ABSTRACT

MORLEY, JAMES WALTER. Overwintering Ecology of Two Intra-annual Cohorts of Juvenile Bluefish and their Relative Importance to the Adult Population. (Under the direction of Jeffrey A. Buckel).

Bluefish Pomatomus saltatrix are an ecologically and economically important species along the eastern United States. Uncertainties exist about juvenile life stages after the first growing season, especially the first winter. Historically, two intra-annual cohorts, from separate spring- and summer-spawning events, contributed to the adult population. However, the importance of the summer cohort appears to have declined. I first test the hypothesis that the summer cohort experiences negative size-selective mortality during winter, which would limit its contribution. Length distributions of juveniles from before and after winter showed that the mean length of the summer cohort increased during winter. Changes in length were due to selective mortality during a severe winter, and growth during a mild winter. Despite evidence for winter mortality, summer-spawned bluefish were commonly caught at age-1. I next examined the relative contribution of both cohorts to the adult population using archived scales from North Carolina fisheries. Cohort origins of adults were determined by back-calculating length at age-1. One third of adults consisted of summer-spawned fish, which contrasts with previous research from the northeast United States. I determined that the discrepancy was a result of sized-based migration of age-1 and age-2 bluefish; smaller individuals did not migrate north. Although I found summer-spawned fish contributing to the adult population, there were some years where the cohort was only a minor contributor. I next examined potential mechanisms for this variability. Spring-spawned bluefish were found to have an energetic advantage over the summer cohort because of high energy reserves during fall. However, spring-spawned fish depleted a much larger percentage of stored energy during winter. The average energy content of summer-spawned bluefish was related to winter severity, with fish suffering a greater energetic deficit during a severe winter. Next, the overwintering diet of each cohort was described for multiple year classes, and foraging ecology was examined by modeling gut fullness using generalized additive models. Striped anchovy were the most important prey for overwintering bluefish, and anchovy abundance was the primary predictor of
gut fullness. Thus, prey abundance is important for juvenile bluefish that feed to offset energy loss during winter.

I conducted an experiment to examine how winter temperatures affect the behavioral interaction between bluefish preying on two sizes of live bay anchovy *Anchoa mitchilli*. Capture success was not affected by temperature, but larger prey were dropped more frequently after capture, and drops were more common at lower temperatures. At the coldest treatment, no large prey were attacked because bluefish had difficulty handling large prey at low temperatures. This suggests that low winter temperatures restrict bluefish to smaller prey. I conclude that, contrary to results from sampling adults in the northeast United States, summer-spawned bluefish can be a substantial source of recruits to the adult population. However, severe winters can lead to a greater loss of smaller summer-spawned fish due to a greater depletion of energy levels.
Overwintering Ecology of Two Intra-annual Cohorts of Juvenile Bluefish and their Relative Importance to the Adult Population

by
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A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirement of the Degree of Doctor of Philosophy

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Chapter 1. Introduction

The first overwintering period is often a critical time for temperate fishes. For many species, the first winter can be the last period of high mortality before year class strength is stable. For example, young-of-the-year (Y0Y) abundance during the fall is often not correlated to age-1 abundance due to overwinter mortality (Hunt 1969; Conover and Ross 1982; Hurst and Conover 1998; Wiedenmann and Essington 2006). Winter mortality is often size selective, and smaller individuals are more at risk (Conover and Ross 1982; Griffiths & Kirkwood 1995; Hurst and Conover 1998; Chapter 2). This is because the allometries of both energy storage and metabolism favor large body size when fish rely on stored energy for winter survival (Hurst 2007). In many systems predation is another important source of mortality during winter (Santucci and Wahl 2003; Garvey et al. 1998), and it is often selective for smaller fish (Sogard 1997). Due to size-selective mortality, winter can alter the structure of a year class; individuals that are spawned earlier in the year, or that have a higher growth rate, may have an advantage during harsh winters (Conover 1990; Cargnelli and Gross 1996; Ludsin and DeVries 1997; Hurst and Conover 1998). In this dissertation, I examine how the overwintering period structures the recruitment of bluefish. I also examine the foraging ecology of bluefish during winter, and how it relates to energy storage patterns.

Bluefish *Pomatomus saltatrix* is a temperate migratory species of great economic and ecological importance on the United States east coast. A great deal of research has been conducted on bluefish during the last twenty-five years (Wuenschel et al. 2012). The motivation for this research was initially from a population crash that occurred during the late 1980s, which was associated with a large increase in recreational fishing pressure. A primary objective for the first management plan for bluefish (1990) was to increase understanding of the stock. Much of the research has been directed towards the reproductive strategy of adults and the ecology of juvenile recruits during their first growing season. Further, much attention has been directed at comparing the early life
history of the two major intra-annual cohorts, one from a spring-spawning period and one from summer spawning. The many smaller-scale studies conducted on YOY bluefish culminated in an ambitious United States coast-wide survey of juvenile bluefish recruitment and habitat use (Wuenschel 2012); this dissertation was conducted in conjunction with that coast-wide survey.

Available evidence supports the single-stock model for the U.S. east coast bluefish population. First, both genetic and morphometric comparisons of spring- and summer-spawned bluefish have revealed no differences between cohorts (Graves et al. 1992; Austin et al. 1999). Second, Chiarella and Conover (1990) sampled adult bluefish in spawning condition during summer off the northeast U.S. coast. However, these fish originating from the spring-spawning period, suggesting the cohort a bluefish originates from does not affect its own spawning strategy. The authors determined the spawning origin of adult bluefish by back-calculating age-1 length using scales (see Chapter 2). Last, studies on bluefish migration and reproduction both support a model for the population where bluefish exhibit a cessation of spawning during late spring coincident with the migratory period, which gives rise to two intra-annual cohorts (Shepherd et al. 2006; Robillard et al. 2008; reviewed in Wuenschel et al. 2012).

It has become clear that critical information is missing in bluefish life history that links our knowledge of juvenile recruitment with the spawning population. Conover et al. (2003) sampled adult bluefish and found that the majority of the population was made up of spring-spawned fish, despite juvenile abundance surveys that suggested recruitment was mostly summer-spawned fish. Further, Wiedenmann and Essington (2006) compared the abundance of YOY at the end of their first growing season with subsequent age-1 abundance and found evidence for density dependent mortality. Both of these studies suggested that important year class structuring was taking place during the overwintering period in the South Atlantic Bight (North Carolina to Florida). In response to this, Morley et al. (2007) sampled overwintering bluefish over two years off the coast of North Carolina. They found the summer cohort
to survive winter, and to be common off the North Carolina coast as age-1 fish. These results did not support the hypothesis of Conover et al. (2003), which stated that the summer cohort was experiencing high winter mortality. The work of Morley et al. (2007) suggested either summer cohort survival varied at a decadal scale, or that the sampling design of Conover et al. (2003) was biased in some way.

In Chapter 2, I resolve important issues regarding the relative contribution of the two intra-annual cohorts of bluefish to the adult population. Three year classes of juvenile bluefish were sampled during the overwintering period. This sampling was designed to look for evidence of size-selective mortality of the summer cohort during winter, using monthly length distributions. My results support previous work that summer-spawned fish appear to survive winter and that they are abundant off North Carolina as age-1 fish (Morley et al. 2007). However, during severe winters, year class structuring does occur, as evidence for significant size selective mortality of the summer cohort was found. This suggests that body size is important for survival for overwintering bluefish.

I also had the unique opportunity to examine how size-based migration strategies among age-1 and age-2 bluefish can lead to spatial sampling bias of adult bluefish. I examined an archived collection of age-1+ bluefish scales taken from North Carolina fisheries during the late 1990s. The collection years of these archived scales overlapped with Conover et al. (2003) samples, which used scales to estimate the spawning origin (spring versus summer) of adult fish by back-calculating length at age-1. Conover et al. (2003) used samples taken mostly from the northeast United States and found poor contribution from the summer cohort. Conversely, the North Carolina samples showed the summer cohort to represent one third of adult fish when averaged over six year classes. The discrepancy between studies is due to the smaller summer-spawned fish being less likely to migrate north at age-1 and age-2. Although I found summer-spawned fish to contribute substantially to the adult population in most years,
there were some year classes where summer spawning only made a minor contribution and I examined potential mechanisms for this variability.

Energy storage dynamics are of particular interest when examining survival of overwintering fishes (Hurst 2007). However, the indicators of energetic status commonly used, such as condition factor or hepatosomatic index, are often biased in representing the status of the entire fish (e.g. Sutton et al. 2000; Plante et al. 2005). In Chapter 3, I relate multiple indicators of energetic status to total body energy to develop a predictive model for total body energy based on a cost effective energetic indicator. I also examined how the proximate composition of bluefish (lipid, protein, and water) changes across a range of energetic states.

The predictive equation developed in Chapter 3 was used to examine the energetic status of bluefish throughout the overwintering period for three year classes in Chapter 4. The seasonal patterns in energy storage differed greatly between the spring- and summer-spawned bluefish. While spring-spawned fish rely on large amounts of stored energy from the fall, summer-spawned fish maintain relatively low energy stores throughout winter. During a severe winter, the summer cohort had the lowest energy reserves observed during this study, which may explain the relatively intense size selective mortality that year class experienced.

In Chapter 4 I also examined overwinter foraging ecology for bluefish, because winter feeding is probably important for conserving energy stores. The overwintering diet of juvenile bluefish was dominated by striped anchovy Anchoa hepsetus, which are common prey on the continental shelf in the South Atlantic Bight. Next, I used generalized additive models to examine how multiple abiotic and biotic factors affect winter feeding. The only factor that explained significant variation in bluefish gut fullness during the overwinter period was the abundance of anchovy prey; when prey are available during winter, bluefish appear to feed, even at the minimum temperature where bluefish are caught.
One of the goals of my dissertation was to enhance our understanding of feeding at low winter temperatures, because limited information exists on this important topic. In Chapter 2, I give results for a winter feeding and growth experiment, which showed that bluefish are able to feed at temperatures below the minimum values at which they are caught in the field. However, live prey were not used in this experiment, so it remained uncertain if bluefish are able to capture live prey near their thermal minimum. The results from Chapter 4 showed that bluefish sampled during the winter near their thermal minimum are able to feed. Given the importance of winter feeding for the summer cohort to survive winter, I conducted an experiment using two sizes of live prey to examine how predator-prey interactions are affected by low temperatures. The results of this experiment were surprising. While temperature had a strong effect on multiple aspects of predator-prey encounters, the ability of bluefish to catch bay anchovy *Anchoa mitchilli* prey was not impaired at low temperatures. However, as temperatures declined, bluefish had increasing difficulty handling and ingesting captured prey. This was especially apparent with relatively large prey. For example, while bluefish readily attacked and consumed small prey near their thermal minimum, larger prey were never attacked at this temperature. This suggests that low winter temperatures restrict the prey field. As prey abundance is important for winter feeding (Chapter 4), reducing prey availability could have important energetic implications for overwintering fish.

This dissertation makes important contributions in multiple areas. It resolves uncertainties about the management of the United States bluefish population (Shepherd and Nieland 2010). Juvenile abundance is used to forecast year class strength for bluefish, and it was previously suggested that the summer cohort not be considered due to poor survival (Conover et al. 2003). My results show that summer spawning does contribute to the population. Further, work presented in Chapter 2, in conjunction with the recent coast-wide survey (Wuenschel et al. 2012), reveals biases with stock assessment estimates of juvenile abundance that are associated with sampling gear and
survey location. This dissertation also provides an example of how innovative approaches can be used to link knowledge of early life stages with adult populations. Examining the growth history or chemical signature of aging structures (e.g., scales and otoliths) of fish can reveal important information about the spawning origins of the adult population (Thorrold et al. 2001; Rooker and Secor 2004; Chapter 2). My research also represents one of the most comprehensive overwintering studies for a marine species. I accomplished this by integrating energy storage dynamics, foraging ecology, and size selective mortality. Further, I provide one of the most detailed studies of how low temperatures affect predator-prey interactions among ectotherms.

In this dissertation, I often use length distributions of juvenile bluefish to label individual fish as summer or spring spawned. This is due to the typical bimodal length distributions that arise from two major spawning periods (Munch and Conover 2000; Wuenschel et al. 2012). Readers may question this approach as individual growth rates may vary a great deal within a cohort. However, this approach is supported by many studies that have used otoliths to determine spawning date of juvenile bluefish collected during summer and fall (e.g., McBride and Conover 1991; Callihan et al. 2008). These studies show that length overlap between the two cohorts is minimal. However, it should be noted that the degree of separation between the spring and summer cohorts does vary spatially and temporally (Munch and Conover 2000). For example, in years when the warming rate of the northeast continental shelf during spring is rapid, summer spawning may begin sooner resulting in a more unimodal length distribution of juveniles (Callihan et al. 2008). However, there was no evidence of this with the 2004-2007 year classes, because length distributions were distinctly bimodal (Chapter 2).

REFERENCES


Chapter 2. Relative contribution of spring- and summer-spawned bluefish cohorts to the adult population: effects of size-selective winter mortality, overwinter growth, and sampling bias

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ABSTRACT

Length distributions of juvenile bluefish *Pomatomus saltatrix* are bimodal, consisting of spring- and summer-spawned fish. Research during the 1990s from the northeast U.S. suggested that the summer cohort contributes little to the adult population and that overwinter mortality may limit their survival. I examined length distributions of juvenile bluefish from before and after winter and found that the mean length of the summer cohort increased during winter. Based on a winter-growth experiment and temperatures from the two winters examined, changes in mean length were due to size-selective mortality for one year-class, and growth for the other. Despite evidence for winter mortality, summer-spawned bluefish were commonly caught at age-1. I reexamined the relative contribution of each cohort to the adult population using archived scales from North Carolina fisheries. Cohort origin of adults was determined by back-calculating length at age-1. One third of adults consisted of summer-spawned fish, which contrasts with previous research from the northeast. The differences in relative cohort abundance between the northeast and southeast U.S. arise from sized-based migration in age-1 and age-2 bluefish (the dominant age groups sampled in these studies) and the size-selective gear and sampling season in which they were collected.

INTRODUCTION

Many species of fish exhibit bet-hedging strategies for reproduction by varying spawning output temporally and spatially (Collins and Stender 1987; Secor 2007). By
spreading out reproductive effort, these species increase the chance of producing offspring that will encounter favorable conditions, because mortality of early life stages in fishes varies temporally (Rice et al. 1987; Rutherford and Houde 1995) and spatially (Scharf 2000; Secor 2007). Variability in mortality is often examined at the larval and early-juvenile stages, and sources of mortality may differ among cohorts spawned at different times of the year. For some species, cohorts spawned later in the season experience warmer temperatures, which enhances survival through increased growth rates and a subsequent reduction in the time larvae and juveniles spend at small-vulnerable sizes (Rice et al. 1987; Rutherford and Houde 1995). However, making conclusions about juvenile mortality that are based solely on these early-life stages may be misleading; cohorts spawned early in the season typically reach a larger size by the end of the year, and as a result may have an advantage in surviving winter (Cargnelli and Gross 1996; Ludsin and DeVries 1997). One of the most effective ways to examine the relative value of different intra-annual cohorts, or spawning regions, is to sample adult fish and determine their temporal or spatial spawning origins using scales or otoliths (Cargnelli and Gross 1996; Thorrold et al. 2001; Conover et al. 2003). However, obtaining an unbiased sample of adult fish can be challenging, especially in marine systems (Hilborn and Walters 1992).

Bluefish Pomatomus saltatrix is a migratory species that has historically been difficult to manage due to their complex life history and bet hedging reproductive strategy (Wilson and Degnbol 2002; Conover et al. 2003; Wuenschel et al. 2012). Adult bluefish are batch spawners with a prolonged spawning season (Robillard et al. 2008). Based on catches of eggs and larvae, spawning occurs throughout most of the year (Kendall and Walford 1979; Smith et al. 1994; Hare and Cowen 1996), but the majority of juveniles come from two spawning periods. The first group of juveniles each year is the spring-spawned cohort, with hatch dates typically between late March and early May (McBride and Conover 1991; Taylor et al. 2007; Callihan et al. 2008). The spring cohort originates from spawning in the South Atlantic Bight (SAB), which is defined by
Cape Hatteras, North Carolina and Cape Canaveral, Florida (Kendall and Walford 1979; Robillard et al. 2008). Juveniles from the spring cohort occur along the entire east coast, because of larval transport in the Gulf Stream (Kendall and Walford 1979; Hare and Cowen 1996; Wuenschel et al. 2012). The second group of juveniles is the summer-spawned cohort, with hatch dates in late June and July (McBride and Conover 1991; Taylor et al. 2007; Callihan et al. 2008). Summer spawning of bluefish mostly takes place off the northeast coast (Smith et al. 1994; Robillard et al. 2008), so juveniles from the summer cohort mostly occur in coastal habitats of the Middle Atlantic Bight (MAB), which is defined by Cape Cod, Massachusetts and Cape Hatteras, North Carolina (Wuenschel et al. 2012). The distinct spring and summer spawning periods result in bimodal length distributions of juveniles, and the two cohorts are easily identified (Munch and Conover 2000; Able et al. 2003). During the fall, juveniles in the MAB migrate south to overwinter in the SAB (Shepherd et al. 2006; Morley et al. 2007; Wuenschel et al. 2012).

Recent evidence suggests that the overwinter period of juvenile bluefish may be important in determining year-class strength. Wiedenmann and Essington (2006) compared abundance of young-of-the-year (YOY) bluefish during the fall migration period with age-1 abundance the following year. They found an asymptotic relationship suggesting density dependent mortality during the winter. Morley et al. (2007) addressed several questions related to overwintering in juvenile bluefish. Due to the large difference in size between spring and summer-spawned fish, their overwintering strategies differ. Summer-spawned fish accumulate less energy stores during the fall, and they incur a greater energetic deficit during severe winters. Also, evidence for negative size-selective mortality of the summer cohort was found. This suggests that winter mortality may be greater for the summer cohort. However, Morley et al. (2007) was only able to examine one year-class for size-selective mortality, so its prevalence and the influence of winter severity is uncertain. Further, the assumption
that YOY bluefish do not grow during the winter has not been experimentally tested; if growth occurs, analysis of size selective mortality would be biased.

If mortality rates differed greatly between the spring and summer cohorts during winter, their relative abundance in the adult population may not be related to their abundance as juveniles. The difference in size between cohorts at the end of their first year allows the cohort identity of adult fish (age-1+) to be determined by back-calculating length at the time of first annulus formation (i.e. length at age-1). Lassiter (1962) was the first to do this using scales from adult bluefish captured off North Carolina in 1960-61; around one third of his adult fish belonged to the summer cohort. Chiarella and Conover (1990) examined the 1981-84 year-classes using adults from the MAB and found almost entirely spring-spawned fish. However, recruitment of the summer cohort was relatively low for those year-classes (Conover et al. 2003). Most recently, Conover et al. (2003) examined the 1991-98 year-classes using adults from the MAB. They also found poor contribution by summer-spawned fish, which was surprising because recruitment of the summer cohort was strong during those years. They hypothesized that decadal scale changes in overwinter mortality of the summer cohort may influence their contribution to the adult population. For example, low winter mortality of juvenile summer-spawned fish during Lassiter’s (1962) study may explain why he found more of them in the adult population. An alternative explanation is that the sampling location for adults influences estimates of the relative contribution of the spring and summer cohorts. For example, summer-spawned fish may be less likely to migrate north into the MAB at younger ages due to their smaller size. The majority of adults examined by Lassiter (1962) and Conover et al. (2003) were age-1 and 2.

In this chapter I estimate the relative contribution of two intra-annual cohorts to the adult bluefish population and examine mechanisms responsible for differential contribution. First, I expand upon the work of Morley et al. (2007) in looking for evidence of size-selective winter mortality of the summer cohort over multiple years, by
comparing length distributions from before and after winter. To ensure the accuracy of this approach, I tested the assumption that growth does not occur during the winter with a laboratory experiment. The second component of this chapter tested the hypothesis that the relative abundance of spring- and summer-spawned bluefish in the adult population differs between the MAB and SAB. I examined archived scale samples from adult bluefish collected off North Carolina and compared the relative cohort abundance from the same year-classes that were sampled in the MAB by Conover et al. (2003). I also examined how size-based migration strategies of age-1 and 2 bluefish may lead to spatial and temporal sampling bias when estimating relative cohort contribution to the adult population. Last, the back-calculation technique assumes that the first annulus is formed on scales at the beginning of the second growing season. I validate this assumption with marginal increment analysis using scales from age-1 bluefish.

**METHODS**

*Size-selective overwinter mortality of field caught bluefish*

Field sampling to examine the potential for size-selective mortality in juvenile bluefish was conducted monthly with 20-minute duration otter trawls (30-m head rope) between November 2004 and April 2008. During this period, there was a hiatus in sampling between January and October 2007. Trawling was conducted in Onslow Bay, North Carolina on two transects, each oriented perpendicular to shore. Tows were conducted at three knots, parallel to shore, at six different locations on each transect: 0.4, 0.8, 1.6, 3.2, 5.6 and 8 km from shore. Temperature was recorded throughout the sampling period at 30 min intervals with a data logger placed on the Suloid wreck in Onslow Bay, which is located 15 km from shore (P. E. Whitfield and G. G. Purifoy Jr., NOAA Beaufort Laboratory, North Carolina, unpublished data).

Negative size-selective winter mortality of the summer-spawned cohort was examined for the 2004, 2005, and 2007 year-classes. The sampling hiatus in spring of
2007 prevented examination of the 2006 year-class. I compared length frequency histograms from catches before and after winter. An increase in the mean fork length (FL) and a decrease in variance over the winter indicate higher mortality of small individuals, provided no growth occurs. Based on examination of monthly length distributions, growth of the summer cohort ceases in November and resumes in April or early May (Morley et al. 2007; Wuenschel et al. 2012). I pooled fish caught between November and January for the pre-winter length distributions because the timing of the largest catches of summer-spawned juveniles varied among years. Further, the southward migration of YOY in the fall is size dependent, and the larger individuals from the summer cohort arrive to North Carolina earlier (Morley et al. 2007; Wuenschel et al. 2012). Therefore, by pooling multiple months a better representation of cohort length structure is achieved. Post winter length distributions came from sampling in late March and April. To ensure growth had not resumed prior to post winter sampling, scale samples from a subset of fish were examined to determine if an annulus had formed.

The cohort structure of age-1 bluefish from the 2004 and 2005 year-classes was examined by scale analysis (see below for methods); FL at annulus-1 was back-calculated, and estimated age-1 length distributions were compared to observed age-1 lengths taken from April trawl-catch data. For the 2004 year-class, I used scales collected from trawl caught fish between June and September of 2005. For the 2005 year-class, trawling did not take place in June, so scales were collected between July and September of 2006. Additional samples were obtained by hook and line for the 2004 year-class between June of 2005 and January of 2006 from Beaufort Inlet and near Cape Lookout, North Carolina. Scales were taken from the area between the lateral line and the spinous dorsal fin.

**Winter consumption and growth experiment**

Summer-spawned juvenile bluefish were caught by 30 m beach seine in Bogue Sound, North Carolina between November 16 and December 2, 2006. Fish were
transported to the Center for Marine Sciences and Technology (CMAST) Fisheries Research Laboratory and held in 540 L circular tanks with flow through seawater from Bogue Sound. During the acclimation period fish were fed daily to satiation with thawed Atlantic silversides *Menidia menidia*. Ambient light from windows was used throughout the experiment. On January 30, 2007 fish were anesthetized with MS-222, measured for FL (mm), weighed (0.01 g), and randomly allocated to six tanks, with six fish per tank. Three temperature treatments were used, 10, 12, and 14.5°C, with two replicate tanks for each. Mean FL was 152 mm (range 136-170); there was no significant difference in initial mean FL between treatments (ANOVA: SS(treatment) = 3.90, SS(error) = 67.3, $F_{[2,3]} = 0.086$, $P = 0.92$). Temperatures in tanks were adjusted from ambient conditions of 11.5°C to treatment levels at 1°C per day. Data loggers recorded temperature in each tank at 10 min intervals.

In order to compare my work to a prior study, bluefish were fed, and consumption was determined, in a similar fashion as the maximum consumption experiments conducted by Hartman and Brandt (1995). Fish were fed twice daily to satiation using thawed *Menidia menidia* at 0800 and 1700 h. The weight of food added to each tank was recorded. After the second feeding, uneaten food was removed with a dip net and weighed. I chose to remove uneaten food only once per day, unlike Hartman and Brandt (1995) who did so twice, in order to minimize stress for the fish. However, I developed a correction factor to account for weight change in uneaten food from remaining submerged in the tanks. Using tanks without fish, I duplicated my feeding protocol and created a regression comparing weight of food added to weight retrieved

$$FA = 0.045 + 0.908 \cdot FR \quad r^2 = 0.99, N = 24$$

where $FA$ is weight (g) of food added to tank, combining the morning and evening, and $FR$ is weight of food removed at the end of the day. Using this equation I estimated the
initial weight of uneaten food, and subtracted that value from the weight of food added to the tank to get daily consumption per tank.

On day 22 of the experiment one fish from a 14.5°C tank died. Based on the FL of this fish, it was easily identified from the initial lengths of fish for the tank, and it was eliminated from growth calculations. A fin-clipped bluefish (155 mm) from an extra tank, was added to replace the mortality; this fish was not included in growth analyses. Consumption values for all tanks on this day were excluded from analyses. The experiment ended on March 1, after 30 days. Fish were sacrificed with a lethal dose of MS-222 and were measured and weighed. I used the change in mean FL and weight from initial values in each tank to determine daily growth rate in length and mass. The effect of temperature on growth rate in length was examined using ANOVA; growth rate was log_{10} transformed to meet assumptions of homogeneity of variance. The effect of temperature on growth rate in mass was examined using a nonparametric Kruskal-Wallis test, because transformations did not improve the heteroscedasticity of residuals.

I determined daily mass specific consumption ($C_{max}$) per tank by dividing consumption by the collective mass of bluefish, divided by the number of days. As bluefish were not weighed during the experiment, I used the geometric mean of initial and final bluefish weights. ANOVA was used to compare mean daily $C_{max}$ across temperatures. To determine if a seasonal effect on consumption exists, I compared my results from February, with predictions from a $C_{max}$ versus temperature model derived from experiments conducted between May and December (Hartman and Brandt 1995). Hartman and Brandt’s (1995) model yields $C_{max}$ values based on bluefish mass and temperature; I used a 50.9 g bluefish with their model, which was the mean mass of all fish during my experiment.
**Does the relative abundance of spring- and summer-spawned bluefish in the adult population differ between the MAB and SAB?**

To compare the relative contribution of spring- and summer-spawned bluefish to the adult population using samples taken from the SAB versus the MAB, I obtained archived scales from adult bluefish collected by the North Carolina Division of Marine Fisheries (SAB samples), and back-calculated age-1 length to compare with Conover et al. (2003; MAB samples). The SAB samples came from commercial and recreational catches throughout North Carolina every month of the year between 1997-2000. These sampling years were chosen because they would allow me to examine many of the same year-classes of Conover et al. (2003). Catches from multiple fishing gears were sampled: gill net (N = 635), beach seine (N = 282), hook and line (N = 95), trawl (N = 82), and pound net (N = 33). FL of bluefish were measured, sex was determined, and scales were removed from the area below the lateral line and posterior to the base of the pectoral fin. During scale analysis each sample was aged and FL at annulus-1 was back-calculated (see below for methods). While bluefish have been shown to lay down an annulus each year (Wilk 1977; Robillard et al. 2009), using scales to age older fish is difficult. Based on comparisons between scale impressions and sectioned otoliths, age estimates of bluefish from scales appear accurate up until age-5 (Chiarella and Conover 1990; Sipe and Chittenden 2002; Robillard et al. 2009), so I excluded age-6+ fish.

Back-calculated age-1 length distributions were examined by year-class, and each was partitioned into spring and summer-spawned fish based on the location of the antimode between the two cohorts. For some year-classes the location of the antimode was not clear, so I used a minimum and maximum value (175 and 210 mm FL) to represent a range in relative cohort contribution. The minimum and maximum values for the antimode were based on results from the present study and from previous studies that have shown bimodal back-calculated age-1 distributions (Lassiter 1962) or actual length distributions of bluefish at the end of winter (Morley et al. 2007). To determine how sampling region affects estimates of summer cohort contribution, I
combined year-classes to get an average back-calculated length distribution for SAB and MAB caught fish. To correct for differences in sample size between year-classes I determined the percent abundance for each 10 mm length bin within each year, and then determined the mean percent across year-classes for each length bin. Only the year-classes that overlapped between my study and Conover et al. (2003) were used. DataThief software (Tummers 2006) was used to extract data from MAB bluefish using length distributions from Conover et al. (2003) and Gilmore (2000).

The archived scales were also used to gain a better understanding of the seasonality of cohort-specific abundance in the SAB. I plotted monthly mean back-calculated age-1 length separately for age-1, age-2, and pooled ages-3-5 bluefish. For age-1 fish that had not yet formed an annulus, I used FL at capture. I used 3-factor ANOVA to examine differences in back-calculated age-1 length between season of capture (winter: January-April versus summer: June-September), age groups (age-1 versus age-2), and gear of capture (gill net versus combined beach seine and trawl). Age-3+ fish were excluded because they were absent from summer catches. Gear type was included in the model because differences in fishing gear among seasons and ages may bias results. Variance among treatments was not homogenous according to Levene’s test ($P < 0.01$), and transformations of the response variable were not helpful. However, ANOVA has been shown to be robust to violations of the homogenous variance assumption, provided that treatment means are not correlated to variance (Lindman 1974); I found no such relationship (slope not different from zero: $t_{[6]} = 0.263, P = 0.80$), so I proceeded with the analysis.

If summer spawning in the MAB is the primary reason adult bluefish migrate north, then immature age-1+ fish may remain in the SAB. Male bluefish mature at a smaller size than females (Salerno et al. 2001; Robillard et al. 2008) so I would expect the ratio of males to females to decrease during summer in the SAB. Using the data on bluefish sex that was provided with the archived scales, I compared the male to female ratio of adult bluefish sampled in winter (January – March) versus summer (July –
September) using a chi-square test of association with a 2x2 contingency table. This analysis was restricted to bluefish between 340 and 440 mm because in this range a majority of males are mature, but most females are immature (Salerno et al. 2001; Robillard et al. 2008). Significant results would suggest maturity influences migration, which would have important implications for relative cohort abundance in the MAB versus SAB.

**Scale analysis**

To back-calculate FL at age-1, actual scales were examined (i.e. not pressings) with a microfiche reader at 32x magnification. Between eight and twelve scales were examined per fish. Regenerated scales and scales that showed evidence of curling, by not entirely coming into focus, were excluded. Only fully formed annuli were counted when aging. Measurements of scales were made in the same manner as Conover et al. (2003), which were from the focus to the first annulus \( S_I \) and to the margin \( S_c \). The measurements were made in the anterior field of the scale at a 20° angle from the dorsoventral axis.

For back calculating length at age-1 I used the Fraser-Lee equation

\[
L_1 = \alpha + \left[ (L_c - \alpha) / S_c \right] \cdot S_I
\]

where \( L_1 \) is the length at age-1, \( L_c \) is the length at capture, and \( \alpha \) is the intercept from a regression of length against scale size (Fraser 1916; Lee 1920). Carlander (1982) advocated using standard intercept (\( \alpha \)) values within a species to facilitate comparison among studies. For the 2004 and 2005 year-classes I took scale samples from the same dorsal region as Lassiter (1962), so his value of \( \alpha = 10.49 \) mm was used for those samples. For archived scales I used \( \alpha = 5.02 \) mm, as determined by Conover et al. (2003), because they used scales taken from the same lateral region. Size at age-1 was calculated with two different scales and the average of the readings was used. For 15% of samples, the age-1 length estimates from the two scales differed by over 15 mm. For
these samples a third scale was examined and the two closest readings were used, or the sample was rejected; less than 5% of samples were rejected.

To confirm the timing of annulus formation on age-1 bluefish scales, I conducted a marginal increment analysis using archived scales of age-1 fish, which included the 1996 – 1999 year-classes. Marginal increments were calculated as the distance between the scale margin and either the focus, or the annulus if present. The marginal increment from two scales per fish was measured and the mean value was used. Bluefish from each half-month were pooled and mean values were plotted against date.

To ensure that my results are comparable with previous studies that used slightly different methods to back-calculate length, I conducted two regression analyses. First, a subset of age-1 bluefish from the 2005 year-class were used to compare back-calculated lengths from both dorsal and lateral scales, each with the appropriate $\alpha$ value. Second, a subset of archived scales from fish between age-1 and age-5 (mean age = 2.4) were used to compare estimated length at age-1 using acetate scale impressions versus estimates using actual scales. For each comparison, regression was used to compare age-1 length estimates and determine if the relationship differed from 1:1; t-tests were used to determine if the intercept differed from zero and if the slope differed from one.

**RESULTS**

**Size-selective overwinter mortality of field caught bluefish**

Water temperatures varied among the three winters (Fig. 2.1). The 2004 year-class experienced the harshest conditions with 73 d below 12°C and 40 d below 10°C. The 2005 year-class experienced 59 d below 12°C and the temperature never dropped below 10°C. The 2007 year-class experienced a mild winter, with only 11 d below 12°C.

The antimode between the spring and summer-spawned cohorts during the late fall months ranged between 175 and 195 mm (Fig. 2.2a-c). Based on spring catches, winter survival of the summer cohort was evident for each year-class (Fig. 2.2d-f).
Based on scale examinations from April caught bluefish, growth in the spring of 2006 had started before my sampling took place, so the 2005 year-class was not included in the size-selective mortality analyses. Evidence for size-selective mortality was found in one of the other two year-classes. The mean length of the 2004 year-class increased from 144.8 to 149.9 mm over the winter (t-test: $P < 0.001$) and the variance decreased ($F$ test: $P = 0.012$), which suggests size-selective mortality occurred. Mean length of the 2007 year-class increased from 153.9 to 159.9 mm (t-test: $P < 0.001$), but the decrease in variance was not significant ($F$ test: $P = 0.188$), which indicates that size-selective mortality did not occur.

Age-1 bluefish collected during trawl sampling were dominated by the summer cohort and had back-calculated length distributions that were similar to actual lengths from the previous winter. For the 2004 year-class the summer cohort comprised 57% of age-1 fish and had a mean back-calculated length of 155 mm (Fig. 2.2g), which was similar to the mean value of 150 mm for the summer cohort in early spring (Fig. 2.2d). The summer cohort made up 85% of age-1 fish from the 2005 year-class and had a mean back-calculated length of 153 mm (Fig. 2.2h). Growth had resumed prior to spring sampling of the 2005 year-class (Fig. 2.2e), but the mean length of the summer cohort in late-fall was also 153 mm (Fig. 2.2b). The estimated contribution by the summer cohort depended on sampling gear used for age-1 fish; only 20% of line caught fish were from the summer cohort (Fig. 2.2g). However, this apparent difference in sampling gear may be a result of different sampling dates between gears; the hook and line caught fish that were sampled between June and August ($N = 12$) had a mean back-calculated FL of 177 mm, whereas the fish sampled between late October and January ($N = 42$) had a mean age-1 FL of 232 mm.

**Winter consumption and growth experiment**

Mean temperatures by treatment were 10.0, 11.9, and 14.3°C. Bluefish fed at all temperatures (Fig. 2.3a), and mean daily $C_{max}$ showed a significant temperature effect (Table 2.1). The coldest temperature treatment had significantly lower $C_{max}$ than higher
temperatures (Bonferroni test: \( P < 0.01 \)), but the two higher temperatures did not differ significantly \( (P = 0.17) \). When compared to \( C_{\text{max}} \) values from the Hartman and Brandt (1995) model, which was parameterized during the summer and fall months, my February values were much lower (Fig. 2.3a). Further, I saw very little increase in consumption between 11.9 and 14.3°C, while \( C_{\text{max}} \) values from the Hartman and Brandt (1995) model almost doubled. Growth rates were low for all treatments (Fig. 2.3b and c), and I saw no significant effect of temperature on growth in length (Table 2.1), or mass (Kruskal-Wallis test: \( H_{[2]} = 4.597, P = 0.1 \), although a positive trend in growth with increasing temperature was evident. At the lowest temperature mean FL increased by 1.2 mm over the 30-day experiment, despite a loss of body mass; length increased by 3.6 mm at 14.3°C.

**Does the relative abundance of spring- and summer-spawned bluefish in the adult population differ between the MAB and SAB?**

Marginal increment analysis showed that age-1 bluefish lay down their first annulus in May and early June (Fig. 2.4). However, it was evident that a majority of fish began to lay their annulus down in late April, but these typically are not fully formed around the circumference of the scale and so were not counted. Thus, back-calculated sizes at age-1 represent bluefish lengths during spring of their second year.

I was able to examine the relative cohort contribution of adult bluefish to the North Carolina fishery from the 1994 through 1999 year-classes. Back-calculated age-1 length distributions were distinctly bimodal for the 1995 year-class, which had 30% summer cohort contribution (Fig. 2.5b), and the 1999 year-class, which had 39% summer cohort contribution (Fig. 2.5f). I was not able to objectively locate the antimode in the four remaining year-classes so minimum and maximum values were used. Despite the lack of a clear antimode, summer cohort contribution appeared to be important for the 1994 year-class, ranging between 21 and 45% (Fig. 2.5a), and the 1998 year-class, ranging between 28 and 59% (Fig. 2.5e). Back-calculated distributions for the two remaining year-classes did not appear bimodal; summer cohort
contribution ranged between 2 and 38% for the 1996 year-class (Fig. 2.5c), and between 5 and 27% for the 1997 year-class (Fig. 2.5d). The relative cohort contribution did not appear to be related to the age of fish that were examined (Fig. 2.5). For example, the 1995 and 1999 year-classes had similar distributions despite examining different aged fish. Further, contribution of the summer cohort appeared much stronger in the 1998 year-class compared to the 1996 and 1997 year-classes, despite examining similarly aged fish.

Five of the six year-classes I examined (1994-98) were also sampled by Conover et al. (2003). Back-calculated length distributions from these pooled year-classes were not bimodal for either SAB or the MAB (Fig. 2.6). However, the two distributions differed significantly (Kolmogorov-Smirnov test: $D = 0.235$, $P < 0.01$). The distribution from North Carolina had a greater proportion of smaller bluefish suggesting that the relative abundance of summer-spawned bluefish is greater in southern regions. The mean age-1 length of North Carolina bluefish from these five year-classes was 227.8 mm, and 32.2% were below 200 mm. The mean age-1 length of MAB bluefish from the same year-classes was 256.0 mm and only 14.5% were below 200 mm.

Season of capture, age of fish examined, and fishing gear influenced the back-calculated age-1 lengths of adult bluefish caught off North Carolina (Table 2.2). However, both the effect of gear and season were dependent on age. The only significant difference between gear types occurred with age-1 fish during the summer, where gill net caught fish had larger back-calculated lengths than trawl and beach seine caught fish (Bonferroni post-hoc: $P < 0.05$; Fig. 2.7a). Further, among gill net caught adults, age-2 bluefish had smaller back-calculated lengths than age-1 fish within each season ($P < 0.05$; Fig. 2.7a and b). Within each gear type, there was a significant decline in back-calculated age-1 lengths from winter to summer for both age-1 and 2 bluefish ($P < 0.05$), with the exception of gill net caught age-2 fish (Fig. 2.7a and b). This suggests that the larger individuals from each age-class are more likely to migrate from the area during the spring. Age-3+ bluefish were not included in the ANOVA because
only two individuals were sampled during the summer months, presumably due to low catches of larger bluefish at this time (Fig. 2.7c). The seasonal and age related-trends in back-calculated length described here would probably be more pronounced for other year-classes. This is because 57% of the age-1 samples and 71% of age-2 samples for this analysis came from the 1996 and 1997 year-classes; these two year-classes showed the least evidence of contribution by the summer cohort.

My hypothesis that maturity (based on males maturing earlier than females) influences migration strategy was supported. The ratio of males to females declined significantly from winter to summer ($N_{\text{winter}} = 109; N_{\text{summer}} = 164; \chi^2_{[1]} = 5.94; P = 0.02$). During winter, 40.4% of sampled bluefish between 340 to 440 mm were male, compared to only 25.6% in summer.

The regression equation comparing back-calculated FL at age-1 from dorsal versus lateral scales was not significantly different from a 1:1 relationship ($y = -7.09 + 1.03\cdot x; N = 24; r^2 = 0.98; t$-test for intercept = 0: $t_{[22]} = 1.28, P = 0.21; t$-test for slope = 1: $t_{[22]} = 1.0, P = 0.33$). The mean difference between age-1 size estimates from the two scale regions was only 2 mm. Thus, my results for the 2004 and 2005 year-classes (Fig. 2.2g and h) are comparable to the archived scale samples (Fig. 2.5) and previous studies that used lateral scales. The regression equation comparing back-calculated size at age-1 from scale pressings versus actual scales was also not significantly different from a 1:1 relationship ($y = 5.94 + 0.96\cdot x; N = 41; r^2 = 0.95; t$-test for intercept = 0: $t_{[39]} = 0.68, P = 0.50; t$-test for slope = 1: $t_{[39]} = 1.06, P = 0.30$). The mean difference between back-calculated length estimates from pressings and actual scales was only 3.3 mm. Thus my use of actual scales to back-calculate length does not bias comparisons with previous studies that used scale pressings.


DISCUSSION

Size-selective overwinter mortality of field caught bluefish

Based on empirical findings, Wiedenmann and Essington (2006) hypothesized that overwinter mortality may be an important source of mortality in juvenile bluefish. Further, Conover et al. (2003) hypothesized that winter mortality may limit contribution of the summer cohort to the adult population. I did find evidence of size-selective overwinter mortality within the summer cohort in one of the two year-classes examined, but this mortality did not result in complete loss of the cohort. The mean length of the summer cohort increased by 5-6 mm during the winter for the two year-classes I examined. Based on the results from my winter growth experiment, the temperature between sampling intervals must be considered to determine if this shift in mean length could have been caused by growth. For the 2004 year-class the increase in mean length was probably due to size-selective mortality. This is because 97% of my pre-winter lengths for that year-class came from January, by this date water temperature had already declined to 12°C. Further, the mean water temperature between my January and April catches was 10.9°C, a temperature at which maximum growth in mass is negative. There is also no evidence to suggest that the antimode between the spring and summer cohorts increased during that time. Conversely, for the 2007 year-class there was a much greater chance that winter growth biased my results. This year-class experienced a mild winter, and 80% of the pre-winter lengths came from November when water temperatures were still mild. Further, my sampling in spring took place in late-April. Morley et al. (2007) found greater evidence for size-selective mortality in the 2002 year-class, which increased by 11 mm. The 2002 year-class experienced a severe winter (76 d < 12°C, 38 d < 10°C) that was similar to the 2004 year-class, and the fish collected in early spring were in poor energetic condition. The impact that size-selective mortality has on the contribution of the summer cohort to the adult population is uncertain. Ricker (1969) demonstrated that it takes a large degree of selective mortality to produce a detectable increase in mean length and
decrease in variation. This would suggest that although I only observed small increases in mean length over the winter, size-selective mortality may be important at the population level.

The source of mortality for overwintering juvenile bluefish is uncertain and may involve multiple biotic factors that interact with winter severity and duration. Acute cold stress is probably not important; in the lab, juvenile bluefish have been shown to survive temperatures as low as 6°C for short periods (Slater et al. 2007). Further, juvenile bluefish appear to effectively avoid water below 11°C (Morley et al. 2007; Wuenschel et al. 2012). However, low temperatures appear to force summer-spawned juveniles farther from shore, potentially into less suitable habitat (Morley et al. 2007). The exhaustion of energy reserves is often cited as a cause of size-selective winter mortality (Hurst 2007) and it may be of importance to the summer cohort because they maintain lower energy stores throughout the winter (Morley et al. 2007). Further, there is evidence that the summer cohort experiences a greater energy deficit than spring-spawned fish during harsh winters, suggesting size-selective mortality would be stronger in those years (Morley et al. 2007). Predation can also be a major source of mortality during the winter (Santucci and Wahl 2003; Garvey et al. 1998), and it is often selective for smaller fish (Sogard 1997).

Winter consumption and growth

The results from the laboratory experiment can be interpreted with respect to winter temperatures in which bluefish are caught. The temperatures that bluefish experienced during my experiment ranged from the lower limit (10°C) at which bluefish have been caught (Munch 2000; Wuenschel et al. 2012), up to mild winter temperatures experienced by the summer cohort (Morley et al. 2007). During winter on the continental shelf of the SAB, water temperatures are generally uniform along isobaths, and near-shore temperatures generally range between 10 and 14°C between North Carolina and northern Florida (Atkinson et al. 1983). There is also a strong cross-shelf temperature gradient associated with the Gulf Stream (Atkinson et al. 1983).
However, the summer cohort has been shown to stay within 2 km of shore during winter unless temperature drops below 11°C, which may force them farther from shore (Morley et al. 2007). Some summer-spawned fish are caught as far south as Florida during winter where temperatures are milder (Wuenschel et al. 2012), but the largest catches of this cohort off North Carolina are typically in December (Morley et al. 2007; Wuenschel 2012) so only a small portion may reach Florida. Further, based on ten years of trawl survey data in the SAB, densities of YOY bluefish in April and May are more than an order of magnitude greater off the Carolinas than off Georgia and Florida (South Carolina Department of Natural Resources 2000).

The goal of my experiment was to determine the physiological maximum capacity for growth during the winter, under ideal conditions of unlimited food and low activity. In the wild, growth would likely be lower at these temperatures because prey may be limited and fish would be expending more energy to forage, avoid predators, and migrate. The trends in growth I observed have important implications for analyzing size-selective mortality during winter. Bluefish held at 10°C were able to feed, but not enough to maintain body weight and growth in FL was within the bounds of measurement error. This supports Hartman and Brandt’s (1995) study, which found a negative scope for growth at this temperature. Physiological limitations of feeding and growth at this low temperature would explain why survey catches of juvenile bluefish below 11°C are rare (Wiedenmann and Essington 2006; Morley et al. 2007; Wuenschel et al. 2012). At 11.9°C growth in mass was negligible, suggesting that fish in the wild at this temperature probably do not grow. At the highest temperature tested, one of the treatments showed marked growth in length and mass. While the growth observed at 14.3°C is low compared to what is found during the spring and summer (McBride and Conover 1991; McBride et al. 1995), it still may lead to an increase in the mean length of overwintering fish. Therefore, when looking for evidence of size-selective mortality, the temperature between sampling dates needs to be considered.
The difference in $C_{max}$ values between my experiment (conducted in winter) and Hartman and Brandt's (1995) experiment (conducted during summer and autumn) suggests a seasonal effect on consumption that is independent of temperature. This supports many laboratory studies that have shown fish to exhibit endogenous-seasonal patterns in appetite (Metcalfe et al. 1988; Simpson et al. 1996), metabolism (Evans 1984; Karas 1990), growth (Metcalfe et al. 1988; Karas 1990), and activity (Sandstrom 1983) that occur independently of temperature or food availability. The adaptation of submaximal consumption in overwintering fishes may result from a feeding strategy designed to maintain adequate energy reserves for winter survival rather than growth (Bull et al. 1996; Hurst and Conover 2001). Further, the strategy of reduced feeding motivation and growth during winter may be the result of consistent prey limitations (Foy and Paul 1999), or poor growth efficiency at low temperatures (Hardewig and van Dijk 2003; Bermudes et al. 2010).

**Does the relative abundance of spring- and summer-spawned bluefish in the adult population differ between the MAB and SAB?**

The relative abundance of spring- and summer-spawned bluefish in the adult population differed between samples obtained from the SAB versus the MAB. Estimates of summer cohort contribution from the SAB were greater than MAB estimates by more than a factor of two. The magnitude of this difference was influenced by collection gear, season of capture, and ages of fish sampled. I will discuss how these three factors influenced my estimates of relative cohort contribution, and how they bias comparisons among previous studies. For my SAB samples, I found contribution of the summer cohort to be 21% or more in four of the six year-classes examined from archived scales. This is a conservatively low estimate because (1) it is based on the lowest value that has been observed as an antimode (see Methods) between the summer and spring cohorts during the winter and (2) a majority of my scale samples came from bluefish captured by gill net, which is a gear that may select for larger age-1 and age-2 individuals (Lucena and O’Brien 2001; this study). When I pooled age-1 lengths from the 1994-1998 year-
classes, the resulting distribution was not bimodal, despite strong summer cohort contribution in three of the five years. This is probably the result of small interannual differences in both the mean length attained by the summer cohort by the end of their first year, and the location of the antimode between cohorts. Conversely, Lassiter (1962) found a bimodal age-1 distribution when he pooled year-classes. However, he only sampled during two years, and two thirds of his bluefish were age-1. Therefore his pooled distribution would have been biased towards only one or two year-classes.

Size-selectivity of sampling gear can lead to misleading information about population size structure (Hilborn and Walters 1992), and this is important to consider when comparing my results with previous studies that have examined bluefish cohort structure. Bottom trawls and seines tend to select for smaller individuals, while gill nets are more selective for an intermediate length, depending on the mesh used (Hilborn and Walters 1992). These general statements have been confirmed for bluefish; Lucena and O’Brien (2001) examined commercial bluefish catches off Brazil and found bottom trawls to select for the smaller individuals in each age class, while gill nets selected for the larger age-1 and 2 fish, and smaller individuals from older ages. My data support the conclusion that gill nets select larger age-1 individuals compared to trawl and seine caught fish. However, there was no effect of gear among back-calculated lengths for age-2 fish. The mean length of the spring and summer cohort differs by 120 mm at age-1 and 70 mm at age-2 (Lassiter 1962). Therefore the potential for gear selectivity biasing estimates of relative cohort contribution in bluefish is strong, especially considering that over half of the scale samples used in Lassiter (1962), Conover et al. (2003), and my study came from age-1 and 2 fish. The National Marine Fisheries Service autumn bottom trawl survey, which is designed to sample juvenile fishes, provided 54% of the bluefish samples used by Conover et al. (2003). The smaller summer-spawned bluefish would have been more vulnerable to this gear, especially at age-1 as I showed with trawl sampling of age-1 fish from the 2004 and 2005 year-classes. Therefore, their results that suggest poor contribution of the
summer cohort are surprising. Further, my examination of the year-classes from the 1990s using bluefish captured off North Carolina found a much higher contribution of summer-spawned fish, despite the fact that a majority of samples came from commercial gill nets. Therefore, I believe the differences between my estimates of summer cohort contribution off North Carolina and those of Conover et al. (2003) from the MAB are conservative, and probably the result of size-based migration strategies among age-1 and 2 bluefish.

Bluefish migration patterns are related to size (Shepherd et al. 2006), and adults occupy progressively more northern and offshore waters as they age (Salerno et al. 2001). Tagging studies have shown that age-1 bluefish may migrate north, or remain in the SAB for their second growing season (Lund and Maltezos 1970; Shepherd et al. 2006), but the proportion of age-1 fish that migrate north and how it differs by cohort is uncertain. Age-1 bluefish appear more abundant off North Carolina than in the MAB during summer months (Wuenschel et al. 2012), which suggests a large proportion may remain in southern regions. The energetic cost of migration decreases with increasing size (Nottestad et al. 1999), so the smaller summer-spawned bluefish may be less likely to migrate north at age-1 and 2. Evidence for size-based migration can be seen in my analysis of seasonal trends in mean back-calculated length. There was a decline in mean age-1 length from winter to summer in both age groups. This suggests that larger age-1 and 2 individuals (i.e., spring-spawned bluefish) are more likely to migrate north than smaller individuals. Additional support for this hypothesis comes from my hook and line samples of adult bluefish from the 2004 year-class; age-1 bluefish collected during summer months were mostly from the summer cohort, and those collected in fall and winter were mostly spring-spawned fish. By age-3, a majority of the bluefish population appears to migrate north each year, based on the lack of samples collected in North Carolina during the summer from older fish. Different migration strategies between spring and summer-spawned bluefish at younger ages leads to important
consequences for studies that examine population level cohort structure, especially when a majority of samples come from age-1 and age-2 fish.

Maturity stage is probably an important factor influencing migration because a majority of summer spawning takes place in the MAB (Kendall and Walford 1979; Collins and Stender 1987; Smith et al. 1994). The difference in size at age between cohorts would lead to summer-spawned fish reaching maturity at a later age. Male bluefish reach 50% maturity at 339 mm (Salerno et al. 2001) and females at 480 mm (Robillard et al. 2008), and growth rate does not differ by sex. Based on cohort specific age and growth data, spring-spawned fish reach maturity at age-1 for males and age-2 or 3 for females (Lassiter 1962; Chiarella and Conover 1990); male summer-spawned fish typically reach maturity at age-2 and females at age-3 or age-4 (Lassiter 1962). My analysis of seasonal changes in bluefish sex ratio shows that the relative abundance of mature males declines in summer off North Carolina. This supports the hypothesis that maturity stage influences migration and may result in the smaller summer-spawned fish remaining in the SAB at age-1 and age-2. Thus catches of younger adults in the MAB would be biased towards the spring cohort.

Lassiter’s (1962) study off North Carolina has been used as a historic baseline for comparison among studies that examine cohort structure of adult bluefish. However, my study has revealed that Lassiter’s (1962) sampling was biased towards catching summer-spawned bluefish. Age-1 and age-2 fish comprised 78% of his samples, and based on the information he provided, most of these were collected by beach seine or bottom trawl. Further, his sampling was biased towards the summer months; as I discussed above, the mean back-calculated length of age-1 and 2 fish declines during summer as the larger individuals move out of North Carolina and most likely migrate north. Also, Lassiter’s samples were only 35% male, which is consistent with my results on seasonal changes in bluefish sex ratio. My trawl caught age-1 bluefish from the SAB in the summer matches best with the timing, location, and gear
type used by Lassiter (1962); back-calculated lengths of those age-1 fish have greater than 50% contribution by summer-spawned bluefish.

Assumptions of back-calculating length with scales

The use of the back-calculation technique to quantify relative cohort contribution in bluefish was originally based on the bimodal length distribution Lassiter (1962) found for fish at age-1, which resembled length distributions of YOY bluefish during the fall migration (Chiarella and Conover 1990). Because Lassiter’s age-1 distribution was bimodal, it was assumed that the spring and summer cohort remain distinctly different in length when their first annulus forms during spring. I found the mean FL attained by the summer cohort at the end of their first growing season to be consistently near 150 mm. Further, my laboratory and field results, combined with previous studies that examined length distributions throughout winter (Morley et al. 2007; Wuenschel et al. 2012), confirm that winter growth does not disrupt the bimodal pattern of spring and summer-spawned bluefish.

Another important assumption for using this technique is that the first annulus is formed on scales at the beginning of the second growing season in early spring. This information is needed so we know that back-calculated age-1 distributions represent the lengths fish attained at the end of their first year and are comparable to cohort structure during the fall. I have confirmed that annuli typically start to form in late-April, and are complete in May and early June. This is supported by Wilk (1977), who gave anecdotal evidence of age-1 annulus formation on scales to occur in May. Similarly, Robillard et al. (2009) found annulus formation in otoliths to be complete in June for age-1 bluefish. Although annulus formation is not complete until May or June, the growing season of most age-1 bluefish begins earlier in the year. Based on monthly length frequency distributions during the early spring, growth appears to resume in late April or early May (Morley et al. 2007; Wuenschel et al. 2012). Annulus formation is associated with early-spring growth, but they are not completely formed, and thus countable based on my criteria, until later in spring.
The use of the back-calculation technique on bluefish to estimate relative cohort contribution has been validated in two ways. First, Conover et al. (2003) conducted an experiment where spring- and summer-spawned bluefish were collected as YOY and held in tanks until the end of their second growing season. In blind tests using scales to back-calculate length, the cohort identity was correctly identified in 29 of 30 fish. The second validation comes from my comparison of YOY length distributions from the overwintering period of the 2004 and 2005 year-classes with subsequent back-calculated length distributions from age-1 fish. For each year-class I observed similar mean summer cohort lengths between back-calculated age-1 distributions and actual lengths from sampling at the end of the first growing season (Fig. 2). Last, support for use of the back-calculation technique on older bluefish comes from the 1995 year-class (Fig. 2.4b); I found a distinctly bimodal distribution and the scale samples came from age-2 – age-4 bluefish, suggesting the accuracy of the technique is not limited to age-1 fish.

**Management Implications**

The relative contribution of the summer cohort to the adult population has been an important topic for bluefish research and management (Munch and Conover 2000; Conover et al. 2003; Shepherd and Nieland 2010). Juvenile abundance indices are used in bluefish stock assessments to help forecast changes in population size, which helps inform decisions on annual fishing quotas (Shepherd and Nieland 2010). During some years, the summer cohort can dominate juvenile recruitment in the MAB (Conover et al. 2003), which is where a majority of juvenile abundance indices are derived (Shepherd and Nieland 2010). It has been suggested that juvenile abundance indices may be improved by reducing the importance of summer-spawned fish (Conover et al. 2003). This is based on evidence from year-classes that had high abundance of summer-spawned juveniles, but poor contribution to the adult population. While my study supports the conclusion that the spring cohort contributes more to the population, the summer cohort consistently comprises around one third of adults captured in the SAB.
The abundance of juvenile summer-spawned bluefish should still be considered when constructing recruitment indices.

One of the surveys used in bluefish stock assessments to calculate juvenile abundance indices is the Northeast Fisheries Science Center (NEFSC) fall bottom trawl, which is conducted mostly in the MAB (Shepherd and Nieland 2010). Length data from this survey have been used to separate juvenile abundance into spring- and summer-spawned bluefish (Munch and Conover 2000; Conover et al. 2003; Wiedenmann and Essington 2006). Based on these cohort-specific juvenile abundance indices, it appears that the relative cohort abundance of adult bluefish is not always in agreement with their abundance as YOY (Conover et al. 2003). For example, juvenile abundance of the summer cohort was low in 1995 and high in 1997, but this was not reflected in back-calculated age-1 lengths from adult fish in my study. The reason for this lack of agreement is important to resolve because it suggests that juvenile abundance may not always be an effective recruitment indicator. A number of explanations are plausible. First, overwinter mortality may decouple recruitment indices from contribution to the adult population (Wiedenmann and Essington 2006). Second, the abundance of summer-spawned juveniles may be over represented by the NEFSC survey. NEFSC sampling excludes most of the SAB, where recruitment of the summer cohort is minimal, but spring-spawned recruitment occurs along the entire east coast resulting in the potential for greater recruitment of the spring cohort (Wuenschel et al. 2012). There is also evidence from the MAB that spring-spawned fish may continue using estuarine habitat into the early fall when the NEFSC survey operates (Taylor et al. 2007). Further, due to size selectivity of bottom trawls (Hilborn and Walters 1992; Lucena and O’Brien 2001), summer-spawned fish may be more vulnerable to capture by the survey. This problem of estimating the juvenile abundance of species with protracted spawning using size-selective gear has been described for largemouth bass (Pine et al. 2000). Third, using a single trawl survey to estimate juvenile abundance of each cohort may result in a poor estimate in some years. The NEFSC survey operates in
a large area, and regional bluefish recruitment within the MAB varies interannually (McBride and Conover 1991; McBride et al. 1995). Further, bluefish stock assessments also use trawl surveys off Connecticut, New Jersey, and Delaware to help estimate juvenile abundance (Shepherd and Nieland 2010). Recruitment indices from these state-level surveys do not always agree with NEFSC survey abundance estimates. Future efforts to compare cohort-specific juvenile abundance with relative contribution to the adult population would benefit from multiple juvenile surveys and adult collections along the entire east coast.

ACKNOWLEDGEMENTS
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Table 2.1. Analysis of variance models with temperature (10.0, 11.9, and 14.3°C) as the independent variable and juvenile bluefish consumption (g·g⁻¹·d⁻¹) and growth (log(mm·d⁻¹)) as dependent variables.

<table>
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<th>SS</th>
<th>F</th>
<th>P</th>
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<tr>
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<tr>
<td>Error</td>
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<tr>
<td>Growth rate</td>
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<td></td>
</tr>
<tr>
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<tr>
<td>Error</td>
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**Table 2.2.** Analysis of variance using North Carolina caught bluefish with capture gear (gill net versus combined beach seine and trawl), season of capture (winter versus summer) and bluefish age at capture (age-1 versus age-2) as independent variables and back-calculated age-1 length as the dependent variable.

<table>
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<th>SS</th>
<th>F</th>
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Figure 2.1. Ocean-bottom temperatures in Onslow Bay, North Carolina 15 km from shore during three winter sampling periods.
Figure 2.2. Length frequency distributions of bluefish caught by bottom trawl off North Carolina. The 2004, 2005, and 2007 year-classes are represented in column 1 (a, d, g), 2 (b, e, h), and 3 (c, f) respectively. Plots in the first row (a, b, c) show pre-winter lengths from each year-class, the second row (d, e, f) shows post-winter, and the third row (g, h) shows back-calculated lengths to the first annulus of trawl caught age-1 bluefish. Some samples were also obtained by hook and line in (g). The dashed lines show the location of the antimode used to separate the spring and summer cohorts. No dashed line is present in (e) because growth had resumed prior to collection and no analysis was performed. The %-value in (g) and (h) shows the relative contribution of summer-spawned bluefish to age-1 adults.
Figure 2.3. Results from a 30-day consumption and growth experiment conducted at three temperatures during winter with juvenile bluefish (mean FL = 152 mm). (a) Mass specific consumption ($C_{\text{max}} \pm \text{SE}$) where feeding occurred twice daily to satiation. The line shows $C_{\text{max}}$ values of the same sized bluefish during the summer and fall, as determined from a laboratory derived weight and temperature predictive consumption model (Hartman and Brandt 1995). Mean ($\pm \text{SE}$) daily growth in length (b) and weight (c) were also calculated.
Figure 2.4. Marginal increments of scales from age-1 bluefish from the 1996-1999 year classes caught off North Carolina with commercial and recreational gear. Values shown are means (±SE) from the first and second half of each month.
Figure 2.5. Back-calculated age-1 length distributions of adult bluefish caught off North Carolina with commercial and recreational gear for the (a) 1994, (b) 1995, (c) 1996, (d) 1997, (e) 1998, and (f) 1999 year-class. For each year-class, the range in age of fish used to back-calculate length is shown, along with the mean age in parentheses. The two dashed lines indicate the potential location of the antimode and thus the potential range in relative summer-cohort abundance. For distributions where the location of the antimode was obvious (b and f), only one dashed line is present.
**Figure 2.6.** Pooled back-calculated age-1 length distributions of adult bluefish from the 1994-98 year-classes for North Carolina fisheries (positive axis) and fish captured from the Mid Atlantic Bight from a fishery-independent trawl survey and commercial fishery (negative axis). Values for each length bin are mean percentages across year-classes. Data from the Mid Atlantic Bight were taken from Conover et al. (2003).
Figure 2.7. Monthly mean (± SE) back-calculated age-1 lengths of bluefish from the 1994-99 year-classes, sampled from two fishing gears from the North Carolina fishery at (a) age-1, (b) age-2, and (c) age-3 through 5.
Chapter 3. Comparing multiple predictors of energy content in juvenile bluefish

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ABSTRACT

The need to estimate energetic status in fish is common, but determining energy content is costly. I examined the relationship between energy density of wild juvenile bluefish Pomatomus saltatrix (115 to 310 mm) and multiple indicators of energetic status, to develop regression models for predicting bluefish energy content. Energy density was strongly related to percent dry mass of whole fish ($r^2 = 0.99$), but poorly related to residual wet mass ($r^2 = 0.33$), a commonly used condition index. Energy density was related to three hepatosomatic indices: liver wet mass ($r^2 = 0.45$), liver dry mass ($r^2 = 0.64$), and liver energy content ($r^2 = 0.70$). Energy density was nonlinearly related to percent dry weight in muscle tissue ($r^2 = 0.93$) and energy density in muscle tissue ($r^2 = 0.83$). A biphasic pattern of lipid storage was found in muscle tissue, with very little lipid stored until after a threshold in percent dry weight was exceeded. Using my predictive equation, energy content in whole bluefish can be determined using the percent dry weight of muscle tissue. However, this predictive model should only be used for wild-caught bluefish because laboratory-held fish exhibited higher lipid:protein ratios in muscle tissue.

INTRODUCTION

In fisheries research the need to quantify the energetic status of individual fish is important for many applications, including the examination of seasonal energy storage
for overwinter survival (Jonas et al. 1996; Morley et al. 2007) or reproduction (Jonsson et al. 1997; Dhieb et al. 2006), interannual trends in population energetic status (Lambert and Dutil 1997a), and bioenergetics modeling (Elliott 1976). However, determining the energy content of fish can be time consuming. For larger individuals, whole fish must be homogenized so that smaller subsamples can be processed. The total energy content can then be obtained by multiplying the energy density of subsamples with the weight of the fish. For many species, predictive equations based on percent dry weight have been developed that estimate energy density (e.g. Hartman and Brandt 1995; Ciancio et al. 2007). Percent dry weight (100-dry weight/wet weight) is determined by drying whole fish or homogenized subsamples. While using percent dry weight to predict energy density is more efficient, it is still a time consuming process for larger fish. Due to the difficulties in determining energy content, studies often instead use indicators of energetic status, and assume that indicators are proportionally related to the actual energetic status of whole fish.

Two commonly used indicators of energetic status in fish are condition indices and tissue-specific sampling. Condition indices use the length and weight of a fish to determine its relative mass for a given length. The use of condition indices are time and cost effective, and condition typically displays a positive relationship with energy density of whole fish (Pangle and Sutton 2005; Rennie and Verdon 2008). However, for some species there is a great deal of variation in energy content for a given value of condition (Jonas et al. 1996; Trudel et al. 2005). Tissue-specific indicators are often more closely related to energy density than condition indices (Plante et al. 2005; Trudel et al. 2005; Kaufman et al. 2007). Some common examples include using the energy or lipid density of muscle tissue, or the hepatosomatic index, which is the relative mass of the liver to body weight. The best tissue to be used as an energetic indicator often depends on where energy is stored by the species of interest (Lambert and Dutil 1997b; Kaufman et al. 2007; Jacobs et al. 2008). Fish primarily store energy as lipid, in the form of triglycerides, and storage depots differ among species (Love 1980; Sheridan
Many species store and utilize lipid throughout the body (Lizenko et al. 1975; Jorgensen et al. 1997; Slater et al. 2007), while others utilize more specific tissues such as muscle, liver, or mesenteric fat (Love 1980; Sheridan 1988). An important drawback to using tissue-specific indices is that they do not incorporate the relative mass of the fish. For example, it is possible for two fish to appear similar in energetic status based on tissue-specific indicators, despite large differences in their relative mass.

Validation studies should be conducted before using indicators of energetic status for two reasons. First, validation studies allow predictive equations to be developed that estimate energy content in whole fish based on simple indicators. By using predictive equations one can incorporate both the mass and energy density of fish and eliminate the bias associated with condition and tissue-specific indicators. Second, investigators should determine how closely related indicators are to energy density of whole fish and if that relationship is linear. If the relationship is nonlinear, changes in the indicator would not proportionately represent changes in the whole fish. Validation studies have been conducted using both laboratory-held and wild-caught fish. However, the accuracy of energy density predictions for wild fish made using equations that were developed using laboratory fish has received little attention. Despite a lack of knowledge about physiological differences between laboratory and wild fish, predictive equations for energy content or body composition have been published using laboratory fish (Brown and Murphy 1991; Jacobs et al. 2008).

Bluefish Pomatomus saltatrix are an economically important species with a worldwide subtropical distribution (Juanes et al. 1996). Along the U.S. east coast and Gulf of Mexico, bluefish are one homogenous population (Graves et al. 1992). Large seasonal changes in energy content have been observed in this population due to energy storage during the fall and depletion during the winter (Morley et al. 2007). Wiedenmann and Essington (2006) suggested that winter mortality of juvenile bluefish may be a key factor in determining recruitment success. Energy storage dynamics of juvenile bluefish may influence survival during the overwinter period, so the validation
of an accurate energetic indicator is important. Hartman and Brandt (1995) found that percent dry weight of whole bluefish was good predictor of energy density. However, simpler means of energy determination have not been examined. Despite the lack of validation, tissue-specific sampling and condition indices have been used to examine seasonal trends in bluefish energy storage (Dhieb et al. 2006; Morley et al. 2007; Slater et al. 2007).

In this study, I examine multiple indicators of energetic status for juvenile bluefish, and develop predictive equations for energy content in whole fish. I relate whole bluefish energy density with a suite of indicators: percent dry weight of whole fish ($%DW$), a condition index, three variations of the hepatosomatic index ($HSI$), percent dry weight of muscle tissue ($%DW_{muscle}$), and energy density of muscle tissue. I also examine the relationship between lipid:protein content and $%DW$ for both whole bluefish and muscle tissue. Last, I examine differences in body composition between laboratory-held and wild-caught bluefish by comparing the ratio of lipid:protein content in the muscle tissue of each.

**METHODS**

Juvenile bluefish from the 2006 and 2007 year-classes were used to compare whole fish energy density with energetic indicators. Bluefish were caught in November 2006 and between November 2007 and April 2008 by bottom trawl on the continental shelf of North Carolina. Upon capture, bluefish were placed in plastic bags and packed in ice. In the laboratory, fish were measured for fork length (mm), wet weighed (0.01 g), sealed in plastic bags, and stored in a freezer at -15°C. Fish to be used for analyses were chosen from a large collection of frozen samples to maximize variation in length and date of capture. This was done by randomly selecting a bluefish from each 25 mm length bin during every month of sampling. The resulting sample ($n = 33$) ranged in fork length from 115 to 310 mm. When processed, whole livers and a sample (between 1 and 4 g) of white muscle tissue, cut from the area between the anterior dorsal fin and
the lateral line, were removed. These tissues were weighed (0.001 g) and placed in a drying oven at 60°C (Lantry and Gorman 2007) for three days then reweighed to determine $%DW_{muscle}$ and dry weight of liver. For two bluefish, some desiccation of muscle tissue had occurred during freezer storage, so muscle samples were not taken from these individuals and regressions involving muscle had a lower sample size ($n = 31$). Stomach contents were removed from the remaining bluefish carcass and weighed (0.001 g), and this weight was subtracted from initial wet weight. The carcass was then cut into smaller pieces and placed in a separate drying oven at 60°C until weight loss ceased; this took between 10 and 15 days. Dry weights of the muscle tissue sample, liver, and carcass were combined to get a total dry weight, and to determine $%DW$.

Energy content of dried tissues and carcass was determined using proximate composition analysis. Dried bluefish carcasses were homogenized in a food processor, and two 1.5 g subsamples were taken for processing. Dried muscle and liver samples were cut into smaller pieces with dissecting scissors. Neutral lipids were then quantified by Soxhlet extraction with petroleum ether for four hours; this is an effective technique to estimate triglyceride content (Dobush et al. 1985). The extracted samples were then combusted in a furnace at 450°C for 24 h to determine the mass of remaining organic material and inorganic ash content. For carcass subsamples, if lipid content of the two differed by over 6%, a third subsample was processed. This occurred with six bluefish. Proximate composition of the whole carcass was determined by extrapolation, using the mean value of the subsamples. To determine proximate composition of whole fish, data from muscle tissue, liver, and carcass were combined. Carbohydrates are often ignored in proximate analyses due to low concentrations in fish (Craig 1977; Black and Love 1986; Jonsson et al. 1997), so I considered the remaining organic material after lipid extraction to be protein. The energy content of tissues and whole fish was calculated using the energy equivalents of lipid (39.54 kJ/g) and protein (23.64 kJ/g) that were shown by Henken et al. (1986) to accurately estimate energy content when compared to methods of bomb calorimetry.
I examined the relationships of seven different energy indicators to energy density of whole fish. First, energy density was related to \%DW of whole fish. Next, a condition index was developed. To do this, I combined length and weight data from the 33 bluefish described above, with data from 1,112 bluefish from the 2001 and 2002 year-classes. Sampling for the 2001 and 2002 year-classes is described in detail by Morley et al. (2007). Briefly, juvenile bluefish were collected by bottom trawl and hook and line in coastal waters of North Carolina between September 2001 and June 2002, and September 2002 and June 2003. All weight data were adjusted for gut contents. Data used from the 2001 and 2002 year-classes were within the same length range as the 33 bluefish being used for energy analyses. Least squares regression was used on log_{10} transformed data to create the following equation:

\[
\log_{10}(WW) = -5.501 + 3.264\cdot\log_{10}(FL) \quad r^2 = 0.98, n = 1145
\]  

(1)

where \(WW\) is wet weight and \(FL\) is fork length. The residual value of \(\log_{10}(WW)\) for the initial 33 bluefish was used as an estimate of condition for these fish.

I next correlated energy density with three types of \(HSI\).

\[
HSI = (L/WW)\cdot100
\]  

(2)

Where \(L\) is the liver wet weight, dry weight, or energy content for the three types of \(HSI\) index.

Last I examined the relationships between energy density of whole fish and both \%\(DW_{\text{muscle}}\) and energy density of muscle tissue. These two relationships appeared nonlinear and I used four functions that appeared to fit the data well, using non-linear regression analysis. First, a linear model was fit:

\[
ED = \beta_0 + \beta_1\cdot M
\]  

(3)
where $ED$ is energy density of whole fish, $\beta_0$ is the intercept, $\beta_1$ is the slope, and $M$ is either $\%DW_{\text{muscle}}$ or energy density of muscle tissue. An asymptotic curve was fit with an added parameter for the intercept:

$$ED = \beta_0 + (\text{ED}_{\text{max}} \cdot M) / (k + M)$$  \hspace{1cm} (4)

where $\text{ED}_{\text{max}}$ is the asymptote, and $k$ is the level of $M$ where $ED$ is half of the asymptotic value. A sigmoidal curve was fit:

$$ED = (\text{ED}_{\text{max}} \cdot M^q) / (k^q + M^q)$$  \hspace{1cm} (5)

which is identical to equation 4, but without an intercept parameter and with added parameter $q$. Last, a piecewise regression function was fit where two linear segments are joined at a breakpoint:

$$ED = \begin{cases} 
\beta_0 + \beta_1 \cdot M & \text{for } M \leq \alpha \\
\beta_0 + \beta_1 \cdot M + \beta_2(M - \alpha) & \text{for } M > \alpha 
\end{cases}$$  \hspace{1cm} (6)

where $\beta_1$ is the slope of the first segment, $\beta_2$ is the difference in slope between the first and second segments, and $\alpha$ is the breakpoint. To determine the most suitable model using muscle tissue, I calculated $AIC_c$ values for each function as

$$AIC_c = [n \cdot \log_e(\text{RSS} / n) + 2 \cdot K] + [2 \cdot K(K + 1) / (n - K - 1)]$$  \hspace{1cm} (7)

where $n$ is sample size, RSS is the residual sum of squares, and $K$ is the number of estimated parameters including the error term (Burnham and Anderson 2002). This is a modified version of AIC with an adjustment for small sample sizes. For each model I
also calculated a $\Delta AIC_c$ value, which is the difference between the $AIC_c$ of a given model and that of the best model, and the $AIC_c$ weight ($w$), which is the probability that a given model is the best one (Burnham and Anderson 2002).

To explain the mechanism causing the nonlinear relationship in energy density between whole fish and muscle tissue, the ratio of lipid:protein content in relation to percent dry weight was examined for both whole bluefish and muscle tissue. For muscle tissue, in addition to the 33 bluefish processed for this study, I again included data from the 2001 and 2002 year-classes. The muscle energy data from these year-classes has previously been used to examine seasonal trends (Morley et al. 2007). The laboratory procedure used for these samples was identical to this study, with the exception that whole fish energy content was not determined.

To determine if my results for field-caught fish are comparable to results derived from fish held in the lab for a prolonged period I compared the lipid:protein content in muscle tissue of laboratory-held bluefish with the wild-caught fish described above. The laboratory bluefish were part of a winter feeding and growth experiment (Chapter 2). Briefly, juvenile bluefish (n = 36) ranging in fork length from 136 – 170 mm were captured by beach seine in late November 2006. Fish were held in 540 L flow-through tanks at ambient temperatures and fed once daily to satiation. On January 30, bluefish were randomly assigned to six different tanks and each tank was gradually adjusted to one of three temperatures: 9.5, 12.0, and 14.5°C. Bluefish were then fed twice daily to satiation until March 1 when the experiment was ended and fish were sacrificed. Whole fish energy content was not determined for these samples, but the procedure for analysis of muscle tissue was identical to the present study.

**RESULTS and DISCUSSION**

A strong linear relationship was found between $\%DW$ and energy density of whole fish ($ED = -2.91 + 0.35 \cdot \%DW; r^2 = 0.99; n = 33; P < 0.001$). This relationship is similar to that found by Hartman and Brandt (1995) found for bluefish ($ED = -3.79 +$
0.37 \% DW; \( r^2 = 0.98; n = 13 \). However, in their study calorimetry was used to
determine energy content. The difference between my model and that of Hartman and
Brandt (1995) is greatest at low levels of \% DW, and the two models converge as \% DW
increases. For example, at 19\% dry weight the two expected values of energy density
differ by 13\%; at 34\% dry weight, expected values differ by only 1.5\%. The reason my
expected values are higher may be due to the difference in using proximate composition
analysis versus calorimetry to determine energy content. I assumed that the leftover
organic matter after lipid extraction was entirely protein, but other organic molecules
would be present in small amounts. Also, the difference between models may be
variation in the relationship occurring at the spatial or interannual level.

My condition index was significantly related with energy density, but explained
relatively little variation \( (r^2 = 0.33, n = 33, P < 0.001; \) Figure 3.1). For a given value of
condition, energy density varied by up to a factor of two. Other studies have also found
that condition to explained little variation in energy content (Jonas et al. 1996; Trudel et
al. 2005). Condition often relates poorly to energy because of the amount of variation
in water content (Sutton et al. 2000; Plante et al. 2005; Rennie and Verdon 2008) and
lipid content (Copeland and Carline 2004; Trudel et al. 2005; Rennie and Verdon 2008)
for a given value of condition. Further, the relationship between condition and energy
or proximate composition can vary seasonally (Neumann and Murphy 1992; Pangle and
Sutton 2005; Trudel et al. 2005) and among different populations (Copeland and
Carline 2004; Kaufman et al. 2007). The use of condition will often depend on what
amount of variation is considered acceptable. However, for many studies that conduct
lethal sampling, validating an alternative using tissue-specific indicators may increase
the accuracy of conclusions about energetic status.

All three HSI indices were related more strongly with energy density than the
condition index (Figure 3.2). However, HSI using liver wet weight still explained less
than half of the variation in energy density of whole fish \( (r^2 = 0.45, n = 33, P < 0.001; \)
Figure 3.2a). Taking the added step of using liver dry weight resulted in a substantial
improvement ($r^2 = 0.64$, $n = 33$, $P < 0.001$; Figure 3.2b). The use of liver energy content resulted in a small improvement over liver dry weight ($r^2 = 0.70$, $n = 33$, $P < 0.001$; Figure 3.2c). Residual values for all HSI relationships were not evenly distributed; fish with high values of energy density had a large range of HSI. While bluefish use the liver as an energy storage depot, and high concentrations of lipid are observed there, it accounts for a relatively small proportion of the energy in whole fish (Slater et al. 2007). The effectiveness of HSI as an indicator probably varies among species. Kaufman et al. (2007) examined multiple populations of walleye and generally found that condition indices performed better than HSI. Using HSI as an energy indicator may be more effective for species that use the liver as a primary storage site of lipids, such as Atlantic cod (Lambert and Dutil 1997b).

Based on $r^2$ values and residual patterns, both energetic status indicators using muscle tissue were better predictors of energy density in whole fish than condition or HSI (Figure 3.3). Based on $\Delta$AIC$_c$ scores, the piecewise, sigmoid, and linear functions using $\%DW_{\text{muscle}}$ as the predictor were good candidate models to estimate energy density of whole fish (Table 3.1). Of these candidate models I chose to proceed with the piecewise function because it was ranked as the best model and had the highest model weight ($w$). In the piecewise model, $\%DW_{\text{muscle}}$ is linearly related with energy density in whole fish up to a breakpoint value of 26.4% (Figure 3.3a). Beyond this breakpoint, energy increased more slowly with further increases in $\%DW_{\text{muscle}}$. The relationship between energy density in muscle tissue and whole fish was also best explained with a nonlinear model (Table 3.1; Figure 3.3b), but all four models based on muscle energy density ranked poorly compared to models based on muscle percent dry weight. Thus my results revealed that the additional laboratory costs required to determine energy density in muscle tissue does not result in a more accurate predictor of total energy content. Using muscle tissue has been shown to be an effective indicator for other species. Kaufman et al. (2007) found that lipid content of walleye muscle tissue was more closely related to somatic proximate composition than HSI. Jacobs et al. (2008)
found that proximate composition of both the abdominal wall and whole fillets was effective at predicting energy content in whole striped bass. Further, the ability to use water content in muscle tissue to estimate energy content in whole fish has been shown for winter flounder (Plante et al. 2005), and coho and Chinook salmon (Trudel et al. 2005).

There is an important distinction to be made between using tissue-specific indicators as a proxy for energetic status, versus using predictive equations derived from indicators to determine energy content in whole fish. I advocate the second approach using predictive equations for two reasons. First, theoretically two fish of the same length and energy density can vary greatly in mass. In this situation, a study that only reports energy density values, or that uses an energetic indicator, will not fully account for the difference in energetic status between these two fish. For example, Einen et al. (1998) found that proximate composition of Atlantic salmon to remained fairly stable when they were starved, while body mass declined. Simpkins et al. (2003) found that HSI remained stable in rainbow trout starved for over 100 days, despite substantial loss of body lipid and protein. The second reason deals with tissue-specific indicators that are nonlinearly related to energy content of whole fish, as I have shown with muscle tissue in bluefish. In this situation, changes in the indicator will not be equally proportional to changes in energetic status. Therefore, for juvenile bluefish I recommend using the predictive equation to convert values of $\%DW_{\text{muscle}}$ to energy density (Table 3.1), and then multiplying by the wet weight of the fish to determine total energy content.

The nonlinear relationship between energy density of whole fish and muscle tissue (Figure 3.3b) is due to the non-uniform way bluefish store lipid throughout the body. When whole fish are examined, the ratio of lipid:protein content increases linearly with $\%DW$ ($r^2 = 0.94, n = 33, P < 0.001$; Figure 3.4a). However, in muscle tissue I observed a biphasic relationship between lipid:protein content and $\%DW_{\text{muscle}}$. A piecewise function was used to describe this relationship ($r^2 = 0.89, n = 724, P < 0.01$;
Figure 3.4b). Below the breakpoint of 23.1%, the ratio of lipid:protein in muscle tissue remains near zero, and newly deposited tissue is almost entirely protein. As a result of mostly protein being deposited initially in muscle, the energy density of the whole fish increases faster relative to the energy density of muscle (Figure 3.3b) as a result of lipids being stored in non-muscle tissues first. Once $\%DW_{\text{muscle}}$ exceeds the threshold of 23.1%, added organic mass in muscle tissue is mostly lipid and energy content of muscle increases rapidly.

Few studies have examined the relationship between tissue-specific energy content and energy in whole fish. To my knowledge, the bi-phasic pattern of muscle lipid storage has not been identified in other species. Aside from the skeleton, white muscle accounts for the largest mass of tissue in most fishes, and in bluefish white muscle is the largest depot of storage lipid (Slater et al. 2007). Therefore it may seem surprising that the nonlinear pattern in the lipid:protein ratio I observed in white muscle is not observed in whole fish. This is likely due to my technique of examining only a specific section of white muscle. To my knowledge, the pattern of lipid storage within muscle tissue has only been examined for Atlantic salmon (Einen et al. 1998; Nanton et al. 2007). Each of these studies observed heterogeneous lipid distribution in muscle tissue, with higher lipid concentrations occurring in the more ventral areas including the abdomen. Jacobs et al. (2008) also found higher concentrations of lipid in the abdomen muscle compared to the remaining muscle in striped bass. The lipid:protein ratio of bluefish white muscle may increase linearly with percent dry mass when it is examined in its entirety. Future validation studies with other species may benefit from examining muscle taken from multiple regions, including the abdominal wall.

Juvenile bluefish held in aquaria for four months during the winter developed much higher lipid:protein ratios in their muscle tissue when compared to wild bluefish (Figure 3.4b). Only muscle tissue was examined, so it is uncertain if lipid content is higher throughout the body of laboratory bluefish. However, I advise against using my
predictive equation on laboratory bluefish because these results suggest that whole body energy content of captive fish would be underestimated. Research on other species suggests that the lack of activity in laboratory fish probably leads to altered body composition. Simpkins et al. (2003) held rainbow trout in the laboratory and some fish were forced to swim continually. These active trout had lower lipid densities for a given value of $\%DW$ when compared to sedentary fish. Further, the relationship between condition and lipid density differed between active and sedentary fish. Similarly, studies with walleye and bluegill have shown the relationship between condition and body composition to differ between laboratory and wild fish (Copeland and Carlisle 2004; Copeland et al. 2010).

Juvenile bluefish recruit along the entire U.S. east coast and during the fall and early winter they migrate south to overwinter (Wuenschel et al. in review). My sampling area off NC is near the northern limit of the juvenile bluefish overwintering range (Morley et al. 2007; Wuenschel et al. in review). By sampling during the migratory period I was able to collect juveniles that initially recruited over a large geographic area. Further, by sampling between November and April I was able to capture juvenile bluefish across the full range in energetic status (Morley et al. 2007). The $\%DW_{\text{muscle}}$ values for the present study (2006-07 year-classes; Figure 3.4b) encompass nearly the entire range that has been observed for juvenile bluefish (Morley et al. 2007; Chapter 4). Therefore the energy storage patterns I described are applicable to the whole population. However, the accuracy of my predictive model for larger bluefish (> 310 mm) is uncertain. Previous research examining whole bluefish suggests that the relationship between water content and energy, lipid and protein content is consistent across size (Hartman and Brandt 1995; Hartman and Margraf 2008). However, it is uncertain if lipid storage patterns in specific tissues changes in larger mature adults. Also, the accuracy of models predicting energy content may vary among populations (Kaufman et al. 2007). Future studies using my predictive models on larger bluefish, or with populations other than those along the U.S. east coast, would
benefit from determining whole fish energy content on a limited sample to validate the model.

I am confident that my predictive equations will perform well with independent data since the physiology of energy storage in fish appears relatively simple. For example, a majority of the variation ($r^2 > 0.9$) in energy content of fish can be explained using only percent dry weight (Hartman and Brandt 1995; Ciancio et al. 2007). In this study I confirmed that patterns of energy storage in bluefish are consistent between studies; my relationship of percent dry weight to energy density in whole fish was similar to that reported by Hartman and Brandt (1995), despite using different methods to determine energy content. Further, I found similar patterns of lipid storage for bluefish muscle tissue across multiple year-classes (Figure 3.4b).

Energetic indicators are commonly used in fisheries science, but in most cases the relationship of indicators with the actual energetic status of the fish is not determined. Validation studies like this one should be the first step when examining the energy dynamics of a species. Predictive models can be developed to estimate the total energy content of fish, which is an unbiased and cost-efficient method of quantifying energetic status. Using percent dry weight of whole fish is an effective way to estimate energy content, but this is a labor-intensive task with larger fish. Tissue-specific sampling is a promising alternative to using percent dry weight of whole fish for predicting total energy content. The predictive model for bluefish energy content developed in this study will allow future studies that examine energy dynamics to standardize their methodology, and results between studies will be more comparable.

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Table 3.1.—Models of energy density (kJ/g wet mass) for whole bluefish (ED) using either percent dry weight in muscle tissue (%DW<sub>M</sub>) or energy density in muscle tissue (ED<sub>M</sub>) as a predictor variable (n = 31). The mean (SD) of each predictor was %DW<sub>M</sub> = 22.51 (2.76), and ED<sub>M</sub> = 4.67 (1.09). K = number of parameters, RSS = residual sum of squares, AIC<sub>c</sub> = Akaike information criterion corrected for small sample size, ΔAIC = AIC<sub>c</sub> difference from the best model, w = mean AIC<sub>c</sub> weight.

<table>
<thead>
<tr>
<th>Function</th>
<th>Equation</th>
<th>K</th>
<th>r&lt;sup&gt;2&lt;/sup&gt;</th>
<th>RSS</th>
<th>AIC&lt;sub&gt;c&lt;/sub&gt;</th>
<th>ΔAIC</th>
<th>w</th>
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<tbody>
<tr>
<td>Percent dry weight muscle (%DW&lt;sub&gt;M&lt;/sub&gt;)</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>ED = -5.45 + 0.51 %DW&lt;sub&gt;M&lt;/sub&gt;</td>
<td>3</td>
<td>0.91</td>
<td>6.1</td>
<td>-43.8</td>
<td>1.5</td>
<td>0.18</td>
</tr>
<tr>
<td>Asymptote</td>
<td>ED = -16.51 + (48.59 %DW&lt;sub&gt;M&lt;/sub&gt;) / (25.75 + %DW&lt;sub&gt;M&lt;/sub&gt;)</td>
<td>4</td>
<td>0.91</td>
<td>5.7</td>
<td>-43.0</td>
<td>2.3</td>
<td>0.12</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>ED = (12.40 %DW&lt;sub&gt;M&lt;/sub&gt;&lt;sup&gt;1.15&lt;/sup&gt;) / (22.56&lt;sup&gt;1.15&lt;/sup&gt; + %DW&lt;sub&gt;M&lt;/sub&gt;&lt;sup&gt;1.15&lt;/sup&gt;)</td>
<td>4</td>
<td>0.92</td>
<td>5.4</td>
<td>-44.8</td>
<td>0.5</td>
<td>0.31</td>
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<tr>
<td></td>
<td>-7.15 + 0.59 %DW&lt;sub&gt;M&lt;/sub&gt; for %DW&lt;sub&gt;M&lt;/sub&gt; ≤ 26.42</td>
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<tr>
<td>Piecewise*</td>
<td>ED = -7.15 + 0.59 %DW&lt;sub&gt;M&lt;/sub&gt; - 0.42 (%DW&lt;sub&gt;M&lt;/sub&gt; - 26.42) for %DW&lt;sub&gt;M&lt;/sub&gt; &gt; 26.42</td>
<td>5</td>
<td>0.93</td>
<td>4.8</td>
<td>-45.3</td>
<td>0</td>
<td>0.39</td>
</tr>
<tr>
<td>Energy density muscle (ED&lt;sub&gt;M&lt;/sub&gt;)</td>
<td></td>
<td></td>
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<tr>
<td>Linear</td>
<td>ED = 0.47 + 1.20 ED&lt;sub&gt;M&lt;/sub&gt;</td>
<td>3</td>
<td>0.79</td>
<td>14.2</td>
<td>-17.3</td>
<td>27.9</td>
<td>0.00</td>
</tr>
<tr>
<td>Asymptote</td>
<td>ED = -42.33 + (56.95 ED&lt;sub&gt;M&lt;/sub&gt;) / (0.79 + ED&lt;sub&gt;M&lt;/sub&gt;)</td>
<td>4</td>
<td>0.83</td>
<td>11.5</td>
<td>-21.2</td>
<td>24.1</td>
<td>0.00</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>ED = (9.87 ED&lt;sub&gt;M&lt;/sub&gt;&lt;sup&gt;1.12&lt;/sup&gt;) / (3.91&lt;sup&gt;1.12&lt;/sup&gt; + ED&lt;sub&gt;M&lt;/sub&gt;&lt;sup&gt;1.12&lt;/sup&gt;)</td>
<td>4</td>
<td>0.83</td>
<td>11.3</td>
<td>-21.8</td>
<td>23.5</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>-2.32 + 1.87 ED&lt;sub&gt;M&lt;/sub&gt; for ED&lt;sub&gt;M&lt;/sub&gt; ≤ 5.18</td>
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</tr>
<tr>
<td>Piecewise</td>
<td>ED = -2.32 + 1.87 ED&lt;sub&gt;M&lt;/sub&gt; - 1.14 (ED&lt;sub&gt;M&lt;/sub&gt; - 5.18) for ED&lt;sub&gt;M&lt;/sub&gt; &gt; 5.18</td>
<td>5</td>
<td>0.82</td>
<td>11.6</td>
<td>-18.1</td>
<td>27.2</td>
<td>0.00</td>
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</table>

* Additional information for piecewise model with %DW<sub>M</sub> as predictor. The mean square of the regression = 301.21 while the mean square error = 0.179. The estimates of the regression parameters (SE) were β<sub>0</sub> = -7.15 (1.04), β<sub>1</sub> = 0.59 (0.05), β<sub>2</sub> = -0.42 (0.19), and α = 26.42 (1.08); refer to equation 6 in methods section for parameter definitions.
Figure 3.1. Energy density of whole bluefish in relation to condition. Condition is expressed in terms of residuals from a regression of $\log_{10}$ (wet weight) on $\log_{10}$ (fork length) with data from multiple year-classes of bluefish ($n = 1,145$).
Figure 3.2. Energy density of whole bluefish in relation to hepatosomatic indexes based on (A) liver wet mass, (B) liver dry mass, and (C) liver energy content.
Figure 3.3. Energy density of whole bluefish in relation to (A) percent dry weight of white muscle tissue fit with piecewise regression and (B) energy density of white muscle tissue fit with a sigmoidal function. The functions fit to the data were chosen based on lowest $AIC_c$ score.
Figure 3.4. Lipid:protein ratios in relation to percent dry weight for (A) whole bluefish and (B) white muscle tissue of laboratory-held and wild-caught bluefish, with piecewise regression function fit to wild-caught bluefish data.
Chapter 4. Overwinter energy storage dynamics and foraging ecology of juvenile bluefish

ABSTRACT

The seasonal energy storage dynamics and foraging ecology of overwintering juvenile bluefish *Pomatomus saltatrix* were examined on the North Carolina continental shelf. Bottom trawling was conducted monthly to sample bluefish and prey between October and June over six years. Monthly changes in the allometric relationship between length and total body energy were examined for three year classes to examine differences in energy use between spring- and summer-spawned bluefish. Overwintering diet of each cohort was described for three year classes. Winter foraging was also examined by modeling gut fullness with multiple abiotic and biotic factors using a generalized additive model approach. Spring-spawned bluefish, due to their larger body size, were found to have an energetic advantage during winter because of energy reserves accumulated during the fall. However, the spring-spawned fish also deplete a much larger percentage of stored energy during the winter. The energy reserves of summer spawned bluefish were related to winter severity; fish suffered a greater energetic deficit during a severe winter, but during a mild winter energy reserves peaked later in the season. Striped anchovy *Anchoa hepsetus* were the most important prey for overwintering bluefish and anchovy abundance was the primary predictor of gut fullness in winter foraging models. Thus, feeding appears to be related to prey abundance, and winter feeding is probably important for offsetting energy loss during winter. The availability of prey may be critical during winter months.
INTRODUCTION

Juvenile fishes in temperate regions have multiple physiological adaptations for winter survival. The best known of these is the buildup of energy reserves during the fall. Energy reserves are important during winter as prey may be less abundant than in other seasons (Adams et al. 1982; Foy and Paul 1999), and temperatures may fall below the minimum value at which fish are able to maintain body weight by feeding (Jobling 1994). The storage and use of energy is related to length. Energy reserves increase disproportionately with length, giving larger fish an energetic advantage. Further, mass-specific metabolism decreases as mass increases, so larger fish deplete energy more slowly. Therefore, it is no surprise that overwinter starvation experiments consistently show that larger fish have a survival advantage (Thompson et al. 1991; Schultz et al. 1998; Biro et al. 2004; Bystrom et al. 2006). However, these same studies show that when food is provided, the effect of body size is much reduced or eliminated. Indeed, survival of juvenile fishes during winter appears to depend on more than just the amount of energy stored during the fall (Hurst 2007).

Although winter feeding is often overlooked, field-based overwintering studies typically have found it to be important (e.g. Foy & Paul 1999; Hurst & Conover 2001b; Eckmann 2004). Smaller individuals may feed more frequently in order to make up for inadequate energy reserves. For example, feeding among smaller members of a year class is one reason why some field-based studies have shown that the rate of energy depletion increases with size, contrary to metabolic expectations (Paul et al. 1998; Eckmann 2004; Biro et al. 2004). While numerous studies have observed that winter feeding is important, to my knowledge no statistical attempt has been made to determine which factors are important to winter feeding. Moreover, there are no foraging ecology studies of bluefish during winter.

Bluefish Pomatomus saltatrix are an economically important coastal species that have been shown to experience year class regulation through juvenile winter mortality (Wiedenmann and Essington 2006). Among temperate species, bluefish produce one of
the broadest length ranges of young-of-the-year, exceeding 250 mm (Wuenschel 2012). This is due to their extended spawning season and rapid growth rate. A majority of juveniles belong to one of two distinct intra-annual cohorts, one from a spring-spawning period and the other from summer spawning (Wuenschel et al. 2012; Chapter 2). Morley et al. (2007) examined overwinter changes in muscle energy density from each cohort and determined that the larger spring-spawned fish had an energetic advantage during winter. However, a more effective method of estimating energetic status of bluefish has recently been developed, which will allow for a more complete understanding of winter energy use between the two cohorts (Chapter 3).

Few studies have integrated energy dynamics and foraging ecology of overwintering fishes. Here I examine seasonal changes in energy storage for spring- and summer-spawned bluefish during the overwintering period, using a new method to determine total body energy (Chapter 3). Next, I describe the overwintering diet of each cohort; there is currently no published information on juvenile bluefish winter diet. Last, I examined how multiple abiotic and biotic variables affect overwinter feeding using a generalized additive model (GAM) approach. The use of GAMs is an effective way to examine stomach content data because these data often do not conform to parametric statistics, and nonlinear relationships between feeding and predictors are common. (Adlerstein and Wellemann 2000; Porter et al. 2005; Voss et al. 2008).

**METHODS**

*Field sampling*

This study is based on data and samples collected from two bottom trawl surveys conducted in Onslow Bay, North Carolina (between Cape Lookout and Cape Fear); these surveys were part of a larger effort to gain a better understanding of bluefish recruitment along the U.S. east coast (Wuenschel et al. 2012). The nearshore continental shelf in this region is an important overwintering area for juvenile bluefish (Morley et al. 2007; Chapter 2). The first trawl survey is described in detail by Morley et
al. (2007). Briefly, a 12 m headrope bottom trawl was used monthly between October 2001 and May 2002, and between October 2002 and June 2003. Four transects in Onslow Bay were sampled each month during the 2001-02 sampling season and two were sampled during the 2002-03 season. Each transect was perpendicular to shore and consisted of five trawl stations located 0.4, 0.8, 1.6, 3.2, and 8 km from shore. Two twenty-minute hauls were conducted, parallel to shore, at each station. Catches were sorted and the abundance of each species was determined, along with thirty length measurements per species. Bluefish were bagged and stored on ice to be processed in the lab.

The second survey utilized a 30 m headrope bottom trawl and was conducted monthly between November 2004 and April 2008. During this period there was a hiatus in sampling between January and October of 2007. Two transects were sampled in the same fashion as described above, but an additional trawl station was added at 5.6 km from shore, and only one haul was performed at each station. The trawl catch was processed as described above. For this study, I only used data from the overwintering period (October to June). I extended the overwintering period beyond the actual winter season (i.e., December 21 – March 21) in order to include both the buildup of energy reserves prior to winter and the recovery of energy during spring (Morley et al. 2007). Further, examining an extended winter season provided a greater range in variables, such as temperature and date, that were used in the winter feeding model (see below).

**Seasonal energy storage**

In the lab, total body energy was determined for a subsample of bluefish from each month. Bluefish were chosen to obtain a representative sample across the entire length range of fish sampled. Energy was determined using the method outlined in Chapter 3. Briefly, bluefish were weighed wet (WW; 0.01 g) and a sample of dorsal-white muscle tissue was excised from each fish. Muscle samples were dried to determine %dry weight (%DW). Total body energy (TBE) was calculated with the following equation.
\[ TBE = WW \cdot \begin{cases} -7.15 + 0.59 \cdot DW \text{ (for } \%DW \leq 26.42) \\ -7.15 + 0.59 \cdot DW - 0.42 \cdot (\%DW - 26.42) \text{ (for } \%DW > 26.42) \end{cases} \]  \hfill (eq. 1)

Temporal changes in the relationship between length and energy content were examined by calculating the allometric relationship between fork length (FL) and TBE for each month and year-class. The allometric equation is a power function

\[ TBE = a \cdot FL^b \]  \hfill (eq. 2)

where \( a \) and \( b \) are estimated parameters. This equation can be linearized by the following transformation.

\[ \ln(TBE) = \ln(a) + b \cdot \ln(FL) \]  \hfill (eq. 3)

When the allometric scaling exponent \( b \) is greater than three, total energy increases disproportionately with length and larger fish have an energetic advantage. Conversely, when \( b \) is less than three, smaller fish will have an energetic advantage (Post and Parkinson 2001; Hurst 2003). Analysis of covariance (ANCOVA) was used for each year class, to determine if the scaling exponent changed significantly during the overwintering period.

To better visualize changes in energy storage patterns during the overwintering period, the allometric equations for each month and year class were used to determine the predicted TBE for a typical individual from each cohort. A representative length for each cohort was used to determine predicted values (150 mm for summer cohort, 250 mm for spring cohort). To get standard errors for these predicted values, an empirical bootstrap technique was used. First, I used the parameter values \( a \) and \( b \), from the log-transformed linear allometries (equation 3), and fit the data with a power function (equation 2). Next, the residual values from these models were randomly reassigned to predicted values and the power function was refit. This was repeated 2000 times, and
for each iteration predicted values for bluefish of 150 and 250 mm were calculated. Standard error values were determined from these 2000 iterations.

**Diet analysis**

Stomachs were removed from bluefish and stored in a 10% seawater buffered formalin solution for later examination. Stomachs from at least eight individuals from each cohort (spring and summer) were examined per trawl sample, unless the catch was smaller than eight, in which case all stomachs were examined. During diet examination, stomach contents were removed, blotted dry, and individual prey items were weighed (0.001 g). Fish prey were identified to species if possible, while invertebrate prey were divided into four categories: *Loligo* sp. (squid), bivalves, crustaceans, and polychaetes. Diet analysis was conducted for the 2001, 2002, and 2004 year classes. Stomach examination was also conducted for the 2005, 2006, and 2007 year classes, but these data were only used for gut fullness estimates and the modeling of winter feeding (described below).

I used the method of Buckel et al. (1999) to describe bluefish diet during the overwintering period (October – June). This method treats the bottom trawl hauls as the sampling units, as opposed to individual fish, and each haul is weighted according to the number of bluefish caught. The estimate of mean proportional contribution of each prey type \((k)\) by weight \((W_k)\) was calculated by

\[
W_k = \frac{\sum_{i=1}^{n} M_i q_{ik}}{\sum_{i=1}^{n} M_i}
\]

(eq. 4)

where

\[
q_{ik} = \frac{w_{ik}}{w_i}
\]

(eq. 5)

and where \(n\) is the number of trawl hauls containing bluefish, \(M_i\) is the number of bluefish captured in haul \(i\), \(w_{ik}\) is the weight of prey type \(k\) from haul \(i\), and \(w_i\) is the total
weight of identifiable prey from the stomachs examined from haul \( i \). Diet was also described using the mean percentage of stomachs (frequency of occurrence) that contain each prey type \( F_k \), and was calculated by

\[
F_k = \frac{\sum_{i=1}^{n} M_i p_{ik}}{\sum M_i}
\]

(eq. 6)

where

\[
p_{ik} = \frac{m_{ik}}{m_i}
\]

(eq. 7)

and where \( m_{ik} \) is the number of stomachs in haul \( i \) containing prey type \( k \), and \( m_i \) is the number of stomachs examined from haul \( i \). Both \( W_k \) and \( F_k \) were expressed as a percentage for the results.

**Modeling winter feeding**

I used GAMs to examine the relationship between both abiotic and biotic predictor variables with bluefish gut fullness during the overwintering period. A GAM is a type of generalized linear model that uses multiple-localized smooth functions of predictor variables (Wood 2006). GAMs are effective at modeling nonlinear relationships with data that do not conform to parametric statistics. They can incorporate multiple predictor variables, and can model individual predictors as linear or smoothed functions within a single model. The response variable was mean gut fullness, square-root transformed, of juvenile bluefish within a trawl haul. Multiple transformations of the dependent variable were considered and the square-root was superior based on residual analysis. This analysis was conducted using R-software (version 2.15.2) with the ‘mgcv’ package and the Gamma distribution and log link function. I used the cubic spline smoother \( s \) for all smoothed functions. The degree of
smoothness ($\gamma$) was set at 1.4 in order to reduce the chance of overfitting some predictors (Wood 2006).

Six predictor variables were examined with the GAM analysis. The first was date of sampling to examine seasonal effects, which was modeled as Julian Day Number. The second predictor was water temperature, which was measured for each haul. Third, was number of bluefish captured in the haul. This predictor was included to determine if density-dependent feeding was evident. The fourth predictor was median fork length of bluefish examined within trawl hauls. This was included because maximum gut fullness declines with increasing size in fish, due to the allometric scaling of stomach size (Buckel et al. 1995). The fifth predictor was a measure of prey abundance; the number of *Anchoa* sp. captured in the haul was used. Anchovy are the most important prey for bluefish during the overwintering period (see results). Last, I included median *Anchoa* sp. total length, as a measure of prey size. These biotic predictors are affected by the type of bottom trawl used. Therefore, data from the 12- and 30-meter trawls were examined separately.

Prior to model selection, correlation among predictor variables was examined using Pearson’s product-moment correlation. Significantly correlated predictors were not included in the same model. During model selection, a full model containing all non-correlated predictors was formulated first. Then multiple reduced models were examined by sequentially removing predictors based on their $P$-values. Akaike information criterion values, adjusted for smaller sample sizes ($AIC_c$), were calculated for each model. Also, the $\Delta AIC$ was calculated for each model, which is the difference in $AIC_c$ value between a given model and the model with the lowest $AIC_c$ score. Models with $\Delta AIC$ values below two were considered for the final model selection (Burnham and Anderson 2002). Among these potential models I used standard residual analyses and model diagnostics (i.e. amount of deviance explained and $P$-values of predictors) to select the final model.
RESULTS

Seasonal energy storage

The 2001, 2002, and 2007 year classes were examined for seasonal energy storage (Table 4.1). The 2004 and 2005 year classes were excluded because muscle samples from these years had desiccated during freezer storage. ANCOVA results showed that there was significant temporal variation in the allometric scaling exponent within each year class (Table 4.2; interaction term significant for each year class). The seasonal pattern of allometric scaling was similar across the three overwintering periods examined (Figure 4.1). During the fall, larger juvenile bluefish possessed a large energetic advantage as the scaling exponent ranged between 3.9 and 4.3 among these three year classes. However, as the season progressed the allometric slope declined. The reason for this decline becomes apparent when the predicted body energy contents for typical summer- and spring-spawned bluefish are compared (Figure 4.2). First, the summer cohort appears to peak in total energy up to two months later than the spring cohort. Second, spring-spawned fish use a much larger proportion of their energy reserves during the winter. For example, a typical 250 mm bluefish from the 2001 year class used nearly half of its body energy between November and April (Figure 4.2). During that same overwintering period, a typical 150 mm bluefish only used about 17% of its peak energy stores.

It is worth mentioning two anomalous data points. First, for the 2002 year class, the allometric slope was unusually high during early spring (Figure 4.1). This was due to the summer cohort having low energy stores following the winter that year (Figure 4.2). Second, the 2007 year class had an allometric slope below three in January, although the slope was not significantly lower than three (t-test, $t = 0.676, P = 0.25$). The reason for the low allometric slope during winter that year was because the summer cohort increased energy reserves later into the season (Figure 4.2).
**Diet analysis**

The diet of spring-spawned juvenile bluefish during the overwintering period was dominated by teleost fishes (Table 4.3). However, squid were an important diet component of the 2004 year class. For both diet metrics (%W and %F), anchovies were the most important prey, making up more than half of diet weight for these year classes. Striped anchovies (*A. hepsetus*) were the most important prey species, although bay anchovies (*A. mitchilli*) were also consumed. The importance of other prey species varied among year-classes. Three species of clupeid were identified, and they were a minor contributor to consumed prey during two years. Silversides (*Atherinopsidae*), spot (*L. xanthurus*), and pinfish (*L. rhomboides*) also made minor contributions, each in one year class. Also, for one year class, a species of myliobatiform (rays) was a minor diet item; in each case, the whip-like tail was found in pieces.

Teleost fishes dominated the diet of the summer cohort as well (Table 4.4). The diet of the 2001 year class was over 90% anchovies by weight, mostly striped anchovy; pinfish also were a minor component of diet that winter. The 2002 year class had a broader diet and anchovies, clupeids, pinfish, and spotted hake (*U. regia*) were important when examining diet by weight. However, the importance of clupeids, pinfish, and spotted hake was much lower when considering frequency of occurrence. Anchovies were the dominant consumed prey for the 2004 year class. Clupeids were also important when considering diet by weight during the winter of 2004, especially Atlantic menhaden (*B. tyrannus*) and Atlantic thread herring (*O. oglinum*). In summary, for both cohorts and all year classes, anchovies were dominant prey, which validates the use of anchovy catch as a metric for prey abundance in my examination of winter feeding.

**Modeling winter feeding**

For the GAM analysis conducted with data from the 12-meter trawl sampling, a number of predictor variables covaried (Table 4.5). As a result, models with more than two predictor variables were not possible; all combinations of non-correlated predictor
variables were examined (Table 4.6). Two models fit the data well, but the model using anchovy abundance as the sole predictor was chosen for the final model (Table 4.7). The second model also included bluefish abundance as a predictor, but this only marginally increased the amount of deviance explained. Bluefish exhibited lower gut fullness values when anchovy abundance was low (Figure 4.3). As anchovy abundance increased from low levels, gut fullness increased rapidly and then leveled out.

For the 30-meter trawl GAM the only predictor variables that were significantly correlated were temperature with both date and median bluefish length (Table 4.5). Therefore, two full models were tested, one that excluded temperature and the other excluding date and median bluefish length (Table 4.6). Three potential models fit the data well, one with anchovy abundance as the sole predictor while the other two combined anchovy abundance with either temperature or bluefish abundance. The final model used anchovy abundance and temperature (Table 4.7) and was chosen because it explained the most deviance (Table 4.6). The GAM for the 30-meter trawl resulted in a similar pattern with anchovy abundance as the 12-meter trawl (Figure 4.4), but the model explained relatively little variability in the data. Temperature exhibited a slight decreasing linear effect on gut fullness (Figure 4.4). However, temperature made only a minor contribution to model performance.

DISCUSSION

Seasonal energy storage and winter feeding

Winter severity appears to play an important role in the energy dynamics and survival of juvenile bluefish during the winter. For example, the 2002 year class experienced a severe winter with temperature dropping earlier in the season than normal (Morley et al. 2007; Chapter 2). Both the spring and summer cohorts appeared to store less energy during the fall that year. This lower peak in energy reserves did not appear to affect the spring cohort as there was little difference between year classes later in the winter. However, the summer cohort had the lowest recorded energy
reserves of this study following that winter. Further, Morley et al. (2007) found evidence for negative size selective winter mortality for this year class. The remaining two year classes from this study experienced mild winters, and the 2007-2008 winter was the mildest between 2001 and 2007 (Morley et al. 2007; Chapter 3). During this winter the summer cohort was able to continue building energy reserves into early January. Further, this year class showed evidence of winter growth (Chapter 3).

The analysis of bluefish energetic allometries shows how different sized fish within a year class can have very different overwintering strategies. Bluefish may represent an extreme example of this, because the modal lengths of the spring and summer cohorts may differ by over 100 mm (Wuenschel et al. 2012; Chapter 2). In fishes, the allometry of energy storage typically gives individuals of greater length an energetic advantage during the fall (Hurst 2007). This may result from relatively small individuals devoting a larger portion of ingested energy for growth, because smaller fish face a greater mortality risk from predation compared to starvation (Post and Parkinson 2001). Juvenile bluefish are consistent with this hypothesis. Spring-spawned juveniles are able to build up a large amount of energy stores during the fall and these are depleted throughout the winter season. The rapid depletion of energy by the spring cohort, as compared to summer-spawned fish, is counterintuitive given that mass-specific metabolism decreases with increasing fish size (Jobling 1994). However, greater winter energy depletion with increasing fish size has been reported for several other species (Paul et al. 1998; Eckmann 2004; Biro et al. 2004).

There are multiple reasons why winter energy depletion may be more rapid among larger juvenile bluefish. First, spring-spawned fish may be more active than summer-spawned fish during winter. Second, there is evidence that spring-spawned fish mostly occur in warmer offshore waters during the winter (Morley et al. 2007). The higher temperatures offshore would increase their metabolic energy use. Last, spring-spawned bluefish may have a “defended energy level” (Mrosovsky & Sherry 1980), which is described as a target energy level an individual maintains, that is based on
anticipated energy needs to survive the winter. This target energy level declines as the winter season progresses. Atlantic salmon and striped bass show evidence for this energetic strategy during winter (Bull et al. 1996; Hurst & Conover 2001a). The lack of variation in energy stores between year classes for a 250 mm bluefish supports this hypothesis. Surplus energy may go towards somatic or skeletal growth. Surplus energy probably does not go towards gonad development. This is because females do not mature until about 480 mm and males at about 340 mm (Salerno et al. 2001; Robillard et al. 2008). Further, the overwinter development of gonads was not apparent during fish dissections. Notwithstanding, some of the largest age-1 fish may spawn during spring, but these individuals would probably not occur in my sampling area.

Bluefish in the summer cohort exhibit very different energy storage patterns during winter compared to spring-spawned fish. They achieve peak energy at a later date, which may result from two things. First, the energetic cost of migration is much greater in smaller fish (Nottestad et al. 1999), which would reduce the rate of building up energy reserves in smaller individuals. Second, because summer-spawned fish have less of an energetic buffer against starvation (Slater et al. 2007), they may continue foraging intensely during late-fall and early-winter. Summer-spawned bluefish also use a much lower proportion of stored energy during the winter. This may result from three things. First, the summer cohort shows evidence of occupying cooler-nearshore waters during winter (Morley et al. 2007). This may be a behavioral adaptation that reduces energy depletion, which has been shown experimentally with other species (Morgan & Metcalfe 2001; van Dijk, et al. 2002). Unfortunately, the spatial scale of the two trawl surveys used in this study were not large enough to conclusively address this hypothesis. Second, smaller bluefish may be less active during winter. A winter reduction in activity (Sandstrom 1983) and metabolism (Evans 1984; Karas 1990) has been shown for other species. Summer-spawned bluefish have been experimentally shown to experience a reduction in appetite during winter, which suggests decreased activity and energy use (Chapter 2). However, comparable data for spring-spawned fish
is not available, so a cohort comparison is not possible. The last potential explanation for the energy depletion pattern of the summer cohort is that these fish depend on winter feeding to prevent energy loss.

Evidence for the importance of winter feeding and prey abundance for juvenile bluefish is shown in the GAM analysis. If feeding during the winter was not important, temperature or date would be expected to explain most of the variation in gut fullness. However, neither of these factors played an important role in winter-feeding models. Temperature was included in the final model for fish collected with the 30 m trawl, but its effect was small and suggested that gut fullness is actually higher at lower temperatures. The main factor that explained variation in gut fullness was prey abundance; when anchovy catch was low, gut fullness was negatively affected. This suggests that bluefish feed when prey are available during winter. While studies examining overwintering fish typically ignore the importance of winter feeding (Hurst 2007), those that have examined it have found it to be important (Hurst & Conover 2001b; Eckmann 2004; Bell 2012). Even more rare are studies that have examined winter prey abundance (Hurst 2007). To my knowledge, only two other field-based studies have demonstrated a link between prey abundance and either winter survival or energy storage patterns (Adams et al. 1982; Foy and Paul 1999).

The GAM results showing that prey abundance is important for winter feeding are biased in three ways. First, it is likely that in many instances substantial time (perhaps many hours) would have passed between when a bluefish consumed a prey item and when it was captured by the trawl. So prey abundance from trawl data would not always reflect what the fish experienced when feeding. While prey abundances were typically similar between trawl stations on a given transect, order of magnitude differences were not uncommon, suggesting that prey aggregations were often patchy. However, it is possible that bluefish stay in close proximity to anchovy aggregations, which would help mitigate biases due to differences in timing between bluefish feeding and prey abundance sampling. Second, measuring anchovy abundance with the 30 m
bottom trawl was probably less accurate than estimates made with the 12 m trawl. The 30 m trawl often had catches exceeding 500 kg; the size of these catches made it easier for small prey fish to be underrepresented because large catches were subsampled by volume. This is probably the reason for the poor model performance with the larger sampling gear. Last, only anchovy were considered as prey for this analysis. However, other species did play a minor role in bluefish overwintering diet. Because of the above three biases, the importance of prey abundance for overwinter feeding is probably more important than my results indicate.

Temperature is well known to affect consumption rate in fishes (Jobling 1994). However, the GAM analysis on winter feeding showed temperature to be of little or no importance. This illustrates a limitation with using gut fullness as a metric for consumption. As temperature declines, the rate of digestion slows in fishes (Jobling 1994). For example, with bluefish, it takes seven hours for a meal to be 75% digested at 30°C, compared to 11 hours at 21°C (Buckel and Conover 1996). Maximum consumption experiments on bluefish have also demonstrated a temperature effect on consumption (Buckel et al. 1995; Harman and Brandt 1995). There are two reasons why temperature probably did not explain much variation in gut fullness data. First, the temperature effect on consumption has been shown to be less pronounced during winter (Chapter 2). Second, while temperature may slow digestion, overwintering bluefish may still initiate foraging when their stomachs are empty. This would result in temperature not explaining much variation in the data.

**Diet analysis**

Many diet studies have been published on juvenile bluefish. This body of research has shown bluefish to be opportunistic predators, feeding on different prey species based on availability (e.g. Friedland et al. 1988; Buckel and Conover 1997; Gartland et al. 2006). These studies have shown that juvenile bluefish >70 mm are largely piscivorous, and important prey are consistently pelagic zooplanktivores (e.g. *Anchoa* sp., Atherinopsidae, Clupeidae; Marks and Conover 1993). Prior to this study,
little was known about bluefish diets during the winter, or south of Cape Hatteras, North Carolina. My results have shown that winter diets of bluefish on the continental shelf are similar to bluefish diets farther north during summer and fall. However, my results suggest that bluefish are consistently more dependent on a single prey, striped anchovy, during winter. Other studies rarely show a single species consistently making up more than 50% of diet by weight (e.g. Friedland et al. 1988; Buckel and Conover 1997; Gartland et al. 2006). Similarly, a different population of bluefish off Brazil also had a more restricted diet during winter and anchovies were the most important prey (Lucena et al. 2000).

Most studies examining the life history or trophic importance of anchovies on the U.S. east coast have been conducted with bay anchovy (*Anchoa mitchilli*) (e.g. Vouglitois et al. 1987; Scharf et al. 2002). Scharf et al. (2003) showed that bay anchovy are relatively easy for bluefish to capture. Further, low temperatures do not inhibit bluefish ability to capture bay anchovy (Chapter 5). It is unknown if striped anchovy are also relatively “easy prey.” Morphologically, the two species are difficult to distinguish when compared at the same size. However, very little research has focused on the ecology of striped anchovy. This is surprising, as they may have the greatest biomass on the continental shelf of the southern United States (SEAMAP 2000), which makes this species an important trophic link from zooplankton to piscivores in the region.

**Conclusions**

This study supports the works of Wiedenmann and Essington (2006) and Morley et al. (2007), which suggest that the winter season is important in regulating year class strength in bluefish. It is evident that bluefish have adapted physiologically to a stressful winter season by accumulating energy during the fall and also by suppressing appetite (Chapter 2), which suggests a reduction in metabolism as seen in other species (Evans 1984; Karas 1990). These adaptations suggest that winter poses a risk of mortality associated with the depletion of energy reserves. However, my results suggest that it may only be in severe winters when the summer cohort risks starvation. During
severe winters bluefish endure extended periods with temperatures below critical levels where weight cannot be maintained; I showed experimentally that this critical temperature is between 10 and 12°C (Chapter 2). However, in the wild active-metabolic energy use would be much greater, so the minimum temperature for maintaining body weight is probably higher. Further, during winter, smaller juvenile bluefish may have a more restricted prey field due to difficulties in consuming relatively large prey at low temperatures (Chapter 5). Also, the energetic cost of handling prey is probably much higher at low temperatures due to increased handling time and an increased reliance on severing prey in half prior to ingestion.

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REFERENCES


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Table 4.1. Sample sizes and lengths of bluefish used for energy allometry analysis. Also provided are $r^2$ values for regressions of ln(total body energy) against ln(fork length).

<table>
<thead>
<tr>
<th>Sampling dates</th>
<th>$n$</th>
<th>Fork lengths (mm)</th>
<th>Mean length (mm)</th>
<th>Standard deviation</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2001 - 2002</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct 18 – Nov 20</td>
<td>82</td>
<td>158 – 300</td>
<td>218</td>
<td>28.43</td>
<td>0.93</td>
</tr>
<tr>
<td>Nov 25 – Dec 21</td>
<td>97</td>
<td>127 – 297</td>
<td>212</td>
<td>48.49</td>
<td>0.96</td>
</tr>
<tr>
<td>Jan 5 – 15</td>
<td>23</td>
<td>125 – 276</td>
<td>158</td>
<td>36.47</td>
<td>0.90</td>
</tr>
<tr>
<td>Feb 2 – 13</td>
<td>44</td>
<td>124 – 248</td>
<td>187</td>
<td>38.85</td>
<td>0.96</td>
</tr>
<tr>
<td>Apr 2 – 8</td>
<td>41</td>
<td>135 – 273</td>
<td>183</td>
<td>29.59</td>
<td>0.94</td>
</tr>
<tr>
<td>Apr 30 – May 7</td>
<td>53</td>
<td>117 – 236</td>
<td>191</td>
<td>34.79</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>2002 – 2003</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct 18 – Nov 25</td>
<td>56</td>
<td>136 – 255</td>
<td>208</td>
<td>52.59</td>
<td>0.98</td>
</tr>
<tr>
<td>Dec 12 – Jan 5</td>
<td>50</td>
<td>106 – 242</td>
<td>165</td>
<td>30.94</td>
<td>0.95</td>
</tr>
<tr>
<td>Mar 22 – Apr 1</td>
<td>55</td>
<td>131 – 304</td>
<td>210</td>
<td>51.61</td>
<td>0.96</td>
</tr>
<tr>
<td>Apr 13 – May 10</td>
<td>36</td>
<td>147 – 269</td>
<td>221</td>
<td>42.53</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>2007 – 2008</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov 12 – 13</td>
<td>82</td>
<td>140 – 281</td>
<td>193</td>
<td>30.96</td>
<td>0.96</td>
</tr>
<tr>
<td>Dec 6 – 7</td>
<td>59</td>
<td>115 – 236</td>
<td>171</td>
<td>33.53</td>
<td>0.94</td>
</tr>
<tr>
<td>Jan 13 – 14</td>
<td>46</td>
<td>123 – 232</td>
<td>155</td>
<td>25.63</td>
<td>0.91</td>
</tr>
<tr>
<td>Apr 25</td>
<td>80</td>
<td>129 – 247</td>
<td>190</td>
<td>28.94</td>
<td>0.93</td>
</tr>
</tbody>
</table>
Table 4.2. Analysis of covariance models for the allometric analysis of juvenile bluefish total body energy during the overwintering period. FL = fork length.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2001 year class</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month</td>
<td>1.18</td>
<td>5</td>
<td>10.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ln(FL)</td>
<td>102.94</td>
<td>1</td>
<td>4781.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Month*ln(FL)</td>
<td>1.35</td>
<td>5</td>
<td>12.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>7.06</td>
<td>328</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2002 year class</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month</td>
<td>0.44</td>
<td>3</td>
<td>6.10</td>
<td>&lt;0.001</td>
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<tr>
<td>ln(FL)</td>
<td>120.55</td>
<td>1</td>
<td>5058.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Month*ln(FL)</td>
<td>0.44</td>
<td>3</td>
<td>6.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>4.50</td>
<td>189</td>
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<td></td>
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<tr>
<td><strong>2007 year class</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Month</td>
<td>0.86</td>
<td>3</td>
<td>13.12</td>
<td>&lt;0.001</td>
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<tr>
<td>ln(FL)</td>
<td>76.16</td>
<td>1</td>
<td>3478.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Month*ln(FL)</td>
<td>0.89</td>
<td>3</td>
<td>13.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>5.67</td>
<td>259</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.3. Diet and summary of stomach sampling from overwintering (October – June) spring-spawned juvenile bluefish. %W = percent by weight; %F = percent frequency of occurrence; FL = fork length.

<table>
<thead>
<tr>
<th>Prey item</th>
<th>2001</th>
<th>2002</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%W</td>
<td>%F</td>
<td>%W</td>
</tr>
<tr>
<td>Anchoa sp. (total)</td>
<td>76.5</td>
<td>38.2</td>
<td>69.5</td>
</tr>
<tr>
<td>Anchoa hepsetus</td>
<td>44.3</td>
<td>31.6</td>
<td>69.0</td>
</tr>
<tr>
<td>Anchoa mitchilli</td>
<td>2.2</td>
<td>0.7</td>
<td>5.0</td>
</tr>
<tr>
<td>Anchoa sp.</td>
<td>30.1</td>
<td>8.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Clupeidae (total)</td>
<td>6.2</td>
<td>8.6</td>
<td>16.6</td>
</tr>
<tr>
<td>Sardinella aurita</td>
<td>0.5</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Brevoortia tyrannus</td>
<td>0.0</td>
<td>0.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Opisthonema oglinum</td>
<td></td>
<td></td>
<td>6.6</td>
</tr>
<tr>
<td>Clupeidae</td>
<td>5.7</td>
<td>7.6</td>
<td>8.2</td>
</tr>
<tr>
<td>Atherinopsidae</td>
<td>1.1</td>
<td>1.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Scomberomorus sp.</td>
<td>1.9</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Leiostomus xanthurus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lagodon rhomboides</td>
<td>2.4</td>
<td>0.3</td>
<td>12.2</td>
</tr>
<tr>
<td>Orthopristis chrysoptera</td>
<td>1.8</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Peprilus sp.</td>
<td>0.4</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Larval fish</td>
<td>1.2</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Unidentified fish remains</td>
<td>NA</td>
<td>37.0</td>
<td>NA</td>
</tr>
<tr>
<td>Myliobatiformes</td>
<td>6.7</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Squid (Loligo sp.)</td>
<td>0.3</td>
<td>0.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Bivalve</td>
<td>1.5</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Crustacean</td>
<td>0.1</td>
<td>1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Polychaete</td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.7</td>
</tr>
<tr>
<td>Total stomachs examined</td>
<td>220</td>
<td>98</td>
<td>131</td>
</tr>
<tr>
<td># with prey in stomach</td>
<td>175</td>
<td>86</td>
<td>89</td>
</tr>
<tr>
<td>Mean FL (mm)</td>
<td>207.2</td>
<td>214.0</td>
<td>221.4</td>
</tr>
<tr>
<td>Standard Deviation FL</td>
<td>21.17</td>
<td>23.82</td>
<td>32.60</td>
</tr>
<tr>
<td>Range FL (mm)</td>
<td>176 - 276</td>
<td>181 - 278</td>
<td>176 - 318</td>
</tr>
</tbody>
</table>
Table 4.4. Diet and summary of stomach sampling from overwintering (October – June) summer-spawned juvenile bluefish. %W = percent by weight; %F = percent frequency of occurrence; FL = fork length.

<table>
<thead>
<tr>
<th>Prey item</th>
<th>2001</th>
<th>2002</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%W</td>
<td>%F</td>
<td>%W</td>
</tr>
<tr>
<td><em>Anchoa sp.</em> (total)</td>
<td>91.8</td>
<td>29.7</td>
<td>28.0</td>
</tr>
<tr>
<td><em>Anchoa hepsetus</em></td>
<td>70.8</td>
<td>19.3</td>
<td>20.1</td>
</tr>
<tr>
<td><em>Anchoa mitchilli</em></td>
<td>1.9</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td><em>Anchoa sp.</em></td>
<td>19.0</td>
<td>9.3</td>
<td>8.0</td>
</tr>
<tr>
<td>Clupeidae (total)</td>
<td>0.6</td>
<td>0.7</td>
<td>19.3</td>
</tr>
<tr>
<td><em>Brevoortia tyrannus</em></td>
<td>0.6</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td><em>Opisthonema oglinum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clupeidae</td>
<td>0.6</td>
<td>0.7</td>
<td>18.7</td>
</tr>
<tr>
<td>Atherinopsidae</td>
<td>0.7</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td><em>Lagodon rhomboides</em></td>
<td>4.3</td>
<td>3.5</td>
<td>37.0</td>
</tr>
<tr>
<td><em>Pepirius alepidotus</em></td>
<td></td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td><em>Pepirius triacanthus</em></td>
<td></td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td><em>Urophycis regia</em></td>
<td>12.5</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Larval fish</td>
<td>1.2</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Unidentified fish remains</td>
<td>NA</td>
<td>24.3</td>
<td>NA</td>
</tr>
<tr>
<td>Squid (<em>Loligo sp.</em>)</td>
<td>1.8</td>
<td>0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Bivalve</td>
<td>0.5</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Crustacean</td>
<td>0.6</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Polychaete</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

| Total stomachs examined    | 207 | 185 | 138 |
| # with prey in stomach     | 137 | 142 |  61 |
| Mean FL (mm)               | 153.2 | 156.8 | 146.9 |
| Standard Deviation FL      | 14.81 | 17.30 | 14.30 |
| Range FL (mm)              | 117 - 189 | 106 - 199 | 110 - 175 |
Table 4.5. Pearson’s correlation matrix for predictor variables used in the generalized additive models (GAMs) with bluefish gut fullness as the response variable. The GAMs based on data from both the 12-meter and 30-meter bottom trawls are shown. Asterisks indicate significance ($P < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Date</th>
<th>Temperature</th>
<th>#Bluefish</th>
<th>Median bluefish length</th>
<th>#Anchovy</th>
<th>Median anchovy length</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>12-meter trawl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.17</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#Bluefish</td>
<td>-0.13</td>
<td>-0.25*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median bluefish length</td>
<td>-0.26*</td>
<td>0.43*</td>
<td>-0.02</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#Anchovy</td>
<td>-0.32*</td>
<td>0.47*</td>
<td>-0.09</td>
<td>0.42*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Median anchovy length</td>
<td>0.25*</td>
<td>-0.30*</td>
<td>0.05</td>
<td>-0.24*</td>
<td>-0.34*</td>
<td>1</td>
</tr>
<tr>
<td><strong>30-meter trawl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.28*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#Bluefish</td>
<td>-0.17</td>
<td>-0.21</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median bluefish length</td>
<td>-0.01</td>
<td>0.49*</td>
<td>-0.10</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#Anchovy</td>
<td>0.08</td>
<td>0.22</td>
<td>-0.07</td>
<td>0.18</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Median anchovy length</td>
<td>-0.13</td>
<td>-0.15</td>
<td>0.11</td>
<td>0.04</td>
<td>-0.16</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 4.6. Generalized additive models examining the influence of five predictor variables on bluefish gut fullness. Predictor variables that are not in parentheses were modeled as linear functions. Predictors preceded by an “s” were smoothed functions. AIC<sub>c</sub> = Akaike information criterion corrected for small sample size, ΔAIC = difference in AIC<sub>c</sub> value between each model and the best-fit model.

<table>
<thead>
<tr>
<th>Model</th>
<th>Total degrees of freedom</th>
<th>% Deviance explained</th>
<th>AIC&lt;sub&gt;c&lt;/sub&gt;</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>12-meter trawl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date + s(temperature)</td>
<td>4.9</td>
<td>15.2</td>
<td>-736.0</td>
<td>20.5</td>
</tr>
<tr>
<td>Date + #bluefish</td>
<td>3</td>
<td>2.8</td>
<td>-725.0</td>
<td>31.5</td>
</tr>
<tr>
<td>s(temperature)</td>
<td>2</td>
<td>6.8</td>
<td>-731.6</td>
<td>24.9</td>
</tr>
<tr>
<td>Median bluefish + #bluefish</td>
<td>3</td>
<td>14.1</td>
<td>-723.4</td>
<td>33.1</td>
</tr>
<tr>
<td>Median bluefish</td>
<td>2</td>
<td>13.9</td>
<td>-725.5</td>
<td>31.0</td>
</tr>
<tr>
<td>s(median anchovy) + #bluefish</td>
<td>4.9</td>
<td>9.9</td>
<td>-685.9</td>
<td>70.6</td>
</tr>
<tr>
<td>s(median anchovy)</td>
<td>3.92</td>
<td>9.9</td>
<td>-687.9</td>
<td>68.6</td>
</tr>
<tr>
<td>s(#anchovy) + #bluefish</td>
<td>5.9</td>
<td>30.0</td>
<td>-755.1</td>
<td>1.5</td>
</tr>
<tr>
<td>s(#anchovy)</td>
<td>4.9</td>
<td>29.7</td>
<td>-756.5</td>
<td>0</td>
</tr>
<tr>
<td><strong>30-meter trawl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s(#anchovy) + temperature + #bluefish + median anchovy</td>
<td>5.6</td>
<td>8.5</td>
<td>-479.2</td>
<td>71.2</td>
</tr>
<tr>
<td>s(#anchovy) + temperature + #bluefish</td>
<td>4.8</td>
<td>9.9</td>
<td>-547.1</td>
<td>3.3</td>
</tr>
<tr>
<td>s(#anchovy) + temperature</td>
<td>3.7</td>
<td>9.6</td>
<td>-548.8</td>
<td>1.6</td>
</tr>
<tr>
<td>s(#anchovy)</td>
<td>2.6</td>
<td>8.8</td>
<td>-550.4</td>
<td>0</td>
</tr>
<tr>
<td>s(#anchovy) + date + #bluefish + median anchovy</td>
<td>6.2</td>
<td>10.2</td>
<td>-479.2</td>
<td>71.2</td>
</tr>
<tr>
<td>s(#anchovy) + date + #bluefish + median bluefish</td>
<td>5.6</td>
<td>11.5</td>
<td>-546.8</td>
<td>3.6</td>
</tr>
<tr>
<td>s(#anchovy) + date + #bluefish</td>
<td>4.6</td>
<td>11.1</td>
<td>-548.3</td>
<td>2.1</td>
</tr>
<tr>
<td>s(#anchovy) + #bluefish</td>
<td>3.6</td>
<td>9.0</td>
<td>-548.5</td>
<td>1.9</td>
</tr>
</tbody>
</table>
### Table 4.7. Results of the predictor variables used in the final generalized additive models for the 12-meter and 30-meter trawls.

<table>
<thead>
<tr>
<th>Smooth terms</th>
<th>Variable</th>
<th>Degrees of freedom</th>
<th>Parametric coefficients</th>
<th>12-meter trawl</th>
<th>30-meter trawl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anchovy abundance</td>
<td>3.89</td>
<td>Intercept</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Anchovy abundance</td>
<td>1.74</td>
<td>Intercept</td>
<td>0.01</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Temperature</td>
<td>-0.0002</td>
<td>0.0003</td>
</tr>
</tbody>
</table>


Figure 4.1. Allometric slopes (±SE) of total body energy (kJ) for juvenile bluefish from three year classes during the overwintering period on the North Carolina continental shelf.
Figure 4.2. The predicted total body energy (±SE) for a 250 mm (top panel) and 150 mm (bottom panel) bluefish from three year classes during the overwintering period. Predicted values were determined from allometric equations of total energy versus fork length. Note the difference in y-axis scale between the top and bottom panels.
Figure 4.3. Generalized additive model of the effect of anchovy abundance on bluefish gut fullness on the North Carolina continental shelf during the overwintering period. Bluefish and anchovy were collected with a 12-meter bottom trawl between 2001 and 2003 (n = 109). The y-axis is the effect of anchovy abundance on bluefish gut fullness and the tick marks on the x-axis indicate data points in the model. Shaded area is two standard errors.
**Figure 4.4.** Generalized additive model of the effect of anchovy abundance (top) and temperature (bottom) on bluefish gut fullness on the North Carolina continental shelf during the overwintering period. Bluefish and anchovy were collected with a 30-meter bottom trawl between 2004 and 2008 ($n = 82$). The y-axis is the effect of the variable on bluefish gut fullness and the tick marks on the x-axis indicate data points in the model. Shaded area is two standard errors.
Chapter 5. The effects of temperature and prey size on predator-prey interactions between bluefish and bay anchovy

ABSTRACT

Little is known about the behavioral responses of fishes at low temperatures. Of particular interest are predator-prey interactions, because feeding at low temperature is necessary for the overwinter survival of many species. I conducted an experiment to examine how low temperature affects the behavioral interaction between bluefish and two sizes of bay anchovy prey. Temperature had an effect on multiple characteristics of predator-prey encounters including the approach distance of bluefish towards prey, attack and escape speeds, and prey handling time. The reaction distance of prey was important in determining the outcome of an attack; anchovy reacting at a greater distance from an attacking bluefish escaped more often. However, reaction distance was not significantly affected by temperature. Further, bluefish capture success was not affected by temperature. Predator-prey interactions depended on relative prey size. Larger prey were dropped more frequently after a successful capture, and drops were more common at lower temperatures for both prey sizes. Further, larger prey were more often severed before consumption, and bluefish became more dependent on severing prey at low temperatures. At the coldest treatment, no large prey were attacked and this was probably because larger prey were very difficult for bluefish to sever at low temperatures. These results suggest that at low winter temperatures bluefish are restricted to smaller prey, and that handling of prey is more energetically costly during winter.

INTRODUCTION

The importance of feeding to offset starvation during winter is often overlooked for temperate fishes (Hurst 2007). Previously, starvation was considered the primary
source of mortality during winter, but there is limited field evidence that conclusively shows starvation occurs in the wild (but see Lambert and Dutil 1997). Many laboratory studies show that the availability of food is critical for winter survival (e.g. Thompson et al. 1991; Biro et al 2004; Bystrom et al. 2006). In the wild, many temperate fishes feed during winter (e.g. Hurst and Conover 2001b; Eckmann 2004) and some are capable of winter growth (Bystrom et al. 2006; Bell 2012). Indeed, the feeding strategy of overwintering fishes is probably more complex than previously assumed (Hurst 2007). For example, detailed studies of winter feeding by striped bass and Atlantic salmon indicate that feeding motivation of these species is not controlled by a simple relationship with temperature, but by anticipated energy needs for winter survival (Bull et al. 1996; Hurst and Conover 2001a and b).

The effect of temperature on feeding has primarily been evaluated from a bioenergetics perspective. Laboratory studies used to estimate bioenergetics parameters typically do not use live prey, so the effects of temperature on feeding only represent physiological capacity (Jobling 1994). However, ecological processes (e.g., predator-prey interactions) may be more important for winter survival than physiological processes (Hurst 2007). Further, behavioral studies on responses of fish to low temperature are lacking. The dynamics of predator-prey interactions at low temperatures are of particular interest. If predators have more difficulty capturing prey at low temperatures, then their energy budget would be negatively impacted. For example, when prey are more difficult to capture, the energetic activity costs of predators increase (Selch and Chipps 2007). Further, increased activity costs can have a large impact on growth, which may affect multiple traits within a population (Rennie et al. 2005; 2012). It follows that increased difficulty in capturing prey at low temperatures would lead to important energetic implications for overwintering fishes, such as an increase in foraging time and greater energy use during foraging.

Bluefish (Pomatomus saltatrix) are a migratory pelagic piscivore found in temperate and subtropical waters in many areas of the world (Juanes 1996). They
exhibit one of the highest consumption and growth rates among temperate species (Juanes and Conover 1994; Hartman and Brandt 1995). For the United States Atlantic population, winter has been hypothesized to be critical for juvenile survival (Wiedenmann and Essington 2006). Juvenile bluefish from this population exhibit a bimodal length distribution during winter, consisting of summer- and spring-spawned cohorts (Chapter 2). The spring cohort consists of larger fish, which are capable of greater energy storage during fall, and they are resilient to starvation (Morley et al. 2007; Slater et al. 2007; Chapter 4). Conversely, the summer cohort maintains relatively low energy reserves, so winter feeding is probably critical (Morley et al. 2007; Chapter 4). In the lab when consuming thawed food, bluefish are capable of feeding and maintaining body weight at typical winter temperatures (Chapter 2). However, it is unknown if low temperatures negatively affect bluefish ability to catch and consume live prey. Further, it is unknown if low temperatures affect bluefish foraging mode. For example, during summer and fall, bluefish feed on a large range of prey sizes (Scharf et al. 2000); larger prey are first severed and then partially ingested (Scharf et al. 1997). If low temperatures affect their ability to consume larger prey, then bluefish may not have as broad a range of prey available to them during winter.

I conducted a laboratory experiment to examine the effect of temperature on behavioral interactions between bluefish and bay anchovy *Anchoa mitchilli*, a common bluefish prey in winter (Chapter 4), using a novel two-camera approach for estimating speed and distance in three dimensions. Predation trials were filmed at a range of temperatures that encompass environmental conditions bluefish experience from fall through spring (Morley et al. 2007; Chapter 2). Two sizes of bay anchovy were used to see if the effect of temperature on behavioral responses of bluefish depends on relative prey size.
METHODS

Bluefish and bay anchovy were collected from estuaries in Morehead City, North Carolina with a 30-m beach seine. Fish were transported to the University of North Carolina-Institute of Marine Science Fisheries Research Laboratory. Bluefish and anchovy were held in separate 540-l circular holding tanks that received flow-through seawater from Bogue Sound and were maintained at 20°C. Throughout the experiment bluefish were fed daily to satiation with either thawed *Anchoa* spp. or live *Anchoa* spp. and *Menidia menidia*. Anchovy were fed twice daily with formulated fish feed. Natural light was provided throughout the experiment.

This experiment was conducted during summer to ensure feeding motivation was high since bluefish consumption is influenced by season (Chapter 2). Feeding trials occurred from late-June through August in 2009, and mid-July through September 2010. Sampling for bluefish and bay anchovy was done periodically throughout the experiment. Predators and prey were acclimated for at least one week prior to use in trials. Two 740-l experimental tanks were used for feeding trials (length: 180 cm, 72 cm wide, 57 cm deep), each fit with a window encompassing one side. Three bluefish between 131-140 mm fork length were used for all trials, and were randomly sampled with replacement from holding tanks. However, individuals were not used in consecutive trials. Trials were conducted with eight bay anchovy from either a small (36-40 mm total length) or large (61-65 mm total length) size group that were randomly sampled without replacement. Anchovy were not handled directly and were kept submerged during measurements. During acclimation in experimental tanks (2-7 days), predators and prey were kept separate with a two-layer partition; one layer was clear and the other opaque, and mesh panels allowed for water circulation. Bluefish were fed mostly live prey while acclimating and were starved 48 h before trials. The partition was positioned so bluefish had access to 75% of the tank.

Bluefish and anchovy in experimental tanks were adjusted from 20°C at 2°C/d to one of five test temperatures: 10, 11, 13, 16, and 20°C; the choice of temperature
treatment for each successive trial was selected randomly. The lowest temperature
treatment is the minimum at which bluefish are caught in trawl surveys (Wiedenmann
and Essington 2006; Morley et al. 2007; Wuenschel et al. 2012); bluefish are not
capable of maintaining body mass at this temperature (Hartman and Brandt 1995;
Chapter 2). The highest temperature is an intermediate value for bluefish consumption
and growth (Buckel et al. 1995; Hartman and Brandt 1995). Three replicate trials were
conducted at each temperature using the smaller prey, but for large prey three replicate
trials were only conducted at 10, 13, and 20°C treatments. Fish were held at the
designated treatment temperature for 24 h prior to the trial. Two fluorescent lights (17
W), were positioned 0.25 m above experimental tanks during acclimation and the trial.

Feeding trials were conducted between 0800 and 1000. The opaque layer of the
partition was removed 5 min prior to the trial. At this time, bluefish and anchovy were
clearly aware of each other; bluefish would occasionally swim aggressively towards the
partition and anchovy would school against the far wall. Trials were recorded with two
video cameras (30 frames/s) positioned perpendicularly, one 1.2 m in front of the
window, the other facing down from 1.04 m above the tank. Trials were ended when all
prey were eaten, or after 30 min.

**Data analysis**

Eight variables characterizing predator-prey encounters were measured during
video analysis (Table 5.1). Distances were determined by using the estimated three-
dimensional coordinates of predator and prey (see below for coordinate estimation).
Speed was estimated using coordinates from sequential video frames; when fish moved
in a straight line, coordinates from start and end points were used. My initial goal was
to determine the functional relationship of predator and prey response variables with
temperature, and examine how these relationships differed between the two prey sizes.
However, bluefish made no attacks on the larger prey at 10°C, which prevented the
examination of functional relationships with larger prey. Therefore, I used 2-factor
ANOVA to examine the effects of prey size and temperature on response variables at the
two temperatures used with both prey sizes (13 and 20°C). Log_{10} transformations were used on response variables when this improved residual distributions. If no effect of prey size was found, the replicate means of both prey sizes were used to estimate functional relationships across the full temperature range examined; if prey size had a significant effect then only trials with small prey were used to estimate functional relationships. I fit a variety of functions between each response variable and temperature; these included linear, asymptotic, exponential, saturation, maximum, and sigmoidal. AIC_c was used to determine the most suitable functional relationship (Burnham and Anderson 2002).

**A novel approach to estimating fish location**

A clear 5 cm-scale grid was affixed to the back and bottom of each experimental tank. During video analysis, cameras were first synchronized using LED lights that flashed before each trial. The *apparent location* of a fish on a given video-frame could be determined in three-dimensions by recording the coordinates of the fish as a point on the grid. The *apparent location* could be determined in the x (left to right) and y (bottom to surface) dimension using the front camera, and the x and z (front to back) dimension using the overhead camera. The units on the grid were further refined into quarters by eye (e.g. of fish *apparent location*: x = 6.25, y = 4.0, z = 2.75). For bluefish, the snout (area anterior to eyes) was used to estimate *apparent location*, and for anchovy the center of body mass was used.

Due to the cone-shaped video field of the cameras, the *apparent location* of a fish is biased depending on its location in the field of view. To correct for this bias, I developed a three-dimensional matrix of coordinate correction-factors for both camera angles. To develop the matrix, a rod was positioned across the top of the tank at x = 1, and a series of eight weights were hung from the rod to y = 1 at designated points along the z-axis. While filming, the rod was repositioned at different coordinates along the x-axis. Then the weights were adjusted to the next y coordinate and the procedure was repeated. In this fashion, I recorded the difference between the *actual location* and
apparent location of the suspended weights for 25% of the x,y,z coordinates in the tank.

When analyzing a video frame, I first noted the apparent location of a fish from each camera (Table 5.2, row 1). Next, I used the correction factor for those coordinates (row 2) to determine the adjusted location (row 3). The matrix of correction factors was then referred to again, this time with the adjusted location coordinates. Occasionally (<10%), the adjusted location would have a slightly different set of correction factors (row 4), so a second iteration was necessary. The new correction factors would be applied to the apparent location, and the actual location would be determined (row 5). For ~25% of actual location estimates, the x coordinate differed between the two camera angles. For over 90% of these instances, the difference was only 0.25 grid-units (i.e. 1.25 cm). When this occurred, the camera angle with the least bias was used. For example, if the fish was near the bottom of the tank, the overhead camera was used to determine the x value.

To test the accuracy of my approach, I randomly generated twenty-five x,y,z coordinates. While filming, a single weight was sequentially positioned at each random coordinate. The videos were then analyzed blind to estimate these locations. To test for systematic bias in the estimation of each dimension (i.e., x, y, and z), a t-test was used to see if the mean difference between actual and estimated values was different than zero. I also calculated the distance between the estimated three-dimensional coordinates and the actual locations in the tank.

RESULTS

When testing my approach for estimating location in the tanks using random points, I found no evidence for systematic bias with estimating the x and z coordinates (t-test for difference between estimated and actual location: x-value, t = 0.47, P = 0.65; z-value, t = 1.36, P = 0.19). Estimates for the y-value were biased towards greater than actual values (t = 2.87, P = 0.01). However, this bias was not large; the mean y-value estimates were 0.4 cm greater than actual values. Further, the average distance
between estimated and actual three-dimensional point locations was only 1.2 cm (SD = 0.98). Thus, I ignored this bias in response variable measurements.

Bluefish typically ate all available prey during trials with small anchovy at the warmest two treatments, and between two and eight prey (out of eight available) at the three coldest treatments. On average, fewer prey were consumed during trials with large anchovy; between two and eight at the highest temperature and between one and four at the 13°C treatment. The larger prey were never attacked at the coldest treatment (10°C), while up to eight anchovy were consumed during small-prey trials at this temperature. Thus, feeding motivation may be high at 10°C, but feeding behavior suggests relatively large prey are unavailable, or less profitable energetically.

Bluefish typically attacked individual prey and would initiate an approach in response to anchovy movement. A majority of bluefish approaches and attacks came at a posterior or lateral angle to the anchovy (Fig. 5.1). The distance at which bluefish initiated an approach to prey was not influenced by anchovy size or temperature (13 vs. 20°C; Table 5.3; Fig. 5.2a). However, across all temperatures, the approach distance of bluefish exhibited a sigmoidal relationship with temperature (Table 5.4). At colder temperatures bluefish initiated approaches when closer to prey (Fig. 5.2a).

The effect of temperature (13 vs. 20°C) on bluefish attack distance was marginally significant but there was no effect of prey size (Table 3; Fig. 5.2b). At most temperatures, bluefish typically formed an S-start position while gliding towards prey, and then lunged with one stroke. However, at the warmest treatment, bluefish often would begin swimming aggressively towards prey from a greater distance. Functions that were fit to these data resulted in non-normal residual patterns, so the relationship of attack distance with temperature was not examined.

Anchovy reaction distance was not influenced by size or temperature when comparing 13 and 20°C treatments (Table 3; Fig. 5.2c). However, at the lowest temperature (10°C), anchovy generally had low reaction distances. Functions that were fit to these data explained little variation ($r^2 < 0.18$) and residual patterns were not
normal. Reaction distance of anchovy had a strong impact on the outcome of predator prey encounters (Fig. 5.3). When individual attacks from all trials were pooled, the anchovy reaction distance from successful bluefish attacks (including dropped prey) was significantly lower than for unsuccessful attacks (Mann-Whitney U test, n = 126, P < 0.01). Anchovy reaction distance has even greater importance considering that when prey reacted early, an approaching bluefish typically did not attack. Bluefish rarely pursued prey after a missed attack.

Bluefish attack speed increased significantly with temperature (from 13 to 20°C), but was not affected by prey size (Table 5.3; Fig. 5.4a). Combining attack speed from both small and large prey trials, I found that multiple functions described the relationship with temperature well (Table 5.4). Based on the AICc weight scores (w), an exponential model was used for the functional relationship between bluefish attack speed and temperature. Anchovy escape speed increased with temperature (from 13 to 20°C), and larger prey were significantly faster than small prey (Table 5.3; Fig. 5.4b). Using only small-prey trials, the relationship between escape speed and temperature was best explained by a saturation model (Table 5.4; Fig. 5.4b).

There was no effect of temperature (13 vs. 20°C) on capture success, but bluefish exhibited significantly lower capture success with larger prey (Table 5.3; Fig. 5.5a). The effect of prey size was not due to bluefish having more difficulty catching larger anchovy, it resulted from larger prey being dropped more often. Further, temperature also appeared to affect the frequency of bluefish dropping prey. At 20°C, 24% of large prey were dropped after capture and no small prey were dropped; at 13°C a larger proportion of prey were dropped, 43% of large and 11% of small anchovy (Fig. 5.5c). When a successful capture was redefined as a bluefish catching an anchovy and holding it in its jaws, there was no effect of prey size (Table 5.3; Fig. 5.5b). The effect of the full range of temperatures on capture success was examined using small-prey trials with linear regression and there was no significant temperature effect for either method of defining capture success (Fig. 5.5a and b; P > 0.33 for both).
At 13 and 20°C, the interaction between prey size and temperature was marginally significant for handling time (Table 5.3; Fig. 5.6a). For small prey, handling time was significantly shorter at 20°C (Tukey HSD test, \( P = 0.01 \)), but the temperature effect with large prey was not significant \( (P = 0.56) \). The effect of prey size at 20°C was marginally significant \( (P = 0.07) \). Based on the AIC\(_c\) weight scores \((w)\), a linear relationship best described how handling time decreases with increasing temperature for small prey (Table 5.4; Fig. 5.6a). Bluefish often severed prey in half before ingestion, which typically increased individual handling time. The proportion of ingested prey that were first severed in half was greater at lower temperatures and for larger prey (Fig. 5.6b). Of anchovy that were severed \((N = 33)\), 91% were oriented so the posterior section was consumed and the anterior section dropped. Further, of the prey that were consumed whole that I was able to determine orientation for \((N = 30)\), 87% were swallowed tail first.

**DISCUSSION**

*Effects of temperature and prey size on predator-prey behaviors*

Understanding what limits consumption at low temperatures is important for understanding the causes of winter mortality. A limited number of studies have examined how temperature affects capture success, and results are varied. The relationship may depend on the temperatures examined and how they overlap with the optimal temperature range of the predator and prey. For example, the ability of yellow perch to capture lake whitefish declines from 18 to 10°C (Yocum and Edsall 1974). In this case the predator had a higher optimal temperature than the prey. Conversely, Arctic grayling, a cold-water species, exhibits higher capture success on the copepod *Heterocope septentrionalis* as temperature drops from 15 to 5°C (Schmidt and O'Brien 1982).

Temperature did not significantly affect bluefish ability to capture anchovy prey. This is surprising as bay anchovy are more cold-water tolerant than bluefish (Vougloitos
et al. 1987; Luo and Brandt 1993). The lack of a relationship between capture success and temperature may be due to bay anchovy being relatively easy prey to catch (Scharf et al. 2003). While bay anchovy are important prey during the overwintering period for bluefish, striped anchovy (A. hepsetus) make up a greater proportion of the diet (Chapter 4). While the evasive ability of striped anchovy is unknown, the two species are very similar morphologically and can be difficult to distinguish at similar sizes. If striped anchovy are also relatively easy to capture across a wide temperature range, then this prey is probably important for juvenile bluefish winter energy dynamics.

By examining a suite of behavioral responses during bluefish and anchovy interactions, I was able to identify the mechanisms responsible for the relationships between temperature, prey size, and capture success. Reaction distance of anchovy was an important factor determining the outcome of predator-prey interactions in this study. Anchovy that reacted at a greater distance from an attacking bluefish were more likely to escape, and bluefish typically did not attack if prey reacted during an approach. The important influence that reaction distance has on capture success has also been shown with rainbow trout preying on goldfish (Webb and Zhang 1994). Further, Scharf et al. (2003) compared the reaction distance of four prey species and found that species with smaller reaction distances suffered higher capture success rates.

To my knowledge, no other study has examined reaction distance at different temperatures. Unfortunately, I was not able to include a functional relationship of anchovy reaction distance with temperature due to poor model fits; poor model performance was mainly the result of the large amount of variation observed at the 16°C treatment (Fig. 5.2c). Despite this, it is worth noting that the reaction distance of anchovy was consistently short at 10°C. It follows that the success of bluefish at catching prey was relatively high at this temperature (Fig. 5.5b). The short reaction distances at 10°C may have been the result of the low attack speeds at this temperature.

This pattern is consistent with Dill’s (1974) theory, which predicts that reaction
distance will decrease if either speed is reduced or size of the oncoming predator is smaller.

The influence of prey size on capture success depended on how a successful capture was defined. Typically, studies define a successful capture as prey being caught and ingested (e.g. Scharf et al. 2003; Staudinger and Juanes 2010). When defined this way, my results are consistent with previous work showing that capture success declines as relative prey size increases (Juanes and Conover 1994; Scharf et al. 2003; Staudinger and Juanes 2010). Scharf et al. (2003) examined the effects of relative prey size on capture success using bluefish as predators and four different prey species. Bluefish had the highest capture success with bay anchovy as prey. They conducted their feeding trials at 19-21°C, and capture success on bay anchovy of the same relative sizes was about twice as high as observed in the present study. Further, the mean reaction distance of anchovy in their study was only 3.1 cm, which is around one third the distance I measured. The difference between studies probably was not related to tank size. While Scharf et al. (2003) used smaller tanks (475 versus 740 l), they found no difference in capture success when comparing 475 and 900 l tanks. It is possible that the method of prey introduction to the feeding-trial tanks used by Scharf et al. (2003) left prey more vulnerable to capture; prey were transferred from a different tank 20 min before trials.

When capture success was defined as catching prey, regardless of prey ingestion, there was no effect of anchovy size. It follows that reaction distance was similar between large and small anchovy, so there was no size advantage in evading capture. However, handling difficulty, indicated by the higher percentage of dropped prey, led to a lower percentage of large anchovy being ingested after capture. At prey:predator length ratios greater than 0.35, bluefish shift from consuming whole prey, to severing prey and partial consumption (Scharf et al. 1997). Indeed, the larger anchovy in my study had a prey:predator length ratio of 0.41 and were more often severed in half prior to ingestion. Severing prey involved more prey manipulation, which would increase the
likelihood of prey escaping during handling. Further, severing prey often required considerable effort by the bluefish, which would increase the likelihood of prey being rejected.

Temperature also appeared to have an influence on prey-handling and ingestion. As expected, handling time increased as temperature declined (Bergman 1987; Barbeau and Scheibling 1994). More interestingly, the percentage of captured anchovy that were either dropped or severed in half prior to ingestion, both increased at lower temperatures. This suggests that prey-handling becomes more difficult as temperature declines and bluefish rely more on severing prey. For example, in trials with large anchovies at 13°C, almost half of captured prey were dropped and nearly all ingested prey were first severed. There was only one failure of a bluefish severing large prey out of fifteen attempts. However, I was unable to determine if bluefish are capable of severing large prey below 13°C because no attacks were made at the lowest temperature. In trials with small prey at 10 and 11°C, I found that only five out of sixteen attempts to sever prey were successful. This suggests that partial consumption of prey is more difficult below 13°C. To sever prey, bluefish rapidly shake them. The contractile velocity of muscle is positively related to temperature (Bennett 1984), so bluefish may have more difficulty at low temperatures generating the sudden burst of lateral speed required to sever prey. In summary, as temperature declines, bluefish depend more on partial consumption, but severing prey becomes more difficult at low temperatures. This would explain why large prey were not attacked at the coldest treatment. Thus, handling difficulty may prevent bluefish from feeding on relatively large prey near the low end of their temperature range.

Studies to measure the effect of temperature on burst swimming speed in fish are typically performed using an artificial stimulus to instigate an escape response (Webb 1978; O’Steen and Bennett 2003). In this study, I examined the role of temperature and prey length on swimming speed more realistically by examining predator-prey interactions. My results on attack and escape swimming velocity are
consistent with previous research showing that maximum velocity to increases nonlinearly with temperature (Wardle 1975; Webb 1978; O'Steen and Bennett 2003). The effect of temperature on swimming speed for bluefish and anchovy was greater at the lower temperatures (i.e., 10-13°C), especially for anchovy; this may have resulted from a behavioral rather than physiological limitation. Webb (1976) found that escape speeds of prey often were sub-maximal when predators did not pursue, which was typical for bluefish-anchovy interactions. By limiting escape speed and distance, anchovy reduce energetic costs and probably reduce the risk of drawing the attention of other predators.

Larger anchovy exhibited higher escape speeds, which is consistent with numerous studies showing that maximum swimming speed increases with length (Wardle 1975; Webb 1976). However, higher escape speeds conveyed no advantage in evading capture for larger anchovy. Fish acceleration is independent of length (Webb 1976; 1978; Domenici and Blake 1993), and given the small reaction distance of bay anchovy, maximum speed probably does not contribute to their ability to evade capture.

The attack speed of fishes does not indicate maximum speed and is probably related to attack distance. Bluefish typically attacked from a short distance relative to their body length. As a result, attacks were often just one propulsive tail flip. Similarly, Harper and Blake (1991) compared acceleration and velocity of attacking versus escaping responses. They show that escape responses exhibit higher acceleration and velocities compared to attacks. The reason predators may attack from a short distance is to minimize the reaction distance of prey; reaction distance is dependent on the size and speed of an approaching predator (Dill 1974). Thus, in order to minimize reaction distance, bluefish attempt to get close to prey before accelerating into an attack. For example, Webb and Skadsen (1980) showed with tiger muskelunge (Esox sp.) feeding on fathead minnows, that attacks from shorter distances were more successful than attacks from longer distances. It is uncertain why bluefish attacked from greater
distances at the warmest treatment. The maximum consumption rate of bluefish increases rapidly with temperature (Hartman and Brandt 1995; Buckel et al. 1995), and given the schooling nature of bluefish, intraspecific competition may limit prey availability at higher temperatures. Consistent with this hypothesis, Buckel and Stoner (2004) found that per capita prey consumption declined with increasing bluefish numbers in a large experimental arena. Prey were never depleted during their feeding trials, and the authors hypothesized that interference competition played an important role in limiting consumption within large groups of bluefish.

**A novel approach to estimating fish location**

Examining predator-prey interactions in experimental settings has been useful for interpreting large-scale patterns observed in the wild (Juanes et al. 2001; Bystrom et al. 2006; Scharf et al. 2009). Accurate and precise estimates of predator-prey behaviors require estimates of velocity and distance between predator and prey (Scharf et al. 2003). Studies that have measured velocity and distance typically do so in two dimensions, using only one camera angle. For benthic animals this is not a problem (Barbeau and Scheibling 1994), but to achieve this for pelagic fish water depth is often restricted (Webb and Zhang 1994; O’Steen and Bennett 2003). Limiting water depth is often not a realistic alternative for larger or more active predators and prey.

Scharf et al. (2003) estimated distance and speed without restricting depth by only examining interactions that appeared to occur within a two-dimensional plane that was perpendicular to the camera. They used a grid on the back of the tank and adjusted apparent velocity of fish across it based on location in the cameras depth of field. This method is limited because only a subset of the total interactions can be used. Further, error is introduced when interactions are not completely occurring on a two-dimensional plane, or if the location within the depth of field is not accurately estimated. My use of two camera angles eliminated these shortcomings and the average error in determining the location of random points within the tank was small. In practice, this novel method is probably more accurate than the average error indicates.
Typically, predator and prey are in close proximity and each have similar correction factors applied to their apparent locations. Further, when calculating speed, correction factors typically change slowly across sequential video-frames. An important negative aspect of my method is an increased amount of time required for video analysis.

Conclusions

Fishes exhibit seasonal adaptations that minimize energy loss during winter including reductions in routine metabolism (Evans 1984; Karas 1990), activity (Sandstrom 1983), appetite (Metcalfe et al. 1988; Simpson et al. 1996), and growth (Metcalfe et al. 1988; Karas 1990). These adaptations occur independently of temperature or food availability suggesting temperate fishes consistently face a negative growth potential during winter. Similarly, bluefish exhibit a reduction of appetite during winter (Chapter 2). However, based on summer and fall bioenergetics (Hartman and Brandt 1995), bluefish have a positive scope for growth at typical overwintering temperatures (Morley et al. 2007; Chapter 2). Further, low temperatures did not inhibit bluefish ability to catch and consume small anchovy prey. A consistent reduction in winter prey availability of suitable size may explain why temperate species exhibit energy conserving adaptations such as a reduction in metabolism. For example, Adams et al. (1982) described a system for largemouth bass where size-dependent mortality during the early spring was strongly mediated by the size-structure and abundance of prey populations.

Temperature can affect behavioral responses in complex ways. Multi-factor experiments can show how temperature interacts with other variables to influence ecological processes at low temperature. For example, brown trout exhibit a decrease in capture success as temperature declines, but this effect is dependent on foraging mode (Watz et al. 2012); trout that hold position in a current longer, as opposed to actively searching for prey, can minimize temperature effects. My experiment has shown that bluefish foraging success at low temperatures depends on relative prey size. The available prey for bluefish in winter may be restricted due to difficulties handling
relatively large prey. This may be particularly important for smaller individuals, which already experience a narrower range of potential prey (Scharf et al. 2000).

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REFERENCES


**Table 5.1.** Definition of variables estimated during feeding trials with bluefish and bay anchovy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capture success</td>
<td>Proportion of attacks resulting in prey ingestion (or prey caught, see text)</td>
</tr>
<tr>
<td>Approach distance</td>
<td>Distance between predator and prey at initiation of movement toward prey</td>
</tr>
<tr>
<td>Approach angle</td>
<td>From prey perspective, 0° (anterior) to 180° (posterior); e.g. at 180° bluefish approached from behind</td>
</tr>
<tr>
<td>Attack distance</td>
<td>Distance between predator and prey at initiation of predator movement from S-start position or the beginning of continued aggressive swimming</td>
</tr>
<tr>
<td>Reaction distance</td>
<td>Distance between predator and prey at initiation of prey movement in response to bluefish approach or attack</td>
</tr>
<tr>
<td>Attack speed</td>
<td>Bluefish speed from initiation of attack until prey is captured or escapes</td>
</tr>
<tr>
<td>Escape speed</td>
<td>Prey speed during successful escapes, from initiation of startle response when attacked until prey stopped swimming</td>
</tr>
<tr>
<td>Handling time</td>
<td>From capture until rapid opercular movement ceased</td>
</tr>
</tbody>
</table>
Table 5.2. Example of the technique used to estimate three-dimensional coordinates (i.e. $x, y, z$) of fish from video-frames using correction factors to adjust the apparent location of fish within the feeding arena.

<table>
<thead>
<tr>
<th></th>
<th>Front cam</th>
<th></th>
<th>Overhead cam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$x$</td>
<td>$y$</td>
<td>$x$</td>
</tr>
<tr>
<td>1</td>
<td>Apparent location</td>
<td>8.00</td>
<td>4.75</td>
</tr>
<tr>
<td>2</td>
<td>Correction factor #1</td>
<td>+1.25</td>
<td>+0.25</td>
</tr>
<tr>
<td>3</td>
<td>Adjusted location</td>
<td>9.25</td>
<td>5.00</td>
</tr>
<tr>
<td>4</td>
<td>Correction factor #2</td>
<td>+1.00</td>
<td>+0.25</td>
</tr>
<tr>
<td>5</td>
<td>Actual location</td>
<td>9.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>
Table 5.3. Analysis of variance tables for response variables measured during bluefish feeding trials at two temperatures (13 and 20°C) and two size classes of bay anchovy prey (36-40 and 61-65 mm).

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Capture success (prey ingested)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prey size</td>
<td>1</td>
<td>0.118</td>
<td>6.189</td>
<td>0.04</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.042</td>
<td>2.203</td>
<td>0.18</td>
</tr>
<tr>
<td>Prey size x Temperature</td>
<td>1</td>
<td>0.040</td>
<td>2.081</td>
<td>0.19</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.153</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Capture success (prey caught)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prey size</td>
<td>1</td>
<td>0.0002</td>
<td>0.004</td>
<td>0.95</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.0024</td>
<td>0.051</td>
<td>0.83</td>
</tr>
<tr>
<td>Prey size x Temperature</td>
<td>1</td>
<td>0.0030</td>
<td>0.064</td>
<td>0.81</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.378</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Approach distance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prey size</td>
<td>1</td>
<td>2.190</td>
<td>0.046</td>
<td>0.84</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>3.580</td>
<td>0.075</td>
<td>0.79</td>
</tr>
<tr>
<td>Prey size x Temperature</td>
<td>1</td>
<td>4.750</td>
<td>0.100</td>
<td>0.76</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>381.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Attack distance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prey size</td>
<td>1</td>
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### Table 5.3. Continued

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<td><strong>(log transformed)</strong></td>
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<td>Error</td>
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Table 5.4. Model selection for relationships between four different response variables and temperature (10-20°C) from feeding trials with bluefish and bay anchovy. $K =$ number of parameters, RSS = residual sum of squares, $\text{AIC}_c = \text{Akaikes information criterion}$ adjusted for small sample size, $\Delta \text{AIC} = \text{difference in } \text{AIC}_c \text{ value between each model and the best-fit model}$, $w = \text{AIC}_c \text{ weight.}$

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<th>Function</th>
<th>Equation</th>
<th>$K$</th>
<th>$r^2$</th>
<th>RSS</th>
<th>$\text{AIC}_c$</th>
<th>$\Delta \text{AIC}$</th>
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<tr>
<td>Linear</td>
<td>$y=16.61+1.10x$</td>
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<td>0.26</td>
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<tr>
<td>Asymptote</td>
<td>$y=65.93(x/14.42+x)$</td>
<td>3</td>
<td>0.30</td>
<td>1005.5</td>
<td>91.42</td>
<td>5.91</td>
<td>0.04</td>
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<tr>
<td>Exponential</td>
<td>$y=45.89(1-e^{-0.09x})$</td>
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<td>991.7</td>
<td>91.12</td>
<td>5.61</td>
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<tr>
<td>Maximum</td>
<td>$y=0.005x^{4.94}e^{-0.29x}$</td>
<td>4</td>
<td>0.46</td>
<td>782.1</td>
<td>88.91</td>
<td>3.40</td>
<td>0.14</td>
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<tr>
<td>Sigmoid</td>
<td>$y=36.91(x^{12.19}/(10.0^{12.19}+x^{12.19}))$</td>
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<td>0.54</td>
<td>670.0</td>
<td>85.51</td>
<td>0</td>
<td>0.76</td>
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<tr>
<td><strong>Attack speed</strong></td>
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<tr>
<td>Linear</td>
<td>$y=22.85+4.67x$</td>
<td>3</td>
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<td>3578.6</td>
<td>111.24</td>
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<td>0.20</td>
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<tr>
<td>Asymptote</td>
<td>$y=361.2(x/42.53+x)$</td>
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<td>3437.2</td>
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<td>Exponential</td>
<td>$y=213.8(1-e^{-0.04x})$</td>
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<td>3424.8</td>
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<tr>
<td>Saturation</td>
<td>$y=147.0((x-7.51)/(3.65+x-7.51))$</td>
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<td>0.68</td>
<td>3096.8</td>
<td>111.51</td>
<td>1.15</td>
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<td><strong>Escape speed</strong></td>
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<tr>
<td>Linear</td>
<td>$y=25.73+5.12x$</td>
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<td>1382.3</td>
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<tr>
<td>Exponential</td>
<td>$y=220.3(1-e^{-0.04x})$</td>
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<td>1363.0</td>
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<tr>
<td>Saturation</td>
<td>$y=142.0((x-8.54)/(1.81+x-8.54))$</td>
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<td>745.8</td>
<td>70.60</td>
<td>0</td>
<td>0.86</td>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>$y=67.38-3.15x$</td>
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<td>81.36</td>
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<td>0.63</td>
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<tr>
<td>Exponential</td>
<td>$y=161.72e^{-0.15x}$</td>
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<td>2115.6</td>
<td>82.42</td>
<td>1.06</td>
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Figure 5.1. Distribution of approach angles of bluefish towards bay anchovy prey. Approaches that resulted in both attacks and non-attacks are shown. Angle is from the anchovy perspective, for example 180° represents a bluefish approaching an anchovy from directly behind and 0° represents head on.
Figure 5.2. Mean (±SE) distance between bluefish and bay anchovy prey of two sizes when (a) bluefish first initiated movement towards prey, (b) bluefish attacked prey, and (c) anchovy reacted to an approaching or attacking bluefish, at different temperature treatments.
Figure 5.3. Bay anchovy reaction distances from successful and missed bluefish attacks. Boxes show 25th percentile, median (solid line), 75th percentile, and the mean (dashed line); bars show the 10th and 90th percentile, and points show outliers. Note, for hits, the median and lower percentiles are the same value.
Figure 5.4. Mean (±SE) swimming speed of (a) bluefish attacking two sizes of bay anchovy, and (b) two sizes of bay anchovy successfully escaping bluefish attacks, at different temperature treatments.
Figure 5.5. Bluefish preying on bay anchovy of two sizes at different temperature treatments, mean values (±SE) are shown. (a) proportion of successful attacks where a successful capture was defined as prey ingested, (b) proportion of successful attacks where dropped prey were also considered a successful capture, and (c) proportion of successfully captured prey that were dropped.
Figure 5.6. Bluefish preying on bay anchovy of two sizes at different temperature treatments, mean values (±SE) are shown. a) prey handling time, and b) proportion of ingested prey that were first severed in half.