Abstract

Biogeographic characterization of fishes from intertidal sandflats in Pamlico River, North Carolina

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The spatial and temporal changes in fish community structure in Pamlico River, North Carolina were analyzed. Salinity and temperature are important water quality parameters influencing fish community structure in estuaries. Fish were collected with an 18 m long bag seine from sandy habitats in Pamlico River, North Carolina. Each station was sampled bi-monthly (August to November) in 2009-2010. The North Carolina Division of Marine Fisheries (NCDMF) Program 123 has been collecting fish from two stations in Pamlico River since 1987. There are two main differences between this study and Program 123. First, Program 123 has a much narrower geographic sampling area than this study. I sampled 18 fixed locations from Washington, North Carolina to the mouth of Pamlico Sound; whereas, NCDMF sampled only two stations near Bath, North Carolina. Second, sampling frequency was higher in this study than in Program 123. I sampled bi-weekly in October and November, 2009 and August to November, 2010; whereas, Program 123 sampled monthly from September to November, 2009
and 2010. Analysis of similarity (ANOSIM), non-metric multidimensional scaling (MDS), and canonical correspondence analysis (CCA) were used to test for differences in fish community structure both spatially and temporally, in Pamlico River, and to test for the correlation of water quality to fish community structure. Analysis of variance (ANOVA) was used to test for significance in water quality among the various sections. Five species (Anchoa mitchilli, Menidia beryllina, Brevoortia tyrannus, Leiostomus xanthurus, and Lagodon rhomboides) accounted for 97.4% of the total abundance in Pamlico River. Temporal and spatial changes in fish community structure were important in Pamlico River. Temperature was the most important environmental factor correlating with temporal fish community structure in Pamlico River. Salinity was the most important factor in spatial fish community structure. Program 123 captured greater catch per unit effort (CPUE) than this study because Program 123 only sampled the Central section, where greatest fish abundance occurred. However, greater species diversity was captured in this study than in Program 123.

Keywords: Fish community structure; Water quality; Pamlico River; Pamlico Sound; North Carolina; Spatial patterns; Temporal patterns
BIOGEOGRAPHIC CHARACTERIZATION OF FISHES FROM INTERTIDAL SANDFLATS
IN PAMLICO RIVER, NORTH CAROLINA

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BIOGEOGRAPHIC CHARACTERIZATION OF FISHES FROM INTERTIDAL SANDFLATS
IN PAMLICO RIVER, NORTH CAROLINA

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CHAPTER 1: INTRODUCTION

The Pamlico Sound Estuarine System, North Carolina

The Albemarle-Pamlico Estuarine System is important to the commercial fishing industry in North Carolina (Stanley 1992). According to Stanley (1992), 80% of the harvested commercial fish in North Carolina are landed in the Albemarle-Pamlico Estuarine System (Stanley 1992). In Pamlico Sound, the commercial landings are mostly invertebrates such as blue crab (*Callinectes sapidus*) and shrimp (*Penaeus* sp.) (Stanley 1992). Because of the importance of Pamlico Sound fisheries to the economy of eastern North Carolina, there is a need to better understand the variation in community structure.

Pamlico Sound, located in eastern North Carolina, is one of the largest estuaries in North America (Epperly and Ross 1986) and the largest lagoonal system in the continent (Stanley 1992). The coastal plain of North Carolina has a humid sub-tropical climate with long, hot summers and short, cool winters causing fish community structure to change seasonally as cool temperate species migrate into the sound during winter and warm sub-tropical species migrate into the sound during summer (Stanley 1992). Mean, winter air temperature in January (coldest month) ranges from 5°C to 10°C, and in July (warmest month) 24°C to 27°C (Stanley 1992).

Pamlico River is the second largest tributary (Neuse River is the largest) of Pamlico Sound (Epperly and Ross 1986). Pamlico River is a continuation of Tar River and runs approximately 65 km from Washington, North Carolina to the western mouth of Pamlico Sound (Tenore 1972; Xu et al. 2008). The width of the river increases from 0.5 km near Washington, North Carolina to 6 km at the mouth of Pamlico Sound, but depth remains constant in the middle
of the river at 4 m (Xu et al. 2008). There are important blue crab and flounder (Paralichthys sp.) fisheries as well as important recreational fisheries in Pamlico River.

**Estuarine Habitat and Fish Usage**

Estuaries represent transitional areas between freshwater and saltwater habitats. Additionally, they provide goods and services for many societies (Costanza et al. 1997). The variable salinity patterns, shallow depths, high turbidity, and food availability make estuaries important areas for many fish species. The high turbidity allows juvenile fish to avoid predation and high concentrations of crustaceans allow fish to obtain food easily during early stages of development (Baltz et al. 1998; Beck et al. 2001; Meng and Powell 1999). The highly variable estuarine environment can cause estuarine fish communities to vary greatly at different spatial and temporal scales (Maes et al. 2004). Because the biological communities in estuaries are well adapted to cope with stress, it is sometimes difficult to quantify the effects of anthropogenic activities on fish communities (Elliott and Quintino 2007). Ninety percent of the world’s population lives in coastal communities, and a large proportion of this population lives near estuarine systems, which has increased point source and non-point source pollution in these systems (Carpenter et al. 1998). Therefore, it is very important to characterize ecological patterns before identifying anthropogenic impacts, so that human impacts can be quantified.

Environmental variability such as seasonal changes in temperature, salinity, and being geologically ephemeral can cause low species biodiversity; however, the main cause for low diversity is salinity because few species can withstand salinity variability. Additionally, osmotic stress and oxygen deficiency further limit taxonomic diversity within estuaries (Costanza et al. 1993; Meire et al. 2005). Although there are low numbers of resident species and low
biodiversity, many marine species of fish and invertebrates benefit from estuaries and use estuaries as nursery habitat because of their high primary productivity (Beck et al. 2001).

Estuaries have a variety of habitats for fishes. In Pamlico Sound and Pamlico River estuary, three habitat types dominate: marsh, seagrass meadows, and soft bottom (sand/mud). These habitats collectively represent a very complex environment for mobile species and it is highly likely that these habitats do not represent isolated units, but are interconnected (Bell et al. 1991). The role of marshes, muddy bottoms (Ross and Epperly 1989; Stokesbury and Ross 1997; Walsh et al. 1999a; Weinstein et al. 1980), and seagrass meadows (Fitzgerald 1998; Gloeckner and Luczkovich 2008) in North Carolina estuaries has been recognized. However the importance of sandy beaches within the surf zone is largely unknown in Pamlico Sound. Surf zones are defined here as: shallow areas influenced by wave and wind activity. Larval and juvenile stages use these shallow water areas as nursery habitat (Elliott and Quintino 2007; Gibson et al. 1998). Additionally, surf zone areas serve as refugia from predators and may also function as feeding areas (Burke 1995; Ross and Lancaster 2002).

Surf zones are used by a large number of juvenile fish primarily because of abundant food resources. Additionally, these open habitats provide a level of protection from predation because of the water turbidity and turbulence (Lasiak 1986). In North Carolina, estuarine dependent fishes including, L. xanthurus and A. mitchilli, contribute to the estuarine soft bottom community (Ross 2003; Ross and Epperly 1989; Walsh et al. 1999b). Although many studies in North Carolina have addressed how a single species or several species groups use these areas, no study to date has characterized the entire fish community. The term community refers to a directly or indirectly interacting assemblage of organisms occupying a particular area or habitat (Menge 1976).
North Carolina has in place a number of management plans to manage coastal fisheries and natural resources. One program developed per the Fisheries Reform Act of 1997 is the Coastal Habitat Protection Plan (CHPP) (Deaton et al. 2010). CHPP is designed to provide protection and enhancement for important fish habitats. Soft bottom habitat “unconsolidated, unvegetated subtidal sediment in estuarine and marine systems” is identified in the plan as an important fish habitat (Street et al. 2005). In particular, the shallow (<2.0m) soft bottom is a very prominent habitat in North Carolina Estuaries and represents 17-37% of the total bottom habitat (Deaton et al. 2010).

Sandy habitats have been studied extensively throughout the world (Barreiros et al. 2004; Bennett and Attwood 1991b; Lasiak 1986; Santos and Nash 1995), and have been considered unstructured homogeneous environments unlike, oyster reefs and vegetated areas. However, these sandy beaches are now recognized as complex and dynamic environments whose physical structure can be influenced by the distribution of invertebrates and the sediment distribution (Barros et al. 2002; McLachlan et al. 1993). Given the prevalence of sandy habitat present in North Carolina estuaries, this habitat type may be as equally important to juvenile fish and fish recruitment as vegetated habitat because like vegetated areas sandy habitat provides protection from predation (water turbidity and turbulence) and abundant food resources (Gregory and Levings 1998). However, to understand the importance of surf zone habitats, research is needed to provide information on the species present and abundance of these species. This study will characterize the fish species utilizing sandy habitats along Pamlico River and Pamlico Sound.

Because of the paucity of information on the fish communities and environmental factors affecting fishes in surf zone habitats in the South Atlantic, this study is designed to improve our understanding of the fish community in North Carolina estuaries. Therefore, this study will be
useful in understanding fish community patterns along sandy habitats and how water quality influences fish community patterns in North Carolina. This study is useful to identify the functional role of sandy beach habitats in Pamlico Sound. This will provide fisheries managers with important information on fish utilization of these areas. Therefore, management decisions such, as habitat specific protection plans, maybe based on information provided in this study.

**Research Objectives**

The purpose of this thesis is to characterize the fish community structure in Pamlico River, North Carolina. This study was divided into two parts: (1) a field survey component where I sampled the fishes present in shallow, sandy beaches along a salinity gradient (three sections: West, Central, and East) in Pamlico River during October and November 2009 and August to November 2010 and (2) a data synthesis component where I used existing juvenile fish survey data (Program 123) from the North Carolina Division of Marine Fisheries (NCDMF) to characterize the fish community structure in Pamlico River to compare sampling methodology. Sampling was conducted by NCDMF monthly (September to November) each year starting in 1987 (NCDMF 2001).

My research hypotheses are: (1) the positive downstream salinity gradient in Pamlico River (from west to east) will structure distinct spatial patterns in the fish community structure, because marine species will be found in the higher saline waters (east) and freshwater species will be found in the lower saline waters (west) (2) the relative abundance of fish will increase toward the East section of Pamlico River because the presence of marine juveniles will increase because of proximity to the Atlantic Ocean, (3) the relative fish abundance will decrease from August to November because it has been well documented that both marine and estuarine fish
species will migrate to deeper waters as temperatures decline thus lowering the relative abundance of shallow sandy habitats, and (4) the species community profile will differ between this current study and Program 123 because this current study includes fish collected from a wider range of salinities and over a longer period of time in Pamlico River.

In Chapter 2, fish community structure and water quality effects on the community are reported for Pamlico River. Spatial and seasonal similarities in the fish community structure were evaluated by characterizing key taxa driving differences both spatially and temporally. This study allowed an examination of small-scale geographic changes, compared to the entire Pamlico River. Non-metric multidimensional scaling (MDS) was used to visualize the results from ANOSIM. MDS and ANOSIM reported similarities in community structure spatially and temporally. Analysis of variance (ANOVA) was used to show differences among section of river and month in Shannon’s diversity index ($H'$), Pielou’s evenness index ($J'$), and water quality. By understanding these changes I was able to infer that seasonal changes affected the fish community structure to a greater extent than spatial differences. Canonical correspondence analysis (CCA) was used to evaluate how water quality relates to differences in fish community structure. With CCA I was able to show what water quality variables effected fish community structure. ANOSIM and MDS were used to compare the findings in Pamlico River with those in NCDMF Program 123. Spatial and frequency differences were compared. These studies were compared to show differences in species diversity based on sampling frequency and distribution. Therefore, improved sampling methodology can be implemented to characterize species diversity.
References


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CHAPTER 2 : BIOGEOGRAPHIC CHARACTERIZATION OF FISH COMMUNITY STRUCTURE ASSOCIATED WITH SHALLOW NEARSHORE SANDY HABITATS IN PAMLICO RIVER, NC

Abstract

The assemblages of small fish associated with shallow, sandy habitats were characterized in Pamlico River, North Carolina during late summer and fall (August to November) 2009-2010. Fish were collected bi-weekly with an 18 m bag seine and were identified to species in the field. A total of 73,845 fish, representing 43 taxa, were collected during this study. Five species (Anchoa mitchilli, Menidia beryllina, Brevoortia tyrannus, Leiostomus xanthurus, and Lagodon rhomboides) accounted for 97.4% of the total abundance. Abundance was greatest in the Central section and lowest in the most upstream West section of the river. The temporal abundance was greatest in August and lowest in October. A. mitchilli, M. beryllina, and L. xanthurus accounted for most of the spatial and seasonal dissimilarity in fish community structure among East, Central, and West sections of the river. Seasonal changes in fish community structure were stronger and more apparent than spatial changes in Pamlico River, likely due to seasonal temperature patterns. Salinity and temperature were associated with significant differences in the fish community structure. Examination of the seasonal changes in fish community structure suggests that temperature has the strongest influence on seasonal changes in the fish community. The data collected in this study were compared to the data generated by the North Carolina Division of Marine Fisheries (NCDMF) juvenile seine survey (Program 123) in Pamlico River. Program 123 currently samples in the Central section of the river. The comparison was used to determine if increasing sampling frequency and geographic scale would capture greater species
diversity and abundance. There were no differences in fish community structure between the present study and NCDMF Program 123. However, greater species diversity and lower catch per unit effort (CPUE) was observed in this study, compared to NCDMF Program 123. Thus, greater spatial sampling for Program 123 is important to characterize species diversity in Pamlico River. The patterns observed in fish community structure appear to be strongly influenced by fish species responses to seasonal changes in temperature.

Keywords: Fish community structure; Water quality; Pamlico River Estuary, North Carolina
Introduction

Many fishes in the surf zones of estuaries use the shallow water as a nursery habitat (Bennett and Attwood 1991a; Gillanders et al. 2003; Robertson and Lenanton 1984a; Ross et al. 1987). These areas tend to be dominated by a large number of larval and juvenile fishes, and are dynamic environments that have little complexity (Blaber and Blaber 1980). These open, surf zone areas tend to be very turbid thus providing juvenile fishes protection from predation and provide abundant food resources (Gregory and Levings 1998; Robertson and Lenanton 1984b). These sandy areas are dominate features of Pamlico River thus are used by many species (Stanley 1992).

Factors that influence the structure of fish assemblages in estuaries have received considerable attention (Araujo et al. 2000; Barreirosf et al. 2004; Desmond et al. 2002). Many of the studies have concentrated on the factors associated with the structure of fauna in seagrass beds. It has been well documented that juvenile fish utilize vegetated habitat, such as seagrass beds and submerged aquatic vegetation (SAV), within estuarine systems for shelter and food (Guidetti 2000; Jenkins and Wheatley 1998; Paterson and Whitfield 2000). Seagrass beds and vegetated areas generally support a greater abundance and diversity of fishes than nearby unvegetated and bare substrata (Castellanos and Rozas 2001; Conrow et al. 1990; West and King 1996). This is because SAV creates habitats that are complex and highly structured. Seagrass beds provide shelter and food and serve as a nursery areas to a large number of fishes (Guidetti 2000). While most of these habitats function as nursery areas, the biota utilizing these areas may differ among habitats (Phillips et al. 1989; Rogers and Van Den Avyle 1983). Likewise, species may utilize a combination of these habitats as nursery areas (Stunz et al. 2002)
The importance of unvegetated habitats has received far less attention than seagrass beds in previous studies (Bennett 1989; Santos and Nash 1995). However, some species may utilize both vegetated and unvegetated substrates at various stages of their life cycle (Jenkins and Wheatley 1998). Those species that utilize open, sandy areas may do so to decrease competition pressure because the density of fish within seagrass meadows tends to be higher than over open habitat (Bennett 1989; Santos and Nash 1995). Therefore, utilizing open areas reduces the number of individuals in which to compete for resources. Understanding fish species’ use of sandy habitats is important because most studies have occurred along seagrass beds and marshes because of the high species diversity found in those areas (Adams 1976; Crabtree and Dean 1982). Because the importance of vegetated areas to fish is well documented, there have been measures to protect wetlands and other vegetated areas (Nixon and Oviatt 1973; Szedlmayer and Able 1996; Tzeng and Wang 1992). Fewer studies have focused on open sandy areas as being important habitat for fish (Santos and Nash 1995). Sandy habitats are known to be important for various fish species, such as Paralichthys sp. (flounder) (Rogers and Van Den Avyle 1983). Also, Bennett (1989) concluded that sandy beach areas may be as important as other estuarine habitat for juvenile fish. Other studies have shown that shallow water habitats, including seagrass beds and open sandy areas, are important to juvenile fish maturation (Ayvazian et al. 1992). Thus, unvegetated, sandy habitats could be as important to juvenile fish as vegetated areas.

Salinity is one of the most important factors in structuring fish communities in many estuaries (i.e. Great Bay, New Jersey, Little Egg Harbor, New Jersey, Tanshui River, Taiwan, Swan, Australia, Humber, England, and Rio de la Plata, South America) (Jaureguizar et al. 2004; Loneragan and Potter 1990; Marshall and Elliott 1998; Szedlmayer and Able 1996; Tzeng and
Wang 1992). Freshwater flow can also influence the structure of fish communities based on the preferred salinity range of several species (Wingate and Secor 2008). Salinity can affect fish community structure because fish have varying salinity tolerances and will avoid, if possible, unfavorable conditions. Therefore, in the western less saline section of Pamlico River there will be more freshwater species than at the mouth of Pamlico Sound (eastern more saline section). (Martino and Able 2003). However, dissolved oxygen and temperature were more important than salinity to fish community structure in the Thames River, England, Elbe, Germany, and Humber, England estuaries because several fish species have optimal dissolved oxygen and temperature ranges for reproduction and metabolic processes (Araujo et al. 2000; Marshall and Elliott 1998). Because water quality varies in estuaries the interaction among water temperature, dissolved oxygen, and salinity can create complex patterns in fish community structure within an estuary (Crabtree and Dean 1982; Garces et al. 2006).

Pamlico River is the second largest tributary of Pamlico Sound, North Carolina (Stanley 1992). Its shallow areas (<2.0m) are dominated by many sandy beaches interspersed by few areas of SAV and seagrass meadows. Pamlico River only contains 87 ha² of dense (70-100% coverage) and patchy (5-70% coverage) SAV. Therefore, sandy beaches are a prevalent habitat for small fishes. These sandy beaches may function in multiples roles including serving as transit routes for many fish species. It is also likely that these areas may function as habitats for many young and immature fishes in the paucity of seagrass beds. However, estuarine surf zones, mostly sandy habitats, have remained largely under studied. In the scarcity of seagrass beds and other vegetated habitat, it is likely that small fishes will utilize open, sandy habitat to avoid predation pressure. While there have been several studies conducted in Pamlico River (Carpenter 1971; Lin et al. 2006; Mallin et al. 1991; Reed et al. 2008; Roelofs and Bumpus 1953;
Stanley and Nixon 1992; Taylor et al. 2009; Xu et al. 2008), the fish assemblage has never been described. Because of the paucity of information on the fish communities and environmental factors influencing these communities in the South Atlantic, this study is designed to improve our understanding of sandy beaches as habitat for small fishes.

NCDMF (Program 123) has been conducting a monthly seine survey from September to November since 1987. The survey, targets red drum (S. ocellatus) from sandy habitats in Pamlico Sound including Pamlico River (NCDMF 2001). This study differs from Program 123 in spatial and temporal coverage. While there are 18 stations located throughout Pamlico River in this study, Program 123 only samples two stations concentrated in the central portion of the river. This study includes one additional month (August) of sampling than Program 123.

The goal of this study is to characterize fish community structure along sandy habitats and to determine how water quality correlates with fish community structure in Pamlico River estuary. The objectives of this study are to: (1) characterize the spatial and seasonal variation in the fish community structure in Pamlico River, (2) determine which abiotic factors best explain spatial and seasonal variation in species composition, and (3) to evaluate whether the NCDMF Program 123 adequately captures the spatial and seasonal variation in fish community structure along sandy beaches within the Pamlico River.

My research hypotheses are: (1) the positive downstream salinity gradient in Pamlico River (from west to east) will structure distinct spatial patterns in the fish community structure, because marine species will be found in the higher saline waters (east) and freshwater species will be found in the lower saline waters (west) (2) the relative abundance of fish will increase toward the East section of Pamlico River because the presence of marine juveniles will increase
because of proximity to the Atlantic Ocean, (3) the relative fish abundance will decrease from August to November because it has been well documented that both marine and estuarine fish species will migrate to deeper waters as temperatures decline thus lowering the relative abundance of shallow sandy habitats, and (4) the species community profile will differ between this current study and Program 123 because this current study includes fish collected from a wider range of salinities and over a longer period of time in Pamlico River.

Methods

Study Area and Site Description

This study was conducted along sandy beach habitats in Pamlico River, North Carolina. Pamlico River estuary is located in eastern North Carolina and is the extension of the Tar River which flows approximately 346 km from Roxboro, North Carolina to the Highway 17 Bridge in Washington, North Carolina (Stanley 1992) (Fig. 2.1). Pamlico River can be characterized as an oligohaline-mesohaline system (Xu et al. 2008). The average depth in the surf zone is 1 m and in the central portion of the river, 4-5 m; salinity in Pamlico River ranges from 0.5 to 15 (Xu et al. 2008). The low salinity in Pamlico River and Pamlico Sound is due to the buffering effect from the barrier islands, which effectively diminish the amplitude of the lunar tidal cycle.

Eastern North Carolina is generally considered to have a humid subtropical climate with long hot summers and short mild winters (Stanley 1992). Daily mean air temperatures for eastern North Carolina are 5-10°C in January, the coldest month, and 24-27°C in July, the warmest month, with annual precipitation in the region averaging approximately 127 cm/year (Stanley 1992). The Pamlico River runs approximately 65 km from Washington, North Carolina to the western mouth of Pamlico Sound (Tenore 1972; Xu et al. 2008). The width of the river
increases from 0.5 km near Washington, North Carolina to 6 km at the mouth of Pamlico Sound, but depth remains constant in the middle of the river at 4 m (Xu et al. 2008). There are important blue crab (*Callinectes sapidus*) and flounder (*Paralichthys* sp.) fisheries as well as important recreational fisheries in Pamlico River, which is why conservation and management of the river are important (Stanley and Nixon 1992; Tenore 1972).

**Field Sampling and Sample Processing**

Study sites include 18 fixed locations (six locations in each section) ranging from the oligohaline section of Pamlico River near Washington, NC (West) to the mesohaline section (Central), and to the mouth of Pamlico Sound (East) (Fig. 2.1). Sites were selected if there was sandy beach, water was shallow (<1.5 m), and free of large debris. Bi-weekly sampling of water quality and fish began in early October through November in 2009 and from August to November 2010. Fish were captured with a bag seine (18.3 m long, 1.8 m high, 6.4 mm bar mesh in the body, and 3.2 mm bar mesh in the bag). The bag seine was deployed at each site where it was then extended 18 meters from shore and returned by a quarter sweep back to shore. All fish were identified to species and the first 50 of each species were measured (total length) and released; the remaining fish were identified, counted, and released. Any fish that were not identified in the field were identified in the lab. At each station, temperature, salinity, oxygen percent saturation and concentration, and pH were obtained using an YSI-Professional meter. Wind speed, wind direction, and air temperature were also measured at each site during the time of fish sampling using a Brunton ADC-PRO wind meter.

NCDMF Program 123 has been sampling monthly from September through November since 1987 (NCDMF 2001). There are two main differences between this study and Program 123. First, Program 123 has a much narrower geographic sampling area than this study. I
sampled 18 fixed locations from Washington, North Carolina to the mouth of Pamlico Sound; whereas, NCDMF sampled only two stations near Bath, North Carolina (Fig. 2.1). Second, sampling frequency was higher in this study than in Program 123. I sampled bi-weekly in October and November, 2009 and August to November, 2010; whereas, Program 123 sampled monthly from September to November, 2009 and 2010. To determine if sampling frequency affected the reported fish community structure, only two stations (located where the NCDMF stations are) from this study were compared with the NCDMF stations. Conversely, to determine spatial sampling contribution to the reported community structure, all 18 stations were compared with the two NCDMF stations. For spatial and frequency comparisons both 2009 and 2010 were included. September through November for both years were included for Program 123 and October and November (2009) and August through November (2010) were included from this study.

**Statistical and Data Analysis**

Environmental data were characterized using principal components analysis (PCA). To detect trends in environmental data, Normalization is necessary because water quality parameters are expressed on different scales (Marshall and Elliott 1998). Therefore, each environmental variable was normalized by subtracting the mean and dividing by the standard deviation. Normalization was carried out in the PRIMER-E software (Clarke and Warwick 2001).

Species diversity was expressed using species richness, Shannon’s diversity, and Pielou’s evenness indices. Shannon diversity index was calculated using the following formula:

\[ H' = -\sum p_i \log (p_i) \]
where \( p_i \) is the proportion of the total count for each sample from the total number of species (Shannon and Weaver 1963). As \( H' \) increases the diversity of species also increases. Pielou’s evenness index ranges from 0 (no evenness) to 1 (total evenness), and is calculated:

\[
J' = \frac{H'}{H'(\text{max})}
\]

where \( H' \) is the Shannon diversity index and \( H'(\text{max}) \) is the maximum possible \( H' \) value (Pielou 1969).

Multivariate statistical analysis were calculated using the Plymouth Routines in Multivariate Ecological Research (PRIMER) package (Clarke and Warwick 1994). To test the null hypothesis that there are no differences in fish community structure along the spatial gradient (West, Central, and East), analysis of similarity (ANOSIM) was conducted on the fish community composition. This procedure was run for 1000 permutations to obtain possible variations of the R value. R is calculated:

\[
R = \frac{(rb - rw)}{1/2M}
\]

where \( rb \) is the difference of mean ranks between groups, \( rw \) is the difference of mean ranks within groups, and \( M = n(n - 1)/2 \) where \( n \) is the total number of samples under consideration (Clarke 1993). The R value ranges between -1 and 1, where 0 is complete similarity among sections and -1 or 1 is complete dissimilarity. Raw abundances were standardized and square root transformed prior to calculation of Bray-Curtis coefficient to down weight abundant species so both rare and moderately abundant species could also influence similarity among the Pamlico River sections. I used non-metric multidimensional scaling to visualize the Bray-Curtis
coefficients. Within PRIMER a similarity percentage (SIMPER) is used to show total
dissimilarity along a downstream gradient and to define species contributing to that dissimilarity.
The similarity percentages (SIMPER) function showed the contribution of each fish species until
90% contribution is determined. Important species are those accounting for 90% contribution to
the community.

Sectional and monthly differences in environmental parameters (water temperature,
dissolved oxygen, salinity, and pH) were analyzed using analysis of variance (ANOVA). This
was also used to test for differences among diversity indices \( H' \) (Jaureguizar et al. 2004).
ANOVA was calculated in SAS Program. When there was a significant difference Dunn’s
method or Duncan’s multiple range test were used to determine pairwise sectional differences in
environmental data and diversity indices.

The association between species abundance CPUE \( \log(\text{CPUE}+1) \) and environmental
parameters was examined using canonical correspondence analysis (CCA). CCA directly relates
species’ occurrences to environmental variables; it is widely used in community ecology and
more recently in fish community structure studies (Hurst et al. 2004; Martino and Able 2003; ter
Braak 1986). CCA was calculated using Canoco v. 4.5 and was used to directly relate fish
community structure and environmental parameters. Water temperature, wind speed, dissolved
oxygen, salinity, and pH were used in the CCA.

**Results**

**Environmental Factors**

Air temperature ranged from 8.10\(^\circ\)C to 44.40\(^\circ\)C throughout Pamlico River. Mean air
temperature was 22.44\(^\circ\)C (SD=10.67\(^\circ\)C). There was no significant difference in pH either among
the sections of river or throughout the sampling period. The pH ranged from 6.84 to 9.15 with a mean of 8.23 (SD=0.36) (Fig. 2.2; Fig. 2.3). Water temperature ranged from 11.40°C to 32.80°C with highest temperatures in the summer months (August and September) and lowest in the fall months (October and November). Mean water temperature was 20.72°C (SD=6.03°C) (Fig. 2.2; Fig. 2.3). There were no significant differences in water temperature among the three sections, but as expected water temperature declined through the sampling season. No significant difference was found spatially but was found temporally with a significant decrease from August to November. Dissolved oxygen ranged from 2.15 mg L\(^{-1}\) to 12.12 mg L\(^{-1}\) and had a mean of 8.40 mg L\(^{-1}\) (SD=1.59 mg L\(^{-1}\)). There were no significant differences in dissolved oxygen throughout the sampling period. No significant difference was found temporally; however, the Central section had significantly higher dissolved oxygen levels than the East or West sections (Mean DO, Central= 8.8 mg L\(^{-1}\), West=8.1 mg L\(^{-1}\), and East= 8.3 mg L\(^{-1}\): Fig. 2.3). Salinity ranged from 0.4 to 21.4; mean salinity was 11.1 (SD=4.5) (Fig. 2.2). Mean salinity was significantly higher in the East Section (14.4) than in the Central (10.6) and West (8.6) Sections (ANOVA, p<0.001: Fig. 2.3). Salinity increased from west to east due to increased proximity to Pamlico Sound and the Atlantic Ocean. Water temperature and air temperature were strongly correlated and were negatively correlated with dissolved oxygen in the principle components analysis. Salinity, wind speed, and conductivity were correlated with pH (Fig. 2.4).

Species Composition and Diversity

A total of 73,845 fish, representing 43 species and 30 families, were collected during the course of this study (Table 2.1). Of the 43 species collected, 14 were commercially important, 15 were of minor commercial importance, and 14 were not commercially important. Six families represented by two or more species (Scianidae, 7; Carangidae, 3; Centrarchidae, 3; Bothidae, 2;
Cupleidae, 2; and Percichthyidae, 2) although the abundances of these families were still considerably smaller than Engraulidae (A. mitchilli) and Atherinidae (M. beryllina). Five fish species (A. mitchilli, M. beryllina, B. tyrannus, L. xanthurus, and L. rhomboides) accounted for 97.41% of the total abundance caught during the study and the remaining 2.59% consisted of the remaining 38 species. Only six species (Amiurus catus, Lepomis macrochirus, Lepomis microlophus, M. beryllina, Micropterus salmoides, and Notropis cummingsae), 15.7% of total abundance, were freshwater species. The remaining 37 species (84.3% of total abundance) were estuarine, estuarine-dependent, or marine species. The mean catch per unit effort (CPUE=#/haul) per month was 97.0. Highest CPUE was recorded in November (155.0) and lowest in October (54.5) (Fig. 2.5). There were no significant differences in monthly CPUE (ANOVA, p=0.80). CPUE was greatest in the Central section of river at 161.2 and lowest in the East at 62.8 (Fig. 2.6).

Shannon diversity index ($H'$) ranged from 1.54 to 1.26 in August and November (species richness was 8.9 and 5.9, respectively). Monthly $H'$ values were significantly different (ANOVA, p=0.045) where species diversity decreased from summer to late fall. Diversity was highest in the East section; however the differences among the sections were not statistically significant (ANOVA, p=0.94). $H'$ ranged from 1.34 (Central) to 1.46 (East) (species richness was 6.8 (Central) and 8.4 (East)) (Fig. 2.7). $H'$ and species richness both increased from West to East. Mean Pielou’s evenness index ($J'$) in Pamlico River was 0.71 thus the number of individuals is fairly even throughout the river. There were no significant differences in the $J'$ values spatially (ANOVA; p=0.11).
Community Composition

Fish community structure varied monthly structure (ANOSIM; p<0.01) (Fig. 2.8). *M. beryllina* and *A. mitchilli* contributed to the differences among months because the abundance of both species decreased from August to November. The fish communities differed among the sections in Pamlico River (ANOSIM 0.085; p=0.001) (Fig. 2.9). The MDS ordination plot revealed distinct spatial groupings (Fig. 2.10). Similarity percent (SIMPER) shows that *A. mitchilli*, *M. beryllina*, and *L. xanthurus* account for a cumulative contribution percent of 92.1, 89.9, and 85.9 for West, Central, and East sections, respectively. These three species also account for the majority of dissimilarity in the groupings of each section of river. Average dissimilarities among the sections were: West-East (57.09%), West-Central (54.98%), and East-Central (54.52%) (Table 2.2 and Table 2.3). Differences in fish community structure between the East and West, and between East and Central Sections were significantly different (ANOSIM; East-West p=0.001; East-Central p=0.031) (Fig. 2.9). The West and Central Sections were not significantly different (ANOSIM; p=0.056) (Fig. 2.9).

Most fish species were strongly correlated with water temperature, salinity, and pH (Fig. 2.11). The first two axes of the CCA analysis explained 20.5% of the species composition and 83.5% of the species-environment correlation meaning additional axes are needed to explain the remaining variability (Table 2.4). There was a strong correlation between water temperature and the abundant estuarine-dependent species. Of the ten most abundant species, four (*C. sapidus, L. rhomboides, Micropogonias undulatus*, and *Penaeus* sp.) were strongly correlated with water temperature. These four species were also strongly correlated with pH but the association between water temperature and community structure was stronger. Two of the ten most abundant species (*B. tyrannus* and *P. dentatus*) were strongly correlated with salinity. The
remaining three fish species (*A. mitchilli*, *L. xanthurus*, and *M. beryllina*) were either positively or negatively correlated with dissolved oxygen even though variability in dissolved oxygen was low (Fig. 2.11).

The comparison between this study and NCDMF showed no significant difference in fish community structure either with sampling frequency or spatial distribution (Fig. 2.12 and 2.13). Differences in sampling frequency between these two studies did not capture significant differences in fish community structure although CPUE was higher in the NCDMF study than in this study (Table 2.5). However, the two NCDMF stations were located in the Central Section where CPUE and total fish abundance were highest in the river. With the inclusion of the West and East Sections, CPUE in this study was lower than in the NCDMF study. Mean $H'$ was higher in this study than in the NCDMF study (This study=1.39; NCDMF=1.08). Thus, while CPUE was lower in this study, $H'$ was higher. Fish community structure was not significantly different between the two NCDMF Program 123 study sites and all study sites in Pamlico River from this study although there were differences in CPUE and $H'$ (Fig. 2.14 and 2.15).

**Discussion**

**Overview**

The general patterns of the fish community structure along sandy beach habitats in Pamlico River show that seasonal variation in temperature was associated with seasonal variation in fish community structure, whereas, spatial variation in salinity caused fish community structure to vary across the Pamlico River. Similar trends have been found in other temperate estuaries (Araujo et al. 2000; Jaureguizar et al. 2004; Marshall and Elliott 1998). Salinity influenced spatial patterns is likely caused by species’ salinity tolerance. Each species has
specific salinity requirements that will limit where it occurs (Costanza et al. 1993; Meire et al. 2005). Therefore, salinity is an important environmental variable affecting spatial distribution of many fish species. The water temperature effect was obvious, fish relative abundance was lower in the fall months (October and November) than in the summer months (August and September). Declines in relative abundance from summer to fall were especially obvious for two estuarine species (*A. mitchilli* and *L. xanthurus*) that migrate from the shallow, surf zone areas to deeper water during the fall as temperatures decrease (Morton 1989; Phillips et al. 1989). In this study, fewer *A. mitchilli* and *L. xanthurus* were sampled during the fall than in the summer. Many species in the surf zone show similar patterns where they move to deeper areas, thus lowering abundance in shallow waters during the fall. Seasonal migrations may be initiated as water temperature drops below a particular threshold temperature that is typically reached during the fall.

**Salinity Correlation with Fish Community Structure**

I found support for the hypothesis that fish community structure varies across the salinity gradient represented within Pamlico River. Fish have preferred salinity ranges and will avoid unfavorable conditions if possible. But predation pressure and competition may cause some fish to live in unfavorable conditions (Laegdsgaard and Johnson 2001). However, some freshwater species have been found in association with marine species (Peterson and Meador 1994). These associations are generally short lived as freshwater species can tolerate high salinities for short periods of time (Peterson and Meador 1994). Freshwater species were not observed in the East section where salinities were routinely above 10. While some freshwater species can tolerate mesohaline conditions (Peterson and Meador 1994; West and King 1996), most freshwater species (*M. salmoides*, *L. microlophus*, and *A. catus*) in Pamlico River sandy habitats were
present in the West section and marine species in the East section; thus there was little
association between freshwater and marine species in Pamlico River. However, there were both
freshwater and marine species present in the Central section (*L. macrochirus*, *P. lethostigma*, and
*M. beryllina*). Thus, in areas where salinity is lower (4-8), community structure consisted of a
mix of freshwater, estuarine, and marine species.

There were significant differences in the fish community structure among the three
sections of Pamlico River which were caused by differences in salinity. Spatial differences can
be caused by salinity gradients as presented by Jaureguizar et al. (2004), which was the case for
this study. Salinity in the East (mean=14.4) section was significantly higher than values
recorded in both Central (mean=10.6) and West (mean=8.6) sections due to the proximity to the
Atlantic Ocean and Pamlico Sound. There was no significant difference in salinity between the
West and Central sections because of the influence of freshwater from Tar River (Epperly and
Ross 1986). The fish community structure followed similar trends as salinity. Salinity has a
greater influence on species diversity than water temperature (Marshall and Elliott 1998).
Greater species diversity was found in the East section than in either the West or Central
sections. This freshwater flow may have caused an increase of freshwater individuals to migrate
further to the east due to decreased salinity. This may have changed the distribution of
ubiquitous estuarine species, such as *A. mitchilli*, which could explain the difference in
community structure spatially. Therefore, changes in the abundance of individual fish among the
three sections caused by salinity changes affected community structure more than changes in the
number of species present.

Salinity may also show slight correlations with seasonal changes in fish community
structure. Because there is higher rainfall during the summer than in the fall in eastern North
Carolina, salinity levels are usually lower in August and September than in October and November according to Stanley, 1992 and Xu et al., 2008. The resulting abiotic conditions may be responsible for the wide distribution of *M. beryllina* (which prefers freshwater (Fay 1983)) in Pamlico River. *M. beryllina* were present throughout most of Pamlico River during the summer months but found mostly in the Central and West sections in the fall months. Therefore, seasonal differences in salinity effected the distribution and abundance of the second most abundant species, *M. beryllina*.

**Spatial Relative Abundance Patterns**

The hypothesis that increases in the relative abundance of fishes along the downstream gradient did not hold true. The highest relative abundance was in the Central section and lowest abundance was in the East section. The high abundance in the Central section was highly influenced by *A. mitchilli* which was found in predominately in the Central section. *M. beryllina* was predominately found in the West section. *A. mitchilli* and *M. beryllina* were the two most abundant species in this study, thus their contribution to relative abundance was higher than any other species. I observed large schools *A. mitchilli* and Young-of-Year (YOY) *B. tyrannus* at the surface in many of the saltmarsh creeks. The frequency and the large number of fish from these areas were responsible for the increase in CPUE. The higher relative abundance of fish in the Central section may also be due to the distribution of predators. Large predatory fish (>300 mm TL) including *P. saltatrix* and *S. ocellatus* were consistently collected in the East section; thus influencing the upstream settlement of prey species (*A. mitchilli*) to the Central section. *P. saltatrix* and *S. ocellatus* were probably most abundant in the East section because both are marine species that prefer higher salinity levels than estuarine species (Stunz et al. 2002).
Water Temperature Correlation with Fish Community Structure

As water temperature declined (October and November) the relative abundance of *A. mitchilli* increased in the East section. In 2009, I observed an increase in CPUE (and variability) with decreasing water temperature from October to November. This was not expected and can only be explained by the outward migration of fishes from adjacent saltmarsh creeks using sandy beach habitats as a migration corridor.

The seasonal changes in fish community structure were likely caused by emigration of marine species back to the ocean and estuarine species moving to deeper waters within the estuary (Fay 1983; Morton 1989; Rogers 1983). While marine species do emigrate as water temperature declines, in Pamlico River marine species played a minor role in the species composition because estuarine residents were present in much greater numbers. The seasonal migration of *A. mitchilli* to deeper water likely caused a decrease in fish CPUE. Some of this migration may be to Pamlico Sound but may also be movement to the deeper waters of Pamlico River. Thus, to test this, more than one sampling methodology is needed to understand migration patterns of estuarine species (Selleslagh and Amara 2008). Because *A. mitchilli* was the most abundant species in Pamlico River, this seasonal migration may have caused increased abundance in the Central and East sections as temperatures declined. The change in *A. mitchilli* distribution led to lower CPUE throughout the river because most marine species and the abundant estuarine species had migrated to deeper water (Morton 1989; Rogers 1983).

Monthly changes in fish community structure from late summer to fall are more dominant than sectional differences. Mean monthly water temperature did not differ significantly among the three sections (West, Central, and East); however, it did change significantly through time. The temporal changes were associated with the expected seasonal
changes (late summer to fall). Thus, water temperature caused the greatest change in fish community structure in Pamlico River and is the most important variable, of those studied, affecting fish community structure in Pamlico River sandy habitats. Seasonal changes in fish community structure have also been found in other temperate estuaries (Araujo et al. 2000; Marshall and Elliott 1998) and have been reported to be closely associated with seasonal water temperature decrease. Water temperature can cue emigration of several fish species, such as *A. mitchilli* and *B. tyrannus* (Desmond et al. 2002; Morton 1989; Rogers 1983). Similar patterns are apparent in other temperate estuaries as in Jaureguizar et al. (2004) where temporal variation patterns in fish community structure are associated with changing water temperature. Water temperature decreased, as expected, from August to November. *A. mitchilli* migrate to deeper estuarine waters during cooler temperatures (Morton 1989) and they became less abundant in our samples later in the sampling season. *B. tyrannus* spawning occurs in greatest abundance off the Cape Lookout, North Carolina shore in November and December (Rogers 1983). Three months after hatching, *B. tyrannus* will migrate to estuaries and reside there until late fall (Nicholson 1978). Juvenile *B. tyrannus* occupy the rivers and saltmarsh creeks, but most adults are found in deeper estuarine waters and in marine waters (Rogers 1983). *B. tyrannus* of all ages emigrate from estuaries in the fall. These migration patterns can cause changes in fish community structure in Pamlico River. Several other temperate estuaries have shown similar patterns (Jaureguizar et al. 2004; Marshall and Elliott 1998; Wingate and Secor 2008). However, Wingate and Secor (2008) found that winter water temperature effects spring and summer fish assemblage in Chesapeake Bay. They found that environmental variables sampled at the same time as fish were not important to the fish community in Patuxent River estuary, Maryland. This supported previous work conducted by Hurst et al. (2004) which showed that previous
environmental factors (water temperature and salinity) were important in explaining the fish assemblage in Hudson River over a 21 year period.

Winter conditions can affect estuarine-dependent species in two ways. First, the winter temperature and water flow can affect nursery habitats thus affecting the species present and changing abundances of estuarine spawning fish (Wingate and Secor 2008). Second, winter temperatures could affect recruitment such as influencing the timing of migration and spawning of adult fish (Wingate and Secor 2008). No work at this point has been conducted year-round on fish communities in Pamlico River or Pamlico Sound. This could aid in understanding community dynamics in the estuary and the effects of environmental variables on these communities. Therefore, while this work has characterized the community structure in Pamlico River during late summer and fall, additional work in Pamlico River estuary should focus on year-round monitoring of fish and water quality to determine if water quality effects fish community structure day to day or if there is a lag in the effect of water quality on fish communities as found by Wingate and Secor (2008).

**Dissolved Oxygen**

Dissolved oxygen levels did not affect the fish community structure within Pamlico River. Because of the shallow water and mixing of oxygen with the water, dissolved oxygen levels rarely fell below 5.0 mg L\(^{-1}\). Dissolved oxygen correlated inversely to water temperature as expected because water is capable of containing more oxygen at lower temperatures. Pamlico River frequently has hypoxic events in mid-summer which have caused large scale fish and invertebrate kills (Stanley and Nixon 1992). I observed only one hypoxic event (dissolved oxygen <3.0 mg L\(^{-1}\)) during this study because sampling occurred in late summer and fall in shallow water. Most hypoxic events in Pamlico River occur in the warmer summer months (Lin...
et al. 2007; Lin et al. 2006; Stanley and Nixon 1992). The lowest recorded dissolved oxygen level in this study was in August 2010 with a value of 2.2 mg L$^{-1}$. Hypoxic events are characterized when dissolved oxygen is below 5.0 mg L$^{-1}$ (Stanley and Nixon 1992). Dissolved oxygen is likely more important to fish in the deeper portion of Pamlico River where stratification can cause bottom water hypoxia (Stanley and Nixon 1992). Due to the decreased likelihood that shallow water can thermally stratify, hypoxic or anoxic events become less common and affect fish communities in surf zones less often than in the deeper parts of the river.

North Carolina Division of Marine Fisheries Program 123

The fish community structure was not significantly different between this study and Program 123. The four most abundant species ($A.~mitchilli$, $M.~beryllina$, $L.~xanthurus$, and $B.~tyrannus$) were identical between Program 123 and this study even though all sampling periods were compared (this study: October and November 2009, August through November 2010; Program 123: September through November 2009 and 2010). The sampling differences and similarity in the most abundant species show that sampling frequency and geographic coverage differences between this study and Program 123 captured similar fish communities along sandy habitats in Pamlico River. The overall dominance of the four species probably masked any subtle differences in community structure between the two studies. This study also had greater spatial coverage in Pamlico River than did Program 123. After comparing all sampling sites from this study with the two located in Pamlico River from Program 123, I found no significant difference in fish community structure. Although greater spatial sampling was expected to show significant differences in fish community structure than small spatial sampling, no significant differences were found. However, I found that greater spatial sampling and increased sampling frequency did capture higher species diversity. Therefore, while both this study and Program
123 captured similar community structure, greater spatial coverage and sampling frequency are needed to capture the species diversity present in Pamlico River. The other main difference is that CPUE was higher in Program 123 than in this study. This was surprising because I expected the CPUE to be similar; especially since I controlled much of the variability in sampling methodology (used same gear and same stations). Therefore, it appears that Program 123 overestimated the CPUE for Pamlico River because sampling was only conducted in the Central section (where CPUE is highest). These differences in CPUE highlight a point made earlier that the outward migration of fish from adjacent saltmarsh creeks during the fall probably occurs in pulses. The overlap of the frequency of sampling and migration pulses will control CPUE and the variability of the estimates. While the NCDMF sampling regime is sufficient for capturing high CPUE, greater spatial coverage and sampling frequency are needed to adequately characterize species diversity along sandy habitats in Pamlico River.

Conclusion

This study can serve as a benchmark for future studies in Pamlico River fish community structure. The fish community structure along Pamlico River has been documented here, which consists mostly of estuarine residents and estuarine-dependent species. As shown in many studies, there is a distinct seasonality to the community structure of fish in Pamlico River. Highest abundance was found in the summer months which are comparable to other estuarine studies (Jaureguizar et al. 2004; Santos and Nash 1995). Year-round sampling is needed in Pamlico River to characterize the winter and spring fish communities and water quality parameters. There could be a lag in time between when water quality is sampled and the effects on the fish community structure (Wingate and Secor 2008). Thus, spring and early-summer environmental conditions may affect late-summer and fall fish communities. As shown in
Wingate and Secor (2008) winter environmental parameters can affect the spring and summer fish assemblage in Chesapeake Bay. To understand these inter-seasonal relationships between water quality and fish community structure in Pamlico River, year-round monitoring must be considered.
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Fig. 2.1. Map of Pamlico River. Red markers represent potential sites not selected by the random number generator. Pink circles represent sites that were included in the study. These stations were divided into three sections (West, Central, and East) based on location in the river. There were six stations within each section. The yellow circles represent North Carolina Division of Marine Fisheries Pamlico River sampling sites.
Fig. 2.2. Bar graph showing monthly mean (±1 SD) water temperature (a), dissolved oxygen (mg L$^{-1}$), salinity, and pH. Water quality was taken bi-weekly using a YSI Professional at each station. Stations were located along sandy habitats in Pamlico River from Washington, NC to the mouth of Pamlico Sound, NC.
Fig. 2.3. Mean water temperature (a), dissolved oxygen (b), salinity (c), and pH (d) (±1 SD) in Pamlico River, North Carolina. Water quality was sampled bi-weekly from August to November (2009-2010) using a YSI Professional at each site in the Pamlico River. Sites were sandy beach habitats in Pamlico River from Washington, NC to the mouth of Pamlico Sound, NC. Bars with different letters are significantly different.
Fig. 2.4. 2-Dimensional Principal Components Analysis of water quality. Water quality was sampled bi-weekly from August to November 2009 and 2010 from stations in Pamlico River, NC. Stations were located on sandy beach habitats from Washington, NC to the mouth of Pamlico Sound, NC. Parameters located closer together on the PC axes are more closely correlated than points further apart. Length of the line correlates to strength of the correlation. WaterTemp= Water Temperature, AirTemp= Air Temperature, DO= Dissolved Oxygen.
Fig. 2.5. Catch per unit effort (CPUE) per month (±1SD). Fish were collected using an 18 m long bag seine in Pamlico River, NC bi-weekly from August to November 2009 and 2010. Stations were located along sandy habitats from Washington, NC to the mouth of Pamlico River, NC.
Fig. 2.6. Catch per unit effort (CPUE #/haul) per section of river (±1SD). Fish were collected with an 18 m long bag seine at sandy habitats in Pamlico River, NC. Stations were divided into three sections based on location in the river from the oligohaline portion near Washington, NC to the mesohaline portion at the mouth of Pamlico Sound, NC.
Fig. 2.7. Mean Shannon diversity index ($H'$) value based on month (a) and section (b). Fish were collected from Pamlico River from August to November 2009-2010 bi-weekly using an 18 m long bag seine.
Fig. 2.8. Non-metric multidimensional scaling based on square root transformation of the data and Bray-Curtis similarity. Fish were collected from Pamlico River from August to November of 2009 and 2010. 2D Stress represents the accuracy of the points’ location. 2D Stress less than 0.20 is usable in ecological data. The colors and shapes represent the month the particular sampling unit was sampled (ANOSIM; p<0.01).
Fig. 2.9. Analysis of similarity of fish community structure based on section of Pamlico River.

Fish were sampled from sandy habitats along Pamlico River, NC using an 18 m long bag seine. Sampling occurred bi-weekly from August to November in 2009 and 2010. As above, the X axis represents R value and the Y axis frequency of a given R value being calculated. All bars in the histogram are possible R values that support the null hypothesis that there is no significant difference in community structure based on section of river.
Fig. 2.10. Non metric multidimensional scaling of sampling units based on species space.

Sampling was conducted in Pamlico River, NC bi-weekly from August to November 2009 and 2010. Sampling locations were located on sandy habitats from Washington, NC to the mouth of Pamlico Sound, NC. Points located closer together are more compositionally related than points located further apart. The 2D Stress is below the 0.20 threshold which means these data can be utilized in ecological studies (Kruskal 1964). The MDS was calculated using square root transformed Bray-Curtis similarity. The various color and shaped point relate to location along the river (West, Central, and East).
Fig. 2.11. Canonical correspondence analysis of environmental variables and fish assemblage in Pamlico River. Fish and water quality were sampled bi-weekly from August to November 2009-2010 along sandy beach habitats along Pamlico River, NC. Red lines and abbreviations (WaterTemp=water temperature and DO=dissolved oxygen) represent environmental data and black triangles and abbreviations represent fish species (fish species codes found in Table 2.1). Lines going in the same direction are positively correlated and lines going in opposite directions are negatively correlated. Longer lines represent stronger correlations and fish species located on a line show strong correlation to that environmental parameter.
Fig. 2.12. Comparison of fish assemblage between NCDMF and ECU studies. NCDMF sampled at two stations in Pamlico River monthly with a bag seine from September to November since 1987, and ECU sampled the same stations bi-weekly from August to November in 2009 and 2010 with a bag seine. The R value is -0.077 (p=0.697) and is shown as the vertical dashed line. The histogram represents potential R values that support the null hypothesis that there is no difference in fish assemblage between the two studies. R values range from -1 to 1 where -1 and 1 represent complete dissimilarity in fish assemblage and 0 represents complete similarity.
Fig. 2.13. Non-metric multidimensional scaling of fish assemblage. Each point represents a seine haul at one of the two stations in Pamlico River, NC. NCDMF sampled monthly from September to November since 1987 although only samples taken in 2009 and 2010 were plotted for comparison. ECU sampled bi-weekly from August to November in 2009 and 2010 from the same locations. Black points represent samples taken by NCDMF and grey points represent samples taken by ECU Biology Department. Each point represents a sample. Points closer together represent more similar fish assemblages than points further apart. 2D stress is 0.05 meaning the location of the points is true. There is not a significant difference between the studies (R=-0.077, p=0.697).
Fig. 2.14. Comparison between this study and NCDMF Pamlico River study sites. The fish collected in this study were obtained bi-weekly from August to November 2009-2010 using an 18 m long bag seine along sandy habitats in Pamlico River, NC from Washington, NC to the mouth of Pamlico Sound. NCDMF sampled monthly from September to November 2009-2010 at two stations located across the river from Bath, NC. The analysis showed no significant difference in fish community structure between this study and the NCDMF study (R= -0.11; p=0.80).
Fig. 2.15. Non-metric multidimensional scaling (MDS) of samples taken from Pamlico River in 2009 and 2010. Fish were collected in this study (ECU) with an 18 m long bag seine bi-weekly from August to November 2009-2010 from sandy habitats in Pamlico River, NC. Sampling occurred at 18 stations in Pamlico River from Washington, NC to the mouth of Pamlico Sound. The NCDMF study (DMF) collected fish monthly from September to November 2009-2010 using a bag seine at two sandy locations in Pamlico River located across from Bath, NC. 2D stress is 0.15 meaning the location of each point is accurate. There is no significant difference in fish community structure between the two studies (R= -0.11; p=0.80)
Table 2.1. Fish species sampled during the study. Fish were collected with an 18m long bag seine from sandy habitats along Pamlico River during late summer and early fall in 2009 and 2010. Relative abundance is a percent of the total fish abundance. The approximate adult size shows the average total length at which these species mature. Preferred environment is where these species would generally be found: F=Freshwater, M=Marine, and E=Estuarine. Economic importance shows if a species is important or not.

<table>
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<th>Common Name</th>
<th>Species/Taxa</th>
<th>Species Code</th>
<th>Relative Abundance</th>
<th>Approx. Adult Size (mm)</th>
<th>Preferred Environment</th>
<th>Economic Importance</th>
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<td><em>Anchoa mitchilli</em></td>
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<td>40</td>
<td>E</td>
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</tr>
<tr>
<td>Inland silverside</td>
<td><em>Menidia beryllina</em></td>
<td>Men ber</td>
<td>15.59%</td>
<td>91</td>
<td>F, E</td>
<td>Minor (Aq)</td>
</tr>
<tr>
<td>Atlantic menhaden</td>
<td><em>Brevoortia tyrannus</em></td>
<td>Bre tyr</td>
<td>1.70%</td>
<td>180</td>
<td>M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Spot</td>
<td><em>Leiostomus xanthurus</em></td>
<td>Lei xan</td>
<td>1.69%</td>
<td>250</td>
<td>M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Ctenophore</td>
<td>Ctenophora</td>
<td>Ctenop</td>
<td>1.04%</td>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Pinfish</td>
<td><em>Lagodon rhomboides</em></td>
<td>Lag rho</td>
<td>0.59%</td>
<td>180</td>
<td>F, M, E</td>
<td>Minor (Bait)</td>
</tr>
<tr>
<td>Shrimp</td>
<td><em>Penaeus sp.</em></td>
<td>Penaeu</td>
<td>0.30%</td>
<td>74</td>
<td>M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Atlantic croaker</td>
<td><em>Micropterus undulatus</em></td>
<td>Mic und</td>
<td>0.25%</td>
<td>300</td>
<td>M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Blue crab</td>
<td><em>Callinectes sapidus</em></td>
<td>Cal sap</td>
<td>0.11%</td>
<td>50 (Carapace)</td>
<td>E</td>
<td>Yes</td>
</tr>
<tr>
<td>Summer flounder</td>
<td><em>Paralichthys dentatus</em></td>
<td>Par den</td>
<td>0.10%</td>
<td>280</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>Red drum</td>
<td><em>Sciaenops ocellatus</em></td>
<td>Sci oce</td>
<td>0.10%</td>
<td>550</td>
<td>M, E</td>
<td>Minor (Com)</td>
</tr>
<tr>
<td>Silver perch</td>
<td><em>Bairdiella chrysoura</em></td>
<td>Bai chr</td>
<td>0.10%</td>
<td>200</td>
<td>F, M, E</td>
<td>Minor (Bait)</td>
</tr>
<tr>
<td>Striped mullet</td>
<td><em>Mugil cephalus</em></td>
<td>Mug cep</td>
<td>0.10%</td>
<td>300</td>
<td>F, M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Kingfish</td>
<td><em>Menticirrh saxatilis</em></td>
<td>Men sax</td>
<td>0.09%</td>
<td>300</td>
<td>M, E</td>
<td>Minor (Bait)</td>
</tr>
<tr>
<td>Fish Name</td>
<td>Scientific Name</td>
<td>Common Name</td>
<td>Percentage</td>
<td>Length</td>
<td>Gender</td>
<td>Notes</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------------------</td>
<td>-------------</td>
<td>------------</td>
<td>--------</td>
<td>--------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Spotted seatrout</td>
<td><em>Cynoscion nebulosus</em></td>
<td>Cyn neb</td>
<td>0.08%</td>
<td>350</td>
<td>M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Mud crab</td>
<td><em>Scylla serrata</em></td>
<td>Scy ser</td>
<td>0.06%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goby</td>
<td><em>Gobiidae</em></td>
<td>Gobiid</td>
<td>0.04%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silver jenny</td>
<td><em>Eucinostomus gula</em></td>
<td>Euc gula</td>
<td>0.04%</td>
<td>150</td>
<td>F, M, E</td>
<td>Minor (Bait)</td>
</tr>
<tr>
<td>Pipefish</td>
<td><em>Syngnathinae</em></td>
<td>Syngna</td>
<td>0.03%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hogchoker</td>
<td><em>Trinectes maculatus</em></td>
<td>Tri mac</td>
<td>0.03%</td>
<td>110</td>
<td>F, M, E</td>
<td>No</td>
</tr>
<tr>
<td>Bluegill</td>
<td><em>Lepomis macrochirus</em></td>
<td>Lep mac</td>
<td>0.03%</td>
<td>190</td>
<td>F</td>
<td>Minor (Aqua) c</td>
</tr>
<tr>
<td>Mummichog</td>
<td><em>Fundulus heteroclitus</em></td>
<td>Fun het</td>
<td>0.02%</td>
<td>89</td>
<td>F, M, E</td>
<td>Minor (Aq) a</td>
</tr>
<tr>
<td>Leatherjacket</td>
<td><em>Oligoplitesurus</em></td>
<td>Oli sau</td>
<td>0.02%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida pompano</td>
<td><em>Trachinotus carolinus</em></td>
<td>Tra car</td>
<td>0.01%</td>
<td>250</td>
<td>M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Grey trout</td>
<td><em>Cynoscion regalis</em></td>
<td>Cyn reg</td>
<td>0.01%</td>
<td>140</td>
<td>M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>River herring</td>
<td><em>Alosa aestivalis</em></td>
<td>Alo aes</td>
<td>0.01%</td>
<td>270</td>
<td>F, M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Jellyfish</td>
<td><em>Cnidaria</em></td>
<td>Cnidar</td>
<td>&lt;0.01%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern flounder</td>
<td><em>Paralichthys lethostigma</em></td>
<td>Par let</td>
<td>&lt;0.01%</td>
<td>500</td>
<td>M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Bluefish</td>
<td><em>Pomatomus saltatrix</em></td>
<td>Pom sal</td>
<td>&lt;0.01%</td>
<td>250</td>
<td>M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Toadfish</td>
<td><em>Thalassothia cirrhosa</em></td>
<td>Tha cir</td>
<td>&lt;0.01%</td>
<td></td>
<td>M</td>
<td>No</td>
</tr>
<tr>
<td>White perch</td>
<td><em>Morone americana</em></td>
<td>Mor ame</td>
<td>&lt;0.01%</td>
<td>130</td>
<td>F, M, E</td>
<td>Minor (Com) b</td>
</tr>
<tr>
<td>American eel</td>
<td><em>Anguilla rostrata</em></td>
<td>Ang ros</td>
<td>&lt;0.01%</td>
<td>350</td>
<td>F, M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Dusky shiner</td>
<td><em>Notropis cummingsae</em></td>
<td>Not cum</td>
<td>&lt;0.01%</td>
<td>45</td>
<td>F</td>
<td>No</td>
</tr>
<tr>
<td>Gray snapper</td>
<td><em>Lutjanus griseus</em></td>
<td>Lut gri</td>
<td>&lt;0.01%</td>
<td>210</td>
<td>F, M, E</td>
<td>Yes</td>
</tr>
<tr>
<td>Ladyfish</td>
<td><em>Elops saurus</em></td>
<td>Elo sau</td>
<td>&lt;0.01%</td>
<td>600</td>
<td>M, E</td>
<td>Minor (Bait)</td>
</tr>
<tr>
<td>Largemouth bass</td>
<td><em>Micropterus salmoides</em></td>
<td>Mic sal</td>
<td>&lt;0.01%</td>
<td>400</td>
<td>F</td>
<td>Minor</td>
</tr>
<tr>
<td>Lizardfish</td>
<td><em>Synodontidae</em></td>
<td>Synodo</td>
<td>&lt;0.01%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigfish</td>
<td><em>Orthopristis chrysoptera</em></td>
<td>Ort chr</td>
<td>&lt;0.01%</td>
<td>300</td>
<td>M, E</td>
<td>Minor (Bait)</td>
</tr>
<tr>
<td>Redear sunfish</td>
<td><em>Lepomis microlophus</em></td>
<td>Lep mic</td>
<td>&lt;0.01%</td>
<td>190</td>
<td>F</td>
<td>No</td>
</tr>
<tr>
<td>Spadefish</td>
<td><em>Chaetodipterus faber</em></td>
<td>Cha fab</td>
<td>&lt;0.01%</td>
<td>120</td>
<td>M, E</td>
<td>Minor (Com) b</td>
</tr>
<tr>
<td>Striped bass</td>
<td><em>Morone saxatilis</em></td>
<td>Mor sax</td>
<td>&lt;0.01%</td>
<td>120</td>
<td>F, M, E</td>
<td>Minor (Com) b</td>
</tr>
<tr>
<td>White catfish</td>
<td><em>Ameiurus catus</em></td>
<td>Ame cat</td>
<td>&lt;0.01%</td>
<td>300</td>
<td>F</td>
<td>No</td>
</tr>
<tr>
<td>Yellowfin jack</td>
<td><em>Hemicaranx leucurus</em></td>
<td>Hem leu</td>
<td>&lt;0.01%</td>
<td>250</td>
<td>F, M, E</td>
<td>Minor (Com) b</td>
</tr>
</tbody>
</table>
a= Aquarium, b= Commercial, c= Aquaculture
Table 2.2. Similarity percentage (SIMPER) of three most important species in each section in Pamlico River, NC. Fish were collected bi-weekly using a beach seine from August to November 2009-2010 along sandy habitats in Pamlico River. Column headings are as follows: Av. Abund= average abundance, Av.Diss= average dissimilarity, Diss/SD= dissimilarity/standard deviation, Contrib%= contribution percentage, and Cum.%= cumulative contribution percentage.

<table>
<thead>
<tr>
<th>Section</th>
<th>Avg. Sim. (%)</th>
<th>Species</th>
<th>Av.Abund</th>
<th>Av.Sim</th>
<th>Sim/SD</th>
<th>Contrib%</th>
<th>Cum.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>West</td>
<td>46.5</td>
<td><em>M. beryllina</em></td>
<td>11.75</td>
<td>23.54</td>
<td>1.76</td>
<td>50.66</td>
<td>50.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. mitchilli</em></td>
<td>12.17</td>
<td>15.99</td>
<td>1.47</td>
<td>34.41</td>
<td>85.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. xanthurus</em></td>
<td>2.41</td>
<td>3.25</td>
<td>0.81</td>
<td>6.99</td>
<td>92.07</td>
</tr>
<tr>
<td>Central</td>
<td>45.2</td>
<td><em>A. mitchilli</em></td>
<td>18.4</td>
<td>20.38</td>
<td>1.43</td>
<td>45.04</td>
<td>45.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>M. beryllina</em></td>
<td>8.52</td>
<td>15.39</td>
<td>1.65</td>
<td>34.02</td>
<td>79.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. xanthurus</em></td>
<td>3.34</td>
<td>4.92</td>
<td>0.82</td>
<td>10.87</td>
<td>89.93</td>
</tr>
<tr>
<td>East</td>
<td>49.6</td>
<td><em>A. mitchilli</em></td>
<td>17.82</td>
<td>30.71</td>
<td>2.07</td>
<td>61.97</td>
<td>61.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>M. beryllina</em></td>
<td>4.76</td>
<td>9.71</td>
<td>1.16</td>
<td>19.6</td>
<td>81.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. xanthurus</em></td>
<td>1.69</td>
<td>2.16</td>
<td>0.94</td>
<td>4.37</td>
<td>85.94</td>
</tr>
</tbody>
</table>
Table 2.3. Paired sections showing average dissimilarity in fish assemblage between them.

Average dissimilarity is showing percent dissimilarity in fish assemblage between two sections. Column headings are as follows: Species= species scientific name, AvAbund= average abundance, AvDis= average dissimilarity, Dis/SD= dissimilarity/standard deviation, Contrib%= contribution percentage, and Cum% = cumulative contribution percentage.

<table>
<thead>
<tr>
<th>Pair</th>
<th>AvgDis %</th>
<th>Species</th>
<th></th>
<th></th>
<th>AvAbund</th>
<th>AvAbund</th>
<th>AvDis</th>
<th>Dis/SD</th>
<th>Contrib %</th>
<th>Cum %</th>
</tr>
</thead>
<tbody>
<tr>
<td>West &amp; East</td>
<td>57.1</td>
<td>A. mitchilli</td>
<td>12.2</td>
<td>17.8</td>
<td>19.7</td>
<td>1.3</td>
<td>34.5</td>
<td>34.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M. beryllina</td>
<td>11.8</td>
<td>4.8</td>
<td>12.3</td>
<td>1.3</td>
<td>21.5</td>
<td>56.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. xanthurus</td>
<td>2.4</td>
<td>1.7</td>
<td>3.3</td>
<td>1.1</td>
<td>5.8</td>
<td>61.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>West &amp; Cen</td>
<td>55.0</td>
<td>A. mitchilli</td>
<td>12.2</td>
<td>18.4</td>
<td>19.4</td>
<td>1.2</td>
<td>35.2</td>
<td>35.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M. beryllina</td>
<td>11.8</td>
<td>8.5</td>
<td>11.9</td>
<td>1.2</td>
<td>21.6</td>
<td>56.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. xanthurus</td>
<td>2.4</td>
<td>3.3</td>
<td>4.9</td>
<td>0.9</td>
<td>8.8</td>
<td>65.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cen &amp; East</td>
<td>54.5</td>
<td>A. mitchilli</td>
<td>17.8</td>
<td>18.4</td>
<td>20.8</td>
<td>1.3</td>
<td>38.2</td>
<td>38.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M. beryllina</td>
<td>4.8</td>
<td>8.5</td>
<td>8.1</td>
<td>1.1</td>
<td>14.8</td>
<td>53.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. xanthurus</td>
<td>1.7</td>
<td>3.3</td>
<td>4.6</td>
<td>1.0</td>
<td>8.4</td>
<td>61.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.4. Summary of results from canonical correspondence analysis (CCA) used to examine the association between water quality variables and fish community structure in Pamlico River, NC. Fish and water quality data were collected bi-weekly from 18 fixed sampling sites from August to November 2009-2010. Values for CCA axes 1-4 are given.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Axis</th>
<th></th>
<th></th>
<th></th>
<th>Total inertia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalues</td>
<td></td>
<td>0.206</td>
<td>0.177</td>
<td>0.03</td>
<td>0.016</td>
</tr>
<tr>
<td>Species-environment correlations</td>
<td></td>
<td>0.637</td>
<td>0.583</td>
<td>0.443</td>
<td>0.381</td>
</tr>
<tr>
<td>Cumulative percentage variance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of species data</td>
<td></td>
<td>11</td>
<td>20.5</td>
<td>22.1</td>
<td>23</td>
</tr>
<tr>
<td>of species-environment relation:</td>
<td></td>
<td>45</td>
<td>83.5</td>
<td>90</td>
<td>93.6</td>
</tr>
<tr>
<td>Sum of all eigenvalues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of all canonical eigenvalues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.5. Five most abundant catch per unit effort (CPUE) species in
Pamlico River for NCDMF and this study (ECU). DMF sampled at two locations in Pamlico River, NC across from Bath, NC monthly from September to November in 2009-2010 using an 18m long bag seine. I sampled at 18 fixed stations in Pamlico River from Washington, NC to the mouth of Pamlico Sound bi-weekly using an 18m long bag seine. CPUE is the mean number of each species collected per seine haul.

<table>
<thead>
<tr>
<th>Species</th>
<th>NCDMF</th>
<th>ECU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anchoa mitchilli</td>
<td>2020.5</td>
<td>552.5</td>
</tr>
<tr>
<td>Menidia beryllina</td>
<td>341.0</td>
<td>110.7</td>
</tr>
<tr>
<td>Leioptomus xanthurus</td>
<td>133.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Brevoortia tyrannus</td>
<td>67.0</td>
<td>12.1</td>
</tr>
<tr>
<td>Anchoa hepsetus</td>
<td>36.0</td>
<td>-</td>
</tr>
<tr>
<td>Ctenophora sp.</td>
<td>-</td>
<td>7.4</td>
</tr>
</tbody>
</table>
APPENDIX A: ANIMAL USE PROTOCOL APPROVAL

East Carolina University.

April 13, 2011

Anthony Overton, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Overton:

Your Animal Use Protocol entitled, "Juvenile Fish Habitat Use in Pamlico Sound NC" (AUP #D256) was reviewed by this institution's Animal Care and Use Committee on 4/13/11. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

[Signature]

Scott E. Gordon, Ph.D.
Chairman, Animal Care and Use Committee

SEG/jd

enclosure