 1991. Physiological consequences of habitat selection. *The American Naturalist* **137**: 91-115.

Hughes, J., Deegan, L.A., Wyda, J.C., Weaver, M.J., Wright, A. 2002. The effects of eelgrass habitat loss on estuarine fish communities of southern New England. *Estuaries* **25**: 235-249.

Jones, A.G., Avise, J.C. 1997. Polygynandry in the dusky pipefish *Syngnathus floridae* revealed by microsatellite DNA markers. *Evolution* **51**: 1611-1622.

Joseph, V., Locke A., Godin J.J. 2006. Spatial distribution of fishes and decapods in eelgrass (*Zostera marina* L.) and sandy habitats of a New Brunswick estuary, eastern Canada. *Aquatic Ecology* **40**: 111-123.

Lazzari, M.A., Able, K.W. 1990. Northern pipefish, *Syngnathus fuscus*, occurrences over The Mid-Atlantic Bight continental shelf: evidence of seasonal migration. *Environmental Biology of Fishes 27*: 177-185.

Lubbers, L., Boynton, W.R., Kemp, W.M. 1990. Variations in structure of estuarine fish communities in relation to abundance of submersed vascular plants. *Marine Ecology Progress Series* **65**: 1-14.

Lyons, D.O., Dunne, J.J. 2003. Reproductive costs to male and female worm pipefish. *Journal of Fish Biology* **62**: 767-773.

Orth, R.J., Heck, K.L. 1980. Structural components of eelgrass (*Zostera marina*) meadows in the lower Chesapeake Bay- Fishes. *Estuaries* **3**: 278-288.

Orth, R.J., Heck, K.L., Van Montfrans, J. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries* **7**: 278-286.

Pimm, S.L., Rosenzweig, M.L. 1981. Competitors and habitat use. *Oikos* **37**: 1-6.

Ripley, J.L., Foran, C.M. 2006. Population structure, growth rates, and seasonal abundance of two *Syngnathus* pipefish species. *Estuaries and Coasts* **29**: 1161-1171.

Roelke, D.L., Sogard, S.M. 1993. Gender-based differences in habitat selection and activity level in the northern pipefish (*Syngnathus fuscus*). *Copeia* **2**: 528-532.

Rosenzweig, M.L. 1991. Habitat selection and population interactions: the search for mechanism. *The American Naturalist* **137**: 5-28.

Ryer, C.H., Orth, R.J. 1987. Feeding ecology of the northern pipefish, *Syngnathus fuscus*, in a seagrass community of the lower Chesapeake Bay. *Estuaries* **10**: 330-336

Schein, A. 2009. The estuarine fish community and food web structure in areas of sea lettuce (*Ulva lactuca*) and eelgrass (*Zostera marina*) within the Stanley River estuary Prince Edward Island. M.Sc. Thesis. University of Prince Edward Island, Charlottetown, PEI.

Takahashi, E., Connolly, R.M., Lee, S.Y. 2003. Growth and reproduction of double-ended pipefish *Syngnathoides biaculeatus*, in Moreton Bay, Queensland, Australia. *Environmental Biology of Fishes* **67**: 23-33.

Target, T.E. 1984. A breeding population of Gulf Pipefish (*Syngnathus scovelli*) in a Georgia estuary, with discussion on the ecology of the species. *Contributions to Marine Science* **27**: 169-174.

Thériault, M-H. 2006. Les effets des effluents d'usines de transformation des produits marins sur la communauté de poissons côtiers et sur deux espèces sentinelles dans le sud du golfe du Saint Laurent. M.Sc. Thésis. Université de Moncton, Moncton, NB.

Vincent, A., Ahnesjö, I., Berglund, A. 1994. Operational sex ratios and behavioural sex differences in a pipefish population. *Behavioral Ecology and Sociobiology* **34**: 435-442

Vincent, A., Ahnesjö, I., Berglund, A., Rosenqvist, G. 1992. Pipefishes and seahorses: are they all sex role reversed? *Trends in Ecology and Evolution* **7**: 237-241.

Vincent, A.C.J., Berglund, A., Ahnesjö, I. 1995. Reproductive ecology of five pipefish species in one eelgrass meadow. *Environmental Biology of Fishes* **44**: 347-361.

Watanabe, S., Kaneko, S., Watanabe, Y. 1999. Immunocytochemical detection of mitochondria-rich cells in the brood pouch epithelium of the pipefish, *Syngnathus schlegeli*: structural comparison with mitochondria-rich cells in the gills and larval epidermis. *Cell and Tissue Research* **259**: 141-149.

Watanabe, S., Watanabe, Y. 2001. Brooding season, sex ratio, and brood pouch development in the seaweed pipefish, Syngnathus schlegeli, in Otsuchi Bay, Japan. *Ichthyological Research* **48**: 155-160.

Wilson, A.B., Vincent, A., Ahnesjö, I., Meyer, A. 2001. Male pregnancy in seahorses and pipefishes (family Syngnathidae): rapid diversification of paternal brood pouch morphology inferred from a molecular phylogeny. *The American Genetic Association* **92**: 159-166.

Wyda, J.C., Deegan, L.A., Hughes, J.E., Weaver, M.J. 2002. The response of fishes to submerged aquatic vegetation complexity in two ecoregions of the Mid-Atlantic Bight: Buzzards Bay and Chesapeake Bay. *Estuaries* **25**:86-100.

CHAPTER 6: GENERAL CONCLUSIONS

6.1 General conclusions

The objectives of this study were to: 1) assess the impacts of macroalgae on eelgrass and the eelgrass fish community; 2) examine diel and monthly variation in the fish community within an eelgrass habitat; 3) explore differences in fish community structure in relation to varying eelgrass habitat conditions; and, 4) use an eelgrass-dependent species to consider the importance of eelgrass habitat.

The results of an initial study to assess some of the consequences associated with increased sea lettuce growth demonstrated that sea lettuce negatively affects the growth of eelgrass and the fish community structure did not differ in habitats dominated by eelgrass vs. sea lettuce. In the 1 m enclosures eelgrass density and biomass decreased greatly throughout the seven week period, while eelgrass within the exclosures thrived. Among the three sampling sites (exclosure, mixed, sea lettuce) the fish community composition did not differ significantly; however, fourspine stickleback were found in significantly higher numbers in eelgrass.

Although the importance of eelgrass habitat to the estuarine fish community is quite apparent, a fuller understanding of utilization of this habitat was investigated by sampling over 24 h time periods to examine potential changes in fish community structure over these intervals. More fish were captured at night than during the day. Interestingly, no species were captured exclusively during either period; only differences in abundance were found. However, the observation that Atlantic silverside YOY and mummichog were more numerous at night and ninespine stickleback during the day provided indirect evidence of movement patterns and leads to questions (and potential explanations) concerning these patterns. Incorporating diel sampling into fish community research is imperative to increase the understanding of how individual species interact within their habitat.

Examining how fish species responded to eelgrass habitats of varying condition (i.e. shoot density, canopy height, percent cover, above-ground biomass, and epiphyte biomass) provides insight as to how habitat degradation affects the overall community. All eight estuaries showed variability in eelgrass habitat condition among sites sampled thus giving a representative view of how fish may respond to eelgrass characteristics. It was expected that areas of lower eelgrass complexity (i.e. lower shoot density/percent cover) would contain lower numbers of fish and fewer species when compared to areas of higher complexity. However, this response was only detected in August when young-of-the-year (YOY) fishes were included in the analysis speaking to the nursery function of this habitat. It is possible that the presence of eelgrass was adequate to sustain the adult community and had extreme differences (i.e. low vs. high complexity) in eelgrass habitat been the main focus, the community response may have been more notable.

The reduced ability of degraded habitat to support individual fish species was apparent from sampling northern pipefish. Stanley River was an ideal estuary in which to assess population and reproductive differences of pipefish living in either eelgrass habitat or in suboptimal habitat where eelgrass had largely disappeared due to sea lettuce growth. This estuary has been undergoing considerable habitat changes in the past several years and it is obvious from one year to the next that eelgrass habitat is declining very quickly and sea lettuce is becoming the dominant vegetation. All pipefish (males, females, YOY) were captured in higher numbers in the eelgrass habitat. Male *S. fuscus* living in the sea lettuce-dominated habitat did not show significantly lower brood pouch somatic indices (BPSI). However, males sampled from the sea lettuce habitat did have significantly lower brood sizes, although this was related to their smaller size. Female gonadosomatic indices (GSI) did not differ between the habitats, although body condition was found to be lower in females sampled from sea lettuce.

6.2 Future considerations

This thesis provides important information concerning how fish communities and individual fish species respond to changes in eelgrass habitat. To expand upon the impacts of sea lettuce on the eelgrass fish community, it would be necessary to increase sample size and frequency of sampling. In our approach, it was encouraging to see that: 1) the exclosure was successful in excluding sea lettuce and could withstand heavy wind/wave action; and, 2) that there were differences in the fish community between the habitats even though they were located so close together. Given that sites were so close, they were under the influence of the same environmental variability, which downplays the significance of water parameters (i.e. temperature, salinity) in driving fish community structure, a proposition that could not be assessed from the study of eight estuaries.

Habitat associations are certainly important as witnessed by the diel sampling endeavour. The differences seen in the fish community between day and night were important; however, this study would benefit from sampling in other estuaries as well. This would eliminate the question: are the findings universal, or the result of some local effect seen only in the Midgell River estuary? It is likely that certain species, such as the mummichog, are responding to changes in food abundance over a 24 h period; however, how generally this occurs is not known. Also, given the fluctuation in water oxygen levels, it is possible that certain species are responding to changes in water parameters throughout the diel periods. Further, it would be useful to seine in slightly deeper waters as well. This would provide more insight into daily movement patterns of various fish species and examine the possibility of predatory species moving inshore during night hours.

To fully examine how the fish community responds to areas of varying eelgrass habitat condition, which is important given the future outlook for continued habitat degradation of PEI estuaries, a smaller scale study (i.e. within a single estuary) would be useful. This would allow fish community differences to be examined in more detail. By exploring eight different estuaries, although all along the north shore of PEI, there existed large spatial variability, and to a smaller extent temporal variability as there was about two weeks between sampling the first estuary and the eighth. It is therefore difficult to determine which factors are having the greatest impacts on fish communities. If temperature, salinity, and DO were similar between sites, as might be found within a single estuary, the observed fish community differences would be more likely a response to the eelgrass habitat rather than more broad scale abiotic factors. It would also be interesting to assess the impacts of eelgrass bed size, bed patchiness, and distance from the edge on the fish community. Although most sites were sampled < 10 m from the edge, not all beds were continuous and certainly were not the same size. These characteristics may well impact the community composition. Further, in some estuaries eelgrass beds were sampled on a gentle slope in which the distance from shore to water 1 m deep was greater than 100 m, while in other estuaries this distance was significantly smaller. Together these features of the estuarine habitat likely have an impact on the fish community.

Spatial variability was likely to be less influential while studying the pipefish. Sampling and collection of pipefish occurred at two sites within the same estuary (approximately 700 m apart), and therefore differences seen in the fish populations were more likely the result of differences in the habitat structure and not water parameters such as temperature, DO, and salinity. One question that needs to be addressed is: how far do these fish move within an estuary? Given the differences between males and females, both in numbers captured and reproductive response to the habitat types, it would be interesting to tag individual fish and trace the movement patterns within the estuary, not only between habitat patches but also within the areas of varying water depth. Male pipefish invest heavily in reproduction and the cost of moving long distances with a full brood pouch would be expected to be high. Therefore it is hypothesized that males would be less likely to move around and search for different habitats and subsequently remain in the habitat in which they originally settle. Female northern pipefish on the other hand tend to be more mobile (Roelke and Sogard 1993), and this variability in habitat quality may not affect them as greatly.

Field studies pose certain challenges when compared to lab studies where most variables can be carefully controlled. Accounting for this variability can be difficult and often comes down to the expertise of the researcher and others in a similar field having spent an exhausting amount of time studying and surveying similar ecosystems. It was clear from this study that nutrient enrichment is impacting the structural complexity of the eelgrass habitat (i.e. amount of eelgrass) within the estuarine environment and consequently the fish are responding to this change. It is obvious that in order to restore the natural habitat, anthropogenic activities within watersheds must be modified. Any future studies in estuaries experiencing the impacts of nutrient enrichment will likely continue to see changes in fish communities over time. It would be expected that as the eelgrass and estuarine habitat quality declines, the fish community will respond such that tolerant species will become more numerous and sensitive species will disappear. Appreciating the current status of estuarine ecosystems and associated fish communities is essential and perhaps a major motivation to increase awareness of the impacts that many activities within upland watersheds are having on coastal environments.

6.3 Literature cited

Roelke, D.L., Sogard, S.M. 1993. Gender-based differences in habitat selection and activity level in the northern pipefish (*Syngnathus fuscus*). *Copeia* **2**: 528-532.

APPENDIX A: FISH NUMBERS THROUGOUT THE SAMPLING PERIOD FOR CHAPTER 2

Appendix A

Table A.1. Number of each fish species captured, adults and YOY (young-of-the-year), in each location (exclosure (EX), mixed (MX), sea lettuce (SL), n = 1 seine haul) on July 20th, August 1st, and August 15th, 2009.

Sampling Date	July 20th			August 1st			August 15th		
Location	EX	MX	SL	EX	MX	SL	EX	MX	SL
Species Common name/ Scientific name									
American eel (*Anguilla rostrata*)	0	0	0	0	0	0	0	0	1
Atlantic silverside YOY* (*Menidia menidia*)	0	0	4	0	2	4	8	6	6
Atlantic tomcod (*Microgadus tomcod*)	0	0	0	0	0	0	0	0	0
Blackspotted stickleback (*Gasterosteus wheatlandi*)	0	3	1	0	0	0	0	0	0
Cunner (*Tautogolabrus adspersus)*	0	0	0	0	0	0	0	0	0
Fourspine stickleback (*Apeltes quadracus*)	42	43	4	43	81	15	74	73	48
Fourspine YOY	61	14	7	101	59	29	97	51	87
Gasterosteus spp. YOY**	773	376	357	8	67	26	13	11	5
Mummichog (*Fundulus heteroclitus*)	6	1	4	3	1	1	5	8	28
Mummichog YOY	0	0	0	0	0	0	6	11	14
Ninespine stickleback (*Pungitius pungitius*)	9	15	9	6	22	1	2	13	2
Ninespine YOY	5	2	1	0	14	0	2	6	0

Table A.1 continued

Northern pipefish (*Syngnathus fuscus*)	12	10	1	1	3	0	0	6	0
Northern pipefish YOY	0	0	0	0	0	0	2	11	3
Threespine stickleback (*Gasterosteus aculeatus*)	8	7	4	0	0	1	1	1	0
Total fish captured	916	471	392	162	249	77	210	197	194
Species richness	5	6	7	5	6	5	6	6	7

*YOY designates young-of-the-year fishes.

**Threespine and blackspotted stickleback are difficult to distinguish as young and therefore are presented here as *Gasterosteus* spp. YOY.

Table A.2. Number of each fish species captured, adults and YOY (young-of-the-year), in each location (exclosure (EX), mixed (MX), sea lettuce (SL), n = 1 seine haul) on August 22nd, August 31st, and September 12th, 2009.

Sampling Date	August 22nd			August 31st			September 12th		
Location	EX	MX	SL	EX	MX	SL	EX	MX	SL
Species Common name/ Scientific name									
American eel (*Anguilla rostrata*)	0	0	0	0	0	0	0	0	0
Atlantic silverside YOY* (*Menidia menidia*)	0	8	0	44	15	6	14	6	3
Atlantic tomcod (*Microgadus tomcod*)	0	0	0	0	0	0	1	0	0
Blackspotted stickleback (*Gasterosteus wheatlandi*)	0	0	0	0	0	0	0	0	0
Cunner (*Tautogolabrus adspersus)*	0	0	0	0	3	0	0	0	0
Fourspine stickleback (*Apeltes quadracus*)	102	110	60	226	310	109	86	81	24
Fourspine YOY	212	156	93	651	524	304	119	103	87
Gasterosteus spp. YOY**	3	12	3	53	206	34	14	21	11
Mummichog (*Fundulus heteroclitus*)	3	12	7	10	10	4	5	3	4
Mummichog YOY	52	17	61	34	25	65	13	13	6
Ninespine stickleback (*Pungitius pungitius*)	2	60	3	126	187	66	52	164	53
Ninespine YOY	1	5	0	22	26	14	3	7	2

Table A.2 continued

Northern pipefish (*Syngnathus fuscus*)	0	2	1	0	2	0	0	0	0
Northern pipefish YOY	19	22	12	68	39	43	9	26	7
Threespine stickleback (*Gasterosteus aculeatus*)	2	0	0	0	20	4	4	10	3
Total fish captured	396	404	240	1234	1367	649	320	434	200
Species richness	5	6	5	6	7	6	7	6	6

*YOY designated young-of-the-year fishes.
**Threespine and blackspotted stickleback are difficult to distinguish as young and therefore are presented here as *Gasterosteus* spp. YOY.

APPENDIX B: FISH NUMBERS AND SIMPER RESULTS FOR CHAPTER 3

Appendix B

Table B.1. Number of each fish species captured, adults and YOY (young-of-the-year), during each time interval (n = 2 seine hauls per time period) on June 22nd, 2008.

Species Common name/ Scientific name	10:00	14:00	18:00	22:00	2:00	6:00
American eel (*Anguilla rostrata*)	0	0	0	0	4	0
Atlantic silverside (*Menidia menidia*)	2	0	0	4	18	2
Atlantic silverside YOY*	0	0	0	0	0	0
Atlantic tomcod YOY (*Microgadus tomcod*)	0	0	0	0	0	0
Blackspotted stickleback (*Gasterosteus wheatlandi*)	56	39	37	20	48	40
Cunner (*Tautogolabrus adspersus)*	2	0	0	0	0	2
Fourspine stickleback (*Apeltes quadracus*)	371	684	758	528	763	784
Fourspine YOY	0	0	0	0	0	0
Gaspereau YOY (*Alosa* spp.)	0	0	0	0	0	0
Gasterosteus spp. YOY**	5	7	0	0	0	0
Mummichog (*Fundulus heteroclitus*)	38	48	59	274	271	53
Mummichog YOY	0	0	0	0	0	0
Ninespine stickleback (*Pungitius pungitius*)	34	39	17	17	20	29
Ninespine YOY	0	0	0	0	0	0

Table B.1 continued

Northern pipefish (*Syngnathus fuscus*)	13	16	10	19	14	51
Northern pipefish YOY	0	0	0	0	0	0
Threespine stickleback (*Gasterosteus aculeatus*)	50	64	53	48	53	75
Winter flounder (juv) (*Pseudopleuronectes americanus*)	0	0	0	0	0	0
Total fish captured	571	897	934	910	1191	1036
Species richness	8	6	6	7	8	8

* YOY designates young-of-the-year fishes.
**Threespine and blackspotted stickleback YOY are difficult to distinguish as very small individuals, therefore all young of this type are designated *Gasterosteus* spp. YOY, which is true for tables B.1-B.6 inclusively.

Table B.2. Number of each fish species captured, adults and YOY (young-of-the-year), during each time interval (n = 2 seine hauls per time period) on June 27th, 2008.

Species Common name/ Scientific name	10:00	14:00	18:00	22:00	2:00	6:00
American eel (*Anguilla rostrata*)	0	2	0	1	0	2
Atlantic silverside (*Menidia menidia*)	0	0	0	1	0	0
Atlantic silverside YOY	0	0	0	0	0	0
Atlantic tomcod YOY (*Microgadus tomcod*)	2	1	0	2	1	0
Blackspotted stickleback (*Gasterosteus wheatlandi*)	32	33	39	34	27	59
Cunner (*Tautogolabrus adspersus)*	2	1	0	2	1	0
Fourspine stickleback (*Apeltes quadracus*)	672	609	721	831	316	668
Fourspine YOY	5	9	1	3	2	1
Gaspereau YOY (*Alosa* spp.)	0	0	0	0	0	0
Gasterosteus spp. YOY	19	28	26	7	5	5
Mummichog (*Fundulus heteroclitus*)	52	40	75	115	22	61
Mummichog YOY	0	0	0	0	0	0
Ninespine stickleback (*Pungitius pungitius*)	25	43	28	28	11	13
Ninespine YOY	0	1	0	0	0	0
Northern pipefish (*Syngnathus fuscus*)	59	39	28	42	27	48

Table B.2 continued

Northern pipefish YOY	0	0	1	1	0	0
Threespine stickleback (*Gasterosteus aculeatus*)	65	58	56	5	23	54
Winter flounder (juv) (*Pseudopleuronectes americanus*)	0	0	1	0	0	0
Total fish captured	933	864	976	1122	435	911
Species richness	8	9	7	10	8	8

Table B.3. Number of each fish species captured, adults and YOY (young-of-the-year), during each time interval (n = 2 seine hauls per time period) on July 11th, 2008.

Species Common name/ Scientific name	10:00	14:00	18:00	22:00	2:00	6:00
American eel (*Anguilla rostrata*)	0	0	0	1	1	0
Atlantic silverside (*Menidia menidia*)	0	10	0	0	1	2
Atlantic silverside YOY	1	5	70	167	256	232
Atlantic tomcod YOY (*Microgadus tomcod*)	0	0	0	0	0	0
Blackspotted stickleback (*Gasterosteus wheatlandi*)	12	19	8	1	5	8
Cunner (*Tautogolabrus adspersus)*	0	1	0	0	0	0
Fourspine stickleback (*Apeltes quadracus*)	292	406	323	293	162	155
Fourspine YOY	271	489	237	265	163	122
Gaspereau YOY (*Alosa* spp.)	0	0	0	0	0	0
Gasterosteus spp. YOY	178	118	85	318	124	14
Mummichog (*Fundulus heteroclitus*)	23	20	51	246	178	24
Mummichog YOY	4	11	3	0	0	10
Ninespine stickleback (*Pungitius pungitius*)	42	26	18	6	7	6
Ninespine YOY	0	0	0	0	0	0
Northern pipefish (*Syngnathus fuscus*)	30	38	27	34	29	22

Table B.3 continued

Northern pipefish YOY	0	4	4	0	0	0
Threespine stickleback (*Gasterosteus aculeatus*)	35	35	22	27	14	22
Winter flounder (juv) (*Pseudopleuronectes americanus*)	0	0	0	0	0	0
Total fish captured	888	1182	848	1358	940	617
Species richness	7	8	6	8	8	7

Table B.4. Number of each fish species captured, adults and YOY (young-of-the-year), during each time interval (n = 2 seine hauls per time period) on July 23rd, 2008.

Species Common name/ Scientific name	10:00	14:00	18:00	22:00	2:00	6:00
American eel (*Anguilla rostrata*)	0	1	0	0	0	0
Atlantic silverside (*Menidia menidia*)	0	2	0	0	0	0
Atlantic silverside YOY	47	74	56	1212	835	4
Atlantic tomcod YOY (*Microgadus tomcod*)	0	0	0	0	0	0
Blackspotted stickleback (*Gasterosteus wheatlandi*)	9	6	2	2	3	1
Cunner (*Tautogolabrus adspersus)*	0	0	0	0	0	0
Fourspine stickleback (*Apeltes quadracus*)	338	370	274	433	363	302
Fourspine YOY	839	766	494	521	414	319
Gaspereau YOY (*Alosa* spp.)	0	0	0	0	0	0
Gasterosteus spp. YOY	807	250	339	138	152	77
Mummichog (*Fundulus heteroclitus*)	17	8	36	208	247	42
Mummichog YOY	87	169	77	46	83	180
Ninespine stickleback (*Pungitius pungitius*)	33	46	29	17	24	18
Ninespine YOY	0	2	2	0	0	1
Northern pipefish (*Syngnathus fuscus*)	8	8	8	9	3	2

Table B.4 continued

Northern pipefish YOY	53	40	34	15	14	15
Threespine stickleback (*Gasterosteus aculeatus*)	19	18	1	9	13	13
Winter flounder (juv) (*Pseudopleuronectes americanus*)	0	0	0	0	0	0
Total fish captured	2257	1760	1352	2610	2151	974
Species richness	7	8	7	7	7	7

Table B.5. Number of each fish species captured, adults and YOY (young-of-the-year), during each time interval (n = 2 seine hauls per time period) on August 6th, 2008.

Species Common name/ Scientific name	10:00	14:00	18:00	22:00	2:00	6:00
American eel (*Anguilla rostrata*)	0	1	0	0	0	0
Atlantic silverside (*Menidia menidia*)	0	0	0	0	0	0
Atlantic silverside YOY	33	209	509	586	829	81
Atlantic tomcod YOY (*Microgadus tomcod*)	0	0	0	0	0	0
Blackspotted stickleback (*Gasterosteus wheatlandi*)	0	0	0	1	0	0
Cunner (*Tautogolabrus adspersus*)	0	0	0	0	0	0
Fourspine stickleback (*Apeltes quadracus*)	195	193	345	231	481	426
Fourspine YOY	286	423	385	348	352	263
Gaspereau YOY (*Alosa* spp.)	0	0	19	0	7	0
Gasterosteus spp. YOY	24	54	59	45	50	45
Mummichog (*Fundulus heteroclitus*)	88	36	55	134	153	72
Mummichog YOY	260	459	284	602	508	225
Ninespine stickleback (*Pungitius pungitius*)	12	6	20	17	6	16
Ninespine YOY	0	13	22	8	5	6
Northern pipefish (*Syngnathus fuscus*)	2	1	4	4	1	2

Table B.5 continued

Northern pipefish YOY	12	39	42	37	44	35
Threespine stickleback (*Gasterosteus aculeatus*)	5	8	9	1	1	11
Winter flounder (juv) (*Pseudopleuronectes americanus*)	0	0	0	0	0	1
Total fish captured	917	1442	1753	2014	2437	1183
Species richness	6	7	7	7	7	7

Table B.6. Number of each fish species captured, adults and YOY (young-of-the-year), during each time interval (n = 2 seine hauls per time period) on August 24th, 2008.

Species Common name/ Scientific name	10:00	14:00	18:00	22:00	2:00	6:00
American eel (*Anguilla rostrata*)	0	0	0	0	0	0
Atlantic silverside (*Menidia menidia*)	0	0	0	0	0	0
Atlantic silverside YOY	7	1	46	458	446	66
Atlantic tomcod YOY (*Microgadus tomcod*)	0	0	0	0	0	0
Blackspotted stickleback (*Gasterosteus wheatlandi*)	0	0	0	0	0	0
Cunner (*Tautogolabrus adspersus)*	0	0	0	0	0	0
Fourspine stickleback (*Apeltes quadracus*)	191	137	85	210	133	166
Fourspine YOY	861	1054	471	776	486	614
Gaspereau YOY (*Alosa* spp.)	0	0	0	0	2	0
Gasterosteus spp. YOY	33	26	4	2	1	6
Mummichog (*Fundulus heteroclitus*)	26	18	4	135	45	42
Mummichog YOY	302	392	554	734	778	183
Ninespine stickleback (*Pungitius pungitius*)	10	4	1	2	0	2
Ninespine YOY	3	8	8	2	0	0
Northern pipefish (*Syngnathus fuscus*)	0	1	1	0	0	0

Table B.6 continued

Northern pipefish YOY	15	10	0	0	3	6
Threespine stickleback (*Gasterosteus aculeatus*)	4	2	1	0	1	1
Winter flounder (juv) (*Pseudopleuronectes americanus*)	0	0	0	0	0	0
Total fish captured	1452	1653	1175	2319	1895	1086
Species richness	6	6	6	5	6	6

Table B.7. SIMPER results of monthly differences in the fish community (adults and YOY) showing species contributing more than 4% to the dissimilarity in the community. Abundances are back-transformed from the fourth root data used in the analysis.

Month Comparison	**Average dissimilarity**	**Species contributing**	**Average abundance/ 100 m^2 month 1**	**Average abundance/ 100 m^2 month 2**	**Contribution to dissimilarity (%)**	**Cumulative contribution to dissimilarity (%)**
June vs. July	38.34%	Fourspine YOY*	0.03	150.06	20.35	20.35
		Silverside YOY	0.00	31.02	15.27	35.61
		Gasterosteus YOY**	0.50	67.85	13.43	49.05
		Mummichog YOY	0.00	5.92	9.94	58.99
		Pipefish YOY	0.00	1.22	6.59	65.58
		Blackspotted	15.06	1.31	5.97	71.55
		Fourspine	261.16	122.96	4.71	76.26
		Mummichog	28.47	23.85	4.58	80.83
June vs. August	56.55%	Mummichog YOY	0.00	183.40	16.97	16.97
		Fourspine YOY	0.03	222.00	16.15	33.12
		Silverside YOY	0.00	60.59	12.58	45.7
		Blackspotted	15.06	0.000003	8.94	54.64
		Threespine	22.17	0.33	6.62	61.26
		Pipefish	10.97	0.04	6.39	67.65
		Pipefish YOY	0.00	4.18	6.36	74.01
		Gasterosteus YOY	0.50	7.23	4.85	78.86
		Fourspine	261.16	97.21	4.29	83.15
July vs. August	29.26%	Mummichog YOY	5.92	183.40	16.53	16.53
		Silverside YOY	31.02	60.59	11.86	28.39
		Gasterosteus YOY	67.85	7.23	9.85	38.24
		Pipefish	5.20	0.04	8.61	46.85

Table B.7 continued

	Blackspotted	1.31	0.000003	7.98	54.83
	Pipefish YOY	1.22	4.18	7.16	61.99
	Threespine	6.39	0.33	7.14	69.13
	Ninespine YOY	0.0004	0.45	5.93	75.06
	Mummichog	23.85	21.37	5.73	80.79
	Ninespine	7.78	1.52	5.3	86.09
	Fourspine YOY	150.06	222.00	5.16	91.25

* YOY designates young-of-the-year fishes.

**Threespine and blackspotted stickleback YOY are difficult to distinguish as very small individuals, therefore all young of this type are designated *Gasterosteus* spp. YOY.

Note: threespine, fourspine, ninespine, and blackspotted are all stickleback species which is true for tables B.7-B.8 inclusively.

Table B.8. SIMPER results of monthly differences in the fish community (YOY excluded from analysis) showing species contributing more than 4% to the dissimilarity in the community. Abundances are back-transformed from the fourth root data used in the analysis.

Month Comparison	**Average dissimilarity**	**Species contributing**	**Average abundance/ 100 m^2 month 1**	**Average abundance/ 100 m^2 month 2**	**Contribution to dissimilarity (%)**	**Cumulative contribution to dissimilarity (%)**
June vs. July	17.07%	Blackspotted	15.06	1.31	20.42	20.42
		Fourspine	261.16	122.96	15.5	35.92
		Mummichog	28.47	23.85	15.45	51.37
		Threespine	22.17	6.39	13.42	64.79
		Pipefish	10.97	5.20	10.44	75.23
		Silverside	0.02	0.002	10.12	85.35
		Ninespine	10.04	7.78	7.52	92.87
June vs. August	34.80%	Blackspotted	15.06	0.000003	25.13	25.13
		Threespine	22.17	0.33	18.69	43.82
		Pipefish	10.97	0.04	18.1	61.92
		Fourspine	261.16	97.21	11.97	73.89
		Ninespine	10.04	1.52	9.57	83.46
		Mummichog	28.47	21.37	8.64	92.09
July vs. August	28.07%	Pipefish	5.20	0.04	20.84	20.84
		Blackspotted	1.31	0.000003	19.19	40.03
		Threespine	6.39	0.33	17.52	57.55
		Mummichog	23.85	21.37	14.18	71.73
		Ninespine	7.78	1.52	13.42	85.15
		Fourspine	122.96	97.21	8.41	93.56

APPENDIX C: FISH NUMBERS, SIMPER RESULTS, AND FIGURES OF SAMPLING LOCATIONS FOR CHAPTER 4

Appendix C

Table C.1. Number of each fish species captured in each of the eight estuaries sampled in June 2009. N = 10 seine hauls in each estuary.

Common name/ Scientific name	Mdl	Trd	Wht	Stn	Bid	Bro	Frl	Kld
American eel (*Anguilla rostrata*)	1	0	4	0	0	0	0	1
Atlantic silverside (*Menidia menidia*)	6	5	5	17	59	1	32	13
Atlantic silverside YOY*	0	0	0	0	0	0	0	0
Atlantic tomcod (*Microgadus tomcod*)	0	0	0	0	1	0	0	0
Atlantic tomcod YOY	0	5	6	3	32	2	4	0
Blackspotted stickleback (*Gasterosteus wheatlandi*)	541	169	34	135	37	157	260	59
Cunner (*Tautogolabrus adspersus)*	0	46	25	30	43	12	84	7
Cunner YOY	0	0	0	0	0	0	0	0
Dace	1	0	0	0	0	0	0	0
Fourspine stickleback (*Apeltes quadracus*)	2804	1663	1783	2673	1127	1353	2325	1589
Fourspine YOY	0	4	23	3	1	0	0	144
Gaspereau YOY (*Alosa* spp.)	0	0	0	0	0	0	0	0
Gasterosteus spp. YOY**	39	32	108	7	0	0	0	216
Grubby (*Myoxocephalus aenaeus*)	0	4	7	2	0	0	3	1
Grubby YOY	0	0	0	0	0	0	0	0
Mummichog (*Fundulus heteroclitus*)	161	52	32	236	437	842	209	74

Table C.1 continued

Mummichog YOY	0	0	0	0	0	0	0	0
Ninespine stickleback (*Pungitius pungitius*)	995	32	41	50	46	8	57	56
Ninespine YOY	0	2	26	0	0	0	0	280
Northern pipefish (*Syngnathus fuscus*)	7	184	7	77	65	1	3	4
Northern pipefish YOY	0	0	0	0	0	0	0	0
Smooth flounder (*Pleuronectes putnami*)	0	0	0	0	0	0	0	4
Smooth flounder YOY	0	0	0	0	0	0	0	0
Threespine stickleback (*Gasterosteus aculeatus*)	412	252	508	295	308	259	460	390
White hake (*Urophycis tenuis*)	0	1	0	0	0	0	0	0
Winter flounder (juv) (*Pseudopleuronectes americanus*)	9	20	11	11	0	2	3	4
Winter flounder YOY	0	0	1	0	0	0	0	0
Total in each estuary	4976	2471	2621	3539	2156	2637	3440	2842
Species Richness	9	12	12	11	9	10	11	12

*YOY designates young-of-the-year fishes. **Threespine and blackspotted stickleback YOY are difficult to distinguish as very small individuals, therefore all young of this type are designated *Gasterosteus* spp. YOY. Estuaries: Mdl, Midgell; Trd, Tracadie; Wht, Wheatley; Stn, Stanley; Bid, Bideford; Bro, Brooks; Frl, Freeland; Kld, Kildare.

Table C.2. Number of each fish species captured in each of the eight estuaries sampled in August 2009. N = 10 seine hauls in each estuary.

Common name/ Scientific name	Mdl	Trd	Wht	Stn	Bid	Bro	Frl	Kld
American eel (*Anguilla rostrata*)	1	0	0	0	0	0	0	1
Atlantic silverside (*Menidia menidia*)	2	0	1	0	3	0	0	11
Atlantic silverside YOY*	22	22	124	401	1045	107	108	345
Atlantic tomcod (*Microgadus tomcod*)	0	0	0	0	0	0	0	0
Atlantic tomcod YOY	0	0	0	0	0	0	2	0
Blackspotted stickleback (*Gasterosteus wheatlandi*)	1	2	2	0	0	3	12	0
Cunner (*Tautogolabrus adspersus)*	0	3	0	1	38	0	6	0
Cunner YOY	0	12	17	0	0	1	8	52
Dace	0	0	0	0	0	0	0	0
Fourspine stickleback (*Apeltes quadracus*)	1230	1332	1219	828	1921	891	1475	1851
Fourspine YOY	840	281	709	255	313	527	278	555
Gaspereau YOY (*Alosa* spp.)	0	1	0	0	0	0	0	0
Gasterosteus spp. YOY**	1107	149	1263	178	339	676	522	1069
Grubby (*Myoxocephalus aenaeus*)	0	0	3	0	0	0	0	5
Grubby YOY	0	1	3	0	0	0	1	0
Mummichog (*Fundulus heteroclitus*)	106	57	31	1186	139	131	41	430

Table C.2 continued

Mummichog YOY	400	159	62	349	735	121	130	304
Ninespine stickleback (*Pungitius pungitius*)	482	78	198	46	287	36	116	288
Ninespine YOY	399	4	2	5	119	36	15	99
Northern pipefish (*Syngnathus fuscus*)	15	13	4	4	3	0	0	0
Northern pipefish YOY	53	140	185	86	435	7	11	14
Smooth flounder (*Pleuronectes putnami*)	0	0	0	0	0	0	0	0
Smooth flounder YOY	0	0	0	9	0	0	0	0
Threespine stickleback (*Gasterosteus aculeatus*)	23	11	24	31	34	54	89	35
White hake (*Urophycis tenuis*)	0	1	0	0	0	0	1	0
Winter flounder (juv) (*Pseudopleuronectes americanus*)	1	5	1	0	0	2	2	1
Winter flounder YOY	0	5	0	0	0	0	2	4
Total in each estuary	4682	2276	3848	3379	5411	2592	2819	5064
Species Richness	9	12	10	9	8	9	12	11

*YOY designates young-of-the-year fishes. **Threespine and blackspotted stickleback YOY are difficult to distinguish as very small individuals, therefore all young of this type are designated *Gasterosteus* spp. YOY. Estuaries: Mdl, Midgell; Trd, Tracadie; Wht, Wheatley; Stn, Stanley; Bid, Bideford; Bro, Brooks; Frl, Freeland; Kld, Kildare.

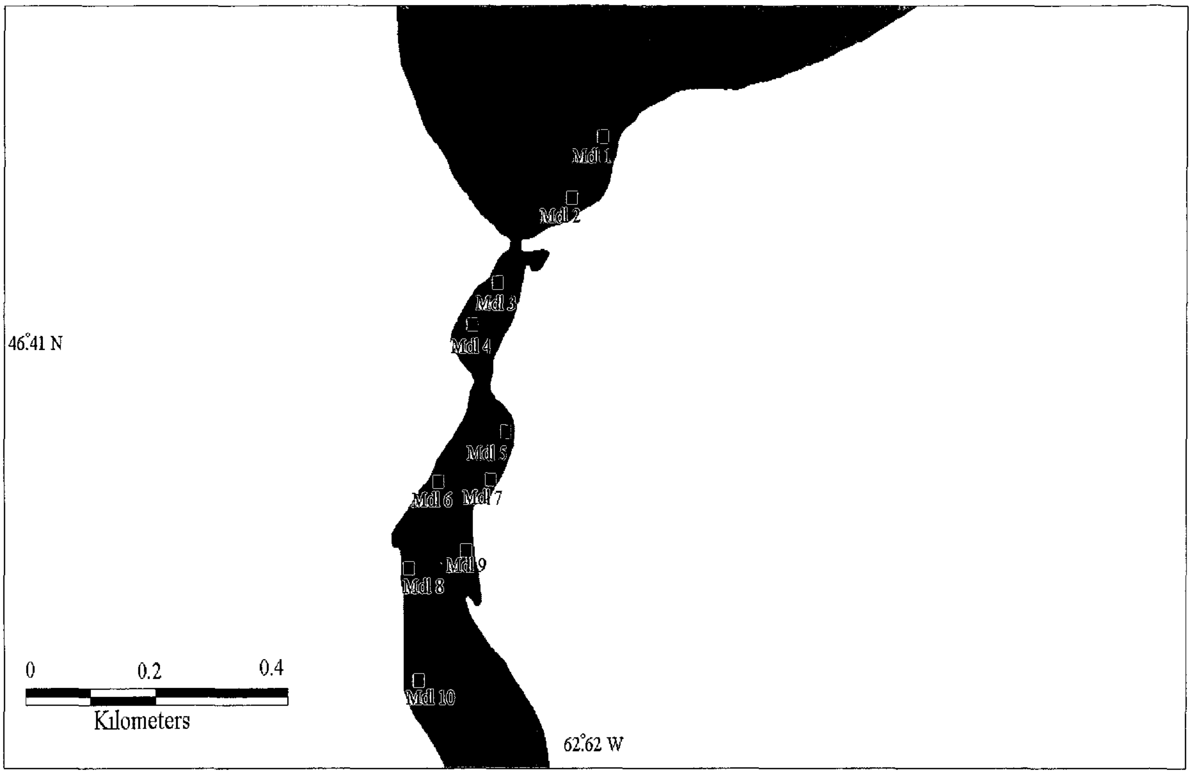

Figure C.1. Sampling sites in the Midgell River estuary.

Figure C.2. Sampling sites in the Tracadie Bay estuary.

Figure C.3. Sampling sites in the Wheatley River estuary.

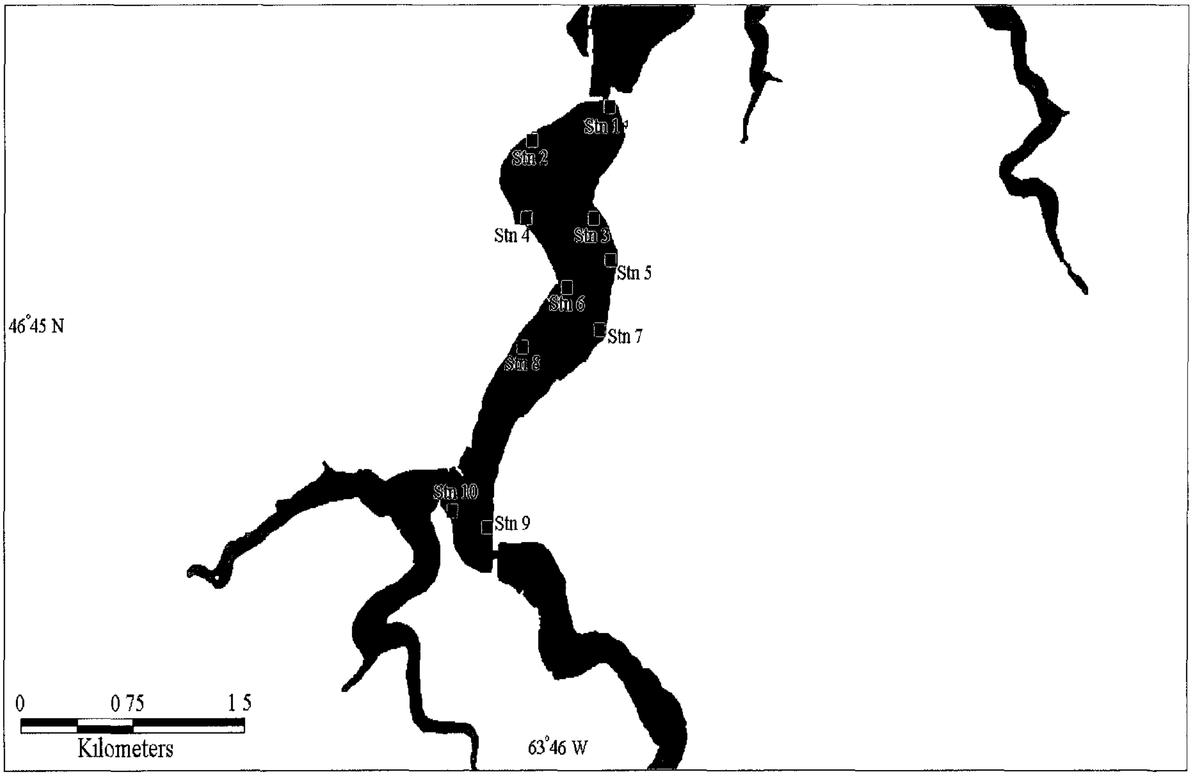

Figure C.4. Sampling sites in the Stanley River estuary.

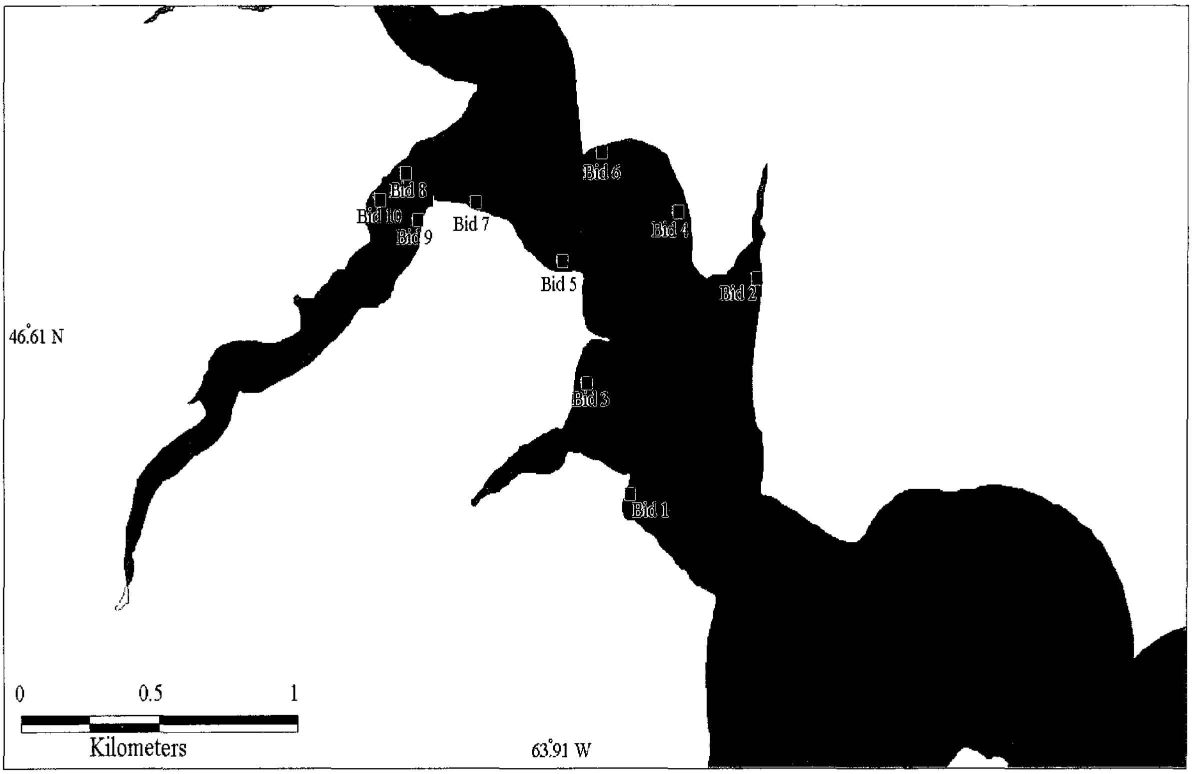

Figure C.5. Sampling sites in the Bideford River estuary.

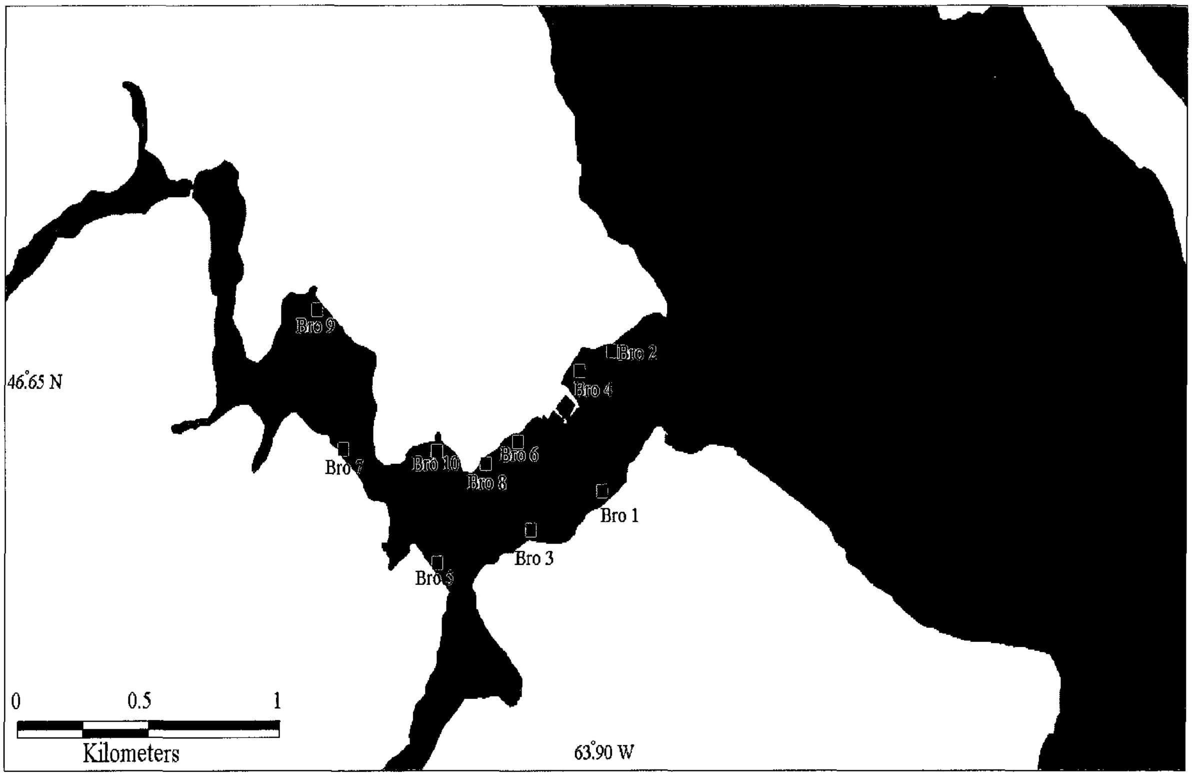

Figure C.6. Sampling sites in the Brooks River estuary.

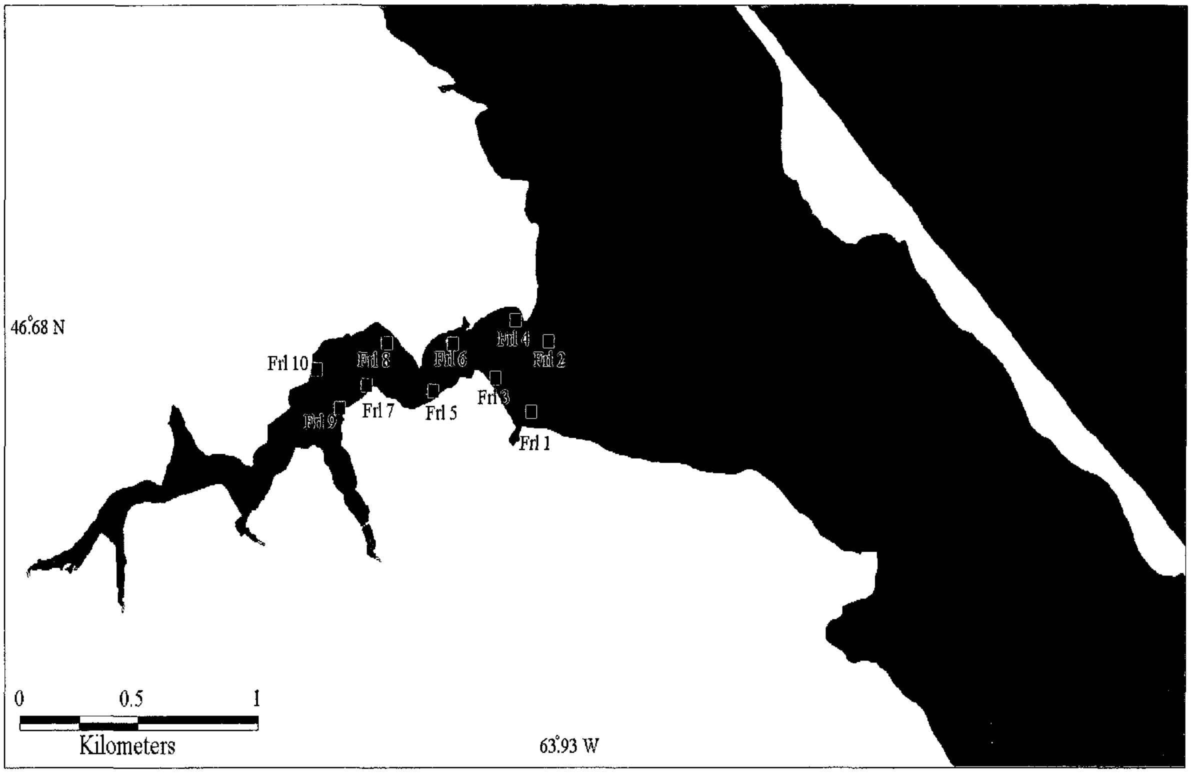

Figure C.7. Sampling sites in the Freeland River estuary.

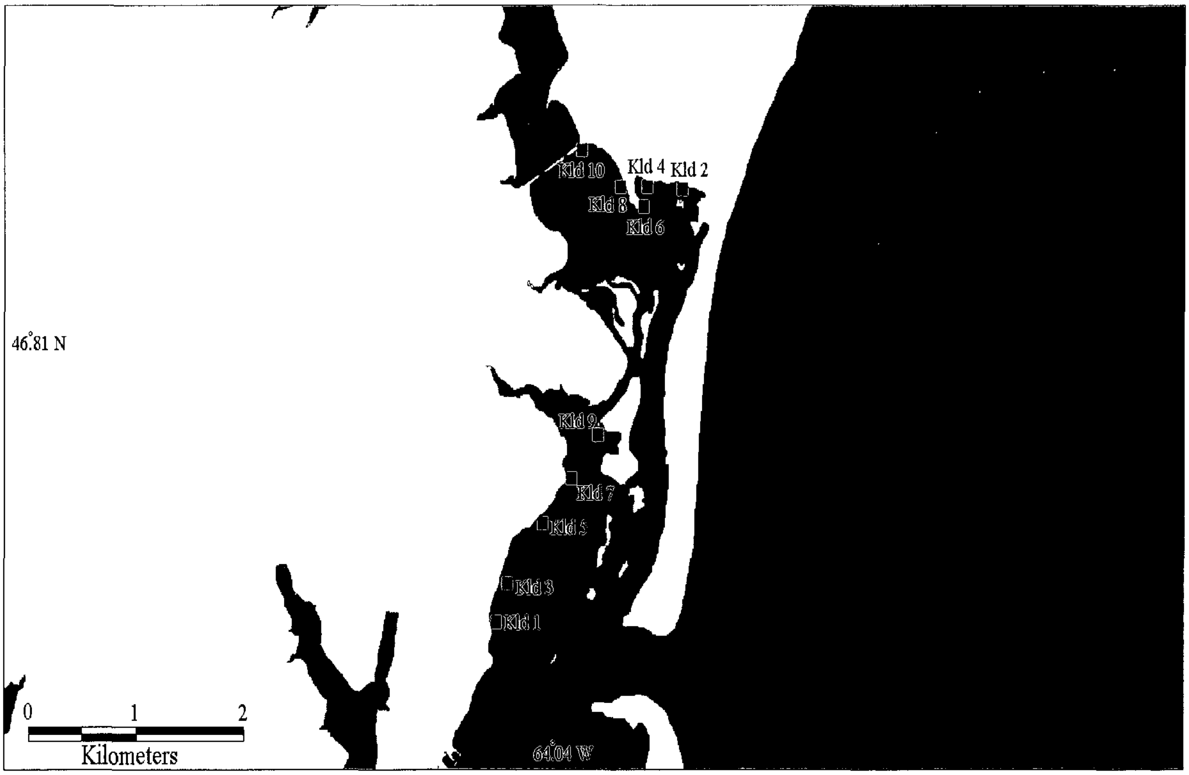

Figure C.8. Sampling sites in the Kildare River estuary.