

ABSTRACT

ELLIS, TIMOTHY ALAN. Assessing Nursery Quality for Southern Flounder, *Paralichthys lethostigma*, through Fish Energy Content and Habitat Abiotic Conditions. (Under the direction of Dr. John M. Miller).

Estuarine nursery habitats are essential to the sustainability of many of the world's fisheries. Anthropogenic perturbations and watershed activities threaten the quality of these vital ecosystems, and the significance of nursery habitat loss has become so distressing that most fishery management strategies now place high priority on the protection and restoration of 'Essential Fish Habitat' (EFH, Magnuson-Stevens Act, NOAA 1996). The inability to accurately evaluate habitat quality and fully understand the factors limiting habitat-specific productivity preclude the success of any management efforts to improve fishery recruitment through the protection and restoration of estuarine nursery habitats. The Albemarle-Pamlico Estuarine System in North Carolina (NC) provides important nursery habitat for many ecologically and economically important species, including southern flounder, *Paralichthys lethostigma*. Abiotic factors, in particular temperature, dissolved oxygen (DO), and salinity, almost certainly affect growth and survival at the levels experienced in NC nurseries, but the relative importance of these factors in determining nursery habitat productivity for southern flounder has yet to be determined. Two separate indicators of nursery habitat quality were compared: fish energy content and habitat abiotic conditions. The hypothesis that fish energy content represents the cumulative effect of the abiotic environment on the fish was tested to see if these independent but potentially related indicators could be used in assessments of nursery habitat quality. When measured on appropriate temporal and spatial scales, total fish energy content provided a measurable response of young-of-the-year (YOY) southern flounder to short-term environmental variability, and the results of this study suggest a strong

potential for the utility of fish energy content in evaluations of nursery habitat quality for the species. Over a continuous 15 week sampling period from May to August 2006, YOY southern flounder energy content varied weekly in a single nursery habitat by a maximum of approximately 8%, and 51% of this weekly variability in energy content was explained by the weekly variability in temperature and DO measured in this habitat. Across multiple nursery habitats sampled biweekly from May to August 2005, the variability in YOY southern flounder energy content ranged from approximately 12 to 37%. The spatial variability in temperature, DO, and salinity measured in these different nursery habitats was often significant but minimal and did not unequivocally explain the measured variability in fish energy content. The results of this study suggest that fish energy content can provide an unbiased measure of the fish's perception of habitat quality and can be an alternative to other, more labor intensive and potentially biased studies that estimate juvenile growth through field enclosures or micro-otolith analyses. The conclusions of this study and the utility of fish energy content as an indicator of nursery habitat quality are likely applicable to other fish species whose juveniles utilize similar estuarine nursery habitats.

**Assessing Nursery Quality for Southern Flounder, *Paralichthys lethostigma*,
through Fish Energy Content and Habitat Abiotic Conditions**

by

Timothy A. Ellis

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the requirements for the
Degree of Master of Science

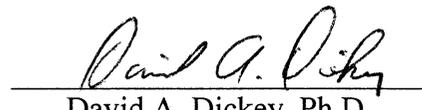
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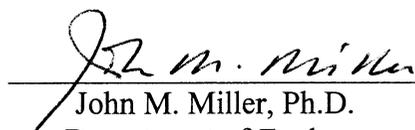
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BIOGRAPHY

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INTRODUCTION

In aquatic ecosystems, habitats that promote the growth and survival of fish in early life stages are generally considered nurseries (Wooton 1992; Gibson 1994). More recently, it has been suggested that these habitats be defined by their level of production (i.e. biomass contributed to the adult/fishable stock) where only highly productive juvenile habitats are considered 'nurseries' or 'effective juvenile habitats' (Beck *et al.* 2001; Dahlgren *et al.* 2006). Although these classification systems will certainly allow for more directed efforts to conserve and manage juvenile fish habitat given limited resources, production as it is commonly measured today may not be an adequate measure of habitat priority for such efforts. Considering that present guidelines for 'Essential Fish Habitat' (EFH, Magnuson-Stevens Act, NOAA 1996) primarily focus on utilization (Able 1999; Musick 1999), the simplest and most often used measure of nursery production by fisheries management agencies is an estimate of juvenile abundance. However, production in nurseries is limited by not only growth and survival but also by larval supply (i.e. initial colonization), immigration, and emigration (Miller *et al.* 1991; Wooton 1992; Gibson 1994; Neill *et al.* 1994). Consequently, abundance-based classifications of production may undervalue many nursery habitats, especially if colonization is limiting and/or movement rates are high (Guindon and Miller 1995). This sentiment of a need for better means of evaluating nursery habitat other than juvenile abundance has been expressed by many (Guindon and Miller 1995; Able 1999; Beck *et al.* 2001; Dahlgren *et al.* 2006). Alternative measures of production, and more importantly, the causes of production variability provide more accurate estimates of a particular habitat's need for conservation and management efforts and are more informative about what specific efforts are needed.

Survival through the larval, early, and late juvenile life stages undoubtedly plays a major role in habitat-specific productivity and ultimately year-class strength (Sissenwine 1984; Bailey 1994; Peterman *et al.* 1988; Bradford and Cabana 1997). Mortality rates during these stages are extremely high and often quite variable (Sissenwine 1984; Anderson 1988; Bradford and Cabana 1997; Sogard 1997). Size-dependent survival during the prerecruit period is driven by high rates of predation and overwinter mortality that generally have inverse relationships to body size (Tanaka *et al.* 1989; Sogard 1992; Van der Veer *et al.* 1994; Sogard 1997). As such, rapid growth is an advantageous early life-history characteristic for many species (Parker 1971; Post and Prankevicius 1987; Tanaka *et al.* 1989; Post and Evans 1989a; Post and Evans 1989b; Van der Veer *et al.* 1994; Sogard 1997; Searcy 2005). Because a rapid growth trajectory is dependent upon adequate energy accumulation for the entire growing season and subsequent allocation of all excess energy towards somatic growth, juvenile growth rates can be, and often are, highly variable (Sissenwine 1984; Weatherley 1990; Sogard 1997).

Growth is only one component of production, but its effects on nursery productivity are widespread, often determining rates of movement and survival. The 'quality' of nursery habitat generally refers to how preferable habitat conditions are to a measure of fish performance (e.g. growth) (Gibson 1994). Early growth in fish has been routinely used as an indicator of nursery habitat quality largely due to its sensitivity to environmental conditions and response to environmental change (Fry 1971; Weatherley 1990; Yamashita *et al.* 2001; Neill *et al.* 2004). The assumption is that higher quality habitat supports higher growth by providing favorable abiotic conditions, refuge from predation, and abundant prey (Sogard 1997). The complexity of growth however, is problematic and can hinder a researcher's

ability to understand the relationship between habitat quality and fish performance. In numerous field studies, high variability in growth rates among individuals from a single habitat made it difficult to detect significant growth differences across multiple habitats (Sogard 1992; Phelan *et al.* 2000; Duchon 2001; Meise *et al.* 2003; Ross 2003; Necaise *et al.* 2005) and led some researchers to conclude that there were no significant differences in quality among these different habitats (Necaise *et al.* 2005). Growth is also difficult to measure in the field. Estimates of actual growth of juvenile fish in a habitat have generally required the use of enclosures or back-calculation techniques via micro-otolith analyses. Both methods are costly and time-consuming, which consequently, are the main reasons why today, estimates of abundance remain the preferred method of habitat assessment by management agencies, despite the repeated concerns of fisheries biologist about their use for such purposes. Given the uncertainty that has been reported in numerous field studies about growth's reflection of habitat quality and the difficulty of measuring actual growth in the field, other options for estimating fish growth and evaluating habitat quality are worth exploring. The energy content of a fish is an indirect measure of growth due to its strong positive correlation with growth rate (Busacker *et al.* 1990; Weatherley 1990). A proximate analysis of the energy content of free-ranging fish can be quicker, easier, and generally cheaper to determine relative to actual growth. Therefore, fish energy content could potentially be a useful indicator of habitat quality.

Variability in juvenile growth is due in large part to habitat effects on energy accumulation and allocation during the early growth period (Weatherley 1990; Post and Parkinson 2001). Energy accumulation and subsequent allocation are dependent upon resource limitations including prey availability, prey type, and competition, and also upon

environmental control of and limitations on fish physiology and metabolism, mainly through temperature, dissolved oxygen (DO), and salinity (Fry 1971; Sissenwine 1984; Miller *et al.* 2000; Neill *et al.* 2004). A measure of variability in fish energy content and the potential sources of that variability across different nurseries could be informative about the processes likely and unlikely limiting growth in those habitats.

Estuaries are among the most physically dynamic and biologically productive ecosystems in the world. For many marine fish species, adequate and abundant estuarine nursery habitat is critical to the growth and survival of early life stages (Weinstein 1979; Hoss and Thayer 1993; Gibson 1994; Able and Fahay 1998; Able 1999). Able and Fahay (1998) suggest that approximately two-thirds of the economically important fish species in the eastern United States depend on estuarine nursery habitat, and in North Carolina, USA, more than 90% of commercial and 60% of sport fishery species rely on estuarine habitat for part, if not most, of their life history (Ross and Epperly 1985; Division of Marine Fisheries, NC, USA). Most temperate estuaries are characterized by seasonal variability in the environmental conditions that determine rates of fish growth, where warmer months are generally the most productive and also the most variable. Temporal and spatial variability in abiotic conditions during these warmer months, notably in temperature, DO, and salinity, are likely factors driving observed habitat-specific differences in juvenile fish growth in temperate estuarine nurseries (Miller *et al.* 1991; Miller *et al.* 2000; Sogard *et al.* 2001). Miller and colleagues (2000) simulated the growth of juvenile red drum, *Sciaenops ocellata*, in four different temperate estuaries, and their results suggest a strong potential effect of abiotic factors on fish growth. Their simulations compared growth rates under constant optimal abiotic conditions to that under actual conditions measured in each estuary, and they

found that summer growth in these estuaries was 40 to 135% of that which would occur under constant optimal abiotic conditions (Miller *et al.* 2000).

Southern flounder, *Paralichthys lethostigma* (Jordan and Gilbert 1884), are ecologically and economically important euryhaline flatfishes that inhabit the estuarine and coastal waters of the southeast United States and the northern Gulf of Mexico. Southern flounder have estuarine-dependent early life stages that utilize nursery habitats located in the upper estuary where salinities are generally lower (Powell and Schwartz 1977; Burke *et al.* 1991). In North Carolina, habitats in the upper regions of the Pamlico River and Neuse River estuaries have been designated (based on juvenile abundance) as primary nurseries for the species (Division of Marine Fisheries, NC, USA). High variability in the abiotic conditions of these nurseries during the summer months can create challenging environments for early growth and survival. As such, southern flounder and their North Carolina nurseries provide an excellent opportunity to study the relationship between fish and their environment, especially fish response to change in abiotic conditions, and to evaluate nursery habitat quality for the species.

Rising population has increased the demand for coastal resources and placed enormous stress on U.S. estuarine ecosystems (Hinrichsen 1998, Peterson 2003). Estuaries are currently threatened by watershed activities including agriculture, industry, forestry, and recreational and urban development. The degradation and loss of estuarine habitat resulting from these anthropogenic activities directly affects fishery production. Without abundant high quality estuarine nursery habitat, juvenile recruitment is limited (Hoss and Thayer 1993; Gibson 1994; Peterson 2003). Greater efforts must be made to protect and restore this rapidly diminishing natural resource. Although, the justifications for such efforts are

becoming increasingly more apparent, their success depends on the ability of fishery biologists and managers to accurately understand and efficiently evaluate habitat quality.

The approach taken in this study was to assess nursery habitat quality from the perspective of the fish, where there are no constraints on fish behavior, such as movement or feeding, beyond what are due to habitat and individual fish characteristics (i.e. no caging). A comparison between the fish's perspective and that of fish biologists, which is based largely upon factors suggested by experimental lab research to be important determinants of growth, should provide better insight as to what factors are *actually* important as well as potential factors that may be important to consider in future studies of habitat quality. In this study, the biologist's perception is based on the measured abiotic environment, specifically temperature, DO, and salinity, and the hypothesis is made that young-of-the-year (YOY) southern flounder energy content will reflect the variability observed in their nursery habitat's abiotic conditions. The objectives of this study were (1) to examine the response of YOY southern flounder energy content to recent change in abiotic conditions encountered in a typical temperate estuarine nursery and (2) to use fish energy content to assess nursery habitat quality for southern flounder across multiple nurseries.

METHODS

Study Species

In North Carolina, the northern limit of the species range, southern flounder are an economically important species and when combined with summer flounder, *Paralichthys dentatus*, are second only to blue crabs, *Callinectes sapidus*, in value of a commercial fishery (Division of Marine Fisheries, NC, USA). North Carolina adults spawn offshore from late

fall through early spring near the continental shelf (Warlen and Burke 1990; Fitzhugh 1993; Fitzhugh *et al.* 1996; Monaghan and Armstrong 2000). Onshore currents transport developing larvae across the shelf and into the Albemarle-Pamlico estuary (Miller *et al.* 1984; Pietrafesa and Janowitz 1988; Miller *et al.* 1991) where they actively select shallow, low salinity habitats to settle out as early juveniles (Powell and Schwartz 1977; Burke *et al.* 1991). Juveniles frequently occupy these primary nurseries beyond their first year of life, however seasonal shifts in habitat conditions in conjunction with a diet shift from benthic invertebrates to piscivory prompt most late juveniles to emigrate out of the relatively shallow creeks and bays by late summer and fall and occupy the deeper waters of the main river and sound bodies (Powell and Schwartz 1977; Fitzhugh 1993; Fitzhugh *et al.* 1996; Division of Marine Fisheries, NC, USA).

Southern flounder in NC are overfished according to the State's current stock assessment (NCDMF 2005). Age-1 fish are largely recruited to the fishery and comprise roughly half of total annual landings, yet only 59% of this age class on average is mature (Safrit and Schwartz 1998; Monaghan and Armstrong 2000; NCDMF 2005). This dependence upon incoming recruits, half of which are pre-spawned, combined with high fishing mortality rates have reportedly reduced the spawning stock biomass to an estimated 5% of that of an unfished stock (NCDMF 2005).

Study Sites

The Pamlico River Estuary (PRE) and the Neuse River Estuary (NRE), located in North Carolina, USA, provide nursery habitat critical for the growth, survival, and successful recruitment of many juvenile fish species (Fig. 1). These low-salinity, muddy-bottom

nurseries are the preferred habitat types for southern flounder and are therefore ideal areas to capture and study this species (Powell and Schwartz 1977; Burke *et al.* 1991).

Watershed activities heavily impact the water quality of both estuaries. Anthropogenic nutrient loading causes eutrophication which has increased the frequency and severity of hypoxia ($DO < 2.0 \text{ mg l}^{-1}$) within these systems in recent years (Stanley 1993; Paerl *et al.* 1998). During the late summer and early fall, thermohaline stratification creates deep hypoxic water within both estuaries (Stanley and Nixon 1992; Paerl *et al.* 1998; Buzzelli *et al.* 2002). Wind-driven upwelling moves large volumes of this deep hypoxic water into the shallow near-shore habitats along each of the main estuary bodies (Reynolds-Fleming and Luettich 2004). Nursery habitats located within the bounds of the shallow creeks of these estuaries, however, may be less affected by these episodic upwelling events due in part to their spatial isolation from the flow of the upwelling event (e.g. their location is opposite of the wind direction) and/or protection from a large influx of water due to a narrow or indirect link between the mouth of the creek and the main river body. Hypoxia more frequently occurs in these shallow estuarine creeks as a result of a diurnal cycle of microbial respiration and photosynthesis (Stanley and Nixon 1992). Oxygen supply is high during the day when photosynthesis increases primary production and becomes limited in the evening when respiration exceeds primary production. Often poor circulation and high turbidity within these creeks can result in prolonged hypoxia of the benthic habitat that can last on the order of hours to days.

Part of this study was conducted in Hancock Creek which is located on the south side of the NRE (Fig. 1). The other portion of the study was conducted in four creeks of the PRE. Back Creek (BC) and East Fork Creek (EF) are located on the north side of the PRE and

Long Creek (LC) and Porter Creek (PC) are located on the south side of the PRE (Fig. 1). These four nursery habitats were selected based on previous work that showed differences in growth of YOY southern flounder among these habitats (Guindon and Miller 1995). Preliminary data collected by the author in the early fall of 2004 also suggested high variability in abiotic conditions (especially DO) among these habitats.

Abiotic Environment

Field Sampling. In order to examine the relationship between the abiotic environment and fish energy content, the environmental history of the fish prior to capture had to be documented. Water quality sondes (Yellow Springs Instruments, YSI, Model 6000) were placed in the nursery habitats to record temperature, dissolved oxygen, and salinity every 30 minutes for the week prior to fish capture. All sondes were placed in depths of 1-1.5 m to target depths heavily populated in the summer months by YOY southern flounder. The sonde recorded the environment of the capture site, and the data were assumed to represent the abiotic conditions experienced by all captured fishes. Sondes were anchored and suspended in the water column approximately 30-38 cm from the substrate. Southern flounder exhibit little vertical movement and are adapted to life on the bottom, so measuring the quality of the bottom water is most relevant to this study. Due to rapid fouling of the sensors, all sondes were cleaned and properly calibrated according to YSI protocol before each weekly deployment to ensure the most accurate measurements possible.

Analyses. The means and range of temperature, DO, and salinity as well as the frequency (number of events) and severity (duration of an event in hours) of hypoxia were summarized for each nursery habitat. For the portion of the study conducted in Hancock Creek in 2006, these abiotic parameters were used as explanatory variables in the analyses of

total fish energy content. For the nursery habitat comparison portion of the study conducted in 2005, differences in mean temperature, mean DO, and mean salinity across habitats were examined using an analysis of variance (ANOVA). Since these data are temporally autocorrelated (i.e. measured every 30 minutes), they violate the ANOVA assumption of independence. Therefore, a repeated measures design was used that incorporated a first-order autoregressive process. This design models the correlation between the repeated abiotic measurements and allows for the error term at a given time (t) to be partially predicted by the preceding error at time (t-1). To examine differences in the severity of hypoxia across habitats, a normal one-way ANOVA was used. Cochran's test for heterogeneity of variances was used to test the assumption that variances in mean length of hypoxia were the same across all sites (Underwood 1997). All statistical tests were performed at an $\alpha = 0.05$ significance level using SAS version 9.1.3 (SAS 2004).

Fish Energy Content

Field Sampling. Free-ranging YOY southern flounder were collected weekly from late May to late August from the four creeks in the PRE in 2005 and from Hancock Creek in the NRE in 2006. All sampling was done using a 1-m beam trawl. Dutch designed beam trawls have been shown to be more efficient at capturing juvenile flatfish than other more conventional gears such as otter trawls (Kuipers *et al.* 1992). In the 2005 portion of the study, only comparable sample sizes among sites were of interest and therefore no effort was made to standardize tow durations and estimate catch-per-unit-effort (CPUE). However, in the 2006 portion of the study conducted in Hancock Creek, five 1-minute tows covering a distance of approximately 20 m each were made to obtain a sufficient sample size and estimate CPUE. If five tows provided an insufficient number of samples, then more tows

were made but not included in the measurements of CPUE. All samples from each creek were collected in the area immediately surrounding the YSI-6000 water quality sonde (less than 75 m away), however, low abundances in Back Creek in 2005 made it necessary to use fish caught away from the sonde placement (100 to 300 m away) to achieve sufficient sample sizes. Samples from 2005 were immediately placed on ice and subsequently frozen. Samples from 2006 were placed on ice and soon processed in the laboratory without freezing.

Laboratory Procedure. Samples collected in the field from 2005 were thawed and processed in the laboratory 3-5 months after capture. Samples from 2006 were processed within 12 h of capture. First, excess water was blotted from the exterior of the thawed fish and the total length (TL) and the wet weight (nearest 0.001 g) were determined. Wet weights were not obtained prior to freezing for a majority of the 2005 samples and the freezing process appeared to lower the post-freezing wet weight measurements by approximately 2-10%. Thus, wet weights for 2005 samples were not used in the analyses presented in the appendix. The otoliths were then extracted, allowed to air dry for 48 h and then stored dry in air-tight vials for future analysis. Next, the stomach contents were removed, weighed, and stored in 95% ethanol for later identification. The wet weight of the fish was corrected for the weight of the stomach contents (WETWEIGHT). The whole fish was then held in a drying oven set at 60°C until a stable weight was reached (at least 48 h). The desiccated sample was then weighed (DRYWEIGHT) and broken down to a semi-homogenized sample using dissection scissors and a mortar and pestle. The weight of a pre-dried alundum extraction thimble was obtained (THIMBLEWEIGHT) and the semi-homogenized sample was placed inside of it. The weight of the thimble and the semi-homogenized sample

together was obtained (THIMBLEDRY) and used to correct for sample lost due to the homogenization process and transfer to the thimble (LOSTSAMPLE). Next, neutral lipids were extracted from the sample using a Soxhlet apparatus and petroleum ether (see Schultz and Conover 1997). In the 4 h extraction process, fresh non-polar solvent constantly drips on the sample allowing non-polar lipids to dissolve into the solvent and drain out of the extraction thimble. Once the extraction process was complete, the sample was dried again for 24 h at 60°C and then weighed (THIMBLELEAN). Finally, the lipid-free sample was placed in a muffle furnace at 450°C for 24 h. The ashed sample, once cooled to 60°C, was then weighed (THIMBLEASH). The variables explained above were used in the following mass difference equations to analyze the total energy content of the fish.

$$\% \text{ Dry Weight} = (\text{DRYWEIGHT} / \text{WETWEIGHT}) * 100$$

$$\text{LOSTSAMPLE} = \text{DRYWEIGHT} - (\text{THIMBLEDRY} - \text{THIMBLEWEIGHT})$$

$$\text{UNCORRECTEDLIPID} = \text{THIMBLEDRY} - \text{THIMBLELEAN}$$

$$\text{UNCORRECTEDLEAN} = \text{THIMBLELEAN} - \text{THIMBLEWEIGHT}$$

$$\text{UNCORRECTEDASH} = \text{THIMBLEASH} - \text{THIMBLEWEIGHT}$$

$$\text{UNCORRECTEDPROTEIN} = \text{UNCORRECTEDLEAN} - \text{UNCORRECTEDASH}$$

$$\% \text{LIPID} = \text{UNCORRECTEDLIPID} / (\text{DRYWEIGHT} - \text{LOSTSAMPLE}) * 100$$

$$\text{Corrected Total Lipid (g)} = (\% \text{LIPID} / 100) \times \text{DRYWEIGHT}$$

$$\% \text{PROTEIN} = \text{UNCORRECTEDPROTEIN} / (\text{DRYWEIGHT} - \text{LOSTSAMPLE}) * 100$$

$$\text{Corrected Total Protein (g)} = (\% \text{PROTEIN} / 100) \times \text{DRYWEIGHT}$$

$$\% \text{ASH} = \text{UNCORRECTEDASH} / (\text{DRYWEIGHT} - \text{LOSTSAMPLE}) * 100$$

$$\text{Corrected Total Ash (g)} = (\% \text{ASH} / 100) \times \text{DRYWEIGHT}$$

The calculation of total protein is based on the assumption that protein is the primary component of lean tissue. Petroleum ether does not dissolve phospholipids, which may compose a minor portion of the lean tissue, or carbohydrates, also considered a minor component of fish tissue (Love 1980). The total energy for the whole fish in kilojoules (kJ) was calculated using the converted caloric values of lipid and protein presented in Brett and Groves (1979). Using the standard conversion of 1 kcal = 4.184 kJ, the lipid energetic value of 9.45 kcal g⁻¹ was converted to 39.54 kJ g⁻¹, and the protein energetic value of 4.80 kcal g⁻¹ was converted to 20.08 kJ g⁻¹ (Brett and Groves 1979).

$$\text{Total Energy (kJ)} = (39.54 * \text{Total Lipid}) + (20.08 * \text{Total Protein})$$

Analyses. To determine if significant differences in total energy content existed in YOY southern flounder caught in Hancock Creek over the summer of 2006, an analysis of covariance (ANCOVA) was used. Fish energy content varies as a function of length (Appendix, Fig. A9). Using ‘Total Length’ (TL) as an independent continuous variable and ‘Week’ as an independent categorical variable, ANCOVA determined the least-squares mean total energy content for all fish in a given week adjusted to a common length. Fish lengths and energy values were ln-transformed to linearize the data prior to use in the ANCOVA model. If the ANCOVA results suggested a significant difference existed among weeks then pairwise comparisons were conducted to determine the significance of differences between adjacent weeks. The resulting significance levels were adjusted by the Tukey-Kramer method of honest significant difference (HSD).

To determine if significant differences in YOY flounder total energy content existed among four different nursery sites at a given time during the early growth season in 2005, ANCOVA was used in the manner just described, using ‘Creek’ instead of ‘Week’ as the independent categorical variable. If the ANCOVA results suggested a significant difference existed between creeks, then pairwise comparisons were conducted to determine which creeks were significantly different at a given time period. The resulting significance levels were also adjusted by the Tukey-Kramer method of HSD. All statistical tests were performed at an $\alpha = 0.05$ significance level using SAS version 9.1.3 (SAS 2004).

Diet

Laboratory Procedure. The diets of YOY southern flounder collected from the four different nursery habitats in 2005 were determined by analyzing the previously weighed and preserved stomach contents under a dissecting microscope. Prey items were identified to the nearest taxonomic group. After analysis, samples were placed back in vials containing 95% ethanol for further preservation. Gut fullness values for YOY southern flounder collected in Hancock Creek in 2006 and the four different nursery habitats in 2005 were also determined.

Analyses. Qualitative comparisons were conducted to determine if differences in diet were apparent among fishes from the four different nursery habitats during the six summer sampling periods of 2005. The frequency of occurrence for each prey category was determined as follows:

$$\% O_i = \frac{100 * O_i}{\sum_{i=1}^n O_i}$$

where O_i is the number of stomachs containing prey of category i , and n is the total number of prey categories for all stomachs (Bowen 1996; Liao *et al.* 2001).

Qualitative comparisons of gut fullness values were also conducted to determine if relationships existed between apparent feeding rate and total fish energy content for YOY southern flounder collected in Hancock Creek during the summer of 2006 and in the four different nursery habitats studied in 2005. Gut fullness for individual fish was determined as follows:

$$\text{Gut fullness} = \frac{\text{weight of stomach contents}}{\text{weight of fish without stomach contents}}$$

Prey Availability

Field Sampling. YOY southern flounder mainly consume mysids during their early growth period (Appendix Table A13, Fig. A14; Fitzhugh 1993). To examine habitat differences in prey availability, mysid densities were quantified for each nursery habitat twice during the 2005 experimental season, once in early June and again in late July. Mysids and other potential prey items were sampled using an epibenthic sled with a 0.19 m² opening and fitted with a plankton net of 100- μ m mesh with a cod end. The epibenthic sled is designed to sample available prey present on the surface of the sediment and in the water column extending roughly 25 cm above the bottom (see Kamermans *et al.* 1995). Five one-minute tows were made in each site, covering a distance of approximately 20 m each. Multiple tows were made in order to cover the entire fish capture site and to obtain an average representative of the entire site. All samples were immediately preserved in 95% ethanol for later analysis in the laboratory.

Laboratory Procedure. Prey samples obtained from the field were first drained of their ethanol using a 100- μ m sieve and rinsed with water. Most samples were counted and identified without the need for subsampling, however, the high density of mysids in some

samples made subsampling necessary. In samples not subsampled, all prey items were counted and identified using a dissecting microscope. The subsampling process was begun by first counting and identifying all large prey items. Next, the remaining sample was placed in 500 ml of water and stirred to uniformly mix the sample throughout the water. Three 50 ml samples were then taken, and prey items in each were counted and identified under a dissecting microscope. The average of the three subsamples was taken and used to estimate the total number of each prey item in the entire sample.

Analyses. To determine if significant differences in mysid densities existed between nursery habitats at a given time, a one-way ANOVA was conducted. Cochran's test for heterogeneity of variances was used to test the assumption that variances in mysid density were the same across sites (Underwood 1997). If the ANOVA results suggested a significant difference existed between sites, then pairwise comparisons were conducted to determine which sites were different. The resulting significance levels were adjusted by the Tukey-Kramer method of HSD. All statistical tests were performed at an $\alpha = 0.05$ significance level using SAS version 9.1.3 (SAS 2004).

RESULTS

Fish Energetic Response to Changing Abiotic Environment

Fish Sampling. Weekly collections of YOY southern flounder were made from Hancock Creek in 2006 from May 25 to August 30. The mean length, range, and number of samples collected for each week are presented in Table 1. Mean fish length increased approximately 30 mm over the 15 week summer sampling period, and YOY sizes differed in the range of 25-59 mm during each week. Weekly means in the total lengths of captured fish

gave apparent growth rates that ranged weekly from 0 to 1.417 mm d⁻¹ with an overall mean apparent growth rate of 0.43 mm d⁻¹ for the 15 week sampling period. CPUE remained steady at approximately 4-5 fish tow⁻¹ (0.2-0.25 fish m⁻²) for much of the summer sampling period (Fig. 2). Peak catches were in mid-June at approximately 7 fish tow⁻¹ (0.35 fish m⁻²), and CPUE decreased steadily after August 1.

Abiotic Environment. Temperature, DO, and salinity in Hancock Creek were continuously monitored every 30 minutes for each week during the 2006 summer sampling period from May 25 to August 30. The means, range, and sample sizes for these abiotic parameters during each week were calculated (Table 2), along with the frequency and severity of hypoxia for each week (Table 3). Mean temperature rose in Hancock Creek from 24°C in late May to 31°C by early August. Mean salinity fluctuated between 6.7 and 14 ppt during the summer sampling period with the lowest salinities occurring during the first week of July and the highest during the second week in August. Mean dissolved oxygen remained constant at 5-6 mg l⁻¹ from late May to late July and then declined to 3-4 mg l⁻¹ for the month of August. The frequency and severity of hypoxia were greatest in Hancock Creek during the month of August (Table 3). Fish in Hancock Creek experienced the worst abiotic conditions in the first and second weeks of August due to the combination of low levels of DO and high temperatures.

Fish Energy Content. To determine if fish energetic condition varied over the 2006 summer sampling period, energy content was determined for all fish collected during each weekly sampling event. ANCOVA allowed for the comparison of least-squares means of total fish energy content adjusted to a common length across time (Table 4). The test for homogeneity of slopes found no significant interaction between 'Week' and 'TL' (p =

0.891). The results of this simple ANCOVA are presented in Table 5. ‘Week’ had a significant effect on fish energy content (ANCOVA, $p < 0.0001$) and explained approximately 51% of the variability seen in fish energy content. Pairwise comparisons of all adjacent weeks were conducted, and resulting significance levels were adjusted using the method of Tukey-Kramer HSD. Weeks where fish had significantly different energy content as compared to fish from the previous week included the weeks of July 6, July 26, August 15, and August 23 (Fig. 3).

An apparent relationship between fish energy content and the abiotic environment experienced by those fish in the week prior to capture existed for this nursery habitat over the summer sampling period (Fig. 4). To test if the measured abiotic parameters had a significant effect on fish energy content and also if the ‘Week’ effect was still significant after accounting for variability in the abiotic environment during each week, a more complex ANCOVA model was developed. The mean temperature, mean DO, mean salinity, number of hypoxic events (#Events), and mean length of hypoxia (MeanEvent) for each week were entered as independent continuous variables and ‘Week’ as an independent categorical variable. The results of this ANCOVA are presented in Table 5. Mean temperature, mean salinity, and the mean length of hypoxia were not significant (ANCOVA, $p = 0.8832, 0.6138, 0.1016$, respectively) and were removed from the model. Mean DO and the interaction of mean DO and mean temperature explained approximately 27 and 21% of the variability in fish energy content, respectively. The number of hypoxic events explained only 1.1% of the variability in fish energy content. The interaction between the number of hypoxic events and the mean length of hypoxia explained only 2.1% of the variability in fish energy content. The effect of ‘Week’ was still significant (ANCOVA, $p = 0.0081$) after accounting for

variability in the abiotic environment and explained an additional 3.9% of the variability in fish energy content.

Although no apparent relationship existed, additional testing was done to determine if the abiotic environmental history of fish two weeks prior to capture had any effect on fish energy content. One-week lags of mean temperature, mean DO, and mean salinity were included in the ANCOVA model as continuous independent variables. Lags of all three abiotic parameters were insignificant (ANCOVA, $p = 0.0703, 0.6696, 0.0641$, respectively) and explained no additional variability in energy content beyond what was explained by the abiotic parameters of the week prior to capture.

Gut Fullness. No apparent relationship existed between the mean gut fullness of YOY southern flounder collected in Hancock Creek during the summer of 2006 and the ANCOVA determined least-squares means of total fish energy content across this time period (Fig. 5) or the abiotic conditions measured over that same time period (Fig. 6). Mean gut fullness was included as a continuous independent variable in the complex ANCOVA model previously described and was found to be insignificant (ANCOVA, $p = 0.8320$).

Nursery Habitat Comparison

Fish Sampling. YOY southern flounder were collected during six sampling events from four different nursery habitats throughout the summer of 2005: late May, early June, late June, early July, late July, and early August. The mean length, range, and number of samples collected during each habitat's sampling events are presented in Table 6. Long Creek was not sampled in late July, and Back Creek was not sampled in early August. No efforts were made to calculate CPUE in these nursery habitats, but southern flounder abundances in East Fork Creek and Porter Creek were much higher than in Back Creek and

Long Creek and correspond to the generally greater number of samples collected in East Fork and Porter creeks.

Abiotic Environments. Temperature, DO, and salinity were measured every 30 minutes in each habitat for the week prior to fish capture. The means, range, and sample sizes for these abiotic parameters in each habitat and for each sampling event were calculated (Table 7), along with the frequency and severity of hypoxia (Table 8). No abiotic data were collected for any habitat during the late May sampling event. Due to probe failures, abiotic data for some creeks were not collected during some sampling events. DO data are missing for East Fork Creek in early June. No abiotic data were collected in Porter Creek during late June or in Long Creek during early August.

The data presented in Table 7 describe the general trends in mean temperature, mean DO, and mean salinity for each nursery habitat throughout the summer sampling season. Back and East Fork creeks were generally warmer than Long and Porter creeks. Maximum water temperature ranged from approximately 23°C in early June to approximately 31°C in early August. East Fork and Long creeks generally had higher salinities than Back and Porter creeks. Salinities across all habitats ranged from upper-oligohaline to lower-mesohaline levels throughout the summer. Mean DO showed no general trends across habitats over time with the exception of East Fork Creek, where mean DO was always high. Back and Porter creeks had the highest variability in DO. East Fork Creek only went hypoxic in early July; however, Back, Long, and Porter creeks went hypoxic and/or anoxic (no DO) during every summer sampling event except for early August.

Beyond a descriptive and qualitative analysis of the abiotic environments of these nursery habitats, a repeated measures ANOVA with autoregressive capability was used to

test for significant differences in these abiotic variables across habitats for a given sampling event. Although the complex repeated measures ANOVA model is designed to account for temporal autocorrelation, some of the time-series data could not be analyzed through meaningful statistical tests because these tests failed to remove the bias associated with the correlation of adjacent measurements (i.e. the autocorrelation of the model was close to 1). The results of these ANOVA are presented in Table 9. For tests with significant results, pairwise comparisons among habitats were made, and the resulting significance levels were adjusted according to the Tukey-Kramer method of HSD.

Early June. In early June, the mean DO level of 2.7 mg l⁻¹ in Back Creek was significantly lower than that of Long Creek (4.9 mg l⁻¹) and Porter Creek (4.1 mg l⁻¹) (ANOVA, p = 0.0001). East Fork and Long creeks had significantly higher mean salinity (6.5 and 6.1 ppt, respectively) than Back and Porter creeks (3.6 and 3.9 ppt, respectively) (ANOVA, p < 0.0001). The repeated measures ANOVA model did not converge (i.e. test was not meaningful) for significant mean temperature differences among creeks. The mean temperatures for Back, East Fork, Long, and Porter creeks were 25.5, 23.2, 23.1, and 24.7°C, respectively.

Late June. In late June, the mean temperature of 26.6°C in Long Creek was significantly lower than that of Back Creek (27.5°C) and East Fork Creek (27.3°C) (ANOVA, p = 0.0011). The repeated measures ANOVA model did not converge for significant mean DO and mean salinity differences among creeks. The mean DO levels for Back, East Fork, and Long creeks were 4.1, 5.2, and 4.0 mg l⁻¹, respectively. The mean salinities for Back, East Fork, and Long creeks were 5.0, 7.7, and 6.6 ppt, respectively.

Early July. In early July, there were no significant differences in mean temperature across habitats (ANOVA, $p = 0.4685$). The mean temperatures for Back, East Fork, Long, and Porter creeks were 30.1, 30.9, 27.5, and 28.7°C, respectively. There were also no significant differences in mean DO across habitats (ANOVA, $p = 0.8084$). The mean DO levels for Back, East Fork, Long, and Porter creeks were 3.3, 5.2, 3.5, and 2.3 mg l⁻¹, respectively. Mean salinity was significantly different across habitats (ANOVA, $p < 0.0001$) where Back and Porter creeks (4.0 and 4.4 ppt, respectively) were lower than East Fork and Long creeks (6.5 and 6.3 ppt, respectively).

Late July. In late July, mean salinity was significantly different among Back (3.1 ppt), East Fork (5.7 ppt), and Porter (4.8 ppt) creeks (ANOVA, $p < 0.0001$). The repeated measures ANOVA model did not converge for significant mean temperature and mean DO differences among creeks. The mean temperatures for Back, East Fork, and Long creeks were 30.6, 30.7, and 28.7°C, respectively. The mean DO levels for Back, East Fork, and Long creeks were 5.7, 7.1, and 3.4 mg l⁻¹, respectively.

Early August. In early August, there was no significant difference in mean DO between East Fork (6.2 mg l⁻¹) and Porter (8.6 mg l⁻¹) creeks (ANOVA, $p = 0.1104$). The repeated measures ANOVA model did not converge for significant mean temperature and mean salinity differences between the two creeks. The mean temperatures for East Fork and Porter creeks were 30.6 and 30.0°C, respectively. The mean salinities for East Fork and Porter creeks were 7.3 and 6.3 ppt, respectively.

Hypoxia. The frequency and severity of hypoxia for each habitat in each time period is summarized in Table 8. Back and Porter creeks generally had more frequent and more severe hypoxic events. Back Creek remained hypoxic for 21 hours in early June and for 47

hours in early July while Porter Creek was hypoxic for 31 hours in late July. ANOVA was used to test for significant differences in the mean length of hypoxia among habitats with more than one hypoxic event, and the results from these tests are presented in Table 10. Cochran's test for heterogeneity of variances was insignificant for late June and late July time periods ($p = 0.1113$ and 0.1427 , respectively). Variances were heterogeneous for early June and early July time periods (Cochran's test, $p = 0.0068$ and <0.0001 , respectively). Log-transformations of the data for these time periods successfully met ANOVA model assumptions (Underwood, 1997). No significant differences were found for any time period except early June when the mean length of hypoxia was greater in Back Creek (5.4 h) than in Porter Creek (1.6 h) (ANOVA, $p = 0.0431$).

Fish Energy Content. To determine if YOY southern flounder energetic condition varied across nursery habitats at different times throughout the summer early growth period, energy content was determined for all fish collected from each habitat during each sampling event. ANCOVA determined the least-squares mean total energy content for all fish in a given creek and given time period adjusted to a common length (Table 11). The results of these ANCOVA are presented in Table 12. The test for homogeneity of slopes found no significant interaction between 'Creek' and 'TL' for late May, early July, late July, and early August sampling periods ($p > 0.05$). Slopes were found to be heterogeneous for early and late June sampling periods ($p < 0.0001$). In early June, Porter Creek had a significantly different slope from those of the other three creeks. The separate-slopes ANCOVA model for these data incorporated one slope for Porter Creek and one common slope for the other three creeks. In late June, East Fork Creek and Porter Creek had similar slopes, Back Creek had a significantly different slope from the other three creeks, and Long Creek had a

significantly different slope for the other three creeks. The separate-slopes ANCOVA model for these data incorporated separate slopes for Back and Long Creeks and a common slope for East Fork and Porter Creeks. 'Creek' had a significant effect on fish energy content in all sampling periods except early May and explained approximately 22 to 46% of the variability seen in fish energy content for those sampling periods where it had a significant effect.

Pairwise comparisons among all creeks were conducted for each sampling period with significant differences among creeks (Fig. 7 b-f). The resulting significance levels were adjusted using the method of Tukey-Kramer HSD. Because of the use of separate slopes ANCOVA models for early and late June sampling periods, the effect of 'Creek' on total fish energy content is dependent upon the 'TL' at which the comparison is made. In other words, comparing mean energy content across creeks at one common length is meaningless. Therefore, least-squares mean fish energy content for each creek were compared at the 25th, 50th, and 75th percentiles of total length (Fig. 7 b,c). In early June, fish energy content was significantly lower in Porter Creek than the other three creeks for smaller fish (approximately 68 mm, 25th percentile). For fish in the 50th percentile (approximately 75 mm), Porter Creek was only significantly different from Back Creek, and for fish in the 75th percentile (approximately 81 mm) there were no significant differences in fish energy content among creeks. In late June, Back Creek fish had significantly lower energy content than fish from the other three creeks for both the 25th and 50th percentile fish (approximately 74 and 83 mm, respectively). There were no significant differences among creeks for large fish (approximately 92 mm, 75th percentile). In early July, fish in Long and Porter Creeks had significantly lower energy content than fish in Back and East Fork Creeks (Fig. 7d). Porter Creek fish had significantly lower energy content than fish in Back and East Fork Creeks in

late July (Fig. 7e) and significantly lower energy content than fish in East Fork and Long Creeks in early August (Fig. 7f).

Habitat Differences in Diet. A stomach content analysis was performed on all fish to determine if diet differences were apparent across nursery habitats. Seven prey categories were found in the 354 flounder stomachs that were analyzed (Table 13). Crab zoeae were only found in two stomachs from Porter Creek, and gastropods were only found in one stomach from East Fork Creek. Mysids were the most frequently occurring prey item in the stomachs of flounder across all nurseries (Fig. 8, Table 13). At least 50% of all full stomachs contained mysids only and approximately 70-95% contained both mysids and other food categories. Next to mysids, amphipods and fish were the most frequently observed prey items in flounder stomachs across all nurseries. There were no apparent differences in the onset of piscivory across nursery habitats (Fig. 9). Beginning at 80-90 mm in total length, flounders were consuming fish prey in every nursery habitat with the exception of East Fork Creek, where only one out of the 83 flounder (approximately 1%) analyzed in this creek that were smaller than 80 mm had the remains of fish in its stomach. Mean gut fullness values were determined for YOY southern flounder collected in each nursery site during each sampling event (Fig. 10 a-f). Apparent differences in mean gut fullness were found across nursery sites for late May, early July, late July, and early August sampling periods. In late May, flounder in Back and Porter creeks had higher gut fullness than flounder in East Fork and Long creeks. In early July, flounder in Back Creek had apparently higher gut fullness than flounder in East Fork, Long, and Porter creeks. In late July, flounder in Porter Creek appeared to have higher gut fullness than flounder in Back and East Fork creeks. In early

August, flounder in Porter Creek appeared to have higher gut fullness than flounder in East Fork and Long creeks.

Habitat Differences in Prey Availability. Mysid densities within each nursery site were estimated in early June and late July. No data were collected for Back Creek in late July. A one-way ANOVA model was constructed for each time period where mean 'Mysid Density' was dependent upon 'Creek' (Table 14). Cochran's test for heterogeneity of variances was insignificant for both time periods ($p = 0.1215$ and 0.0981 , respectively). Significant differences in mysid densities across nursery habitats were found for both sampling periods (ANOVA, $p = 0.0101$ and 0.0005 , respectively). Pairwise comparisons found lower mysid densities in early June in East Fork Creek ($24.9 \text{ mysids m}^{-2}$) as compared to Back and Porter Creeks (116.3 and $104.7 \text{ mysids m}^{-2}$, respectively) and higher mysid densities in late July in Porter Creek ($750.9 \text{ mysids m}^{-2}$) as compared East Fork and Long Creeks (33.9 and $142.8 \text{ mysids m}^{-2}$, respectively) (Fig. 11). Mysid densities increased between months in Porter Creek but remained fairly constant in East Fork and Long Creeks (Fig. 11).

DISCUSSION

Fish Energetic Response to Changing Abiotic Environment

The first objective of this study was to examine the response of YOY southern flounder energy content to recent change in abiotic conditions encountered in a typical temperate estuarine nursery. Hancock Creek had relatively high abundances of YOY southern flounder throughout most of the summer of 2006 and provided important nursery habitat for the species during that time period. The apparent mean growth rate determined

from changes in the mean total length of fish captured each week in Hancock Creek was 0.43 mm d^{-1} . Although the weekly movement of individuals into and out of the capture site can affect the accuracy of this estimate of mean growth rate, YOY southern flounder in Hancock creek during the summer of 2005 were apparently growing at similar rates presented in other field studies. Guindon and Miller (1995) reported a mean growth rate of 0.44 mm d^{-1} for YOY southern flounder caged in NC nurseries. Fitzhugh and colleagues (1996) reported otolith-based growth rates of juvenile southern flounder in NC ranging from 0.35 to 1.5 mm d^{-1} with a mean growth rate of 0.65 mm d^{-1} .

By sampling this nursery habitat on a weekly time scale, significant fine-scale temporal differences in fish energy content were detected and approximately 51% of that variability was explained simply by the timing of each sampling event. These results suggest not only that juvenile fish growth in a single nursery habitat can be highly variable over short time scales but also that these fish were responding to rapid and abrupt changes in their environment. In temperate estuaries, this fine-scale temporal variability in the environment more often corresponds to changing abiotic conditions such as temperature, DO, and salinity as opposed to changing biotic conditions such as food availability (Miller *et al.* 1991). Although it is often difficult in ecological studies to discern the temporal and/or spatial scale in which to measure an organism's response to change in its environment, the measured response often varies on the same scale as the main factors influencing that response (Levin 1992). Therefore, the hypothesis was made that short-term variability in fish energy content should reflect the short-term variability in abiotic conditions of the nursery habitat. When the weekly abiotic variability was included in our ANCOVA model, 'mean DO', 'mean DO*mean temperature', 'the number of hypoxic events', and 'the number of hypoxic

events*mean length of hypoxia', explained approximately 51% of the variability observed in fish energy content. Given those significant abiotic parameters are in the model, all other possible abiotic and biotic factors occurring in Hancock Creek during a given week only explained an additional 3.9% of the variability in fish energy content. These results support the research hypothesis and suggest that weekly variability in the abiotic conditions of Hancock Creek were largely responsible for the variability in fish energy content observed over those weeks.

Temperature. Given that the dominant abiotic factor controlling growth in fish is water temperature (Fry 1971; Brett and Groves 1979; Gibson 1994; Neill *et al.* 1994), it may be surprising that the results from this portion of the field study showed no significant effect of mean temperature on fish energy content. Relative to temperature, the larger weekly variability in salinity and DO in Hancock Creek and the larger response by fish energy content to the weekly variability of those two abiotic parameters masked any overall effect of temperature (Fig. 4). However, fish energy content did increase steadily over the summer early growth period, and given no effects of DO or salinity, the maximum values for energy content were observed on August 1st and 23rd when temperatures were approximately 30°C, near optimum for growth of juvenile southern flounder (Peters 1971; Guindon and Miller 1995; Del Toro-Silva in prep.) and suggest that growth rates were higher at warmer temperatures. These results do not conflict with other field studies that found temperature to be responsible for observed temporal and spatial variability in fish growth (Guindon and Miller 1995; Rooker *et al.* 1997; Baltz *et al.* 1998; Meng *et al.* 2000; Manderson *et al.* 2002; Meise *et al.* 2003), rather they highlight the abiotic complexity of estuaries and the difficulty

in detecting statistically significant effects among such complex abiotic processes and interactions.

Dissolved Oxygen. DO acts as a limiting factor on fish growth by restricting active metabolism at lower concentrations (Fry 1971). The physiological and metabolic responses of fish to low DO are well reviewed (Hughes 1973; Heath 1995), and numerous studies have attributed observed reduction in fish growth to low DO (Bejda *et al.* 1992; Phelan *et al.* 2000; Taylor and Miller 2001; McNatt and Rice 2004; Stierhoff *et al.* 2006; Del Toro-Silva in prep.). DO must be measured on a time-scale physiologically relevant to the fish (e.g. hours) in order to make sensible interpretations of its effects on fish condition and performance. DO is highly variable in shallow estuarine nurseries both temporally and spatially, and point measurements of DO are useless in trying to accurately and completely describe the abiotic environment of these habitats (Miller *et al.* 2000; Taylor and Miller 2001). Mean DO was shown here to have a significant effect on fish energy content and explained approximately 27% of the observed variability. In estuaries, the combination of high temperatures and low DO is extremely important in determining the condition and performance of juvenile fish (Miller *et al.* 2000). Temperature controls the rate of active metabolism; therefore, the effect of DO on growth is not independent of temperature (Fry 1971; Neill *et al.* 2004). Recent studies have shown that the interaction between temperature and DO has a significant effect on growth in juvenile southern flounder and juvenile summer flounder, whereby the minimum oxygen required by the fish to meet metabolic demands increases with increasing temperature (Stierhoff *et al.* 2006; Del Toro-Silva in prep.). The results of this current study found that the interaction of mean temperature and mean DO did have a significant effect on fish energy content and explained approximately 21% of the variability observed. These

field results support the lab results of Stierhoff and colleagues (2006) and Del Toro-Silva (in prep.) and demonstrate that, like the individual effects of temperature and DO on fish performance, the interaction effects can also be quantified in the field.

Typical of a temperate estuarine nursery, Hancock Creek experienced more frequent and severe hypoxia in late summer due to a temperature-induced increase in demand by the ecosystem for oxygen as well as low mixing of the water column attributable to reduced winds (Stanley and Nixon 1992). The propensity for hypoxia generally appeared to be inversely proportional to mean DO for the 15 weeks sampled in the summer of 2006. However, low mean DO was not always an indication of more frequent and/or more severe hypoxia. During the week of August 1-9, mean DO was 4.0 mg l^{-1} , there were 13 hypoxic events, and the maximum length of an event was 17 hours. During the following week, August 9-15, mean DO was 2.9 mg l^{-1} , there were 9 hypoxic events, and the longest event lasted for 9 hours. Variability in the conditions resulting in hypoxia (e.g. reduced winds, high turbidity, high temperatures, and ecosystem-wide oxygen demand) can affect the relationship between mean DO and the propensity for hypoxia. Therefore, lower mean DO does not always suggest more frequent and/or more severe hypoxia. The frequency of hypoxia had a significant effect on fish energy content, as did the interaction of frequency and severity of hypoxia. However, given the other significant parameters in the ANCOVA model (i.e. 'mean DO' and 'mean DO*mean temperature'), the fine-scale variability in hypoxia only accounted for approximately 3% of the observed variability in fish energy content.

CPUE apparently declined as DO conditions worsened (Fig. 2). There are a few possible reasons why this decline in CPUE occurred. Mortality could have increased as a

result of prolonged hypoxia and anoxia, which occurred in the creek throughout late July and early August. Another possibility is that YOY southern flounder moved out of the system as DO conditions deteriorated in search of more suitable habitat. Likewise, the decision to move out of the system could have also been driven by size-dependant emigration processes where larger individuals may move to deeper water to obtain larger prey (Rogers *et al.* 1984; Fitzhugh 1993). YOY southern flounder caught during this time period in Hancock Creek were assumed to be individuals that stayed in the nursery and experienced the DO conditions that were measured. The energy content data on these fishes suggest that staying in the nursery during these unfavorable DO conditions was energetically expensive for the fish.

Salinity. Mean salinity did not have a significant effect on fish energy content, but an apparent relationship was observed between the two. For three weeks during the 2006 sampling period, June 28 to July 19, the variances in salinity measured during each week were the highest across the entire 15 week sampling period with salinity varying by approximately 9 ppt for each of those weeks. Fish energy content was depressed during this time period, potentially due to the high variability in salinity conditions over those weeks. To test this further, the weekly standard deviation in salinity was included as an independent continuous variable in the complex ANCOVA model. Given the other significant abiotic parameters in the model, the weekly standard deviation of salinity had no significant effect on total fish energy content (ANCOVA, $p = 0.8920$).

The effects of mean salinity on fish growth are well known for many species, but the effects of salinity fluctuation, especially over short time scales (i.e. hours to days), are poorly understood (Boeuf and Payan 2001). Osmoregulatory costs have been reported to be as low as 10% to greater than 50% of the total energy budget of a fish (Boeuf and Payan 2001).

Southern flounder are a euryhaline species and, in NC, inhabit nursery areas with a wide range of salinity (Burke *et al.* 1991). The apparent, although statistically insignificant, effect observed here suggests that despite this high tolerance of variable salinity, osmoregulatory costs associated with frequent and rapid changes in salinity may be energetically expensive for juvenile southern flounder and could reduce energy available for growth.

In similar size spot, *Leiostomus xanthurus*, also a euryhaline species, rapid changes in salinity were stressful, but interestingly, the energetic costs incurred from tolerating extreme salinity changes were less than that needed to swim to avoid them (Moser 1987; Moser and Miller 1994). Since swimming costs are greater for flatfish (Duthie 1982), it seems logical that Moser's results would be applicable to the behavior and response of YOY southern flounder to salinity variability. Another interesting aspect of Moser's work is that she found a size effect for the sensitivity of spot to salinity fluctuation. Her results indicate salinity change stresses were greater for 70-90 mm spot versus 40-70 mm and 90-120 mm spot (Moser 1987; Moser and Miller 1994). Moser suggested this size-dependent change in sensitivity to salinity fluctuation may reflect a physiological adaptation by spot to emigrate out of the shallow estuarine nurseries to deeper more physiochemically stable habitats (Moser 1987; Moser and Miller 1994). Southern flounder also exhibit size-dependent emigration out of the shallow nursery habitats to deeper water (Fitzhugh 1993; Fitzhugh *et al.* 1996), and it is possible that the size range of flounder used in this study (mean 70-100 mm TL, Table 1) were the most sensitive to salinity fluctuations over that of smaller and larger individuals. The results of this current study and of the extensive work by Moser suggest that the short-term variability of salinity rather than the mean may be more

physiologically relevant to juvenile fish in regards to short-term growth variability and should be considered in future analyses of fish response to environmental change.

Gut Fullness. The significant effect of the measured abiotic conditions in Hancock Creek on total fish energy content found during the summer of 2006 may have been a result of environmental limitations on fish activity, specifically feeding (Malloy and Targett 1991; Lankford and Targett 1994). If abiotic factors such as highly variable salinity and/or low DO reduced feeding ability, then lower feeding rates could ultimately be the cause of the measured energetic response of YOY southern flounder to change in their abiotic environment (e.g. low total fish energy content). However, no apparent relationships were found between gut fullness and total fish energy content or the abiotic conditions measured over that same sampling period. Mean gut fullness was also statistically insignificant in determinations of total fish energy content. Given the lack of a relationship between gut fullness and total fish energy content found in this study, it is important to consider potential limitations of gut fullness values determined from stomach contents measured at one point in time (i.e. day of capture). Gut fullness values in this study likely only reflect the most recent feeding event and therefore may not represent the temporal scale needed for comparisons to weekly variability in total fish energy content (Baltz *et al.* 1998).

Fish Response in Hancock Creek. The short-term variability in both fish energy content and the abiotic environment of Hancock Creek suggest that there was agreement between how fish and fish biologists perceive nursery habitat quality. However, it is important to interpret these results with caution and consider that the factors influencing fish movement may not be uniform across all species and all nursery habitats. Not much is known about fish behavioral responses to changing abiotic conditions, especially for small

juvenile fish (i.e. <100 mm TL). In Hancock Creek during the summer of 2006, CPUE data suggested that fish did not move out of the habitat in response to highly variable salinity but stayed and suffered an energetic loss as a result. Likewise, in late summer, some fish stayed in the habitat during poor DO conditions, which also resulted in a loss in energy content. There are many possible reasons why fish stayed in the habitat during these unfavorable abiotic conditions. In this study, the abiotic conditions measured in the fish capture site by the YSI water quality sonde were similar to conditions measured by a sonde placed elsewhere in the creek (approximately 1.3 km upstream). This suggests that abiotic conditions were similar across a large part of the creek and that there may have been no other areas with more favorable conditions to serve as refuge from highly variable salinity or low DO. Another possible reason is that flatfish have low resting metabolic costs and swimming requires more energy than that of fusiform fish (Duthie 1982). The energetic costs of staying in poor abiotic conditions for a short period of time may be less than the costs associated with the swimming effort required to successfully find suitable refuge habitat (Moser 1987; Moser and Miller 1994). High predation risk associated with moving out of the shallow water may have also provided sufficient motivation for fish to stay in the nursery despite poor abiotic conditions (Gibson 1994). Lastly, a selective advantage to staying in poor abiotic conditions may exist if these conditions only last a short period of time and resources become more available as a result of some competitors (conspecifics and other species) leaving the habitat during the unfavorable conditions. The results of this portion of the study do not support anything more than speculation as to *why* YOY southern flounder stayed in Hancock Creek during the poor abiotic conditions experienced in the summer of 2006. However, the results do suggest that the captured fish experienced the measured abiotic

conditions, and they support the hypothesis that juvenile fish energy content responds to variability in the abiotic environment of a nursery habitat when measured on appropriate temporal and spatial scales.

Nursery Habitat Comparison

The second objective of this study was to use fish energy content to assess nursery habitat quality for southern flounder across multiple habitats. Four nursery habitats for southern flounder were compared at six times throughout the summer of 2005, from late May to early August, in order to assess habitat quality across a time period relevant to when the habitat is predominantly used by the species as a nursery. Significant differences were found among the four habitats in both fish energy content (fish's perception) and in abiotic conditions (biologist's perception). If fish energy content is largely a reflection of the quality of the abiotic environment, which the results from the study in Hancock Creek suggest, then theoretically, differences in fish energy content among different nurseries compared at similar time periods should be explained by the differences in abiotic conditions of those nurseries. However, the results of these habitat comparisons suggest that some habitats may be more complex than Hancock Creek with respect to variability in abiotic conditions throughout the habitat. This spatial complexity, if not monitored correctly, can make it difficult to understand differences in fish condition observed across different habitats and, ultimately, to assess nursery habitat quality.

Late May. No significant differences in fish energy content among habitats were found during this period. Although no abiotic data were collected, conditions during this time of the year are not highly variable among habitats. Water temperatures are generally

low (22-25°C), and southern flounder show little growth variability at temperatures within this range (Davis 1998; Del Toro-Silva in prep.). DO is also generally high during this time period and should have little effect on fish condition. Given the generally favorable abiotic conditions during late May, it is not surprising that no significant differences in fish condition were found.

Early June. Significant differences in fish energy content were found for smaller fish in the 25th and 50th percentiles of TL, where flounder in Porter Creek had lower energy content (up to approximately 22% difference for 25th percentile fish) than flounder in Back Creek for both size classes. Based on measured abiotic conditions, it is surprising to see that Back Creek flounder not only had higher energy content than flounder in Porter Creek but also that Back Creek flounder had the same energy content as flounder in East Fork and Long creeks. Although no apparent temperature differences were found, mean DO in Back Creek was significantly lower, hypoxia was more frequent, and the mean length of hypoxia was significantly longer. These results suggest that the DO conditions measured in Back Creek were not what the captured fish experienced (i.e. fish energetic condition was higher than expected in these DO conditions).

Late June. Flounder from Back Creek in the 25th and 50th percentiles of TL, had significantly lower energy content (up to approximately 37% difference for 25th percentile fish) than the other three creeks for this time period. There were no apparent differences in the abiotic conditions of these habitats, suggesting that the abiotic environment of Back Creek was misrepresented or other abiotic and/or biotic factors were limiting fish condition in this creek during late June.

Early July. Long and Porter creeks had flounder with significantly lower energy content (up to approximately 16% difference) than flounder from Back and East Fork creeks. DO conditions were not significantly different among these habitats. Although mean temperature was not significantly different, both Long and Porter creeks were approximately 2°C cooler than Back and East Fork creeks. Back and East Fork creeks were at the suggested optimum temperature for growth in southern flounder (around 30 °C) which may explain why fish energy content was higher in those two creeks (Peters 1971; Guindon and Miller 1995; Del Toro-Silva in prep.).

Late July. Flounder in Porter Creek had significantly lower energy content (up to approximately 12% difference) than flounder in Back and East Fork creeks. Back and Porter creeks had similar DO conditions, yet flounder in Back Creek had significantly higher energy content than flounder in Porter Creek. This again suggests that DO conditions measured in Back Creek may not have been what the fish actually experienced. Mean temperatures remained virtually unchanged in these habitats from early July. An apparent temperature relationship, similar to early July, also existed here, with Porter Creek approximately 2°C cooler than Back and East Fork creeks which were near optimum for growth, and fish energy content in Porter Creek was significantly lower than the other two creeks.

Early August. Flounder in Porter Creek again had significantly lower energy content (up to approximately 14% difference) than flounder from other creeks. No abiotic data were collected for Long Creek, however results of data collected from East Fork and Porter creeks suggest no apparent differences in temperature or DO between these two habitats. Both creeks were not hypoxic during this sampling period, and both were at optimum temperatures

for growth. These results suggest that other abiotic and/or biotic factors in Porter Creek were limiting fish condition in early August.

Diet and Prey Availability. Fish energy content can vary due to prey type and prey availability. No apparent differences in diet were found among these four nursery habitats, and mysids were the most frequently occurring prey item in the stomachs of YOY southern flounder from all habitats. In North Carolina, mysid densities are greatest in the upper reaches of the estuaries, where these PRE and NRE study sites were located (Williams 1972; Burke 1995). This study found significant differences in mysid densities among habitats for both early June and late July samples, however, even in low density habitats, mysid abundance did not appear to limit fish energy content. In both early June and late July, East Fork Creek had the lowest mysid densities and flounder of relatively high energetic condition. This suggests that the mysid abundance measured in East Fork Creek, although lower than the other habitats, was not a limiting factor of fish energetic condition. An interesting aspect to note about East Fork Creek is that a larger percentage of full stomachs (approximately 25% more) had mysids only and a smaller percentage (approximately 15-25% less) contained other prey types as compared to the other habitats. One possibility for the lower mysid densities measured in East Fork Creek is that YOY southern flounder fed exclusively on mysids and drove down mysid abundance in the habitat. As a result, the prey field measured may have been misrepresented, and mysid densities may have been higher than what was actually measured. Regardless, prey abundance in this habitat did not appear to be low enough to limit fish energy content. Porter Creek, in both early June and late July, had extremely high densities of mysids relative to the other creeks, yet fish energetic

condition was often the lowest. Prey abundance was apparently not a limiting factor on YOY southern flounder energy content in this creek.

Similar results were found for these same sites in an extensive study by Kamermans and colleagues (1995) that examined the effect of diet on habitat-specific growth rate of caged YOY southern flounder. In that study, the significance of mysid abundance on growth was inconsistent over trials, and the authors concluded that mysid abundance could not unequivocally explain the observed differences in habitat-specific growth rates. Currin and colleagues (1984) also concluded that juvenile fish production in the lower PRE was not limited by food. Food limitation on juvenile fish condition or performance in North Carolina estuaries was not an objective of this study, and no extensive efforts were made to evaluate this relationship, especially given the considerable work done on this topic by Kamermans and colleagues (1995) and Currin and colleagues (1984). However, the results of this current study found no apparent evidence to suggest mysid density affected habitat-specific fish energy content and support the hypothesis put forward by Miller and colleagues (1991) that food likely limits growth at the center of a species range rather than at the edges (North Carolina is the northern edge of the range for southern flounder).

Southern flounder exhibit an ontogenetic shift in diet from benthic invertebrates to fish (Appendix Table A13, Fig. A14,15; Powell and Schwartz 1979; Fitzhugh 1993; Fitzhugh *et al.* 1996). Although the dominant prey found in YOY southern flounder stomachs from this study were mysids, southern flounder will begin to consume fish as early as possible (45-50 mm TL, Appendix Fig. A15). Fitzhugh and colleagues (1996) concluded that the onset of piscivory was largely responsible for the observed bimodal growth in juvenile southern flounder. The higher predator growth rates, and likewise, the higher measures of predator

energy content, that result from a diet shift to piscivory are due to both preconsumptive and postconsumptive processes (Juanes and Conover 1994; Olson 1996; Lankford and Targett 1997). To equal the energy content in one prey fish requires the consumption of a much greater number of planktonic individuals (e.g. mysids) (Thayer *et al.* 1973; Steimle and Terranova 1985). Preconsumptive energetic costs, such as foraging and capture costs, are therefore elevated by feeding on mysids rather than fish and may reduce maximum growth in juvenile southern flounder. Fish prey also has higher energy content per gram versus that of non-piscine prey (Thayer *et al.* 1973; Steimle and Terranova 1985), no exoskeleton, and a greater surface area:volume ratio (Jobling 1987; Lankford and Targett 1997). Therefore, postconsumptive energetic costs, such as digestion and assimilation, may be higher for mysids than for fish prey and could also reduce maximum growth in juvenile southern flounder. The onset of piscivory is largely dependent upon predator morphology, the availability of right-sized fish prey, and rapid growth during the invertebrate-feeding stage (Juanes *et al.* 2002). A difference in the timing of the onset of piscivory among flounder from the different nursery habitats examined in this study could have contributed to the observed habitat-specific differences in fish energy content. The results of this study, however, suggest that the onset of piscivory was similar for flounder in all four nursery habitats and had no apparent effect on the observed habitat-specific differences in YOY southern flounder energy content.

Apparent differences in mean gut fullness were found for YOY southern flounder across nursery sites for late May, early July, late July, and early August sampling periods. However, the patterns of difference in gut fullness for those time periods do not match those of total fish energy content determined over the same periods. In late May, flounder in Back

and Porter creeks had apparently higher gut fullness than flounder in East Fork and Long creeks, yet there were no significant differences in total fish energy content across these nurseries. In early July, flounder in East Fork and Long creeks had apparently similar gut fullness but significantly different total energy content. In late July, flounder in Porter Creek had apparently higher gut fullness than flounder in Back or East Fork creeks, yet total fish energy content was significantly lower in Porter Creek as compared to the other two creeks. Similarly in early August, flounder in Porter Creek had apparently higher gut fullness than flounder in East Fork and Long creeks, yet total fish energy content was significantly lower in Porter Creek as compared to the other two creeks. As mentioned earlier, the lack of a relationship between gut fullness and total energy content of YOY southern flounder collected in Hancock Creek in 2006 and in the four different nurseries compared in 2005 may be due to a disparity in the temporal scales by which total fish energy content and gut fullness were measured.

Other Biotic/Abiotic Factors. Overall, there appeared to be little difference in the measured abiotic conditions experienced by fish in these four different nursery habitats over the summer of 2005 (assuming the abiotic environment of Back Creek was misrepresented). Lower mean temperatures in Long and Porter creeks may have resulted in the lower fish energy content of these two creeks in early and late July, but that remains questionable, especially given that Porter Creek and East Fort Creek were at roughly the same mean temperature in early August, yet flounder in Porter Creek had lower energy content. It is likely that other abiotic and/or biotic factors not considered in this study contributed to the significant differences in fish energy content measured across these different nursery habitats. For example, abundances of juvenile and adult blue crabs, *Callinectes sapidus*,

were higher in Porter Creek than any of the other three creeks studied. Blue crabs and southern flounder are both closely associated with the bottom and may compete for space at high densities. Although predation by blue crabs on juvenile flounder is unlikely (Laughlin 1982; Hines *et al.* 1990; Meise and Stehlik 2003), stress and energetic costs associated with harassment and competition for space may have reduced the capacity of southern flounder in Porter Creek to acquire and assimilate energy (i.e. grow). Another possibility is that water pollution from an industrial phosphate mine that borders the entire eastern shore of Porter Creek could have resulted in the lower fish energy content that was measured in that creek. Elevated concentrations of toxic metals such as cadmium, chromium, zinc, and arsenic have been reported in Porter Creek and other tributaries surrounding the mine (CZR Incorporated 1999). Sublethal concentrations of these metals irritate fish skin and mucous membranes, causing increased spontaneous locomotor activity and oxygen consumption, which ultimately elevates routine metabolism and reduces a fish's capacity to grow (Fry 1971; Heath 1995). Guindon and Miller (1995) also found consistently lower growth rates in Porter Creek as compared to the other nursery habitats using caged southern flounder, suggesting that unmeasured abiotic characteristics of this habitat (e.g. water pollution) are more likely responsible for the lower growth rates rather than unmeasured biotic characteristics (e.g. competition and harassment by blue crabs).

Fish Energy Content as an Indicator

The estimates of energy content were obtained by proximate analysis of total fish lipid and protein content. Lipids make up a minute portion of the dry mass of YOY southern flounder (Appendix, Fig. A4). By approximately 40 mm TL, lipids only constitute about 4% of the dry mass of the fish. Many species, including species of flatfish, require lipid reserves

for metamorphosis which may explain why lipid levels are higher for individuals smaller than 40 mm TL (Fraser 1989; Hossain *et al.* 2003). Protein makes up approximately 80% of the dry mass of YOY southern flounder (Appendix, Fig. A6), and therefore determines the majority of total fish energy content. The rapid somatic growth of early juvenile southern flounder as well as many other species appears to preclude the accumulation of lipids (Suthers *et al.* 1992).

For juvenile southern flounder, energy content proved to be useful in measuring a fish's response to change in its environment and in evaluating nursery habitat quality for the species. The low variability in energy content for individuals at a given size allowed for the detection of differences in fish condition both within a single nursery habitat (Hancock Creek) and across multiple habitats (Back, East Fork, Long, and Porter creeks). In Hancock Creek, weekly variability in fish energy content was largely explained by the weekly variability in temperature, DO, and salinity, suggesting that fish energy content was sensitive to changes in abiotic conditions (i.e. habitat quality).

Fish energy content was shown to be a relatively rapid-response indicator of habitat quality. According to the data, the perspective of the fish, as measured through energy content, was related to the environmental conditions experienced by the fish during the immediate week prior to capture and was independent of previous energetic conditions. This makes sense considering the high plasticity of fish growth, where growth rate quickly slows in response to negative environmental change and quickly returns to maximum levels when favorable conditions return (Weatherley 1990; Ali *et al.* 2003). The major implication of the short time scale of this indicator is that the use of fish energy content in assessments of nursery habitat quality requires multiple sampling events over the entire time period that

habitat is used as a nursery by the species of concern. Therefore, the accuracy of a habitat's assessment is both subject to the frequency at which it is sampled and limited by the time period over which the assessment is conducted.

Perception of Habitat Quality

Fish biologists often perceive habitat quality through some measure of fish performance (e.g. growth) and also through the degree of factors thought to affect that measure of performance. The most common experimental measure of nursery habitat quality is juvenile fish growth. A vast understanding of the relationships between environmental conditions and fish bioenergetics (growth and metabolism) has allowed fish biologists to test a wide range of hypotheses about factors that may affect growth in a nursery habitat. Much of this work in estuaries has involved the use of field enclosures (Sogard 1992; Guindon and Miller 1995; Kamermans *et al.* 1995; Able *et al.* 1999; Meng *et al.* 2000; Phelan *et al.* 2000; Manderson *et al.* 2002; Stunz *et al.* 2002; Necaise *et al.* 2005). Movement of juvenile fish within a nursery, especially recent settlers (i.e. 20-75 mm), is not well known. Because the accuracy of a habitat's assessment depends on quantifying the environmental conditions actually experienced by the fish, field enclosures have been the easiest solution to restricting fish movement. Although these caging studies have provided valuable insight into how natural variability in environmental conditions can affect fish growth, they don't necessarily obtain an unbiased view of habitat quality from the fish's perspective. Caging studies allow for desirable experimental design, help ensure treatments are consistent and/or accurately described for all individuals, and exclude undesirable parameters such as predation. However, when such studies are used to assess habitat quality, biases attributed to caging artifacts (e.g. increased/reduced feeding, altered prey type, altered prey abundance, and

restricted movement), if not correctly accounted for, may alter the habitat effects on measured fish performance and subsequently alter the measure of habitat quality (Peterson and Black 1994; Able 1999; Kellison *et al.* 2003).

There is some evidence that suggests movement rates of recently settled age-0 fish in estuarine nurseries are minimal. Weinstein and O'Neil (1986) found that movement of juvenile spot, *Leiostomus xanthurus* (mean 41.4 mm SL), between adjacent creeks was relatively insignificant and that on average these spot resided in a single nursery for 85 days. Similarly, Miller and Able (2002) found high habitat fidelity for juvenile Atlantic croaker, *Micropogonias undulatus* (41-121 mm SL), throughout most of the summer until fall egress. Able and Hales (1997) also found strong site fidelity for black sea bass, *Centropristis striata* (34-111 mm TL), as did Able and colleagues (2005) in spring and summer months for age-0 tautog, *Tautoga onitis* (25-187 mm TL), and age-0 cunner, *Tautoglabrus adspersus* (24-99 mm TL). YOY winter flounder reportedly move less than 100 m from their nursery sites during the summer months (Saucerman and Deegan 1991), and many appear to remain in their nurseries even when exposed to hypoxia (Howell and Simpson 1994). In this current study, CPUE data and field observations suggest that YOY southern flounder may also have high site fidelity during the summer early growth period. Throughout the summer of both 2005 and 2006, YOY southern flounder in every creek sampled were found in the highest densities in only one or two locations of each creek despite extensive efforts to capture them in other areas of the creek both across different depths and different substrates. Given that post-settlement movement rates appear to be extremely low for a number of juvenile estuarine species (at least for the first 75-100 days), future studies of nursery habitat quality should first consider if cages are necessary or if it would be more beneficial to devote

available resources to more thorough water quality monitoring (i.e. multiple sondes in areas of local high abundance within a single habitat).

The overall approach to this current study was to assess nursery habitat through examining differences in how fish and fish biologists perceive habitat quality. The accuracy of a measure of the fish's perspective depends upon the fish acting naturally in its environment. An obvious advantage to this approach over caging, is that a true measure of habitat-specific fish condition or performance is obtained that gives the best possible estimate of the conditions affecting that measured fish condition or performance (i.e. habitat quality) at a given time (Meise *et al.* 2003). The major disadvantage to a no-cage approach, however, which was realized in this study, is that if there are considerable differences in abiotic conditions across some habitats (i.e. possibly Back Creek), it can make measuring the environmental conditions *actually* experienced by free-ranging fish difficult, hindering the researcher's ability to understand the true factors affecting quality in that habitat (Able 1999; Meise *et al.* 2003). YOY southern flounder in East Fork, Long, and Porter creeks were captured within 0-75 m of the YSI water quality sondes for all sampling events. However, in Back Creek, YOY southern flounder were captured 100-300 m from the YSI water quality sonde. Juvenile southern flounder in this habitat were only located in one area for the duration of the study, however high disturbance by recreational boating in this area prevented safe deployment of expensive monitoring gear. Therefore, the water quality sonde was deployed in the closest safe area that was possible. Although the free-ranging fish approach gave the most accurate estimate of habitat quality for Back Creek from the fish's perspective, the ability of this study to evaluate the factors affecting habitat quality in Back Creek may have been limited by a misrepresentation of the abiotic environment.

The unfortunate circumstances surrounding the YSI water quality sonde placement in Back Creek and the potential significance of this sonde placement provide a relatively easy explanation for why YOY southern flounder in Back Creek were of higher energetic condition than expected. However, habitats with high productivity (e.g. high mysid abundance) are, by nature, prone to nocturnal hypoxia when photosynthesis ceases and higher respiration rates reduce oxygen in the system (Miller *et al.* 2000). The possibility exists that if fish can physiologically withstand the stress of temporarily low DO, they might be able to capitalize on the highly abundant food source when DO conditions improve. Mysid densities in Back Creek were only measured in early June but were the highest of the four nursery habitats that were compared over that same time period. The higher than expected energy content of YOY southern flounder in Back Creek could have been a result of fish maximizing their prey resource despite the physiological limitations incurred as a result of the low DO conditions. This scenario is certainly interesting and worth investigating further. However, given the dramatic energetic responses by YOY southern flounder in Hancock Creek to hypoxic conditions less harsh than those measured in Back Creek, it is more likely that YOY southern flounder in Back Creek did not experience the harsh hypoxic conditions measured by the YSI water quality sonde placed in that creek.

By providing abundant prey and refuge from predation, estuarine nurseries promote the early growth and survival of many juvenile fish species (Hoss and Thayer 1993; Gibson 1994; Sogard 1997; Able and Fahay 1998; Able 1999). However, despite these highly favorable biotic conditions, estuaries have low species diversity (Able and Fahay 1998). The extreme and often harsh variability in summer and early fall abiotic conditions require that temperate species, such as southern flounder, which predominantly utilize estuarine habitats

during this time period as early and late juveniles, are physiologically capable of dealing with such conditions in ways that maintain growth while ensuring survival. The adaptive characteristics of these species that allow for life in estuaries raise an important question to consider when evaluating habitat quality for these species: are statistically significant differences in fish condition or performance biologically significant in terms of nursery quality? Within Hancock Creek, statistically significant differences in fish energy content were found over the continuous 15 week sampling period but the maximum difference observed was only approximately 8%. Fish energy content was also independent of previous energetic states, suggesting that statistically significant drops in energy content were not physiologically difficult to recover from if they were followed by favorable environmental conditions. Greater differences in fish condition were found when multiple nursery habitats were compared. The maximum differences observed in this study between different habitats ranged from approximately 12 to 37% over the entire summer sampling period. The biological significance of these point estimates of habitat quality likely lies in the overall picture (i.e. habitat productivity, see Beck *et al.* 2001). The 8% difference seen in Hancock Creek may be large enough for biological significance if it frequently occurs (i.e. high weekly variability) and reduces the overall growth and survival of fish observed at the end of a growing season. Similarly, habitats consistently performing worse than other habitats over an entire growing season (i.e. Porter Creek) may have reduced overall growth and survival at the end of the season compared to the other habitats. The real value of these point estimates of habitat quality lies in their ability to help biologists and managers understand and describe the causes (e.g. abiotic conditions, colonization, movement, food availability, and survival) of production variability among different nursery habitats. Given the rapid degradation and

loss of estuarine habitat (Hinrichsen 1998; Peterson 2003), it no longer suffices to simply acknowledge differences in nursery-specific productivity without trying to understand why these differences exist, especially if the necessary preemptive and proactive efforts to protect and restore estuarine habitat are to be made.

CONCLUSION

Estuarine nursery habitats are essential to the sustainability of many of the world's fisheries because they promote the growth and survival of early life stages of ecologically and economically important species such as southern flounder. Estuaries are particularly vulnerable to the effects of anthropogenic perturbations and watershed activities, and the degradation and loss of estuarine habitat is occurring at unprecedented rates (Hinrichsen 1998; Peterson 2003). The significance of nursery habitat loss has become so distressing that most fishery management strategies now place high priority on the protection and restoration of 'Essential Fish Habitat' (EFH, Magnuson-Stevens Act, NOAA 1996).

Estimates of juvenile abundance have long been the standard evaluation of habitat quality; however, larval supply, survival, and movement too often result in abundance estimates inaccurately evaluating habitat quality and undervaluing many nursery habitats. As a result, extensive efforts have been made in recent years to find new robust indicators of nursery habitat quality. Although early juvenile growth is only one component of production, much attention has been placed on this parameter since it theoretically encompasses all of the effects of the nursery environment (biotic and abiotic) on the fish. Estimates of actual growth in the field are labor intensive, and high variability in individuals from a single habitat may not allow for detectable differences in quality among different

habitats. Fish energy content is an indirect measure of growth and is relatively easier to measure than actual growth in the field. This study found fish energy content to be a useful indicator of nursery habitat quality for southern flounder both within a single habitat over time and across multiple habitats at a given time. Over a continuous 15 week sampling period from May to August 2006, YOY southern flounder energy content varied weekly in a single nursery (Hancock Creek) by a maximum of 8%. The temporal variability in temperature, DO, and salinity explained 51% of the temporal variability in fish energy content observed in this habitat. Across multiple nursery habitats (Back, East Fork, Long, and Porter creeks) sampled biweekly from May to August 2005, the variability in YOY southern flounder energy content ranged from 12 to 37%. The spatial variability in temperature, DO, and salinity was often significant but minimal and did not unequivocally explain the measured spatial variability in fish energy content. In fact, YOY southern flounder in only one nursery (Porter Creek) were consistently of lower energetic condition as compared to the other nurseries, and the quality of this habitat appeared to be partially limited by an environmental factor unmeasured in this study, possibly water pollution.

The conclusions of this study on juvenile southern flounder and the utility of fish energy content as an indicator of nursery habitat quality are likely applicable to other fish species whose juveniles utilize similar estuarine nursery habitats. Further work is certainly needed that continues to address the role of abiotic factors in estuarine nursery habitat productivity. An important question to answer is should species living in highly variable environments like estuaries be more or less sensitive to abiotic variation. Similarly, are early life stages when fish tend to be smaller and more sedentary even less sensitive to abiotic variability? This study found significant responses in YOY southern flounder energy content

to weekly abiotic variability, though the maximum response observed was only 8%. Southern flounder appeared to be an ideal species for this study, which employed a free-ranging fish approach to assessing habitat quality, because, among other reasons, they seem more likely to be exposed to the abiotic conditions measured by a stationary water quality sonde than other more mobile species. However, from a sensitivity point of view, southern flounder, who are apparently quite capable of adapting to highly variable abiotic conditions, may not be an ideal species for studies addressing the effects of abiotic factors on the condition and performance of juvenile fish in estuaries.

In their prominent paper on the role of nurseries, Beck and colleagues (2001) propose that a measure of the number of juveniles from specific habitats recruiting to the adult/fishable population is likely the best integrative measure of all the factors contributing to habitat-specific productivity, including abundance, growth, survival, and movement to adult habitats, and more accurately identifies essential juvenile habitats where protection is critical. Quantifying the connectivity between juvenile and adult populations likely holds the greatest potential for designation and protection of *current* EFH. However, it does little to address the limitations of production and production capacity of individual habitats (i.e. habitat quality), an important step that precludes the success of any restoration efforts and protects against the loss of habitats with currently low relative production but high productive capacity (e.g. high growth) that may, through restoration or enhancement, be designated as *future* EFH.

The simplicity of single-factor analyses of habitat quality, such as this study, should not discredit their utility in assessments of habitat quality. Although the effects of abundance, growth, survival, and movement on nursery productivity are certainly linked, the

conditions affecting growth alone, for example, are still poorly understood. In dynamic ecosystems such as estuaries, high temporal and spatial variability in the conditions affecting individual factors, such as juvenile fish growth, survival, or movement, make it frequently difficult to make sensible interpretations of the results of relatively simple single-factor studies. A better understanding of the conditions affecting single factors of production, such as growth, may enable researchers to successfully conduct more difficult multifactor assessments of habitat quality.

Despite the extensive estuarine research over the last 20 to 30 years aimed at assessing juvenile fish habitat, scientists still do not fully understand the limitations of habitat-specific productivity and still lack the ability to accurately evaluate and predict nursery habitat quality. Demand for coastal resources threatens estuarine nursery habitats, and fisheries managers must have measures (i.e. justification) with which to protect these essential ecosystems from loss and degradation. Tragically, the ability of scientists to provide managers with further justification for habitat protection, beyond the designation of *current* EFH through abundance estimates, is far exceeded by the ability and ease of developers, agriculture, and industry to destroy fish habitat and habitat quality. In light of this, evaluating and protecting nursery habitat based on productive capacity (e.g. growth potential) rather than actual production (i.e. biomass contributed to fishable stock) may be an important approach for fisheries management agencies to take. Given the current political atmosphere surrounding the “best” use of coastal resources, habitat protection based on potential production rather than realized production will undoubtedly be challenging for fisheries management agencies. Regardless, it is uncertain whether or not scientists will ever fully understand estuarine habitat quality, at least enough to provide fisheries managers with

indisputable proof of EFH, and therefore it is essential to be preemptive and proactive in the efforts to protect and restore these ecosystems that are critical to the sustainability of many of the world's fisheries.

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Table 1. Distribution of total lengths (mm) for young-of-the-year southern flounder collected in Hancock Creek during the summer of 2006 and used in regression analyses.

Period	Mean	SD	Max	Min	n
May 25	67.1	9.1	80	49	14
June 1	70.0	9.5	89	54	17
June 8	72.9	9.1	92	60	22
June 13	76.6	9.0	92	61	29
June 21	74.8	7.0	92	63	35
June 28	81.9	12.9	113	67	20
July 6	86.2	8.0	100	73	29
July 12	88.2	14.3	119	60	32
July 19	88.9	11.0	110	75	19
July 26	96.7	13.1	135	77	23
August 1	97.0	12.9	129	79	41
August 9	90.1	7.8	101	76	16
August 15	98.6	12.5	127	86	11
August 23	101.8	13.9	121	85	6
August 30	98.6	11.6	115	77	10

Table 2. The weekly means, range, and number of observations for temperature, DO, and salinity in Hancock Creek measured continually for 15 weeks during the summer of 2006.

Water Quality	2006 Sampling Period – Hancock Creek														
<u>Sampling Dates</u>															
Begin	5/18	5/25	6/1	6/8	6/13	6/21	6/28	7/6	7/12	7/19	7/26	8/1	8/9	8/15	8/23
End	5/25	6/1	6/8	6/13	6/21	6/28	7/6	7/12	7/19	7/26	8/1	8/9	8/15	8/23	8/30
<u>Temp. (°C)</u>															
Mean	24.0	26.8	26.1	26.7	26.5	28.6	28.8	27.8	30.0	29.8	30.3	31.2	28.2	28.7	28.8
SD	1.2	1.5	1.5	1.0	1.1	1.2	1.2	1.3	1.2	1.4	1.3	1.4	1.5	1.0	1.2
Var.	1.3	2.2	2.2	0.9	1.3	1.4	1.5	1.6	1.5	2.1	1.7	1.9	2.2	0.9	1.5
Max.	26.8	29.6	29.4	28.8	29.0	31.7	31.6	31.1	33.1	33.3	33.1	34.9	31.7	32.0	32.5
Min.	21.3	23.3	23.2	24.2	24.1	26.1	26.3	24.9	27.9	27.4	27.7	28.7	25.5	27.0	26.6
n	335	333	337	242	380	336	384	288	336	337	287	384	288	380	336
<u>DO (mg l⁻¹)</u>															
Mean	5.7	5.8	6.0	6.0	5.2	6.2	5.9	5.3	6.3	4.6	4.7	4.0	2.9	4.2	4.4
SD	1.1	1.8	1.5	1.4	1.6	1.7	1.6	1.5	2.0	2.0	1.9	2.7	1.1	1.7	1.3
Var.	1.2	3.4	2.3	2.1	2.6	2.7	2.5	2.2	4.2	4.0	3.6	7.3	1.2	2.8	1.7
Max.	7.7	11.7	9.3	8.8	10.9	10.4	9.8	9.5	11.1	9.3	10.3	11.5	6.0	9.5	8.4
Min.	2.2	2.7	2.0	2.6	2.3	2.5	1.7	2.1	2.1	1.1	0.1	0.0	0.7	1.4	1.5
n	335	333	337	169	380	336	384	288	336	336	287	384	288	380	336
<u>Salinity (ppt)</u>															
Mean	10.9	10.3	10.8	9.4	9.4	10.0	6.7	9.4	11.2	11.8	12.7	12.6	14.0	12.2	11.4
SD	0.4	1.1	1.1	0.7	1.0	0.6	1.6	1.7	2.1	1.4	1.4	1.0	0.3	0.6	0.9
Var.	0.1	1.1	1.1	0.5	1.1	0.3	2.7	2.9	4.5	2.0	2.0	1.1	0.1	0.3	0.9
Max.	11.7	13.1	14.0	10.9	11.5	11.7	13.4	16.8	16.4	15.0	17.9	15.9	14.9	13.6	13.4
Min.	10.0	7.9	9.7	8.0	8.0	7.3	4.8	7.6	7.8	9.5	10.0	9.6	13.5	10.3	9.0
n	335	333	337	242	380	336	384	288	336	337	287	384	288	380	336

Table 3. The frequency and severity of hypoxia observed in Hancock Creek during the summer of 2006. Only weeks with at least one hypoxic event are shown. The mean, standard error, maximum, and minimum values for length of an hypoxic event (i.e. severity) are given.

Hypoxic Events	2006 Sampling Period – Hancock Creek							
	6/1- 6/8	6/28- 7/6	7/19- 7/26	7/26- 8/1	8/1- 8/9	8/9- 8/15	8/15- 8/23	8/23- 8/30
<u>Frequency (#)</u>								
n	1	2	5	1	13	9	7	3
<u>Severity (hours)</u>								
Mean	0.5	0.8	3.6	6.5	4.2	3.5	2.4	1.0
SE	---	0.3	1.6	---	1.4	1.1	0.5	0.3
Max.	---	1.0	9.5	---	17.0	9.0	4.0	1.5
Min.	---	0.5	0.5	---	0.5	1.0	1.0	0.5

Table 4. ANCOVA determined least-squares means and standard errors of total fish energy content (ln kJ) for young-of-the-year southern flounder collected in Hancock Creek during the summer of 2006 adjusted to a common length (TL = 83.8 mm) across time.

Sampling Period	Total Energy	SE
May 25	2.8440	0.0176
June 1	2.8257	0.0158
June 8	2.8491	0.0138
June 13	2.8403	0.0117
June 21	2.8592	0.0109
June 28	2.8496	0.0138
July 6	2.8121	0.0114
July 12	2.8214	0.0109
July 19	2.8229	0.0142
July 26	2.8680	0.0134
August 1	2.8933	0.0104
August 9	2.8679	0.0155
August 15	2.8224	0.0191
August 23	2.9049	0.0257
August 30	2.8753	0.0199

Table 5. Results of ANCOVA models on total energy content in young-of-the-year southern flounder collected in Hancock Creek during the summer of 2006.

Dependent Variable	Effect	df	Type III SS	F	Pr > F	Partial R²
<i>Simple Model</i>						
ln (Total Energy)	Week	14	48.343	913.01	<0.0001	0.506
<i>Full Model</i>						
ln (Total Energy)	DO	1	25.365	166.09	<0.0001	0.266
ln (Total Energy)	DO*Temperature	1	19.754	129.34	<0.0001	0.207
ln (Total Energy)	#Events	1	1.086	7.11	0.0081	0.011
ln (Total Energy)	#Events*MeanEvent	1	2.041	13.37	0.0003	0.021
ln (Total Energy)	Week	10	3.734	2.44	0.0081	0.039

Temperature, Salinity, and MeanEvent were not significant and were removed from model. (p = 0.8832, 0.6138, 0.1016, respectively)

Table 6. Distribution of total lengths (mm) for young-of-the-year southern flounder collected in the four nursery sites over six sampling periods during the summer of 2005 and used in regression analyses.

Period/Site	Mean	SD	Max	Min	n
<i>Late May</i>					
BC	65.3	7.3	77	54	13
EF	63.8	7.1	80	52	62
LC	57.1	2.9	60	53	7
PC	58.2	8.8	74	47	10
<i>Early June</i>					
BC	83.4	11.8	102	67	7
EF	75.8	14.8	130	55	24
LC	84.8	15.2	103	69	5
PC	68.6	10.0	83	54	13
<i>Late June</i>					
BC	81.3	9.8	98	64	15
EF	86.8	18.1	142	72	15
LC	103.3	16.7	149	82	13
PC	73.7	18.3	131	57	14
<i>Early July</i>					
BC	87.2	2.1	90	84	6
EF	94.4	4.9	103	85	11
LC	110.2	20.2	155	86	15
PC	83.4	10.3	124	63	33
<i>Late July</i>					
BC	103.7	14.0	133	89	10
EF	92.8	14.9	106	72	6
PC	90.0	12.8	125	69	29
<i>Early August</i>					
EF	119.3	24.2	193	102	12
LC	112.2	12.6	134	98	6
PC	101.2	31.5	156	80	5

Table 7. The means, range, and number of observations for temperature, DO, and salinity measured in the four nursery sites over five sampling periods during the summer of 2005. Sampling periods marked with an asterisk (*) are missing data for one abiotic parameter or one nursery site.

Water Quality	2005 Sampling Period – Nursery Habitat Comparison															
	Early June*				Late June*			Early July*				Late July			Early Aug.*	
	BC	EF	LC	PC	BC	EF	LC	BC	EF	LC	PC	BC	EF	PC	EF	PC
<u>Sampling Dates</u>																
Begin	6/3	5/31	5/29	6/4	6/22	6/22	6/21	7/1	7/7	6/29	7/5	7/15	7/14	7/12	7/27	7/27
End	6/10	6/7	6/5	6/11	6/24	6/24	6/28	7/8	7/14	7/2	7/6	7/22	7/21	7/19	8/3	8/3
<u>Temp. (°C)</u>																
Mean	25.5	23.2	23.1	24.7	27.5	27.3	26.6	30.1	30.9	27.5	28.7	30.6	30.7	28.7	30.6	30.0
SD	2.3	1.4	1.1	1.3	0.4	0.6	0.7	1.1	1.6	0.7	0.3	1.1	1.7	1.0	1.4	1.5
Var.	5.1	1.9	1.3	1.7	0.2	0.4	0.5	1.2	2.6	0.5	0.1	1.3	2.8	1.1	2.1	2.3
Max.	29.3	26.7	26.8	28.1	28.7	28.6	27.8	33.3	34.3	29.4	29.5	32.8	34.1	31.5	35.3	34.5
Min.	21.9	21.3	21.4	22.4	26.1	25.9	24.3	26.5	27.5	26.6	28.3	27.8	27.5	26.7	28.3	26.9
n	369	368	371	368	101	99	337	320	357	178	63	360	358	339	337	323
<u>DO (mg l⁻¹)</u>																
Mean	2.7	---	4.9	4.1	4.1	5.2	4.0	3.3	5.2	3.5	2.3	5.7	7.1	3.4	6.2	8.6
SD	1.7	---	1.2	1.3	2.0	1.6	1.6	2.4	1.4	1.6	0.6	2.3	1.6	2.6	1.8	2.9
Var.	2.9	---	1.5	1.8	3.9	2.5	2.4	5.7	2.0	2.5	0.3	5.5	2.5	7.0	3.1	8.3
Max.	7.4	---	8.6	9.0	8.2	7.7	7.7	8.6	8.8	8.0	3.7	11.6	10.6	10.5	10.3	12.0
Min.	0.1	---	1.5	1.1	0.7	2.3	0.0	0.1	1.7	0.7	1.1	0.0	2.9	0.0	2.8	3.6
n	369	---	371	368	101	99	337	320	347	178	63	360	358	339	337	323
<u>Salinity (ppt)</u>																
Mean	3.6	6.5	6.1	3.9	5.0	7.7	6.6	4.0	6.5	6.3	4.4	3.1	5.7	4.8	7.3	6.3
SD	0.3	0.7	0.2	0.3	0.2	0.0	0.1	0.6	0.2	0.3	0.2	0.2	0.4	0.3	0.5	0.2
Var.	0.1	0.5	0.0	0.1	0.1	0.0	0.0	0.3	0.1	0.1	0.0	0.0	0.2	0.1	0.2	0.0
Max.	4.2	8.6	6.5	5.2	5.4	7.7	6.9	4.8	6.8	6.9	4.8	3.7	6.4	5.8	8.6	7.1
Min.	3.0	4.8	5.6	3.4	4.8	7.6	6.5	1.1	5.6	5.7	4.1	2.6	4.1	3.8	6.2	5.6
n	369	368	371	368	101	99	337	320	357	178	63	360	358	339	337	323

Table 8. The frequency and severity of hypoxia observed in each nursery site for each sampling period during the summer of 2005. The mean, standard error, maximum, and minimum values for length of an hypoxic event (i.e. severity) are given. Sampling periods marked with an asterisk (*) are missing DO data for one nursery site.

Hypoxic Events	2005 Sampling Period – Nursery Habitat Comparison														
	Early June*			Late June*			Early July			Late July			Early Aug.*		
	BC	LC	PC	BC	EF	LC	BC	EF	LC	PC	BC	EF	PC	EF	PC
<u>Frequency (#)</u>															
n	17	1	9	2	0	6	5	1	6	9	3	0	8	0	0
<u>Severity (hours)</u>															
Mean	5.4	0.5	1.6	5.5	---	3.5	12.9	4.0	3.2	1.2	4.3	---	10.5	---	---
SE	1.3	---	0.4	3.5	---	1.1	8.7	---	1.1	0.3	1.6	---	3.7	---	---
Max.	21.0	---	4.5	9.0	---	8.5	47.0	---	8.0	3.5	7.5	---	31.0	---	---
Min.	0.5	---	0.5	2.0	---	1.0	0.5	---	0.5	0.5	2.0	---	0.5	---	---

Table 9. Results from repeated measures ANOVA models on means of abiotic parameters observed in the four nursery sites over five sampling periods during the summer of 2005. Dependent variables marked with an asterisk (*) indicate models that failed to converge and therefore gave statistically meaningless results.

Dependant Variable	Effect	df	F	Pr > F
<i>Early June</i>				
Temperature*	Creek	3	---	---
DO	Creek	2	15.99	0.0001
Salinity	Creek	3	67.92	<0.0001
<i>Late June</i>				
Temperature	Creek	2	22.16	0.0011
DO*	Creek	2	---	---
Salinity*	Creek	2	---	---
<i>Early July</i>				
Temperature	Creek	3	1.70	0.4685
DO	Creek	3	0.33	0.8084
Salinity	Creek	3	70.43	<0.0001
<i>Late July</i>				
Temperature*	Creek	2	---	---
DO*	Creek	2	---	---
Salinity	Creek	2	178.29	<0.0001
<i>Early August</i>				
Temperature*	Creek	1	---	---
DO	Creek	1	9.04	0.1104
Salinity	Creek	1	---	---

Table 10. Results of ANOVA models on the severity of hypoxia observed in the four nursery sites over four sampling periods during the summer of 2005. Only creeks with two or more hypoxic events were used in analyses.

Dependant Variable	Effect	df	SS	F	Pr > F
<i>Early June</i>					
Duration of Hypoxia	Creek	1	91.557	4.56	0.0431
<i>Late June</i>					
Duration of Hypoxia	Creek	1	6.000	0.60	0.4698
<i>Early July</i>					
Duration of Hypoxia	Creek	2	459.149	2.50	0.1120
<i>Late July</i>					
Duration of Hypoxia	Creek	1	6.061	0.07	0.7992

Table 11. ANCOVA determined least-squares means and standard errors of total fish energy content (ln kJ) for young-of-the-year southern flounder collected in the four nursery sites over six sampling periods during the summer of 2005 adjusted to a common length for each sampling period. ANCOVA models for late May, early July, late July, and early August sampling periods compared nursery sites at a mean total length. Separate slopes ANCOVA models for early and late June sampling periods compared nursery sites at the 25th, 50th, and 75th percentiles of total length.

Period/Site	Adjusted Total Length Category							
	Mean		25 th Percentile		50 th Percentile		75 th Percentile	
	Energy	SE	Energy	SE	Energy	SE	Energy	SE
<i>Late May</i>								
BC	1.9932	0.0375	---	---	---	---	---	---
EF	1.9983	0.0171	---	---	---	---	---	---
LC	1.9951	0.0520	---	---	---	---	---	---
PC	1.9508	0.0437	---	---	---	---	---	---
<i>Early June</i>								
BC	---	---	2.3519	0.0318	2.6524	0.0290	2.8885	0.0281
EF	---	---	2.2976	0.0167	2.5981	0.0151	2.8342	0.0164
LC	---	---	2.2834	0.0368	2.5839	0.0342	2.8200	0.0333
PC	---	---	2.1270	0.0206	2.5274	0.0249	2.8420	0.0322
<i>Late June</i>								
BC	---	---	2.2742	0.0240	2.7497	0.0199	3.1763	0.0286
EF	---	---	2.4986	0.0222	2.8772	0.0195	3.2168	0.0202
LC	---	---	2.6535	0.0517	2.9813	0.0369	3.2753	0.0259
PC	---	---	2.5410	0.0201	2.9195	0.0227	3.2591	0.0272
<i>Early July</i>								
BC	3.2558	0.0444	---	---	---	---	---	---
EF	3.2222	0.0329	---	---	---	---	---	---
LC	3.0954	0.0349	---	---	---	---	---	---
PC	3.1051	0.0214	---	---	---	---	---	---
<i>Late July</i>								
BC	3.3144	0.0325	---	---	---	---	---	---
EF	3.3396	0.0393	---	---	---	---	---	---
PC	3.2152	0.0183	---	---	---	---	---	---
<i>Early August</i>								
EF	3.9824	0.0218	---	---	---	---	---	---
LC	3.9670	0.0300	---	---	---	---	---	---
PC	3.8405	0.0349	---	---	---	---	---	---

Table 12. Results of ANCOVA models on total energy content of young-of-the-year southern flounder collected in the four nursery sites over six sampling periods during the summer of 2005. Dependent variables marked with an asterisk (*) indicate models incorporating separate slopes.

Dependent Variable	Effect	df	Type III SS	F	Pr > F	Partial R²
<i>Late May</i>						
ln (Total Energy)	Creek	3	2.123	39.36	0.7958	---
<i>Early June</i>						
ln (Total Energy)*	Creek	3	4.241	257.21	<0.0001	0.2362
<i>Late June</i>						
ln (Total Energy)*	Creek	3	9.827	582.91	<0.0001	0.3686
<i>Early July</i>						
ln (Total Energy)	Creek	3	10.011	285.37	0.0008	0.4592
<i>Late July</i>						
ln (Total Energy)	Creek	2	2.168	117.31	0.0047	0.2174
<i>Early August</i>						
ln (Total Energy)	Creek	2	1.564	144.40	0.0104	0.2194

Table 13. Prey categories observed in the stomachs of young-of-the-year southern flounder collected from the four nursery sites during the summer of 2005. Top numbers represent the number of stomachs in each creek containing that prey item. Numbers in parentheses represent the percent occurrence of that prey item in stomachs (with food) analyzed for that creek.

Prey Category	Nursery Site			
	BC	EF	LC	PC
Mysids	26 (55.3)	85 (81.7)	12 (46.2)	48 (52.7)
Crab zoeae	---	---	---	2 (2.2)
Decapods	2 (4.3)	3 (2.9)	2 (7.7)	10 (11.0)
Gastropods	---	1 (1.0)	---	---
Amphipods	15 (31.9)	10 (9.6)	3 (11.5)	18 (19.8)
Isopods	1 (2.1)	1 (1.0)	---	---
Fish	6 (12.8)	7 (6.7)	9 (34.6)	15 (16.5)
# of stomachs with food / total # of stomachs	47/52	104/135	26/41	91/126

Table 14. Results of ANOVA models on mysid density in the four nursery sites for early June and late July of 2005.

Dependent Variable	Effect	df	F	Pr > F
<i>Early June</i>				
Mysid Density	Creek	3	5.40	0.0101
<i>Late July</i>				
Mysid Density	Creek	2	15.09	0.0005

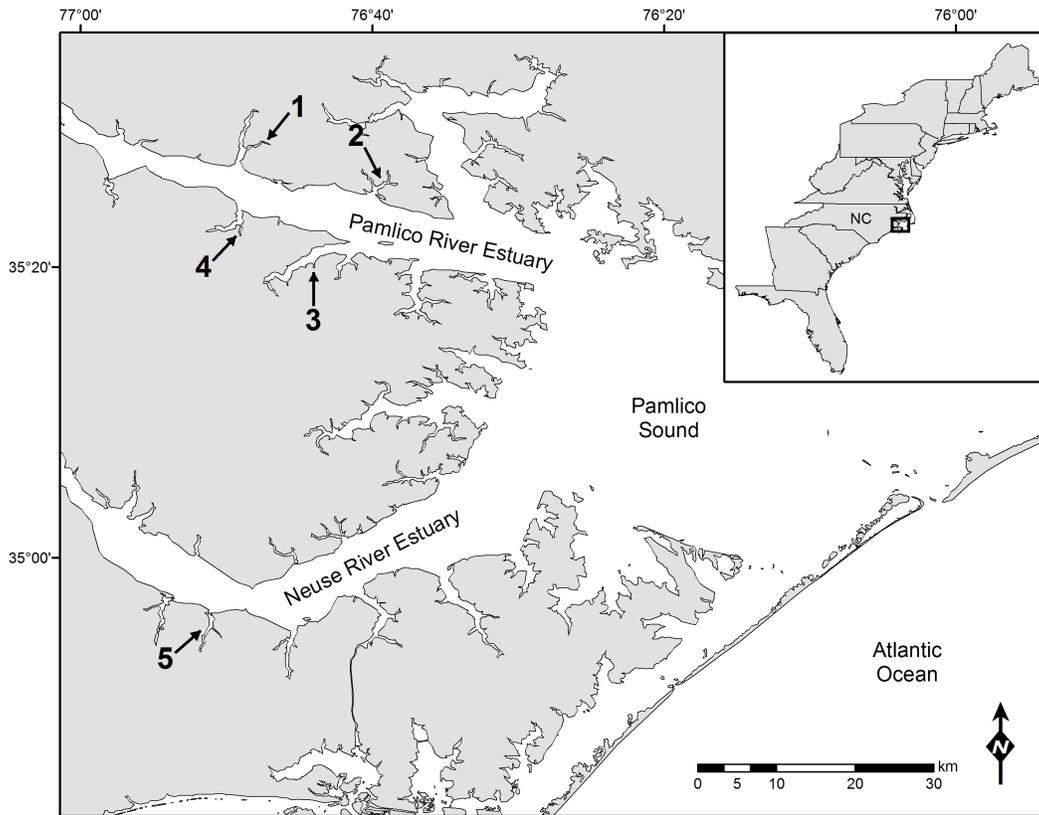


Figure 1. Map of the study sites in North Carolina, USA. Part of the study was conducted during 2005 in four nurseries of the Pamlico River Estuary: Back Creek (1), East Fork Creek (2), Long Creek (3), and Porter Creek (4). The other part of the study was conducted during 2006 in a nursery located in the Neuse River Estuary: Hancock Creek (5).

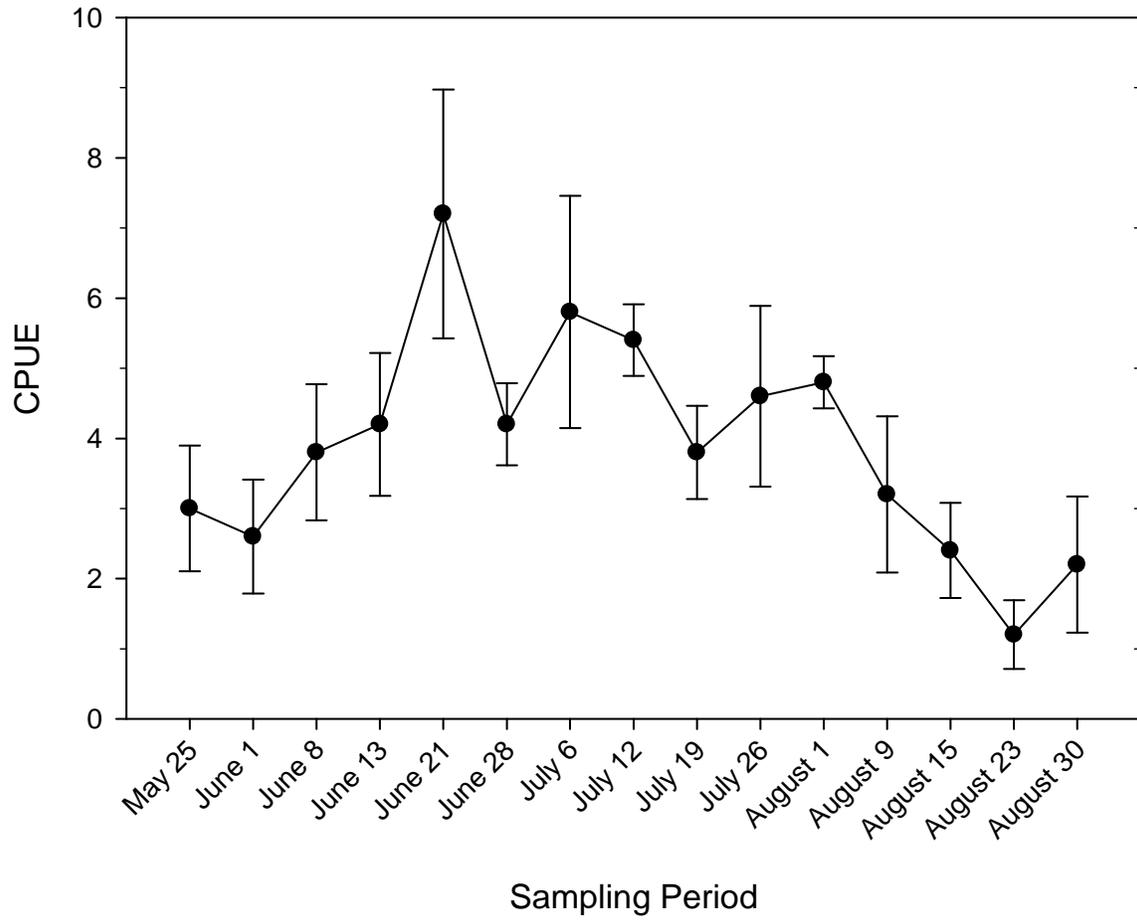


Figure 2. Mean (\pm SE) catch-per-unit-effort (CPUE) of young-of-the-year southern flounder in Hancock Creek during the summer sampling period of 2006.

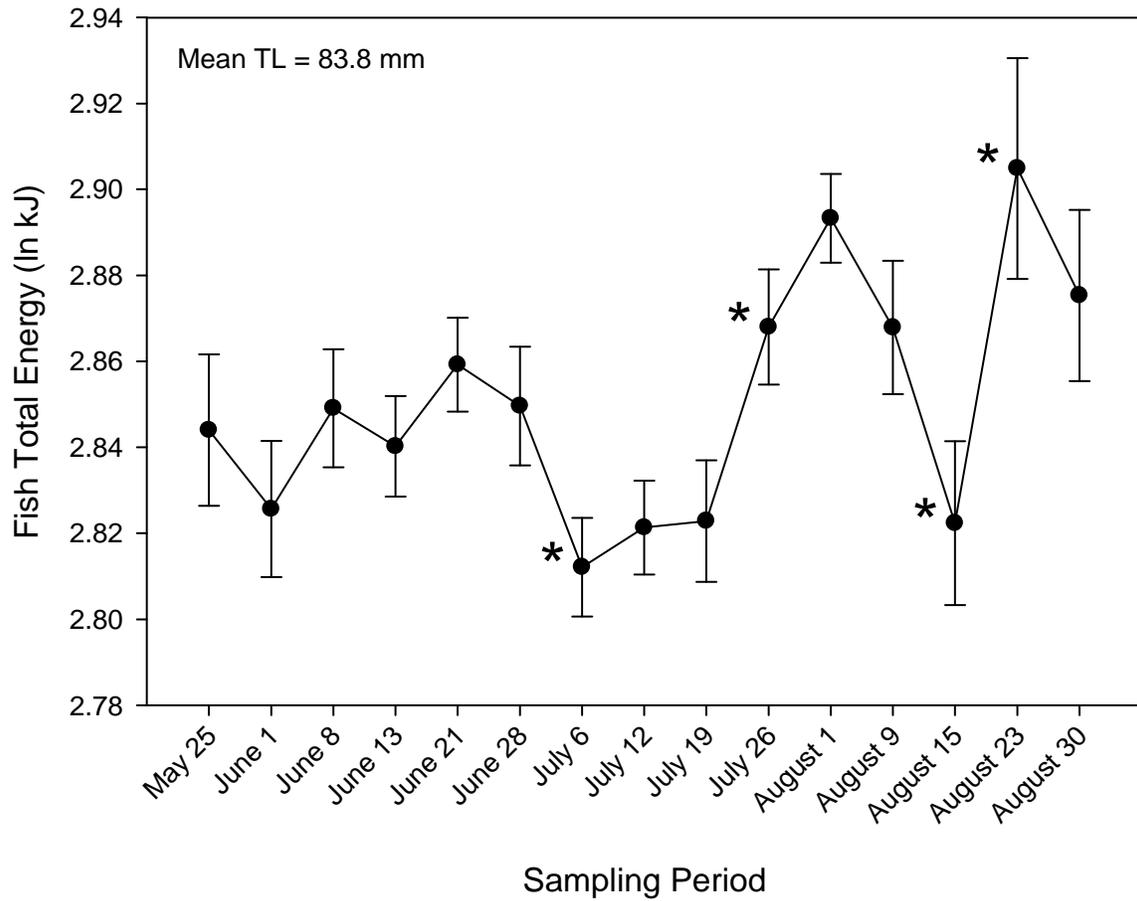


Figure 3. ANCOVA determined least-squares means (\pm SE) of total energy content adjusted to a common length over time for young-of-the-year southern flounder collected in Hancock Creek during the 2006 summer sampling period. LS means marked with an asterisk (*) are significantly different from the ls mean of the corresponding previous week.

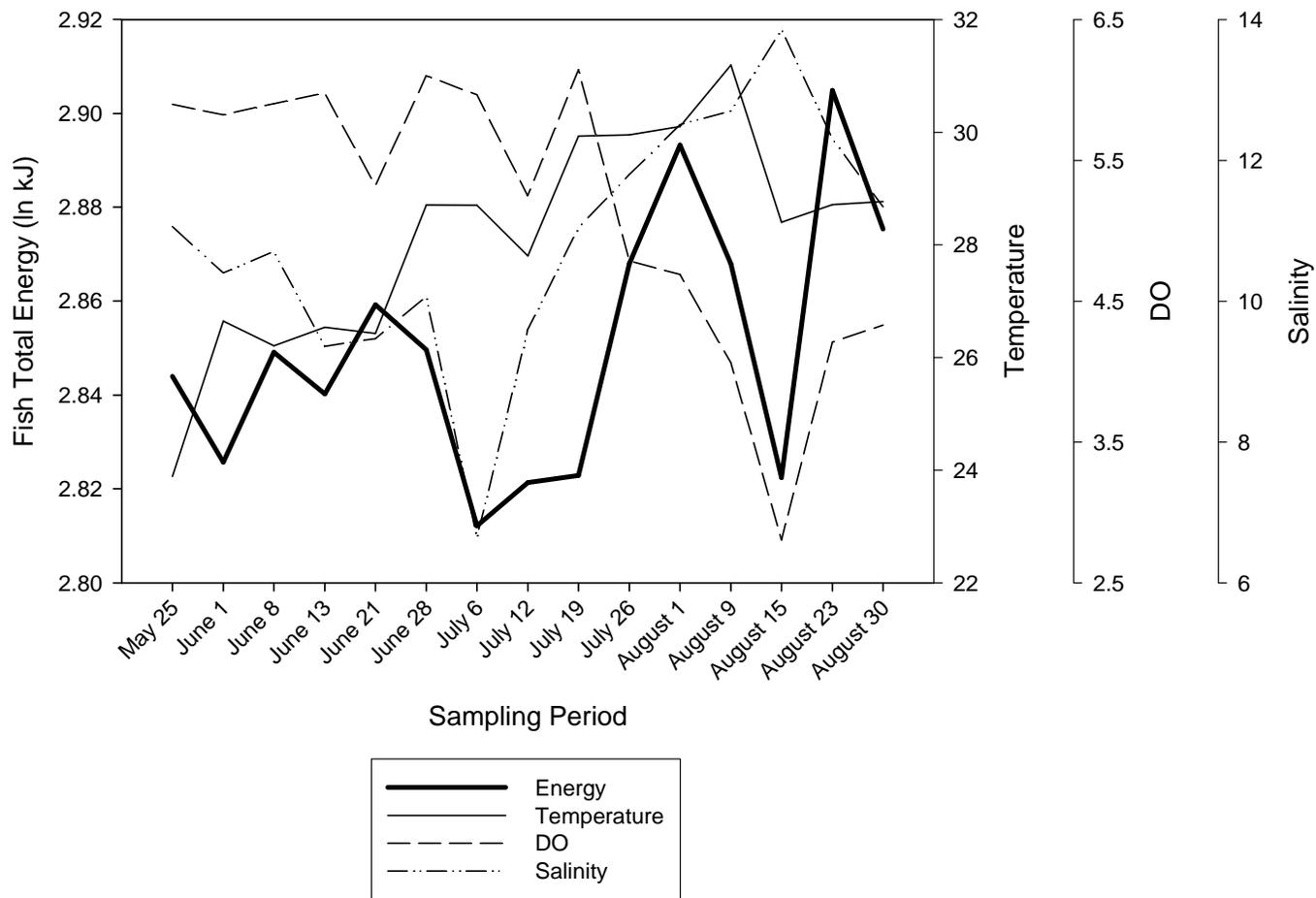


Figure 4. ANCOVA determined least-squares means of total energy content for young-of-the-year southern flounder in Hancock Creek and the weekly variability in mean temperature, mean DO, and mean salinity during the 2006 summer sampling period.

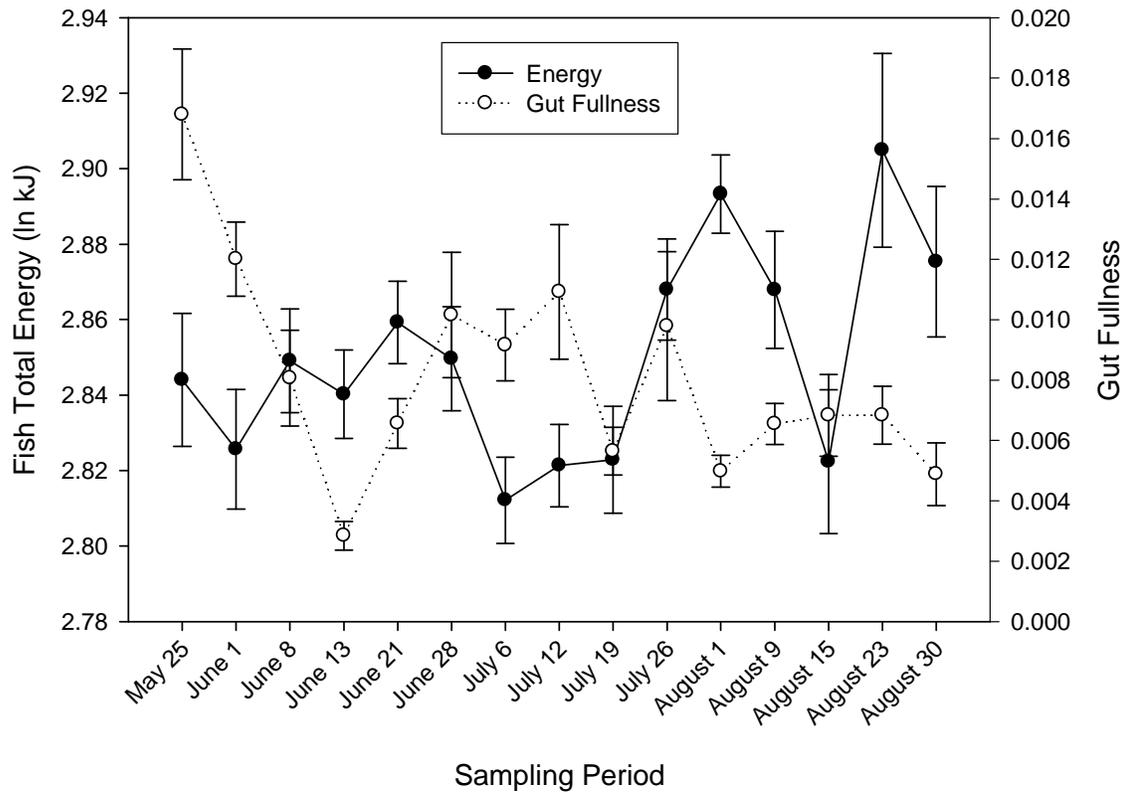


Figure 5. Weekly means (\pm SE) of gut fullness and ANCOVA determined least-squares means (\pm SE) of total energy content for young-of-the-year southern flounder collected in Hancock Creek during the 2006 summer sampling period.

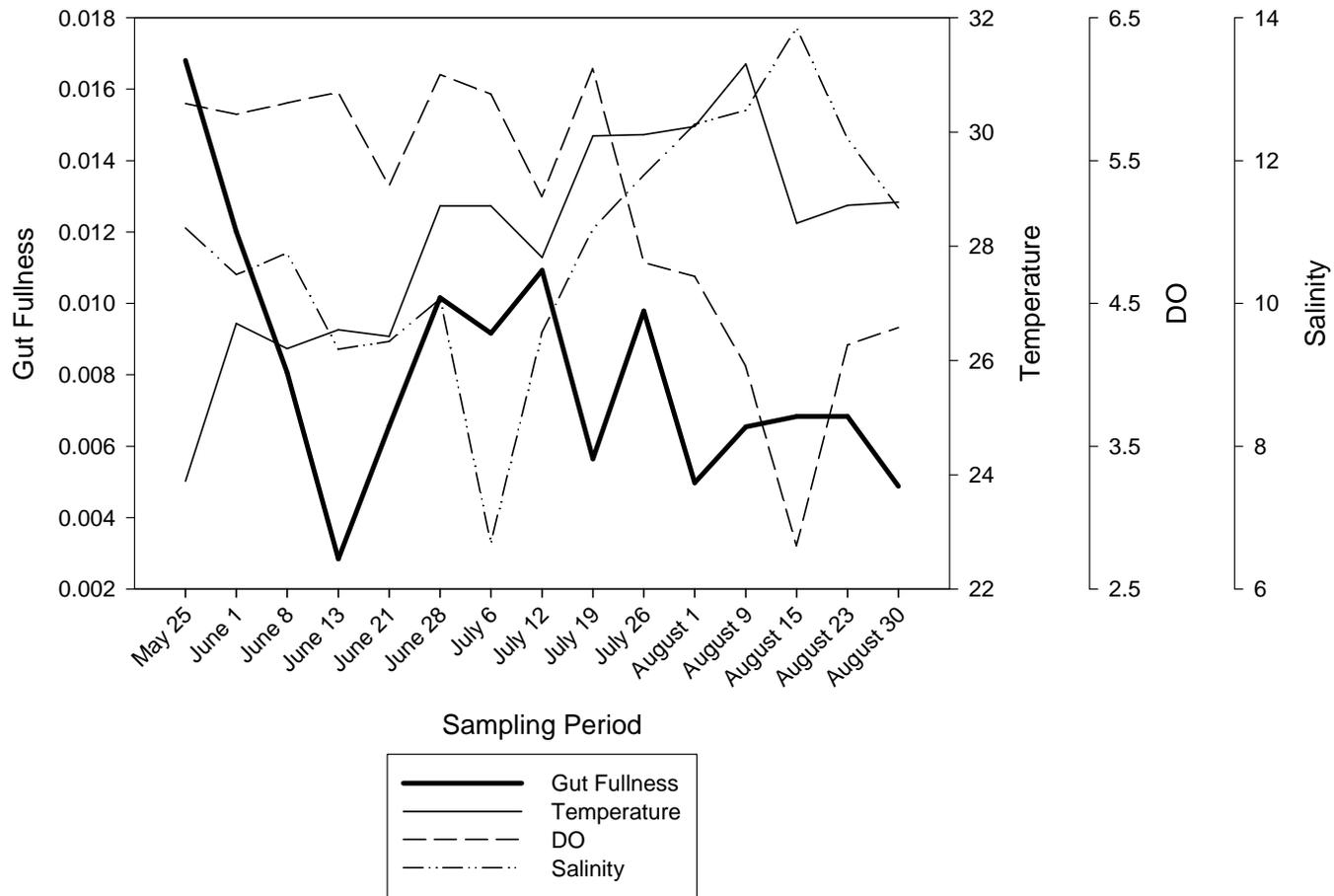


Figure 6. Weekly means of gut fullness for young-of-the-year southern flounder in Hancock Creek and the weekly variability in mean temperature, mean DO, and mean salinity during the 2006 summer sampling period.

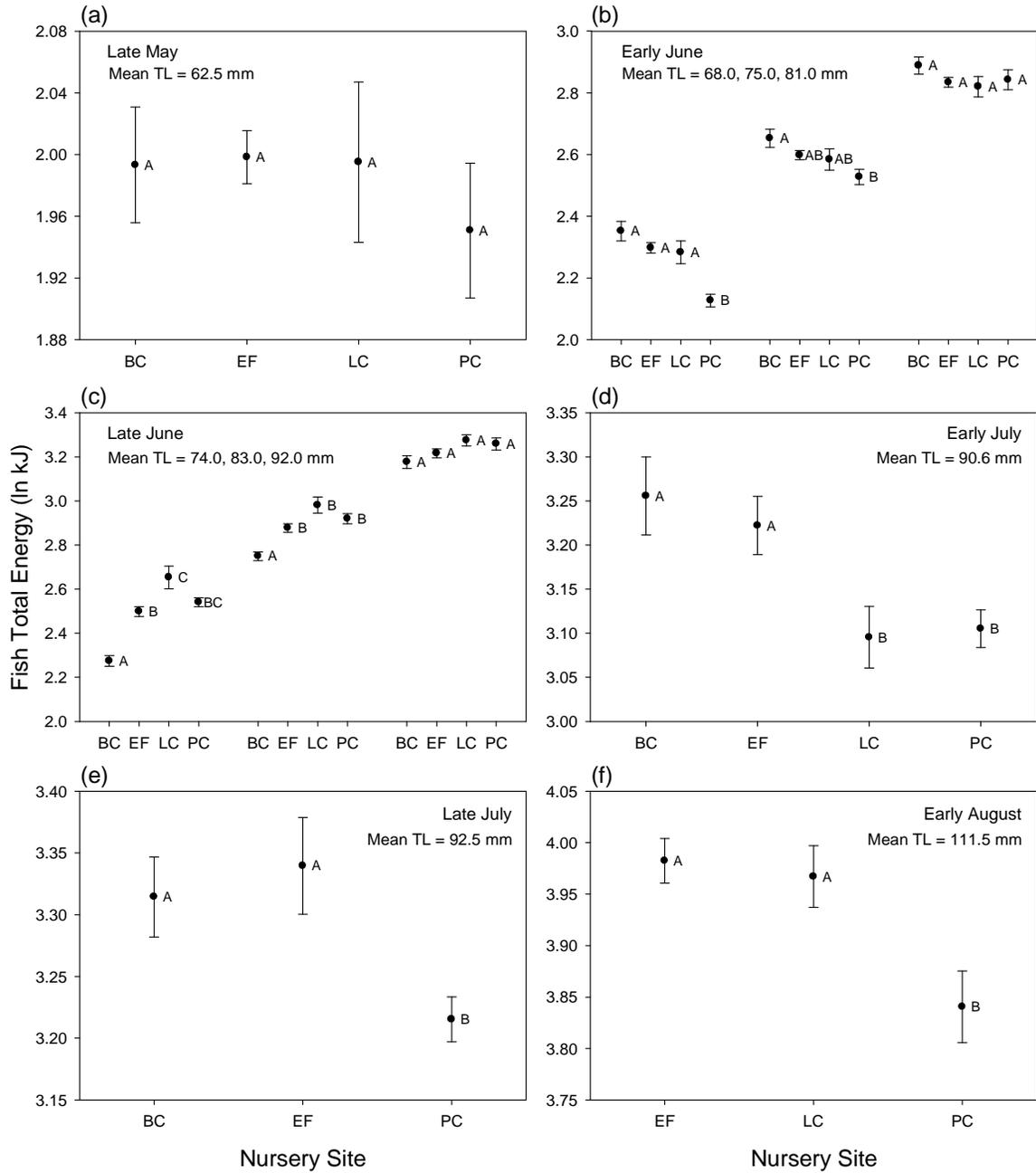


Figure 7. Results of ANCOVA pairwise comparisons of nursery sites for total energy content in young-of-the-year southern flounder collected during the six sampling events in the summer of 2005: Late May (a), Early June (b), Late June (c), Early July (d), Late July (e), and Early August (f). Least-squares means (\pm SE) adjusted to a common length are shown for each nursery site. Mean total length determined by ANCOVA are shown for each graph (a-f). Graphs (b) and (c) depict results of separate slopes models, therefore the three mean total lengths given and three plots represent the 25th, 50th, and 75th percentiles, respectively. Like letters indicate no significant difference ($\alpha = 0.05$) between nursery sites. Sites: Back Creek (BC), East Fork Creek (EF), Long Creek (LC), and Porter Creek (PC).

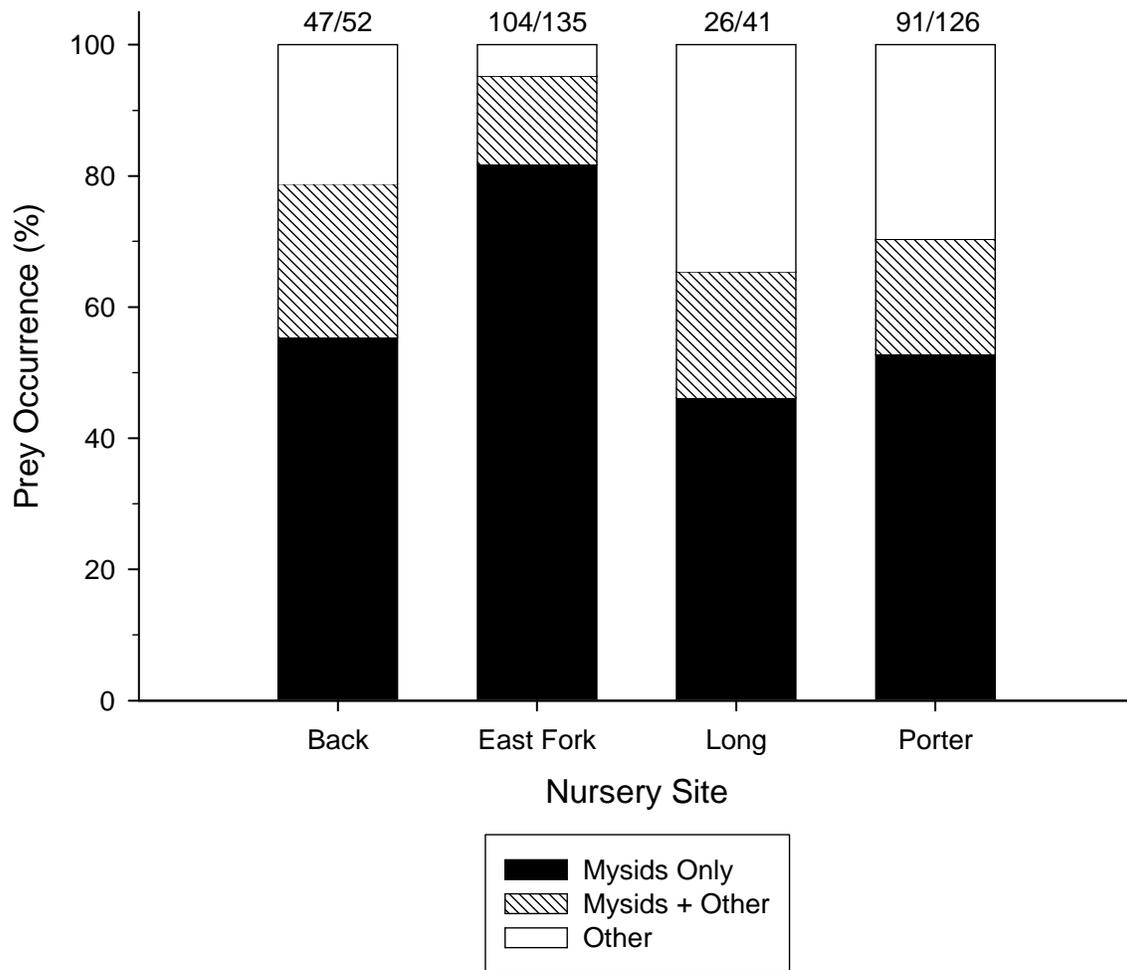


Figure 8. Prey occurrence (% of stomachs analyzed with food present) in young-of-the-year southern flounder stomachs collected in the four nursery sites during the summer of 2005. Numbers above bars indicate the number of stomachs with food present over the total number of stomachs analyzed for each nursery site.

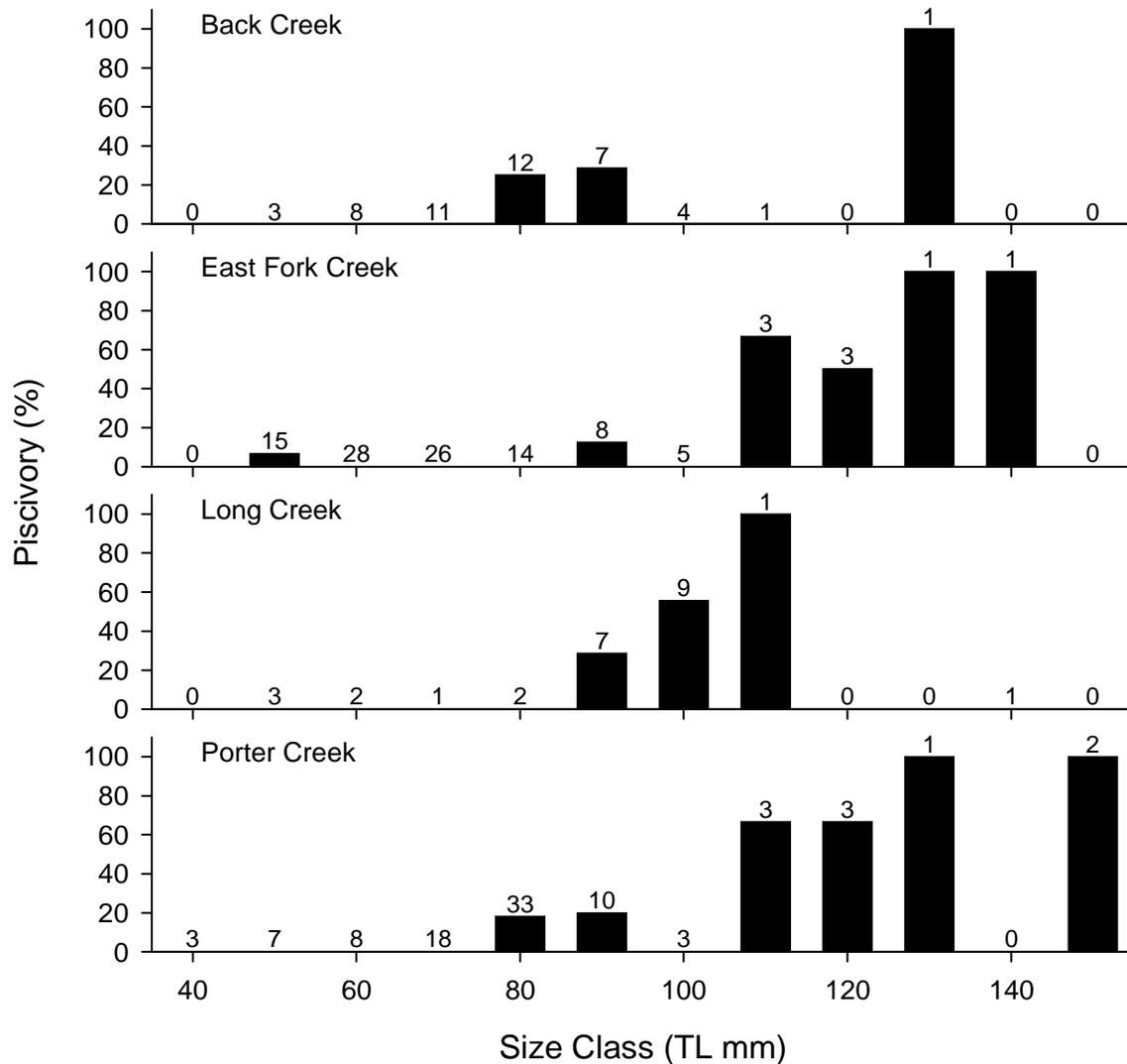


Figure 9. Onset of piscivory (%) for young-of-the-year southern flounder at 10 mm size-classes from 40 to 150 mm for the four nursery sites studied during the summer of 2005. Numbers above bars indicate the number of stomachs with food present analyzed for that size-class in that nursery site.

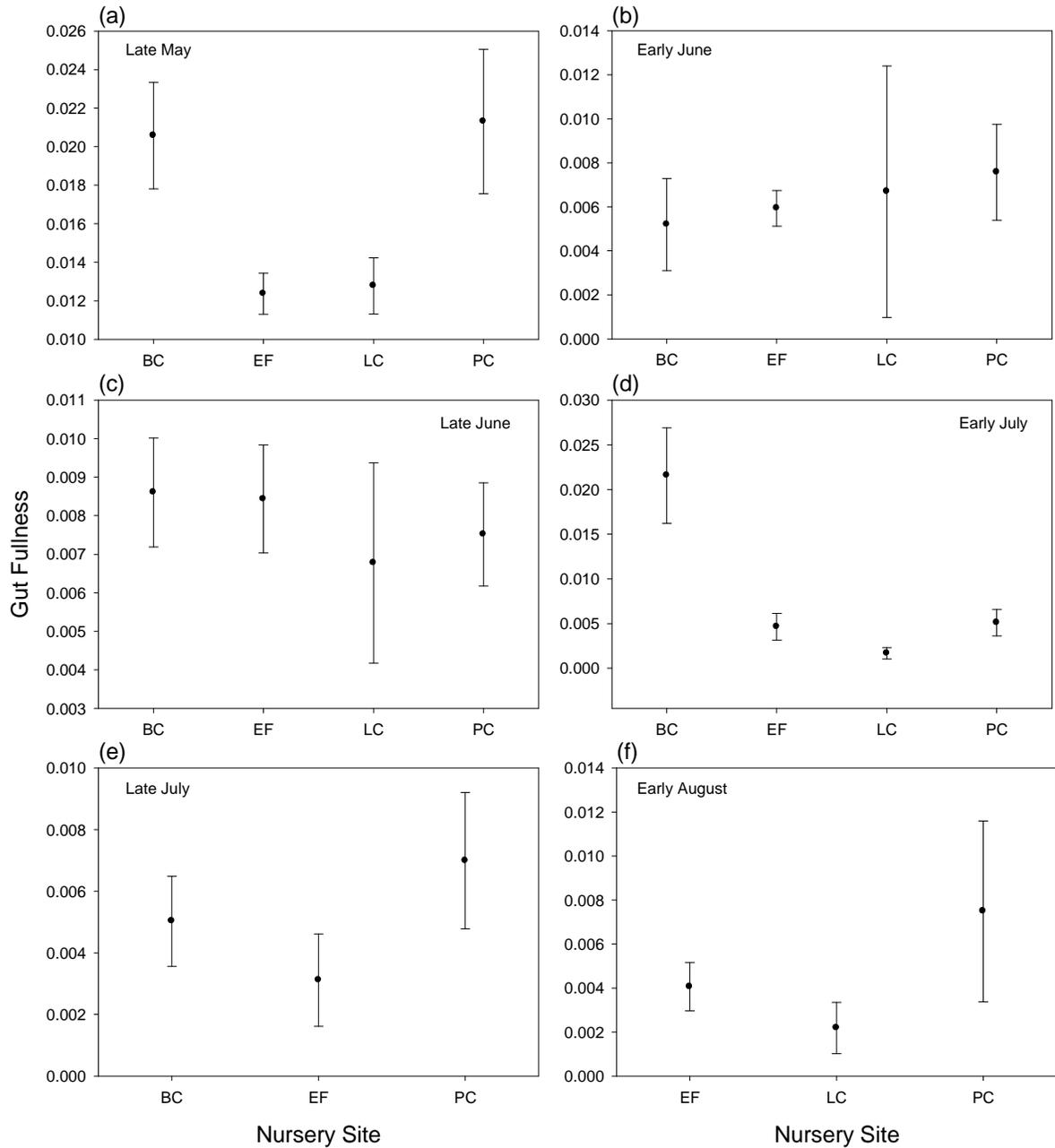


Figure 10. Mean (\pm SE) gut fullness of young-of-the-year southern flounder collected in the nursery sites compared during the six sampling events in the summer of 2005: Late May (a), Early June (b), Late June (c), Early July (d), Late July (e), and Early August (f). Sites: Back Creek (BC), East Fork Creek (EF), Long Creek (LC), and Porter Creek (PC).

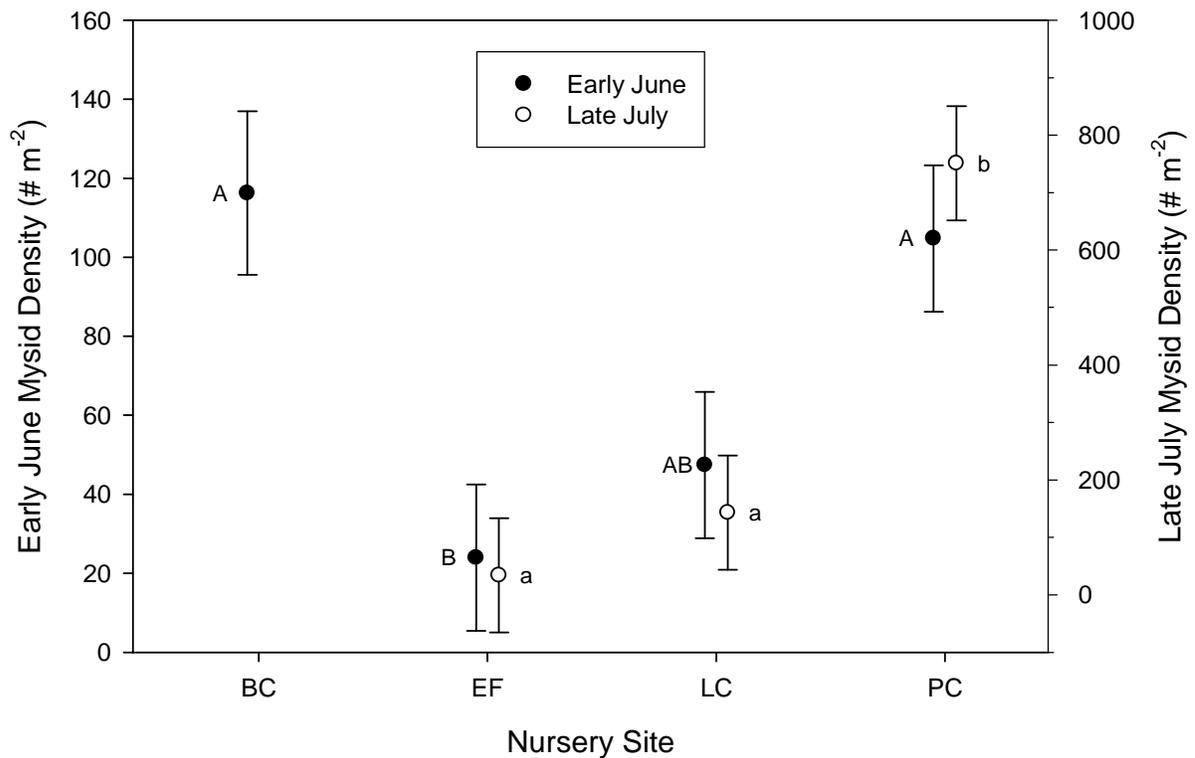


Figure 11. Results of ANOVA pairwise comparisons among nursery sites of mysid density for the two sampling events in the summer of 2005: Early June and Late July. Least-squares means (\pm SE) are shown for each nursery site. Like letters indicate no significant difference ($\alpha = 0.05$) between nursery sites, where upper-case letters correspond to early June samples and lower-case letters correspond to late July samples. Sites: Back Creek (BC), East Fork Creek (EF), Long Creek (LC), and Porter Creek (PC). No data were collected for BC in late July.

APPENDIX

Appendix: Energy Allocation and Diet of YOY Southern Flounder in North Carolina

The basic early life-history characteristics of southern flounder in North Carolina, including size-at-age, growth rates, hatch dates, and habitat preference, have been previously described (Powell and Schwartz 1977; Powell and Schwartz 1979; Miller *et al.* 1984; Pietrafesa and Janowitz 1988; Warlen and Burke 1989; Burke *et al.* 1991; Miller *et al.* 1991; Burke 1995; Fitzhugh 1993; Fitzhugh *et al.* 1996). There are, however, no studies that describe the energetics of wild YOY southern flounder. The purpose of this appendix is to present data collected by the author on the energy accumulation and subsequent energy allocation of YOY southern flounder in North Carolina. Relationships are made between energetic characteristics and body size. Diet data are also presented and can be compared to similar data presented by Fitzhugh (1993).

YOY southern flounder used in these energy and diet analyses were collected in 2005 and 2006 from creeks and bays that are part of the Albemarle-Pamlico Estuarine System (APES) and from other estuarine habitats not considered part of the APES but part of the larger lagoonal system encompassed by North Carolina's barrier islands, which includes the APES (Fig. A1). All sampling sites are classified as primary and secondary nursery areas by the North Carolina Division of Marine Fisheries (NCDMF) according to their juvenile fish abundance indices (Division of Marine Fisheries, NC, USA).

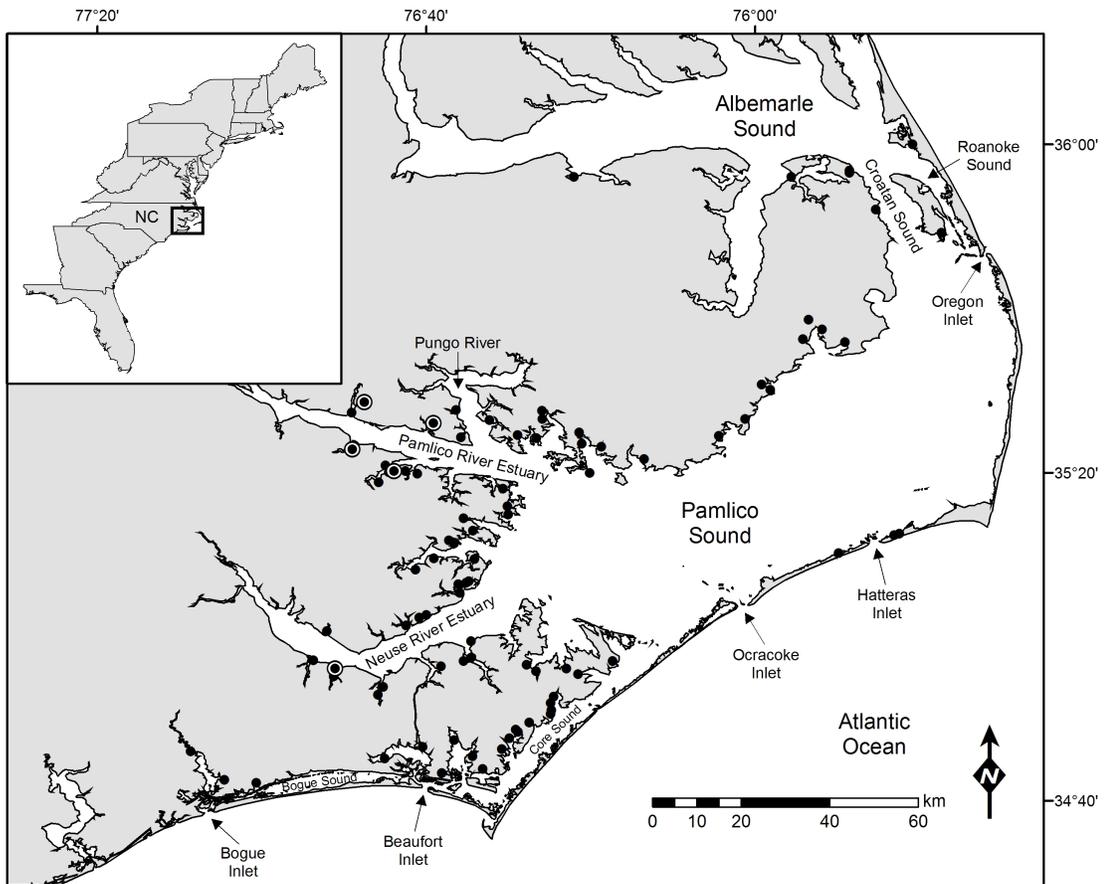


Figure A1. Map of the Albemarle-Pamlico Estuarine System (APES) in North Carolina, USA, and the 2005/2006 collection sites for YOY southern flounder located within the APES and the larger lagoonal system encompassed by North Carolina's barrier islands. Black dots represent NCDMF juvenile sampling sites where southern flounder were collected for use in energy analyses (approximately 35% of total sample). Ocellated black dots represent sites sampled by the author where the majority of southern flounder used for energy analyses were collected (approximately 65% of total sample).

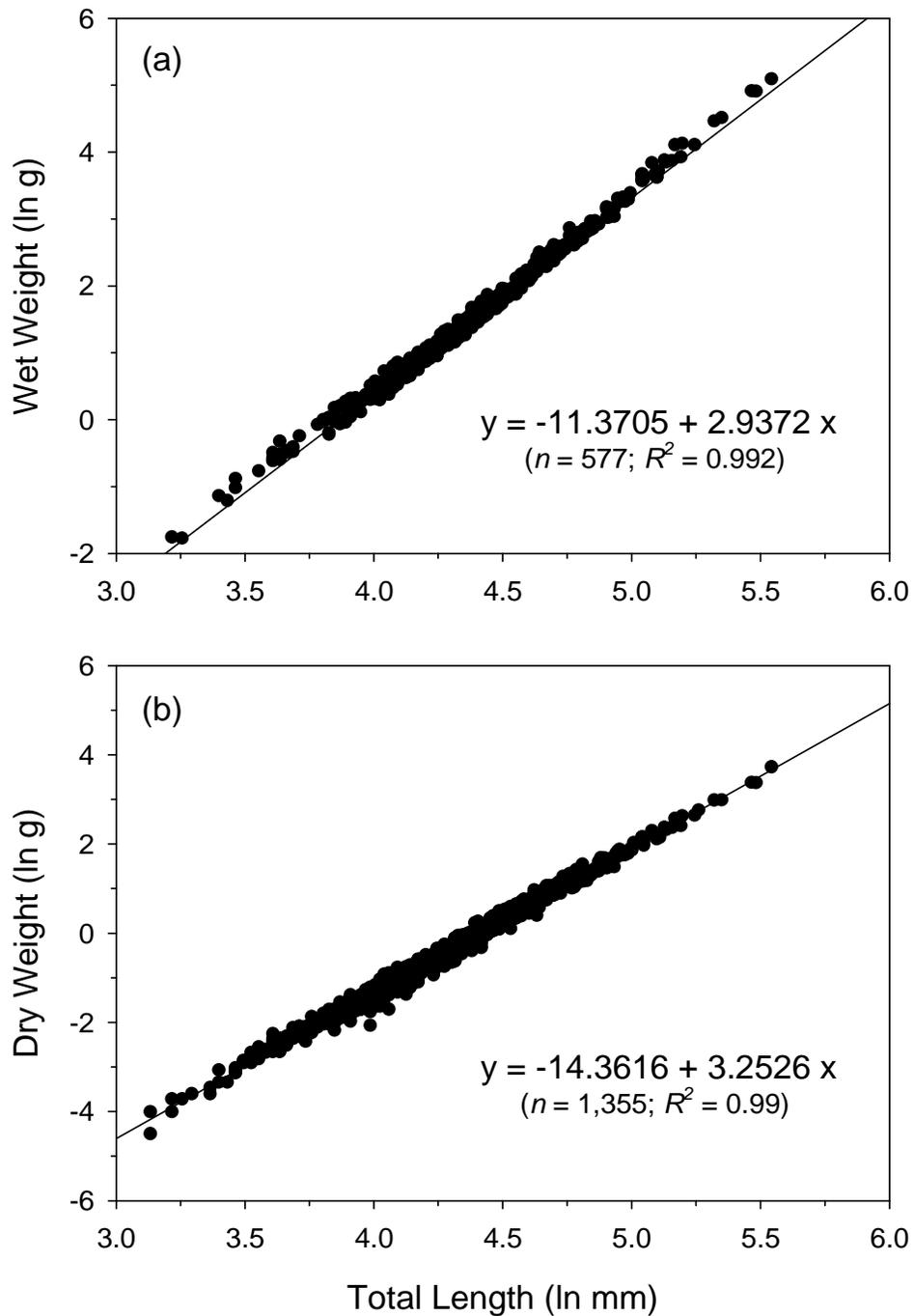


Figure A2. Linear relationships between YOY southern flounder total length and (a) wet weight and (b) dry weight. All data were ln-transformed to linearize the data. The equation of the line, the number of individuals used to establish the relationship, and the fit of the linear regression are shown.

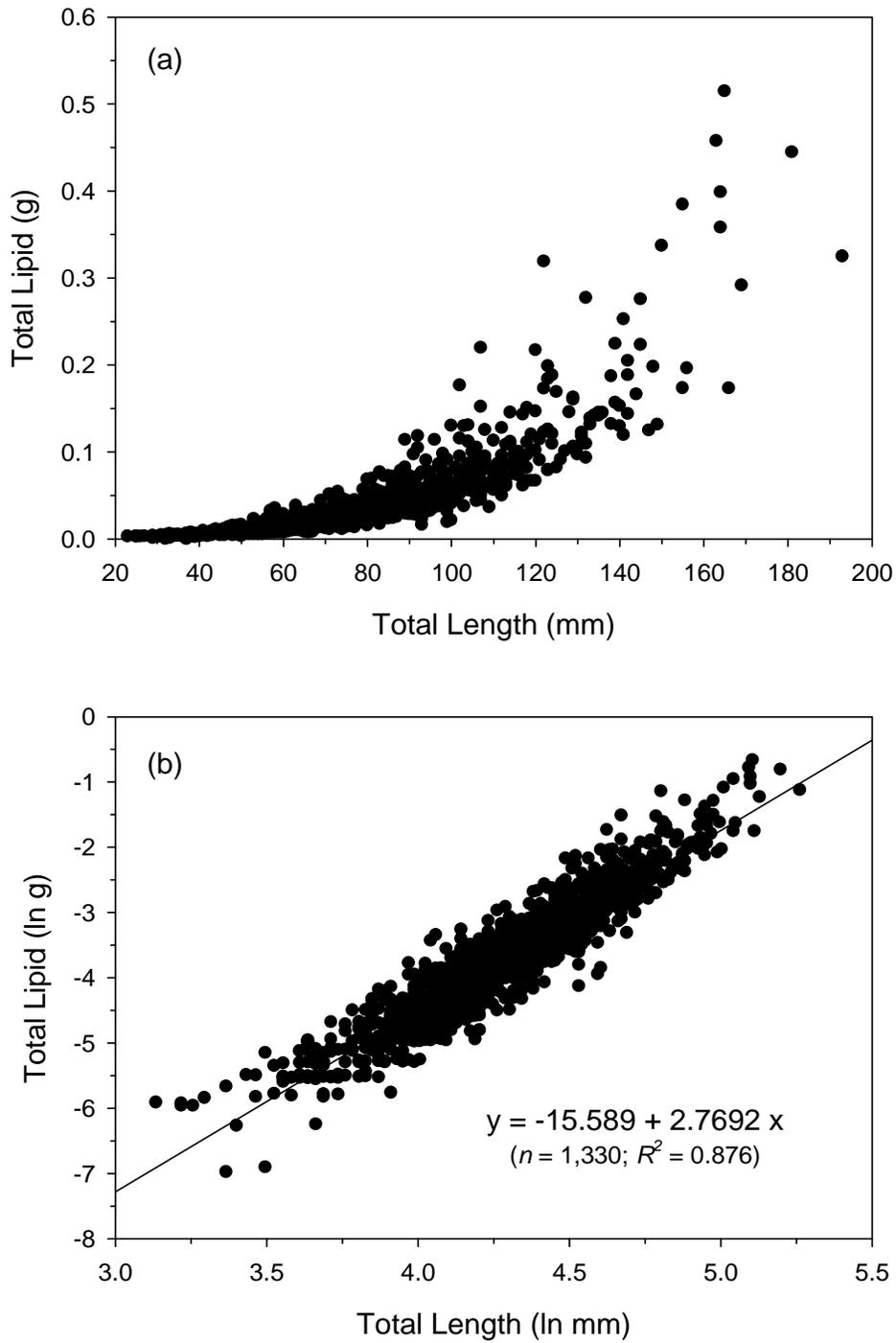


Figure A3. (a) The relationship between YOY southern flounder total lipid (g) and total length (mm). (b) All data were ln-transformed and the equation of the line, the number of individuals used to establish the relationship, and the fit of the linear regression are shown.

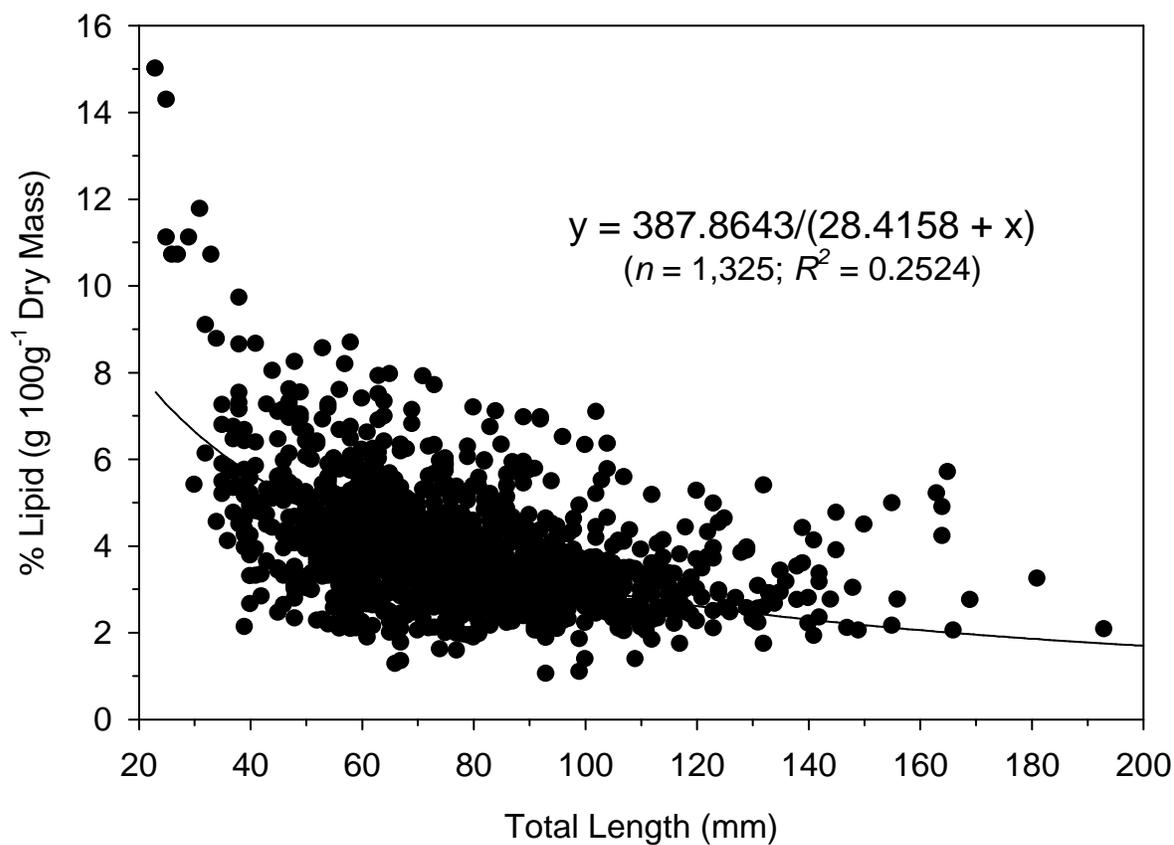


Figure A4. The relationship between the portion of dry mass constituting neutral lipids (%) and the total length (mm) of YOY southern flounder. A two parameter hyperbolic decay relationship was fit to the data. The equation of the line, the number of individuals used to establish the relationship, and the fit of the relationship are shown.

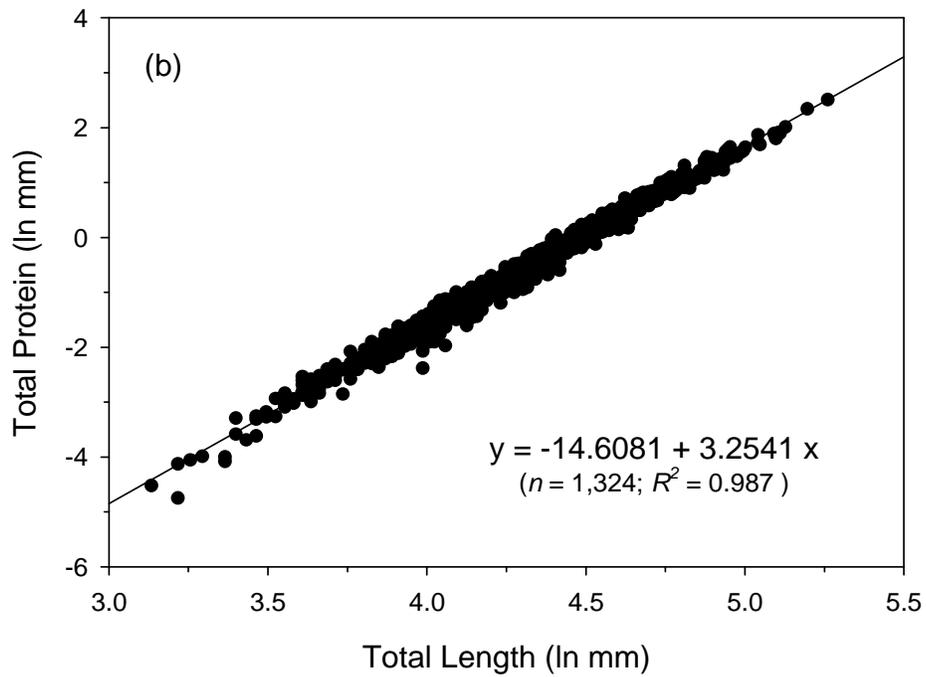
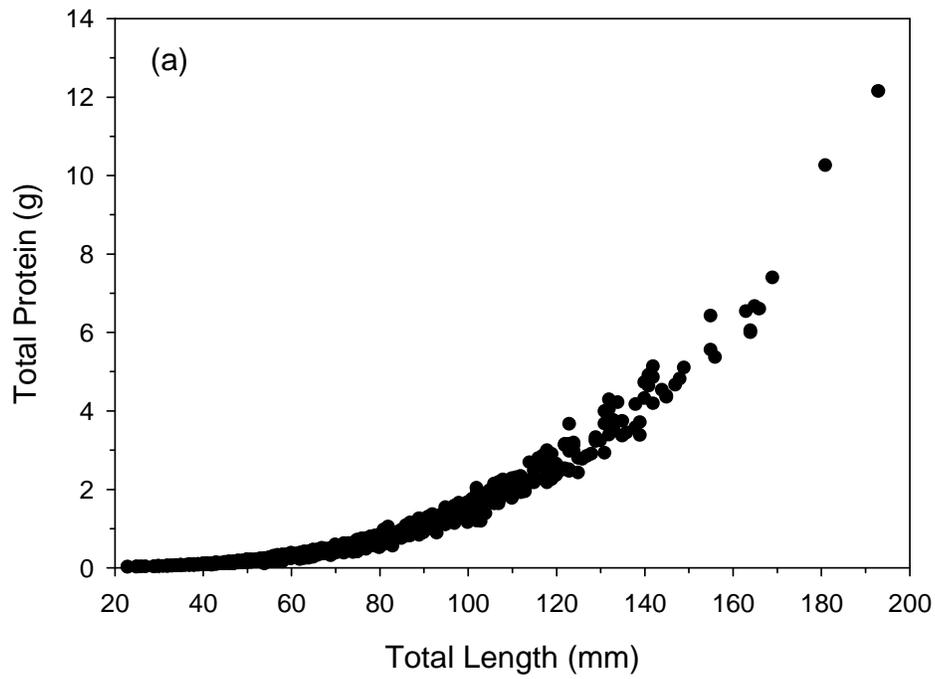


Figure A5. (a) The relationship between YOY southern flounder total protein (g) and total length (mm). (b) All data were ln-transformed and the equation of the line, the number of individuals used to establish the relationship, and the fit of the linear regression are shown.

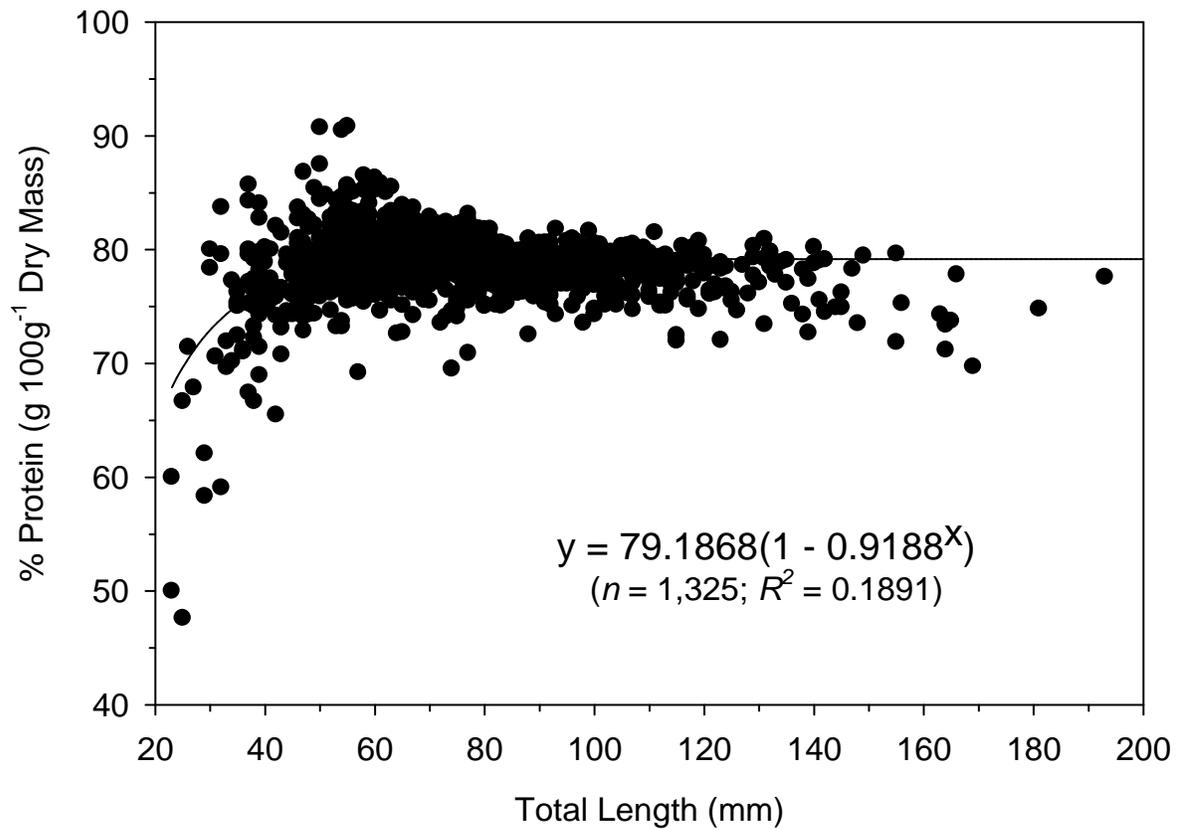


Figure A6. The relationship between the portion of dry mass constituting protein (%) and the total length (mm) of YOY southern flounder. A simple two parameter exponent relationship was fit to the data. The equation of the line, the number of individuals used to establish the relationship, and the fit of the relationship are shown.

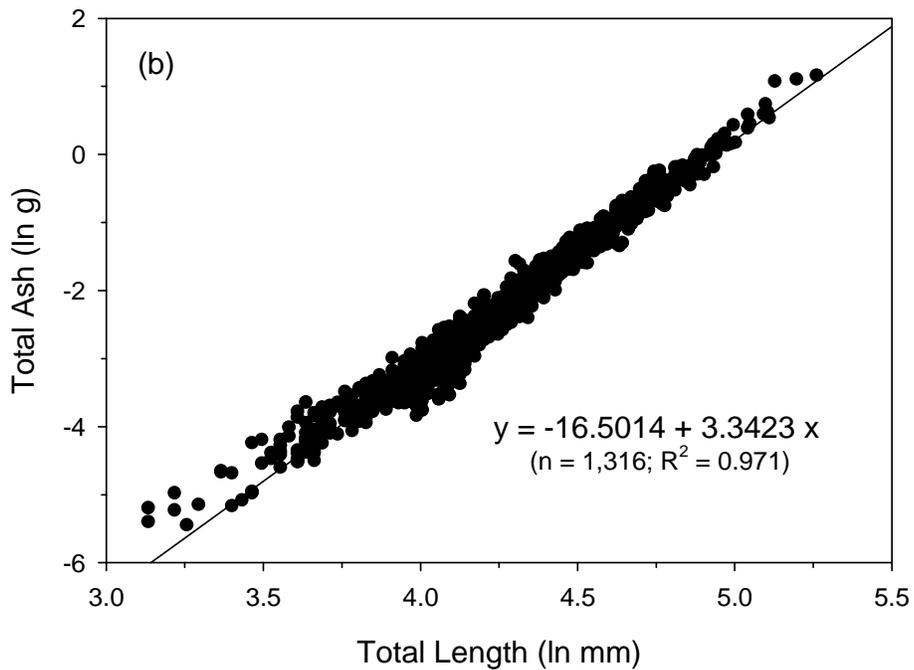
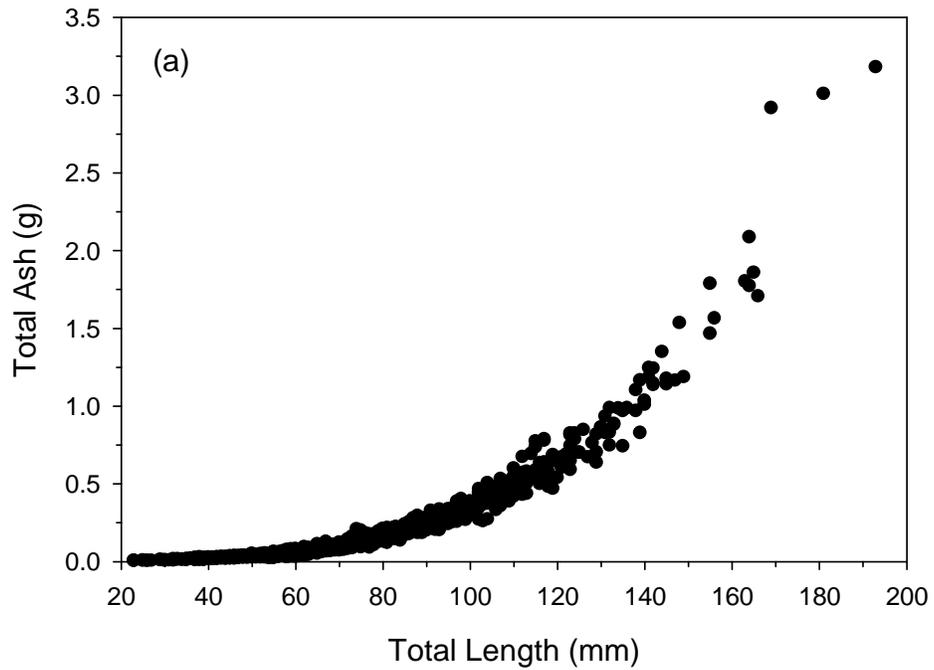


Figure A7. (a) The relationship between YOY southern flounder total ash (g) and total length (mm). (b) All data were ln-transformed and the equation of the line, the number of individuals used to establish the relationship, and the fit of the linear regression are shown.

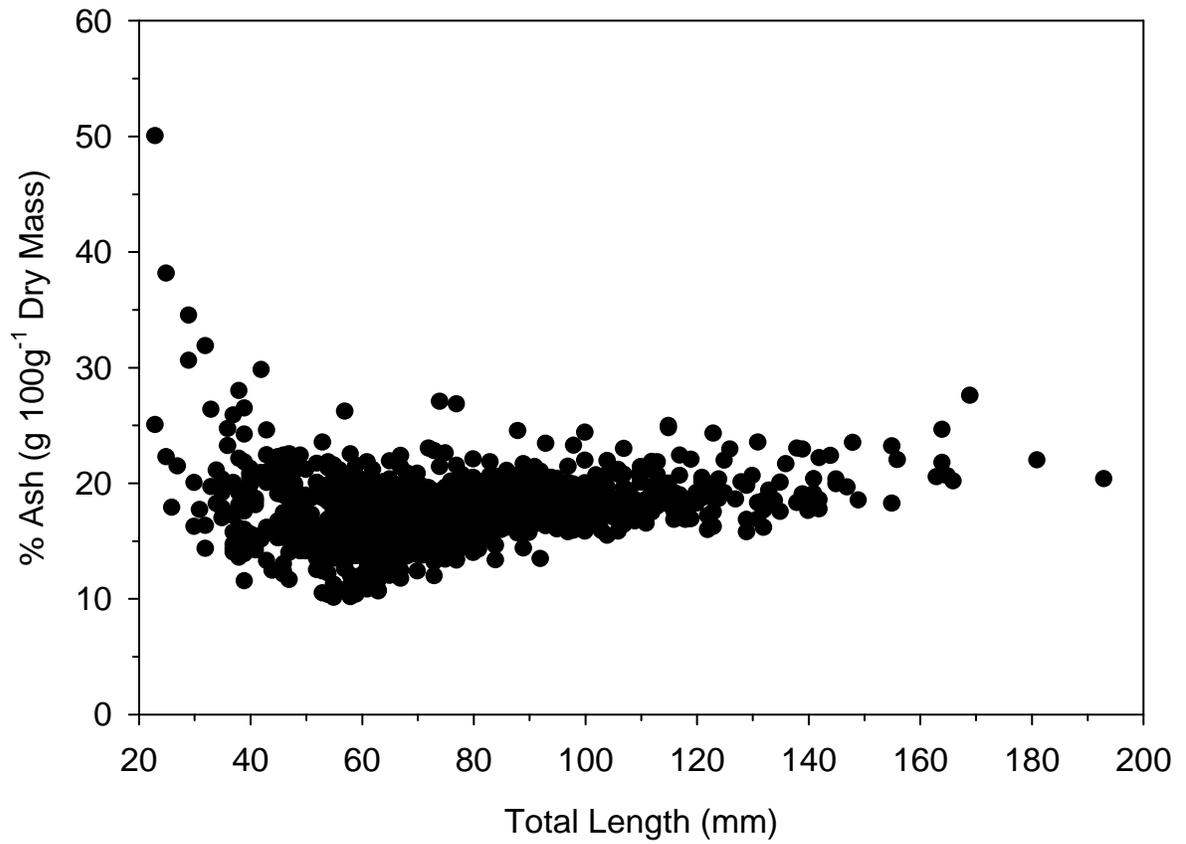


Figure A8. A plot of the portion of dry mass constituting ash (%) versus the total length (mm) of YOY southern flounder. All models fit to the data were unsuccessful at establishing a meaningful relationship between these two variables.

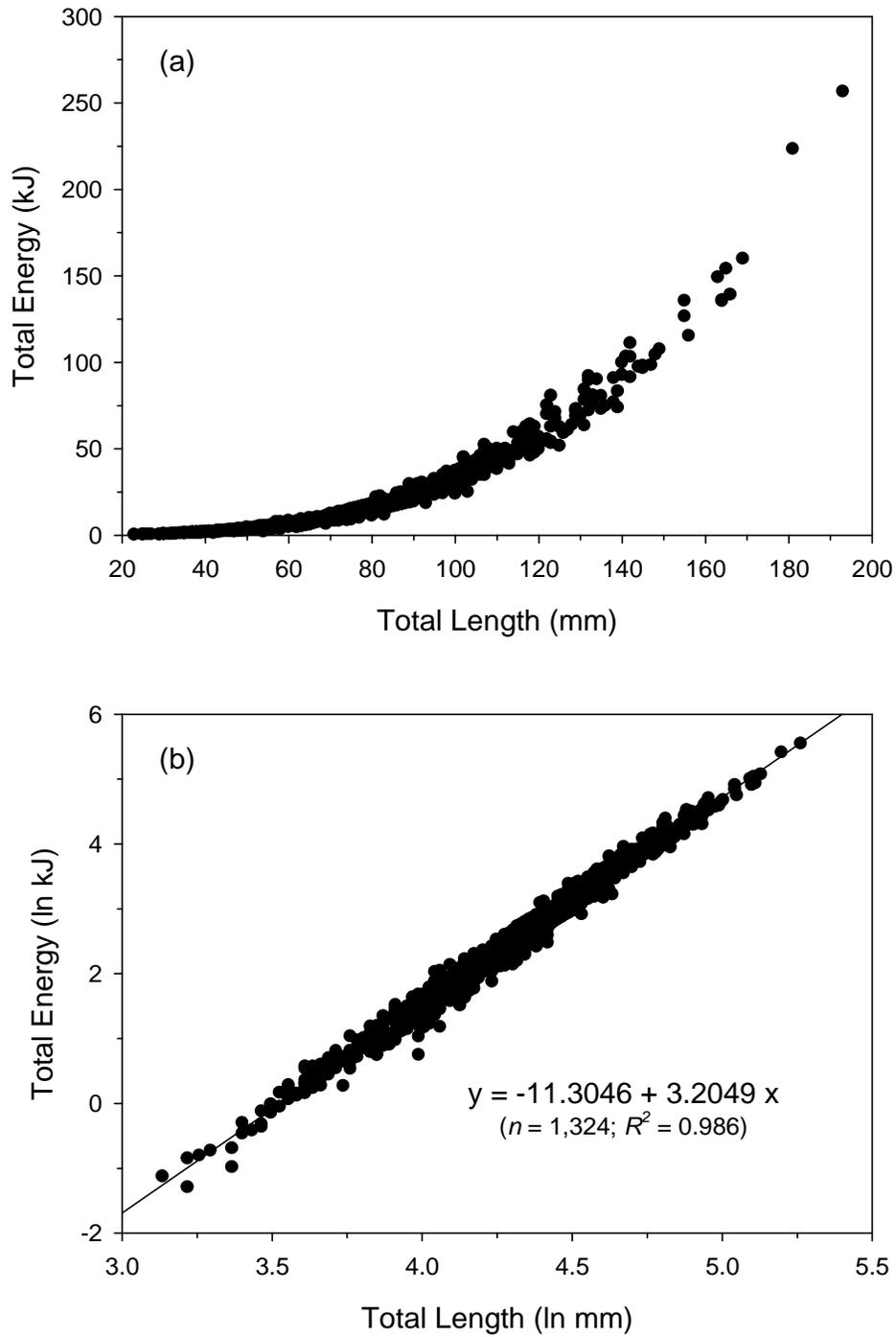


Figure A9. (a) The relationship between YOY southern flounder total energy content (kJ) and total length (mm). (b) All data were ln-transformed and the equation of the line, the number of individuals used to establish the relationship, and the fit of the linear regression are shown.

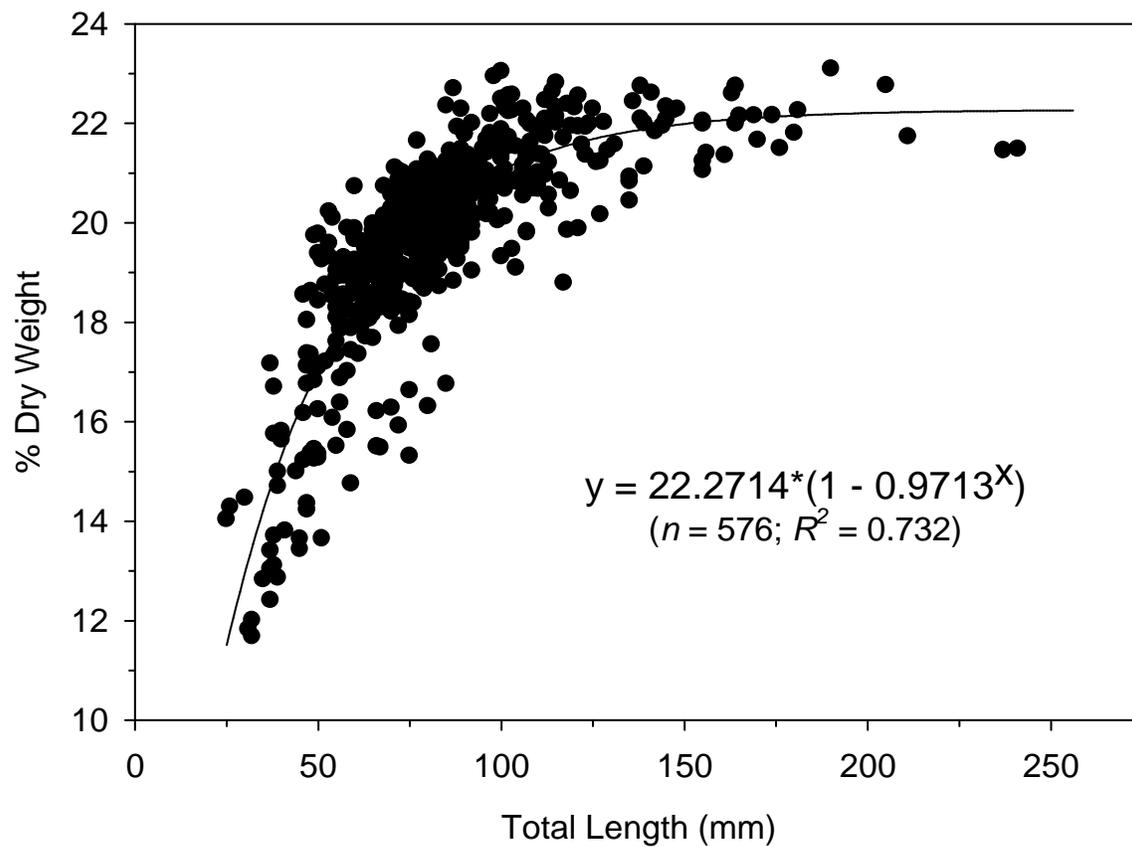


Figure A10. The relationship between percent dry weight and the total length (mm) of YOY southern flounder. A simple two parameter exponent relationship was fit to the data. The equation of the line, the number of individuals used to establish the relationship, and the fit of the relationship are shown.

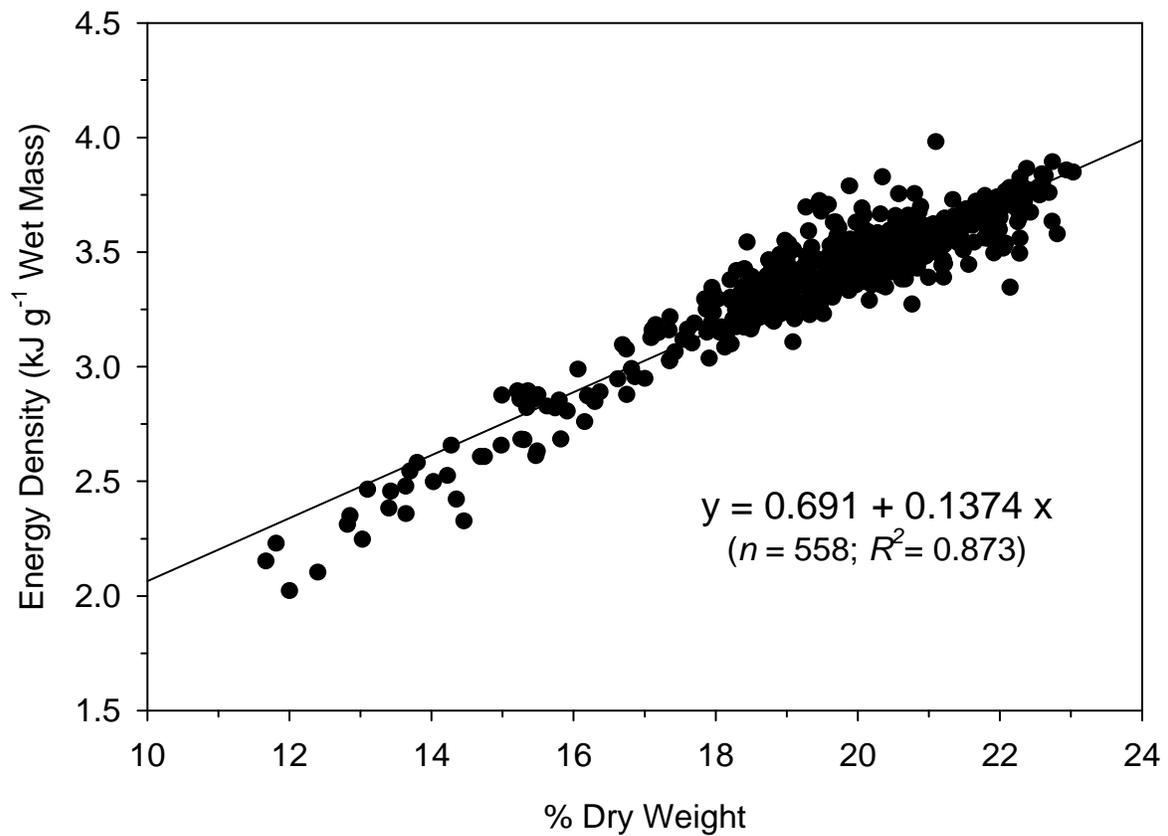


Figure A11. The linear relationship between YOY southern flounder energy density (kJ·g⁻¹ wet mass) and percent dry weight. The equation of the line, the number of individuals used to establish the relationship, and the fit of the relationship are shown.

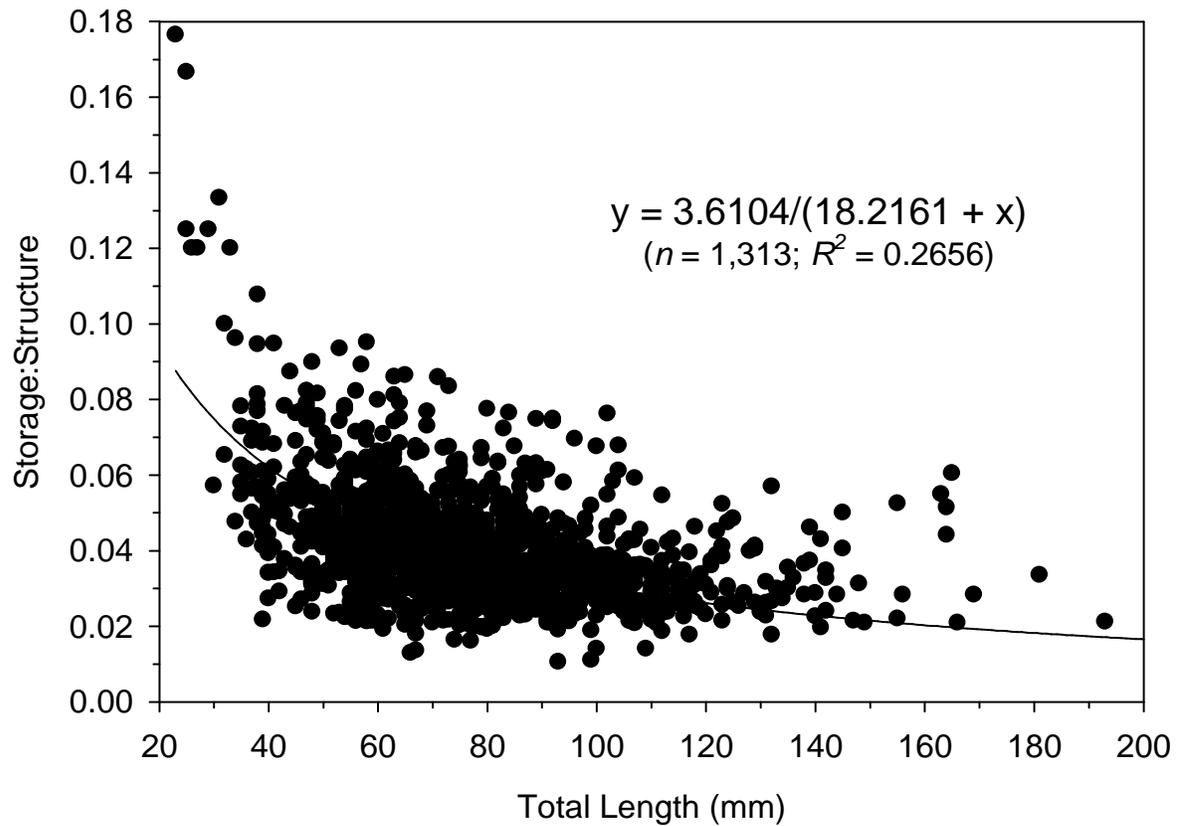


Figure A12. The relationship between the ratio of storage (total lipid) and structure (total protein + total ash) and the total length (mm) of YOY southern flounder. A two parameter hyperbolic decay relationship was fit to the data. The equation of the line, the number of individuals used to establish the relationship, and the fit of the relationship are shown.

Table A13. Prey categories observed in the stomachs of YOY southern flounder collected from nursery sites throughout NC in 2005 and 2006. The data were separated by 30 mm size-classes. Top numbers represent the number of stomachs in each size-class containing that prey item. Number in parentheses represent the percent occurrence of that prey item in stomachs (with food) analyzed for that size-class.

Prey Category	Size Class (TL mm)					
	21-50	51-80	81-110	111-140	141-170	171-200
Mysids	81 (83.5)	504 (90.6)	262 (81.4)	35 (62.5)	6 (35.3)	1 (33.3)
Crab zoea	---	1 (0.2)	1 (0.3)	---	---	---
Decapods	1 (1.0)	22 (4.0)	34 (10.6)	7 (12.5)	1 (5.9)	---
Gastropods	---	1 (0.2)	---	---	---	---
Amphipods	19 (19.6)	72 (12.9)	20 (6.2)	1 (1.8)	2 (11.8)	---
Isopods	---	2 (0.4)	1 (0.3)	---	---	---
Fish	1 (1.0)	30 (5.4)	55 (17.1)	20 (35.7)	11 (64.7)	2 (66.7)
# of stomachs with food / total # of stomachs	97/148	556/640	322/359	56/73	17/24	3/6

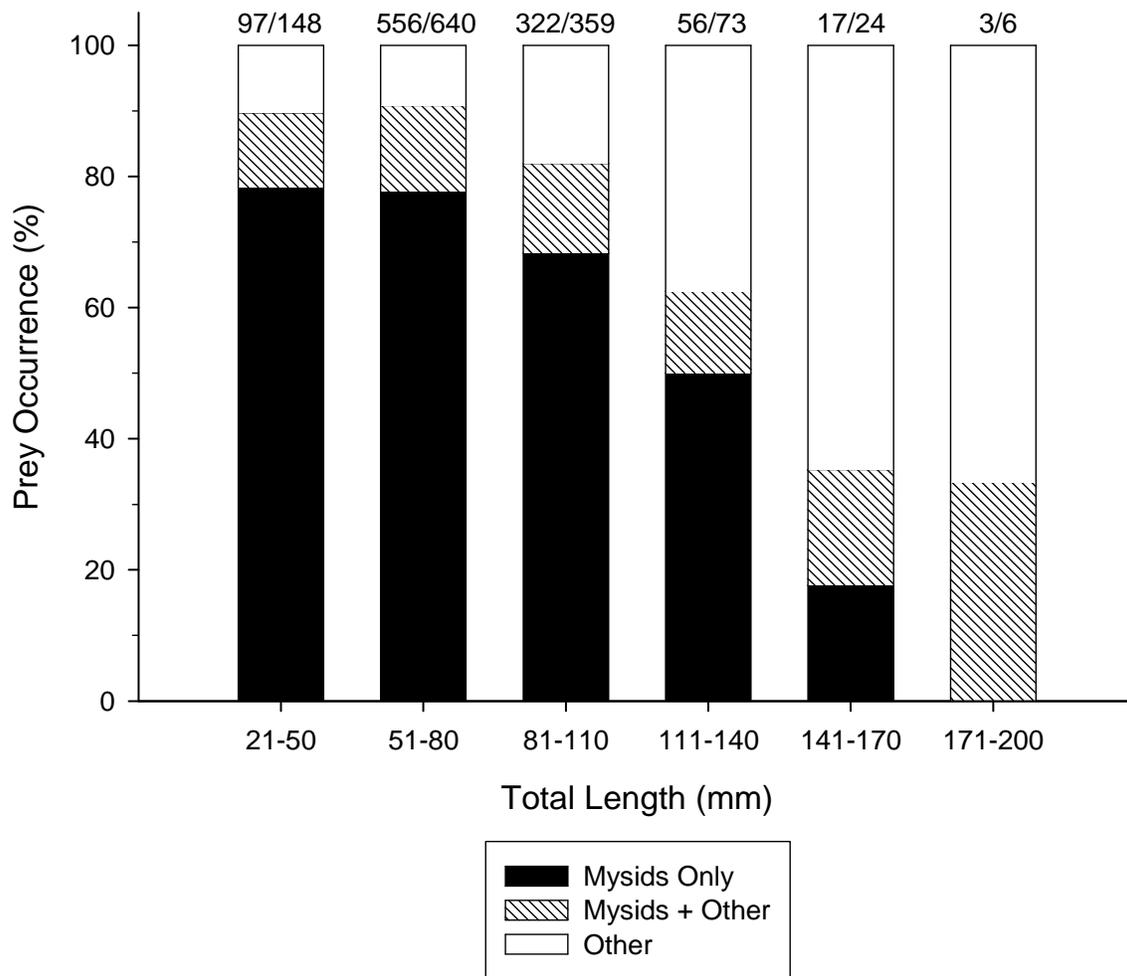


Figure A14. Prey occurrence (% of stomachs analyzed with food present) in YOY southern flounder stomachs (separated by 30 mm size-classes) collected in NC nurseries in 2005 and 2006. Numbers above bars indicate the number of stomachs with food present over the total number of stomachs analyzed for each size-class.

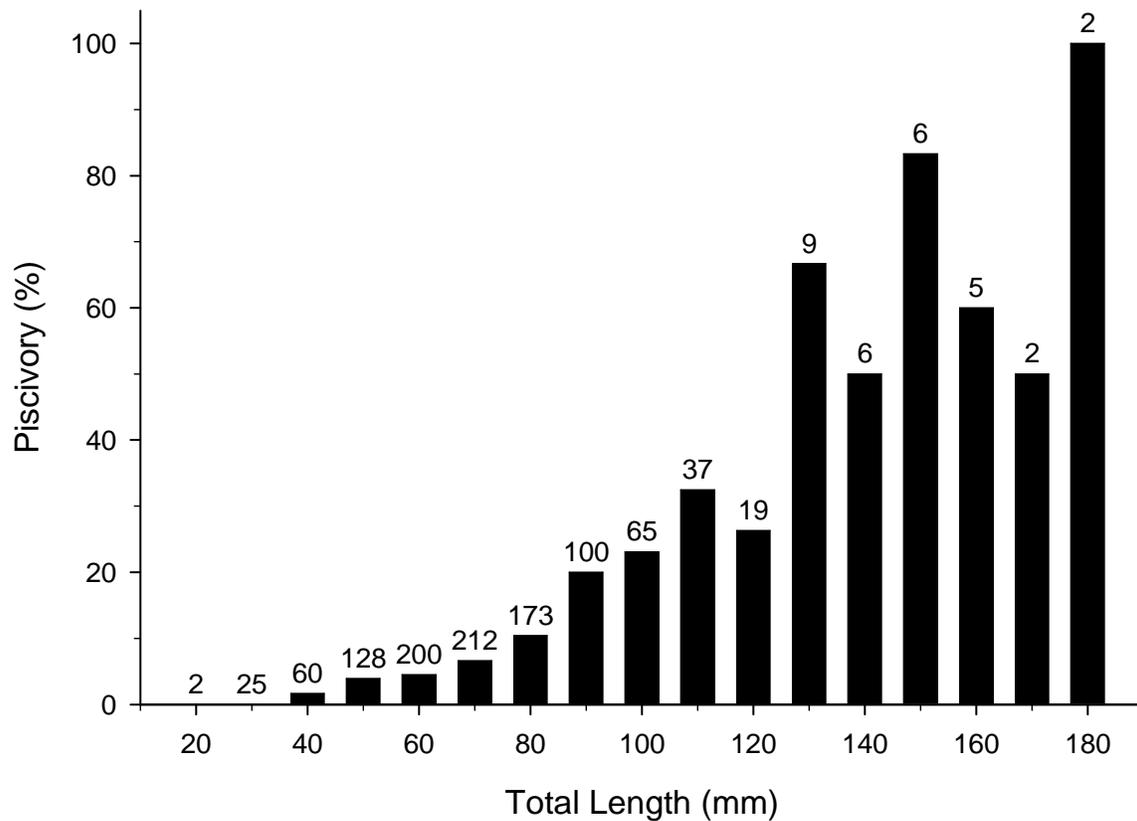


Figure A15. The onset of piscivory (%) for YOY southern flounder at 10 mm size-classes from 20 to 180 mm, collected from NC nurseries during 2005 and 2006. Numbers above bars indicated the number of stomachs with food present analyzed for that size-class.