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Variation in Winter Estuarine Habitat Use by Bluefish in Northeastern Florida with Implications for Growth and Condition

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VARIATION IN WINTER ESTUARINE HABITAT USE BY BLUEFISH IN NORTHEASTERN FLORIDA WITH IMPLICATIONS FOR GROWTH AND CONDITION

A Thesis Presented

By

JOHN S. MURT

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

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VARIATION IN WINTER ESTUARINE HABITAT USE BY BLUEFISH IN NORTHEASTERN FLORIDA WITH IMPLICATIONS FOR GROWTH AND CONDITION

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and to my misses, Lauren, for always being there, keeping me focused and occasionally giving me the little kick that I have needed.
Age and growth were determined from otoliths for 181 juvenile bluefish, *Pomatomus saltatrix*, collected using a variety of gear in northeast Florida during 2003 and 2005. Three distinct cohorts were identified recruiting to the near shore waters during spring, summer and fall. Growth rates were high regardless of cohort or season. To compare pre- and post-recruitment growth rates, models were fit to individual growth trajectories using change point analysis. Post-estuarine growth rates were generally higher. Growth rates and hatching times were within the range of those obtained in other bluefish studies conducted at higher latitudes. As this is the only area where winter recruitment of bluefish has been observed, coastal Florida habitats may be essential for the bluefish stock and will need to be carefully monitored in future studies.

A technique to estimate the lipid content of bluefish was developed using fat stage (subjectively assigned based on mesenteric fat around the stomach), fish length, and fish weight. A highly significant relationship was observed between
fat stage and lipid content in a generalized linear model. The visual lipid content technique provides rapid results, is inexpensive and could be easily implemented into current fisheries sampling methods. Total lipids were also extracted from potential bluefish prey species collected during sampling. Prey lipids ranged from 0.88% to 19.52%. Regular prey species from the MAB; Atlantic silverside and bay anchovy contained 3.49% and 3.19% mean lipids respectively. Highest lipid content was observed in mullet (*Mugil spp.*)(19.52%) and was significantly higher than other available prey species. A previous study indentified a decline in bluefish lipids as winter progressed as well as a prey preference for mullet. We propose mullet are the preferred prey choice due to their high lipid content.
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CHAPTER I

COHORT-SPECIFIC WINTER GROWTH RATES OF YOY BLUEFISH, *Pomatomus saltatrix*, IN NORTHEAST FLORIDA ESTUARIES: IMPLICATIONS FOR RECRUITMENT

**Introduction**

Recruitment to estuaries is considered an important part of the early life history of many fish species (Boehlert and Mundy, 1988). In Chesapeake Bay, the largest estuary in the U.S, some 267 species have been recorded (Murdy et al., 1997). Of these 267 species only 32 are year round residents, while 235 species migrate in and out from both fresh water and marine systems.

Bluefish (*Pomatomus saltatrix*) are a highly migratory species found in semi tropical waters (Briggs, 1960, Juanes et al., 1996). The northwest Atlantic population ranges from Florida to Nova Scotia depending on season (Murdy et al., 1997; Juanes et al., 2002; Collette and Klein-Macphee, 2002). Adult bluefish migrations are coupled with spawning aggregations which first occur during the spring in the South Atlantic Bight (SAB) (Lassiter, 1962). The majority of spring-spawned juvenile bluefish, assisted by the advective current of the Gulf Stream, recruit to the Mid Atlantic Bight (MAB) to feed in estuarine and near shore environments (Nyman and Conover, 1988; McBride and Conover, 1991; Hare and Cowen, 1996). Concurrent with this passive migration, adults actively
migrate north to the MAB and Gulf of Maine. A second spawning event during the summer results in summer-spawned juveniles which also recruit to MAB estuaries (McBride and Conover, 1991). Throughout the summer and fall estuaries provide an abundance of juvenile prey fish (Murdy et al., 1997). High consumption rates allow bluefish to exhibit extraordinarily fast growth before their southerly migration back to the SAB, presumably to over-winter (Juanes and Conover, 1994; Scharf et al., 2004). In late fall, a third, less important, cohort is produced from spawning in northeast Florida (McBride et al., 1993) and recruits directly to SAB estuaries (Clarke, 2006).

Age and growth of bluefish has been studied from Massachusetts (Roemer and Oliveira, 2007) to South Carolina (McBride et al., 1993), with the MAB receiving the majority of study effort (Nyman and Conover, 1988; McBride and Conover, 1991; Able et al., 2003; Takata, 2004; Callihan, 2005). In contrast to the MAB, where spring and summer growth has been well documented, little is known about winter growth of the fall-spawned cohort which recruits directly to SAB estuaries in late fall, or winter growth in general, especially at the southern edge of their range.

The spring cohort is often identified as the dominant contributor to the overall bluefish population (Munch and Conover, 2000). More recently Conover et al. (2003) found a shift in cohort dominance from spring-spawned to summer-spawned in the New York area. Lower recruitment of spring-spawned individuals
to the overall population has been offset by higher summer-spawned recruitment. The fall cohort, which has thus far failed to be detected in MAB sampling and aging studies, could serve a similar role in years of poor recruitment for the spring and summer cohorts.

There is variability in the timing of bluefish recruitment to estuaries, but it generally occurs at lengths between 40 and 70mm (Nyman and Conover, 1988). Hare and Cowen (1995) identified stage specific growth rate effects using otoliths instead of growth rate comparisons among cohorts as has been done previously (McBride et al., 1993; McBride et al., 1995). However, the bluefish used in Hare and Cowen (1995) were pre-recruitment larval and pelagic juveniles, and could not be used to compare estuarine growth to prior oceanic growth. Moreover, no previous studies have compared growth between the two habitats and the consequences for recruitment. Understanding growth rates in both habitats will not answer whether bluefish are estuarine dependent or not (see Able et al., 2003) but will likely shed some light on the growth consequences of variation in estuarine residency on recruitment.

The fall-spawned cohort contributes less to the overall population structure than the spring- and summer-spawned cohorts (McBride et al., 1993). Lower production, as well as being potentially resident to the SAB (Shepherd et al., 2006), has made the fall-spawned cohort less studied than the earlier spawned cohorts. It is essential that the fall cohort be studied and its role understood because it likely has a smaller geographic distribution than the spring and
summer cohorts (perhaps restricted to the SAB). Human development and use of estuarine environments increases every year, impacting this already small range, with northeast Florida being one of the most developed shorelines on the east coast of the United States. This study is the first to analyze growth rates for all three cohorts at the southern end of their range through the fall and winter.

The objectives of this study are to identify whether all 3 YOY bluefish cohorts recruit to estuaries and near shore waters of northeast Florida, to compare growth among cohorts and seasons, and between pre- and post-estuarine entry, and to assess the potential importance of winter growth on cohort-specific recruitment.

**Materials and methods**

**Field sampling**

YOY and age 1+ bluefish were collected in northeast Florida (Fig. 1.1) using gill netting, beach seining, and cast netting techniques. Collections were made in the summer, fall and winter to allow growth rate comparisons among the spring, summer and fall-spawned cohorts. Bluefish were sampled November 9th 2002 - February 24th 2003, and June 6th and 9th 2003 (“Year 1”), October 13th 2003- January 16th 2004 (“Year 2”), and June 26th and June 29th 2005 (“Year 3”). Catch per unit effort for years one and two and spatial distributions are reported in Clarke (2006).
Three sampling locations were identified at each site, inside the inlet, in the mouth of the inlet, and on the ocean beach outside the inlet. Two seine hauls were conducted at each location per month. Lengths (+/- 1mm) were recorded for all species captured in the sampling gears.

Bluefish carcasses less than 160 mm Fork Length (FL) were stored in 95% ETOH, whereas bluefish greater than 160 mm FL had their heads removed in the field and were frozen for future otolith analysis. Year 3 bluefish were preserved whole in 95% ETOH in the field.

**Laboratory Methods - Otolith Processing**

After removing the cranium with a scalpel both sagittae were removed from beneath the posterior end of the brain case for all YOY bluefish. Due to the fragility of bluefish otoliths and the frequency of breakage, one sagitta from each fish was processed for aging, while the remaining otoliths were preserved in 95% ETOH for backup. Otoliths were cleaned with distilled water to remove excess tissue. Sagittae were then glued concave side down to glass microscope slides using Crystalbond 509. Once the glue had set otoliths were sanded down using 600 – 1200 grit wet/dry sand paper. Once the nucleus had been reached otoliths were polished with 0.3 micron levigated alumina polishing compound on a polishing cloth. Due to the difficulty of holding glass slides on a polishing wheel it proved more practical to polish them by hand. Polishing cloths were glued to the worktop and alumina polishing compound was added to them with water. Slides
were placed face down and otoliths were polished in a circular motion. Once one side had been sanded and polished slides were placed on a hot plate. Heating the Crystalbond™ 509 to 80ºC allowed the bonding agent to melt permitting otoliths to be flipped. The same sanding and polishing method was used for both sides of the otolith.

Microstructural analysis of YOY bluefish otoliths has demonstrated daily growth rings in sagittal otoliths (Nyman and Conover, 1988; Roemer and Oliveira, 2007). To enhance the definition of daily growth increments a drop of immersion oil was added to each polished otolith. Otoliths were then viewed and photographed under an Olympus™ BH2 compound microscope with a Canon A95 digital camera and measured using an ocular micrometer to the nearest micron. Digital images were taken at 40 X and 100 X magnifications. Multiple 100 X magnified images had to be taken of the same otolith to allow the whole image to be observed. These images were stitched together using the “merge photo” tool in Adobe™ Photoshop. Once images had been stitched they were imported into Adobe Illustrator and daily rings were counted concurrently on the computer screen as well as under the microscope. A transect was first drawn from the center of the otolith core to the outer tip of the rostrum, this allowed for the longest transect possible. For every seven rings that were counted under the microscope a mark was placed along the transect at the corresponding point using Adobe Illustrator. Bluefish ages were estimated by counting the number of daily increments present on the polished otolith. Otolith daily increment widths
tend to be autocorrelated, therefore interpolation was often needed to estimate the outer increment. Interpolation did not exceed 5% of the total counts deemed acceptable by Campana (1992). All images were saved as illustrator and jpeg files.

Stevenson and Campana (1992) infer that the major source of otolith increment width measurement bias occurs during focus adjustment in light microscopy. Since the focal plane of one increment is not necessarily the same for an adjacent increment, adjustments to the focus can cause apparent width changes in daily increments. By measuring weekly increments the overlap error which occurs during refocusing was minimized as fewer measurements were needed. The otolith jpeg files with the weekly ring counts were opened in Image J™, calibrated using total otolith length and the width between weekly marks was measured. Daily otolith growth was calculated by dividing the weekly growth width by seven. Unfortunately this technique has an averaging effect whereby it assumes that growth remains constant for the whole week, not allowing for daily growth variability. More commonly, daily increments are individually measured in YOY fish (Secor et al., 1992; Stevenson and Campana, 1992) allowing for daily growth variability but increasing daily growth error.
Statistical Analysis

Total increments were counted on one sagittal otolith from each bluefish with each increment representing one day (Nyman and Conover, 1988; Roemer and Oliveira, 2007). One day was subtracted from each total count as the first increment is formed at hatching (Hare and Cowen, 1994). Hatch dates were sorted into bi-weekly hatch bins. For all cohorts, first hatch, last hatch and number of days between first and last hatch were recorded.

Growth Rate Comparisons

Growth rates were compared using analysis of covariance (ANCOVA). The null hypothesis that the growth rates were not different was tested using a significance value of p<0.05. To test whether otolith growth is related to fish length we used linear regression to correlate fish length against otolith length for each cohort.

Oceanic versus Estuarine Growth

Juvenile bluefish recruit to estuaries at fork lengths (FL) of 40 – 70mm (Marks and Conover, 1993) although there is cohort- and population-level variability (Juanes et al., 1996). As no recruitment mark was observed on bluefish sagittae we took the midpoint of the recruitment size range (55mm) and calculated age using the pooled regression equation for fork length versus age, resulting in a recruitment age of 44.67 days or approximately 6 weeks. We considered growth before 6 weeks to be oceanic and any growth after six weeks to be estuarine.
Change point analysis was used to distinguish the point at which a slope diverges from the original slope. If the change point is already known it can be used to test whether there is any difference before and after the change point.

Five models using change point analysis were compared to see which produced the best fit for YOY bluefish growth across cohorts. All intercepts are assumed to pass through the origin. Model 1 \((y=\beta_1 x)\) assumed that growth was the same for all cohorts \((y = \text{otolith radius growth}, \beta = \text{slope}, x = \text{age})\). Model 2 \((y=\beta_1 x)\) plotted individual growth for the three cohorts. Model 3 \((y=\beta_1 x + \beta_2 I(x-x_c)(x-x_c))\) assumed the change point occurred at 6 weeks and plotted the combined data before and after this point \((x_c = \text{age at change point})\). The \(I\) represents an indicator function which depends on \(x > x_c\) to include the second part of the model, if \(x < x_c\) the second part of the model was not added. Model 4 \((y=\beta_1 x + \beta_2 i I(x-x_c)(x-x_c))\) assumed that before the change point growth was the same across cohorts and after the change point growth rates were different \((\beta_2 = \text{slope change})\). Model 5 \((y=\beta_1 x + \beta_2 i I(x-x_c)(x-x_c))\) assumed that individual cohort growth rates were different before and after the change point. The best fitting model was selected using Akaike’s Information Criterion (AIC) which is a measure of the goodness of fit of an estimated statistical model. The AIC is a way of trading off the complexity of an estimated model against how well the model fits the data (Akaike, 1987).
Pre- and post-estuarine recruitment growth was compared for each cohort using a paired t-test. To control for size bias we standardized weekly otolith growth by dividing by total otolith radius for individual cohorts. A p < 0.05 was considered statistically significant.

**Results**

A total of 327 bluefish were available for this study. Of these, 179 YOY and age 1+ bluefish were sampled in years one and two, 132 YOY were sampled during the summer of 2003, and 16 YOY were sampled during the summer of 2005.

Due to difficulty reading daily increments from otoliths of bluefish >110mm FL, only otoliths from bluefish <110mm FL were considered for aging, reducing the sample size to 213 (all YOY). Thirty seven otoliths (17.3% of the fish < 110mm) broke during removal and preparation further reducing the available otoliths to 174, all of which were successfully aged. However, we used cohort-specific regression equations developed for the directly aged otoliths to estimate ages for the 37 samples with broken otoliths. Reader bias was quantified and shown to be less than 10%.

**Hatch Date Analysis**

Daily otolith increments allowed the three cohorts to be clearly differentiated through back calculation (hatch date = capture date – (# of increments – 1) (days)). For the spring-spawned cohort, hatching first occurred on April 8 2003.
and lasted 22 days. The summer-spawned cohort first hatched on August 11, 2003 and lasted for 19 days. Fall-spawned YOY first hatched on October 22, 2003 and lasted for 9 days. The 2005 spring-spawned cohort hatched slightly later than the 2003 spring-spawned cohort, hatching first on April 14, 2005 and lasting for 17 days. Mean length at capture was largest for the summer-spawned cohort at 95.57mm and smallest for the fall-spawned cohort at 47.13mm.

Growth Rate

Juvenile bluefish mean growth rates ranged from 1.35 to 1.52 mm per day during 2003 (Fig. 1.2). However, there were no significant differences in the slopes (p=0.64), but a significant difference in the adjusted means (p<0.0001) with summer-spawned fish being largest and fall-spawned the smallest. Cohort growth rates could thus not be pooled. The otolith length versus age ANCOVA showed similar results, slopes were not significantly different (p=0.70) but the intercepts were (p<0.0001). R² values showed fork length to be good predictor of age (All R² > 0.47) (Fig.1.2) as was otolith length (All R² > 0.57) (Fig. 1.4).

Similarly, no significant difference was detected between spring-spawned growth rates across the 2 years (p = 0.18). However, the spring-spawned 2005 cohort adjusted mean was significantly larger at age than the spring-spawned 2003 cohort (p < 0.0001).
Good relationships were observed between fish length and otolith length for all three cohorts (All $r^2 > 0.68$) suggesting that otolith growth is proportional to somatic growth in this species. However, cohorts could not be pooled because the y intercepts were significantly different ($p<0.0001$).

All indirectly aged samples belonged to spring- (n= 27) or summer-spawned (n= 10) cohorts. Indirectly aged spring-spawned samples were aged using the regression equation $\text{Length} = 1.3565^*\text{(age)} – 0.117$, and indirectly aged summer-spawned samples were aged calculated using the regression equation $\text{Length} = 1.516^*\text{(age)} – 6.3519$, both of which were calculated from directly aged samples (see Fig. 1.2). Hatch dates of the indirectly aged bluefish were then combined with those estimated for the directly aged bluefish (Fig. 1.3).

Oceanic versus Estuarine Growth

Daily incremental otolith measurements showed similar growth among 2003 cohorts (Fig. 1.5), with the 2005 spring-spawned cohort experiencing very little increase in growth between week 4 and 8 (Fig. 1.5 C). The initial observation after the sixth week showed that the spring 2003 cohort continued to grow at a steady rate, whereas the summer 2003 and spring 2005 cohorts growth rates slowed, and the fall cohort growth rate increased (but based on only one week’s growth). All 5 models were fitted to individual bluefish otolith growth (Fig. 1.6). The change point analysis suggested that Model 5 provided the best fit.
demonstrating different growth among all cohorts both before and after recruitment (Fig. 1.7).

Discussion

This study has identified for the first time three cohorts of YOY bluefish recruiting to the near shore waters of northeast Florida. The inclusion of the fall-spawned cohort makes the SAB unique in terms of recruitment as this cohort has not been detected in the MAB, where the majority of the population is present during summer. The growth rates calculated in this study were within the range of other growth studies performed on bluefish across its range, with significant differences in pre- and post-recruitment growth detected for all cohorts. Because of the observed winter recruitment of the fall cohort and continued elevated growth of all cohorts, northeast Florida estuaries are potentially very important to the overall bluefish population.

Hatch date

In contrast to studies in the MAB, where only two cohorts are recognized, we observed trimodal bluefish recruitment. Cohort hatch dates from this study concurred with those produced from nine previous aging studies (Table 1.1) indicating that spring spawning starts in March, continues through April (4 of the studies) or into May (5 other studies). All of our spring-spawned bluefish hatched in April and are thus similar to previous results. Less agreement was found in the summer cohort hatch dates. The earliest hatch date was May (Hare and Cowen,
1996) and the latest was September (Marks and Conover, 1993). Despite the variation in the range of hatch dates, the majority of these studies centralize summer hatching around July and August, which agrees with our August summer-spawned hatching dates. The fall cohort is only recognized in 3 of the studies, and all agree that fall hatching starts in September, with two studies indicating it continues through November and one indicating it carries on through January (McBride et al., 1993). Previous fall hatching dates agree with our observed October fall hatching dates.

The clear separation in the tri-modal cohort distribution (Fig. 1.3) could mean one of two things; that there are three distinct spawning events whereby juveniles recruit to the near shore shortly after the spawn (as proposed for the spring- and summer-spawned cohorts by Kendall and Walford, 1979), or that there is a continuous spawning event starting in the spring and continuing into the fall (as proposed by Hare and Cowen, 1993) and where observed recruitment patterns are a function of survival rates. No overlap was observed between cohort hatch dates in our 2003 samples, however, Takata (2004) identified intermediate hatching between spring and summer cohorts in the MAB during the spring and summer for the same year which would suggest continuous spawning. We also found no overlap between the end of the summer-spawned and start of the fall-spawned hatching with almost two months between hatch dates. It is clear that a larger sample size would produce a wider hatch date distribution for each cohort, but the observed gap between cohorts is sufficiently large that even with an
increased sample size it is unlikely that trimodal recruitment would be better described by continuous spawning.

Growth rate
Mortality during the first winter of juvenile fishes is often high due to thermal stress and starvation (Hurst, 2006). Individuals that are larger at the end of the first growing season likely experience lower winter mortality (Sogard, 1997). The migration from the MAB to the SAB is triggered when water temperatures drop below 15°C. Because northeast Florida estuaries maintain temperatures >20°C beyond November (Clarke, 2006), the southerly migration into these estuaries provides a lengthier growing season during which juvenile bluefish can continue to grow at high rates with the standard benefits of estuarine residency (i.e. low predation and high food resources) (Levin et al. 1997). Our results show that all cohorts grow as well or better in the winter as they did previously either offshore or in MAB estuaries.

It is important to note however that for the three cohorts growth occurred at different times of the year and likely at different temperatures. Spring-spawned bluefish were collected during the summer when water temperatures were the highest, summer-spawned bluefish were collected during the fall when water temperatures were dropping and the fall-spawned cohort was collected during the winter when temperatures were lowest. We would therefore expect that since accumulated water temperatures often control growth (for example as
determined using growth degree days, see Neuheimer and Taggart, 2007), the spring-spawned cohort would have grown fastest and the fall-spawned cohort the slowest. Yet in this study cohort growth increased upon recruitment for the spring- and fall-spawned cohorts and decreased for the summer-spawned cohort.

Scharf et al. (2006) described the spring-spawned cohort growth as the most robust to fluctuations in prey dynamics as their early spawning temporally overlaps with an abundance of prey species. Our results agree with this finding as we observed little variability in growth detected between years. Comparisons could not be made between years for the summer and fall cohorts as we only collected them during one year. Interestingly, Scharf et al. (2006) surmised that summer spawned bluefish growth was more susceptible to prey fluctuations because of its dependence on a more limited diet. Much like the summer cohort in the MAB, the fall cohort in Florida has a limited prey source (Clarke, 2006), as most prey species become either too large for juvenile bluefish to consume or are not present during late fall and early winter, possibly making the fall cohort even more susceptible to prey fluctuations. Scharf et al. (2006) also noted the importance of the relative timing of the spring and summer cohort to growth variability as a consequence of competition for prey. In northeast Florida, the presence of a third cohort suggests that more complex dynamics are possible; as the likelihood of cohort overlap increases so does the potential competition for
shared prey resources. Detailed growth and diet studies over multiple years will be necessary to quantify such dynamics.

Growth rates of juvenile bluefish across their North American range have been shown to be highly variable across years, cohorts and locations (Table 1.2). The eleven studies highlighted in Table 2 have growth estimates ranging from 0.1 – 2.63 mm/d in the wild, with Roemer and Oliveira (2006) estimating a high of 3mm/d in a tank-based study. Our observed growth rates in Florida were within the range for mean growth rates calculated from other latitudes. Although the highly migratory nature of bluefish makes latitude a difficult variable to consider when comparing growth rates across a large geographic area, some latitudinal patterns may be detected across the accumulated bluefish growth data. Excluding the tank-based study, fastest growth is achieved in mid-latitudes, in Maryland for both spring and summer cohorts (Takata, 2004). Otherwise, growth rates decline both north and south of Maryland. Heading north, maximum growth decreased with increasing latitude and heading south, maximum growth decreased with decreasing latitude (Table 1.2).

Oceanic vs. Estuarine growth

The diet transition from planktivory to piscivory occurs when YOY bluefish recruit to estuaries (Marks and Conover, 1993). During the oceanic larval phase, which lasts between 40 and 70 days, growth rates are described as rapid (e.g. Able et al. 2003). However, estuarine growth has been suggested to be faster (McBride
likely due to the ontogenetic feeding shift to the abundant piscine prey, and the nutritional advantage of this prey type over plankton (Juanes and Conover, 1995; Juanes et al., 1994). For the Florida bluefish collected in 2003, the change point inserted at 6 weeks marked a significant change in growth for all cohorts. The spring and fall cohort’s growth rates increased upon recruitment, whereas growth of the summer cohort decreased slightly (Fig. 1.7). However, it is more likely that recruitment of the summer cohort only occurred after 9-10 weeks, after which increased growth was observed (Fig. 1.6). Increased variability in the growth and the timing of estuarine entry of the summer cohort relative to the spring cohort has been noted in northern systems with important implications for cohort-specific recruitment (Scharf et al. 2006).

**Conclusion**

Winter is often a stressful time for many species of marine fish where scarcity of prey is coupled with reduced growth rates and higher mortality (Schultz et al., 1998). The growth rates observed in this study suggest that recruitment to northeast Florida estuaries is important for YOY bluefish during the winter as they continue to achieve high growth rates similar to those attained in the summer. Historically, the spring-spawned cohort has dominated recruitment to the overall population but more recently Conover et al. (2003) identified a shift in population structure to one dominated by the summer-spawned cohort as the overall population has declined. Presently very little is known about the fall-spawned cohort, but its contribution to the population could become more
important if the population decline continues. At present, northeast Florida is experiencing high population growth and development around its inlets, along with development comes increased fishing pressure and habitat degradation. As this is the only area where winter recruitment of bluefish has been observed, coastal habitats may be essential for the bluefish stock and will need to be carefully monitored in future studies. Similarly, winter recruitment of marine species into estuaries can be affected by the dynamics of the resident fauna. For example, Warlen and Burke (1990) observed winter recruitment of predominantly marine species, (Brevoortia tyrannus, Leiostomus xanthurus, Micropogonias undulatus, Lagodon rhomboides and Myrophis punctatus) to North Carolina estuaries, identifying resident estuarine fishes’ lack of fall/winter spawning as potentially less competition for resources for the marine larvae. The interaction between the dynamics of migrating and resident species, especially in the winter when resources are scarce, can therefore have implications for the recruitment of both life history types.
Figure 1.1 Study Area located in northeast Florida between St. Augustine Inlet and New Smyrna Beach.
Figure 1.2  Relationship between size and age for juvenile bluefish. 2003 Spring-spawned fish are depicted by hollow diamonds, 2003 summer-spawned fish by x’s, 2003 fall-spawned fish by solid triangles and 2005 spring-spawned by solid diamonds. Regression equations and statistics are included for each cohort.
Figure 1.3  Bi-weekly hatch dates for 2003 YOY bluefish including directly and indirectly aged fish.
Figure 1.4 Relationship between otolith length and age for juvenile bluefish. 2003 spring-spawned fish are depicted by hollow diamonds, 2003 summer-spawned fish by x’s, 2003 fall-spawned fish by solid triangles and 2005 spring-spawned fish by solid diamonds. Regression equations and statistics are included for each cohort.
Figure 1.5  Mean weekly otolith growth rates for juvenile bluefish. (A) 2003 Spring-spawned fish, (B), 2003 Summer-spawned fish, (C), 2003 Fall-spawned fish, and (D), 2005 Spring-spawned fish. Error bars represent standard error from the mean.
Figure 1.6 Individual bluefish otolith growth used to fit all models. Spring cohort is represented in red, summer cohort is blue and the fall cohort is green.
Figure 1.7 Model 5 \( y = \beta_1 x + \beta_2 I(x-x_c)(x-x_c) \) fitted to 2003 bluefish growth data. A change point is inserted at 6 weeks for estuarine recruitment. Spring cohort is represented by dashed line, summer cohort by dotted line and the fall cohort by solid line. This model was fit to data shown in figure 1.6.
### Table 1.1  Cohort-specific bluefish hatch dates reported in previous studies.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Spring</th>
<th>Summer</th>
<th>Fall</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar + Apr</td>
<td>Jul + Aug</td>
<td></td>
<td></td>
<td>Nyman &amp; Conover, 1988</td>
</tr>
<tr>
<td>Mar to May</td>
<td>Jun to Sep</td>
<td></td>
<td></td>
<td>Marks &amp; Conover, 1993</td>
</tr>
<tr>
<td>Mar to May</td>
<td>Jun to Aug</td>
<td></td>
<td>Sep to Jan</td>
<td>McBride et al. 1993</td>
</tr>
<tr>
<td>Mar + Apr</td>
<td>Aug</td>
<td></td>
<td></td>
<td>Juanes &amp; Conover, 1995</td>
</tr>
<tr>
<td>Mar to May</td>
<td>May to Aug</td>
<td></td>
<td>Sep to Nov</td>
<td>Hare &amp; Cowen, 1996</td>
</tr>
<tr>
<td>Mar to May</td>
<td>Jul</td>
<td></td>
<td></td>
<td>Munch &amp; Conover, 2000</td>
</tr>
<tr>
<td>Mar + Apr</td>
<td>Jun to Aug</td>
<td></td>
<td></td>
<td>Takata, 2004</td>
</tr>
<tr>
<td>Apr</td>
<td>Aug</td>
<td></td>
<td>Oct</td>
<td>This Study, 2008</td>
</tr>
<tr>
<td>COHORT</td>
<td>Spring</td>
<td>Summer</td>
<td>Fall</td>
<td>State/s</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td>0.7-1.3</td>
<td>0.7-1.3</td>
<td>ME</td>
<td>Creaser &amp; Perkins, 1994</td>
<td></td>
</tr>
<tr>
<td>0.4-3.0**</td>
<td>0.7-1.3</td>
<td>MA</td>
<td>Roemer &amp; Oliveira, 2007</td>
<td></td>
</tr>
<tr>
<td>1.3</td>
<td>0.4-3.0**</td>
<td>NY</td>
<td>Nyman &amp; Conover, 1988</td>
<td></td>
</tr>
<tr>
<td>0.71-1.35</td>
<td>0.91-1.47</td>
<td>NY/NJ</td>
<td>McBride &amp; Conover, 1991</td>
<td></td>
</tr>
<tr>
<td>0.1-2.2</td>
<td>0.1-2.2</td>
<td>NJ</td>
<td>Able et al. 2003</td>
<td></td>
</tr>
<tr>
<td>0.19-0.95</td>
<td>0.1-2.2</td>
<td>NJ</td>
<td>Taylor &amp; Able, 2006</td>
<td></td>
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<tr>
<td>1.85-2.49</td>
<td>0.70-2.63</td>
<td>MD</td>
<td>Takata, 2004</td>
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</tr>
<tr>
<td>1.78-1.95</td>
<td>1.98-2.39</td>
<td>MD</td>
<td>Callihan, 2005</td>
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<tr>
<td>1.2-1.9</td>
<td>0.9-1.13</td>
<td>NC/SC</td>
<td>McBride et al. 1993</td>
<td></td>
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<tr>
<td>0.97- 1.35</td>
<td>1.52</td>
<td>FL</td>
<td>This study, 2008</td>
<td></td>
</tr>
<tr>
<td>0.9-2.1</td>
<td>0.9-2.1</td>
<td>East Coast</td>
<td>Juanes et al. 1994</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.2 Cohort-specific bluefish growth rates (mm/day) reported from previous studies. Where cohorts were not identified, the same growth rate was assumed for all cohorts mentioned. * Based on one sample. ** Laboratory study.
CHAPTER II

DEVELOPMENT OF A RAPID, COST-EFFECTIVE METHOD FOR ESTIMATION OF BLUEFISH, *Pomatomus saltatrix*, LIPID CONTENT AND LIPID EXTRACTIONS OF COMMON BLUEFISH PREY IN THE SOUTH-ATLANTIC-BIGHT

Introduction

Seasonal changes in environmental factors can have widespread impacts on marine predators and their prey (Adams et al. 1982). In temperate latitudes, winter is a critical period for many species of fish. Larger fish are less susceptible to over-winter mortality as larger body size allows for proportionally higher lipid storage than smaller body size (Sogard and Olla, 2000). Young-of-the-year fish are therefore more vulnerable to starvation during their first winter than they will be at subsequent life stages. One response to declining temperatures and food resources is to reduce feeding during the winter, living off stored energy reserves accumulated throughout summer and fall (e.g., brook trout, *Salvelinus fontinalis* - Cunjak and Power 1986, Atlantic rainbow smelt, *Osmerus mordax* – Foltz and Norden 1977, Pacific herring, *Clupea pallasii* pallasii – Foy and Paul 1999). Another response to unfavorable temperature and food resources in a given region is to migrate towards the equator where higher temperatures and food resources can be found during winter.

The bluefish, *Pomatomus saltatrix*, a highly migratory pelagic predator of global distribution (Briggs 1960, Juanes et al. 1996), appears to use a combination of both overwintering strategies. The Northwest Atlantic population is a year-round
resident south of Cape Hatteras but seasonal migrations increase its range from Nova Scotia to Florida (Murdy et al. 1997, Juanes et al. 2002, Collette and Klein-Macphee. 2002). During the spring and summer, bluefish migrate north into the Mid-Atlantic-Bight (MAB) and north of Cape Cod with southerly migrations to Florida in the late fall and winter. Bluefish migrations coincide with peak abundances of bay anchovy (*Anchoa mitchilli*), Atlantic silversides (*Menidia menidia*) and Atlantic menhaden (*Brevoortia tyrannus*) during spring in the MAB (Buckel et al. 1998, Juanes et al. 2001, Buckel and McKown 2002). The fall migration south to the SAB, triggered by lower prey availability and dropping temperatures, allows bluefish to continue feeding at high rates (Clarke 2006), likely extending their growing season (see Chap. 1) and may lead to additional storing of energy in the form of lipids to increase overwinter survival. Bluefish can therefore enter the winter larger or in better condition than they would have been in the MAB.

Winter is often a time of reduced growth rate due in part to a reduction in prey availability. A depletion of total body lipids during winter has been reported for many fish species (e.g., Arctic charr, *Salvelinus alpinus alpinus*– Jobling et al. 1998, rainbow trout, *Oncorhynchus mykiss* - Biro et al. 2004), including bluefish overwintering in North Carolina (Morley et al. 2007). Bluefish use stored energy to make up the deficit between a reduction in feeding during winter and their daily energy requirement (personal communication with Jim Morley). Conversely, Clarke (2006) found that bluefish continued to accumulate lipids in northeast
Florida as winter progressed possibly related to a diet switch from bay anchovy and silversides to mullet (*Mugil spp.*). Marais (1990) also reported that mullets contained higher fat levels than other fish species present in a South African estuary. Growth rate has been shown to be directly related to diet, with lipid-rich diets facilitating faster growth in hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus* – Chou and Shiau 1996).

Lipid reserves in fish have been shown to cycle throughout the year, peaking before, and declining after spawning (Blaxter and Hunter 1982, Garcia-Franco et al. 1999, Millan 1999). Lipid content is often assumed to be directly related to condition with higher body lipids ensuring higher over-winter survival in Colorado squawfish (*Ptychocheilus lucius*, Thomson *et al.* 1991); higher egg production in Atlantic cod (*Gadus morhua* -Marshall *et al.* 1999) and northern sardine (*Sardinops sagax* – Morimoto 1996), and better egg quality in the striped trumpeter (*Latris lineate* – Bransden *et al.* 2007). Moreover, Hoey and McCormick (2004) observed significantly higher mortality, due to predation, on low lipid level damselfish (*Pomacentrus amboinensis*) placed on a reef than those containing high body lipids.

Bluefish diets have been well studied in the MAB, predominantly composed of bay anchovy, Atlantic silverside and Atlantic menhaden, the most abundant prey resources available (Buckel *et al.* 1998, Juanes *et al.* 2001, Buckel and McKown 2002). Buckel and Stoner (2000) also identified an increase in selectivity with
increasing density of striped bass (*Morone saxatilis*) prey in the Hudson River. Conversely, Clarke (2006) found that bluefish overwintering in the SAB switched to preying primarily on mullets (*Mugil* spp.) even though common MAB prey fish were more abundant. A difference in prey size selection between MAB and SAB diets was also identified by Clarke (2006), who noted a relative increase in average prey size, >50% of bluefish length, compared to only 0.39% in the MAB (Juanes *et al.* 1993). Clarke’s (2006) results suggest that the increased costs of searching for and attacking such relatively large and scarce prey may be balanced by potential benefits of consuming a diet rich in mullets. Here I compare total lipid content of prey species encountered in northeast Florida to test whether the switch to mullets may be a consequence of higher relative lipid content.

Three lipid content methods have been used in the field at vastly different investment levels. Crossin and Hinch (2005) measured salmon fat content in the field using a Distell fish fatmeter (www.distell.com) which proved to be rapid, reliable and does not require sacrificing the fish. This non-sacrificial method proved ideal for tracking salmon condition over time during their fresh water migration, although at a cost of $6000 might be impractical for smaller studies. A similar approach was adopted by Cox and Hartmann (2005) using bioelectrical impedance analysis to predict lipid content in brook trout (*Salvelinus fontinalis*). This method also was non-sacrificial but the equipment was still expensive with prices of tetrapolar bioelectrical impedance analyzers starting at $1990 (RJL
Simpson et al. (1992) and Adams et al. (1995) used weights and morphometric measurements to predict lipids in Atlantic salmon (Salmo salar) and Arctic char respectively. A sacrificial method, developed by Van der Lingen and Hutchings (2005), estimates lipid content from mesenteric fat deposited around the stomach of anchovy (Engraulis mordax) and sardine (Sardinops sagax). This study investigates the use of Van der Lingen and Hutchings’ (2005) visual estimation technique to produce condition estimates for bluefish in the field. I also explore the use of a red, green blue (RGB) color analysis tool to increase objectivity in visual estimations.

**Goals and Objectives**

The goal of this study was to investigate bluefish condition whilst overwintering in northeast Florida. More specifically this project aimed to: 1) develop a rapid, cost-effective method of assessing bluefish condition. 2) Compare bluefish lipid levels from fall through the winter and 3) Compare the lipid content of available prey fish species as a way to explain bluefish’s preference for mullet in the SAB.

**Methods**

**Collection Methods**

Fifty-two bluefish were available for visual estimation of body lipids. Thirty eight bluefish were sampled in northeast Florida (see Fig. 1.1). Fifteen bluefish were provided by the Triton II, a St. Augustine shrimp trawler, 2 miles off of Matanzas
Inlet, FL (Fig. 2.1b) on 10/28/2005. Recreational fishers provided 23 samples caught from St. Augustine Pier, FL (Fig. 2.1a) on 11/20/2005. The remaining 14 samples were provided by a NMFS cruise on 9/24/2005 off southern New Jersey (station 97). Catch information is presented in table 2.1.

Beach seining was conducted in and around inlets of northeast Florida (Fig. 1.1) (sampling sites and methodology described in chapter 1) to collect prey fish for lipid analyses. I used a 30m beach seine with 7mm stretch mesh wings and a 6mm mesh bag. Monthly sampling was conducted at four fixed sampling sites (Fig. 2.1): St. Augustine Inlet, Matanzas Inlet, Gamble Rogers State Park and Ponce De Leon Inlet. Three stations were sampled in each inlet site during each sampling trip: inside the inlet, in the mouth of the inlet and on the ocean beach. Gamble Rogers State Park was sampled only in the intra-coastal canal. A minimum of two seine hauls were sampled at each station for a total of 114 hauls between October 27th 2005 and January 29th 2006. Where possible, different size classes of each prey species were selected for lipid extractions to allow for variability in lipid content amongst sizes. A total of 480 prey samples from 30 species were used for this study.

Lipid Extraction Methods

Whole frozen bluefish samples were defrosted in the laboratory, weighed (+/- 0.001g) and measured (+/- 1 mm). Using a scalpel an incision was made from the anus to the gills. Blunt probes were used to manipulate the stomach and
intestine in order to examine mesenteric fat deposits. Mesenteric fat deposits were photographed and later assigned a fat stage through color analysis, using Adobe Photoshop. Fish were then homogenized for 2 minutes in a blender. Forty gram samples of homogenized bluefish were placed in a drying oven in individual trays for 24 hours at 105 °C. Before drying fish samples were pre-weighed and moisture content was calculated by dividing dry weight by wet weight. A 2g sample was removed from the dried homogenized fish and ground into a fine powder using a mortar and pestle. It was essential to achieve a fine powder as this allowed the petroleum ether to penetrate the whole sample and remove the total lipids (see below).

Six Aluminum beakers and alundum extraction thimbles were pre-dried in the drying oven for thirty minutes and weighed. Samples were placed into the alundum extraction thimble and thimbles were inserted into the Soxhlet extractor. The aluminum beakers were filled with approximately 30 milliliters of petroleum ether and also inserted into the Soxhlet extractor. The temperature dial on the Soxhlet extractor was set at 95°C for all extractions.

The alundum thimbles containing the samples were boiled in the petroleum ether for two hours to remove all lipids. The alundum thimbles were then raised out of the boiling flasks and rinsed with petroleum ether for twenty minutes. During the rinse the solvent continuously evaporates in the boiling flask and the solvent vapor rises until it condenses and passes back through the sample. The solvent
extracts the lipids and passes through the alundum thimble walls before re-entering the boiling flask. The lipids remain in the boiling flask as they cannot evaporate while the petroleum ether is continuously recycled. After rinsing, the taps were closed, preventing the solvent from re-entering the sample and the boiling flask. The boiling-off period lasted for fifteen minutes and then the machine was turned off. The aluminum boiling flasks containing the lipids and the alundum extraction thimbles containing the extracted samples were removed from the machine and placed in the drying oven for thirty minutes to evaporate any excess solvent. Boiling flasks and thimbles were then removed and allowed to cool for five minutes before being weighed. Lipid weights were calculated using the following equations.

\[
((\text{Alundum thimble} + \text{sample}) - (\text{Extracted alundum thimble} + \text{sample})) - \text{alundum thimble} = \text{lipid weight}
\]

\[
(\text{Boiling flask} + \text{lipids}) - (\text{boiling flask}) = \text{lipid weight}
\]

Total lipids were expressed as a percentage of the dry body mass (%DBW) and were calculated using the following equation.

\[
\frac{\text{Lipid weight}}{\text{pre extraction sample weight}} \times 100 = \text{Lipid \% of dry weight}
\]

Moisture content was expressed as a percentage of wet body mass (%WBM).
1 – (Dry body mass / wet body mass) * 100 = % Moisture content

Regional differences in bluefish lipid and moisture content were compared across locations using ANOVA. Lipid versus length and lipid versus moisture relationships were also compared using linear regression.

Lipid Extraction Verification
The automated Soxhlet extraction system allows six sample extractions to occur simultaneously in individual flasks. To verify that each of the six samples was undergoing identical extractions, six samples were taken from an individual bluefish and run at the same time. This procedure was repeated for a total of six bluefish, yielding lipid results from 36 extractions. Variation in samples was tested using two-way ANOVA (SAS 9.1), testing for a fish effect and a flask effect.

Allocation of Fat Stage
To ensure that staging was as objective as possible I explored color analysis options in Adobe Photoshop to quantify fat stages. This tool allowed me to upload the photo of the bluefish’s open body cavity and using a selection tool click anywhere in the cavity and quantify how much red, green and blue the sample contained. Color analysis (using RGB color codes) proved to be inefficient in fat staging because of difficulties in accounting for fat thickness and
Moreover, when observing several samples (from different locations on the stomach) from each body cavity, RGB color codes were vastly different from one sample to the next, not giving consistent results within samples. The inefficiency of the RGB color analysis meant that I could also not use the area tool, as planned to outline the mesenteric fat to calculate coverage. If fat thickness was homogenous throughout the body cavity, RGB color analysis and the area measuring tool would have been adequate for objective staging.

As an alternative to the RGB analysis, I used the 52 bluefish for visual lipid estimation by first categorizing them into five fat stages depending upon mesenteric fat around the stomach using modified criteria developed to visually estimate fat stages of anchovy and sardine (Van der Lingen and Hutchings, 2005) (table 2.2). Fat stage one represents the least amount of mesenteric fat, with fat stage five representing those fish with the highest amount of mesenteric fat (fig. 2.2). The relationship between fat stage and lipid content was assessed using linear regression.

To assess potential variation between stagers and thus the generality of the method, the author and an independent stager were presented with photographs of the stomach and body cavity of thirty six bluefish. Using the descriptions in table 2.2, we assigned fat stages to the photographs on three separate occasions. Estimating the precision of fat staging across stagers was calculated...
using average percent error (APE) (Beamish and Fournier, 1981). Precision within stagers was also calculated across the 3 separate occasions.

The relationship between fork length (mm), wet body mass (g) and fat stage as predictors of lipid content was analyzed using a generalized linear model. Variance in lipid content was assessed using fat stage as an independent predictor variable, length and weight as dependent variables including all 2- and 3-way interactions.

Extraction of Prey Lipids

Prey were divided by species before extractions. Distinguishing white mullet (*Mugil curema*) from striped mullet (*Mugil cephalus*) juveniles proved to be extremely difficult for fish <40mm. Therefore all mugilids <40mm were categorized as juvenile mullet. All prey species were processed using the same lipid extraction technique used to extract bluefish lipids. Where possible multiple extractions were carried out on the same species but when sample sizes and body sizes were small multiple fish from the same species were combined into one sample. As for bluefish, all lipid results for prey species are presented as % dry weight and used to compare lipid levels among prey species using a t-test.
Results

Lipid Extraction Verification

Average lipids were very similar for each of the six flasks containing samples from individual bluefish (SE < 0.1 for all bluefish, see Fig. 2.3). A two-way ANOVA verified that there was a large fish effect (p < 0.0001) but no flask effect (p = 0.212).

Fifty two bluefish (mean fork length = 268.77 mm ± 9.17 SE) had both mesenteric fat deposits photographed and total lipids extracted. Mean lipid content for bluefish was 10.6% (range = 2.2 - 23.9%). Linear regression showed lipid content to be independent of bluefish length (t-test, p > 0.05). The frequency distribution of bluefish fat stages is approximately normal with most bluefish in fat stage 3 and fewest in stages 1 and 5 (Fig. 2.4). Bluefish caught in New Jersey in September had the highest mean lipid content (13%) followed by Florida bluefish caught in November (10.31%) and October (9%). A barely non-significant difference in lipid content was detected across months (p = 0.06). No significant difference in mean lipid content was detected between bluefish caught in October and November in Florida (p = 0.36). A significant difference in mean lipid content was observed between September and October caught bluefish (p = 0.017) but not between September and November caught fish (p = 0.12). The mean moisture content was 73.6% ± 0.26 and was not significantly different among months (p=0.15). There were only non-significant relationships between lipid
content (LC) and moisture content (MC) \( (LC = -0.244 \times (MC) + 28.63, n = 52, p = 0.48) \), lipid content and fork length (FL) \( (LC = -0.014 \times (FL) + 14.66, n = 52, p = 0.20) \), and moisture content and fork length \( (MC = -0.003 \times (FL) + 72.46, n = 52, p = 0.42). \)

Fat Staging

The mean average percent error between stagers was 15.7% ± 4.2 SE (table 2.4). Differences in staging never exceeded one level between stagers. Precision within stagers increased over successive stagings (1\textsuperscript{st} stager 11.11%, 8.33%, 0%; 2\textsuperscript{nd} stager 19.44%, 13.89%, 8.33%).

Fat Stage versus Lipid Content

A linear fit \( (R^2 = 0.67, p < 0.0001) \) was observed between fat stage and lipid content (Fig. 2.5). The range in lipid content values for each fat stage (Fig. 2.5) illustrates that despite the good fit there is still considerable overlap between fat stages. Fat stage one has a very narrow range because only two specimens were found in this category. All the other fat stages have wider ranges with considerable overlap, although the mean lipid content values increased for each higher fat stage. Standard errors for lipid content remained small for all fat stages except for stage five (Fig. 2.6).

In the generalized linear model fat stage by itself explained 69% of the variance in body lipid content (Table 2.5). Explained variance barely increased with the
addition of morphometric variables. Using fat stage and length with their two way interaction increased the explained little extra variance (71%) at the expense of adding an extra variable. Substituting length for weight produced a minor increase in the explained variance (71.4%). The use of fat stage, length and weight, as well as their three way interaction yielded the highest (84%) explanation of variance in bluefish lipid content but at the expense of adding two extra variables.

Lipid Content of Prey Species
A total of 476 samples were analyzed from 30 different species (table 2.6). I detected a significant difference in lipid content among prey species (t-test, p<0.0001) (Fig. 2.7). Mugil curema had the highest mean lipid content (19.52% ± 1.68 SE) and Sphyraena borealis had the lowest (0.88%). Other common prey species from MAB bluefish diet studies, bay anchovy and Atlantic silverside had intermediate lipid contents of 3.19% ± 0.67 and 3.49% ± 0.58 respectively.
Discussion

Subjective visual assessment is a technique widely used in fish biology. It is an accepted method in assessment of gonadal development, whereby gonads are assigned maturation stages based on key descriptors (e.g., tilefish, *Lopholatilus chamaeleonticeps* - Erickson et al. 1985, Murua et al. 2003), and stomach fullness (Hyslop 1980). Visual assessments are also a widely used technique when assessing coral reef habitat (Mumby et al. 1997). Where possible, reduction in the level of subjectivity will make results more acceptable. Subjectivity in visual estimates can also be decreased through the use of computer programs (i.e. measuring egg diameter or counting number of eggs, see Klibansky and Juanes 2008), although often there are no programs available to measure variability in appearance.

Fat Staging

The technique described to visually estimate lipid content based on the subjective assignment of fat stages related to mesenteric fat deposits may appear to be too subjective. However, I found a large difference between the mesenteric fat of bluefish among stages, so that estimation of mesenteric fat can be an accurate predictor of bluefish lipid content (explaining 69% of observed variability). The use of the visual lipid estimation is also rapid and inexpensive. Sampling surveys often record far more data than are actually being used for a specific study including length and weight in non-invasive studies, and gonadal
development and stomach contents in fatal studies. Incorporating the technique described here would not likely add to the time and cost when fish are already being sacrificed and opened up for stomach and gonad analysis.

The fat staging technique is simple to learn and the APE (15.7 ± 4.2 SE) and reduction in within reader error over successive stagings demonstrates that the technique is also reproducible, making it ideal for large scale studies. The overlap in lipid content values between fat stages (Fig. 2.5) suggests that lipid predictions are coarse, although mean lipid content for a given stage increased with fat stage. Lipid extractions should always be preferable to using the fat staging technique, but where large numbers of samples need to be processed, without the need for lab equipment (i.e. Soxhlet extractor) and extra personnel, visual fat staging is appropriate. Our results suggest that fat stage alone is a good predictor of lipid content in bluefish ($r^2 = 0.69$). Van der Lingen and Hutchings (2005) reported $r^2$ values in their study showing that fat stage alone was a good predictor of lipid content in anchovy ($r^2 = 0.75$), and using fat stage with wet body mass to predict lipid content in sardines ($r^2 = 0.89$, using just fat stage $r^2 = 0.51$). Bias was not assessed in Van der Lingen and Hutchings (2005) study but APE decreased within stagers over successive assessments, suggesting that fat staging experience resulted in increased reproducibility. The successive decrease in APE of this study ($1^{st}$ 19.44%, $2^{nd}$ 16.67%, and $3^{rd}$ 11.11%) would also suggest that experience reduces error and increases reproducibility.
The $r^2$ values reported in Simpson et al. (1992) (0.40-0.94 for Atlantic salmon) and Adams et al. (1995) (0.59-.83 for Arctic charr) from multiple regressions of up to 8 morphometric measurements and fish weights were similar to the results obtained in this study. The biggest advantage of solely using multiple regressions to predict lipid content from morphometric characters is that the fish does not need to be sacrificed and the same technique could be used on the same fish over time. However, the multiple regression method requires greater effort in the field to measure fish. The methods used by Crossin and Hinch (2005) and Cox and Hartman (2005) had high predictability values for Pacific salmon and brook trout lipid content ($r^2$'s of 0.93 and 0.96 respectively) yet the investment in the equipment that was used in these studies would need to be justified and is probably much better suited to lake or riverine systems where the same fish could be sampled over time. The advantages of these methods over visual lipid estimation are that they were both better predictors of lipid content and both are non-sacrificial. The major disadvantage of both these methods remains the cost of the sampling equipment.

Future use of this technique

The visual estimation method is easy to use with high predictability of lipid content. Moreover, developing this method for other fish species could be done with relative ease. However, depending on sampling type and project funding other methods might be more applicable. In studies where fish are being
sacrificed in fisheries surveys for gonad development analysis and stomach content analysis, incorporation of my method could produce a wealth of data on fish condition with very little added expense.

The use of this technique along the whole range of the bluefish migration could lead to the production of bluefish condition maps. Analysis of prey lipids using the same technique would help understand variability in prey condition over time and why bluefish might actively select for one species over another at different times of the year. Bluefish cohorts could then be tracked during migration, addressing the question of whether the YOY are estuarine dependent or not as proposed by Able et al. (2003). Moreover, it could also help identify essential estuarine “refueling stops” upon their southerly migration to the SAB to over-winter. Prey species could also be assessed using the same technique, with seasonal lipid variation helping to explain why predators switch prey.

Energy Storage Dynamics

Larger fish have more capacity for lipid storage than do smaller fish of the same species (Sogard and Olla, 2000). However, an increase in lipid content with body size was not identified in this study, possibly due to the small sample size (n = 52) or small range of bluefish body sizes.

Moreover, too few bluefish were analyzed to fully understand winter energy storage dynamics in this study. A previous study by Clarke (2006) showed lipid
content to be significantly different across months for bluefish, with January being the highest. Morley et al. (2007) reported bluefish lipid levels increasing until November before declining through the winter in North Carolina. This study agreed with both Clarke (2006) and Morley et al. (2007) that bluefish were accumulating lipids before winter but could not verify whether lipids increased or declined through winter due to lack of samples. Furthermore, one would expect to see a decline in lipids, reported here, between bluefish caught in New Jersey in September and those caught in Florida in October given the length of the migration. However, although the decline was statistically significant, a larger decline was expected and could have been offset by continued feeding during the migration.

Prey Lipids

Clarke (2006) identified mullet to be the dominant prey species in bluefish stomachs in northeast Florida, representing 99.55% by weight for age 1+ and 78.33% by weight for spring-spawned YOY. Common bluefish prey in the MAB, striped anchovy (Anchoa hepsetus), bay anchovy and Atlantic silversides were all prevalent in catches for all our winter collections (Clarke 2006). Our results show that lipid content was substantially higher for white mullet (Mugil curema) (19.52%) and juvenile mullet (Mugil spp.) (15.71%) than the other common prey species: striped anchovy (3.77%), bay anchovy (3.19%), Atlantic silverside (3.49%) and squid (3.88%) (Table 2.6). A comparison of Florida mullet lipid levels with mullet from higher latitudes was not possible due to a lack of mullet samples.
from the northern part of their range. Interestingly Marais (1990) also reported high lipid levels in mullets (>8%) compared to other fish species in a South African estuary. However, Marais (1990) found less variability in lipid content across the 10 species studied, ranging between 3% to 9%, than the 30 species analysed in this study (range 0.88% to 19.52%).

Morley et al. (2007) observed a depletion in lipid content of bluefish as winter progressed in North Carolina. The results of my study also showed a significant decline in lipids from bluefish caught in New Jersey during September (13%) to those caught in Florida during October (9%), suggesting that the migration has a negative impact on energy storage dynamics. However, in support of Clarke (2006), a small (but non-significant) increase in bluefish lipid content was observed between October (9%) and November (10.3%) suggesting an increase over the late fall. The prey switching (reported in Clarke 2006), to a diet dominated by mullets, likely promotes faster lipid accumulation, increased energy storage, and higher over-winter survival. Understanding the role of mullet to the over-winter survival of bluefish will require further detailed study. However, the prey lipid results from this study suggest that the higher lipid content of mullet is likely the reason for bluefish prey switching and lipid accumulation.

Fisheries management
Bluefish stocks in Florida receive little management and although it is a desired game fish, very few are taken for food in this region (personal communication...
with Roy Monson, former commercial bait harvester). Current management has designated bluefish as a “restricted species” and requires those caught in the recreational fishery to be 12 inches long to be retained with a bag limit of ten (Florida Fish and Wildlife Conservation Commission). The commercial fishery is restricted to 7,500lbs per boat per day with an Atlantic coast wide quota of 877,000lbs per year (Florida Fish and Wildlife Conservation Commission). A large majority of anglers return bluefish to the water alive yet still talk of the vast declines in number and size over recent years (personal communication with recreational anglers). Results of this study suggest that successful management of bluefish might be achieved if there were greater restrictions on the mullet fishery. Unfortunately, regulations on the mullet bait fishery are weakly enforced in this region. Although a commercial bait fishery can no longer be supported by the low numbers of mullet in north east Florida (personal communication with Roy Monson), recreational anglers armed with cast nets have an almost incalculable impact on their numbers with regulations difficult to enforce due to the number of recreational fishers. Even in the commercial fishery mullet regulations are often ignored with undersized (<11 inches) mullet representing 46% of the commercial catch off the Atlantic coast and 57.2% off the Gulf coast (Munyandorero et al. 2006). The appeal to recreational anglers of the “finger” mullet, named for the finger size of both striped and white mullet present in estuaries, is that they are extremely hardy, and can swim on the hook for many hours (several head-hooked “finger” mullet were retrieved in the beach seine where the hook wound had healed around the hook). Restrictions on recreational
anglers’ mullet catches (50 per day) are loosely enforced (recreational fishers were regularly observed working four rods with three mullet on each, and several buckets containing many more live mullet). Between 1982 and 1995 average mullet harvest on the Atlantic coast of Florida was 349,642 lbs yet has increased 29% since 1996 to average 467,422 lbs annually (Munyandorero \textit{et al}. 2006). Furthermore, from 1967 to 1990, the average annual landings in the commercial striped mullet fishery were 25 million lbs, yet have declined to an average of 8.1 million lbs between 2000 and 2004 (Florida Fish and Wildlife Conservation Commission, http://www.floridamarine.org/features/view_article.asp?id=26636). The small scale commercial fishery for striped mullet roe that still exists has a size limit of 11 inches (FL) and bag limitation of 100 fish per boat per day (Florida Fish and Wildlife Conservation Commission).

Competition with anglers for finger mullet, may force bluefish to feed on less lipid rich prey, not allowing the bluefish to accumulate the reserve lipids required for winter survival. Greater restrictions on mullet takes will help to rebuild the mullet populations in northeast Florida estuaries. Higher mullet abundances will likely result in more bluefish entering the estuaries to take advantage of the high lipid prey. Unfortunately, the majority of anglers regard bluefish as a trash fish and see their reduction as an opportunity to catch red drum and spotted sea trout, both prized table fish.
Surviving winter is essential if a cohort is to contribute to the adult stock the following growing season. Conover et al. (2003) found that the summer-spawned bluefish cohort was more abundant than the spring-spawned cohort from 1992 to 2002, yet the summer cohort appeared to contribute little towards the adult stock based on back-calculated age 1+ scales. This lack of contribution could potentially be due to higher over-winter mortality experienced by the summer cohort relative to the spring cohort (Conover et al. 2003). Furthermore, Sogard (1997) suggested that mortality during the winter is often negatively size-selective, with smaller individuals experiencing higher rates of mortality. Morley et al. (2007) reported that relatively small bluefish (late summer cohort, termed cohort 3) were more susceptible to size-selective winter mortality during severe winters. However, even after severe winters, cohort 3 bluefish recruited to their sampling gear in the spring. In contrast, summer-spawned bluefish showed a remarkable resilience to starvation, with over 90% survival after 4 months in the laboratory without food (Slater et al. 2007). However, low temperatures and reduced prey abundance is the likely trigger for the migration to the SAB. The greater energetic demand of such a migration would need to be replenished before the onset of winter, or feeding would have to continue throughout the winter period to guarantee survival. Replenishment of stored energy through predation on lipid-rich mullet would likely positively affect winter survival of bluefish. However, competition with recreational anglers for available mullet could have wide ranging implications to overwinter survival of bluefish in Florida waters. Alternative prey species, such as the species analyzed here, likely do not
contain the lipid reserves for bluefish to meet their minimum energy requirement for overwinter survival

Finally, incorporation of the visual lipid estimation technique for use on any impacted species of fish could have widespread benefits due to the rapidity of results. Fisheries managers could obtain data on the condition of fish populations whilst field sampling. In the case of the bluefish, once a lipid index (see fig.1 for an example) is produced, management could focus on a percentage of bluefish attaining a certain fat stage. If bluefish visual lipid estimates fail to reach the level set by managers, restrictions could be set in place to reduce the mullet harvested for bait, allowing bluefish greater access to their primary prey.
Figure 2.1. Bluefish sampling sites. (A) St. Augustine Pier, (B) Matanzas Inlet, (C) Gamble Rogers State Park, and (D) Ponce de Leon Inlet. Red dots represent seine sites.
Figure 2.2. Fat staging guide, developed for use at sea, to produce rapid lipid estimates for bluefish.
Figure 2.3. Variation in mean lipid content among flasks in the Soxhlet extraction apparatus for 6 individual bluefish. Error bars represent standard errors of six flasks.
Figure 2.4. Frequency distribution of bluefish fat stages.
Figure 2.5. Relationship between bluefish lipid content measured using Soxhlet extraction and fat stage visual estimation.
Figure 2.6. Mean lipid content for each bluefish fat stage. Error bars represent the standard error from the mean.
Figure 2.7. Lipid content results of 30 potential prey species common in northeast Florida estuaries. Error bars represent the standard error from the mean. Where error bars are absent, only one specimen was available.
<table>
<thead>
<tr>
<th>Location</th>
<th>Capture Date</th>
<th>n</th>
<th>Min L</th>
<th>Max L</th>
<th>Mean L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Station 97 (NJ)</td>
<td>9/24/2005</td>
<td>14</td>
<td>119</td>
<td>221</td>
<td>195.9</td>
</tr>
<tr>
<td>Matanzas Inlet</td>
<td>10/28/2005</td>
<td>15</td>
<td>59</td>
<td>377</td>
<td>260.7</td>
</tr>
<tr>
<td>Flagler Pier</td>
<td>11/20/2005</td>
<td>23</td>
<td>282</td>
<td>353</td>
<td>323.9</td>
</tr>
</tbody>
</table>

Table 2.1. Catch information for bluefish used in development of visual lipid estimation guide. n = sample size, L = bluefish fork length in mm
### Table 2.2. Description of criteria for each fat stage.

<table>
<thead>
<tr>
<th>Fat Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No fat strings visible around the stomach (dark red). Thin, patchy deposits around body cavity.</td>
</tr>
<tr>
<td>2</td>
<td>Thin fat strings running the length of the stomach. Large area of body cavity covered in thin fat layer.</td>
</tr>
<tr>
<td>3</td>
<td>Stomach covered with thin fat layer (pinkish). Thin fat layer covering entire body cavity.</td>
</tr>
<tr>
<td>4</td>
<td>Entire stomach almost covered by thick fat layer (small areas of pink). Thin fat layer covering entire body cavity.</td>
</tr>
<tr>
<td>5</td>
<td>Entire stomach covered by thick white fat deposits. Body cavity also covered in thick layer of fat.</td>
</tr>
</tbody>
</table>

Table 2.2. Description of criteria for each fat stage.
<table>
<thead>
<tr>
<th>Bluefish</th>
<th>Extraction #</th>
<th>Mean</th>
<th>SE</th>
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</thead>
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<tr>
<td>#</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>221</td>
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<td>5.31</td>
</tr>
<tr>
<td>3</td>
<td>199</td>
<td>9.21</td>
<td>9.39</td>
</tr>
<tr>
<td>4</td>
<td>166</td>
<td>3.67</td>
<td>3.92</td>
</tr>
<tr>
<td>5</td>
<td>232</td>
<td>10.96</td>
<td>11.12</td>
</tr>
<tr>
<td>6</td>
<td>175</td>
<td>4.93</td>
<td>4.90</td>
</tr>
<tr>
<td>Mean</td>
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<td>6.89</td>
<td>6.77</td>
</tr>
<tr>
<td>SE</td>
<td>1.14</td>
<td>1.15</td>
<td>1.13</td>
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</table>

<table>
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<tr>
<th>Source of Variation</th>
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<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
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<td>0.025686</td>
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Table 2.3. Verification of lipid extraction procedure. ANOVA results from multiple lipid extractions of 6 bluefish samples.
Table 2.4. Average percent error (APE) % between stagers visually estimating fat stages from photographs of sampled bluefish.
Table 2.5. GLM model outputs using fat stage, length and weight as parameters in the model.
<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Date</th>
<th>Lengths (mm)</th>
<th>MC (%)</th>
<th>LC (%)</th>
</tr>
</thead>
<tbody>
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<td>Fundulus spp.</td>
<td>9</td>
<td>11/16/05</td>
<td>60 – 80</td>
<td>66.91</td>
<td>3.73</td>
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<td>Fundulus spp.</td>
<td>7</td>
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<td>80 – 90</td>
<td>69.41</td>
<td>3.79</td>
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<tr>
<td>Fundulus spp.</td>
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<td>90 – 100</td>
<td>64.32</td>
<td>3.57</td>
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<td>11/16/05</td>
<td>100 – 120</td>
<td>66.29</td>
<td>3.88</td>
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<tr>
<td>Fundulus spp.</td>
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<td>12/27/05</td>
<td>75 – 90</td>
<td>72.38</td>
<td>8.25</td>
</tr>
<tr>
<td>Fundulus spp.</td>
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<td>1/27/06</td>
<td>45 – 75</td>
<td>70.04</td>
<td>2.28</td>
</tr>
<tr>
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<td>64.04</td>
<td>7.11</td>
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<td>11/16/05</td>
<td>40</td>
<td>78.64</td>
<td>4.97</td>
</tr>
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<td>1.97</td>
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<td>25 – 40</td>
<td>90.63</td>
<td>4.70</td>
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<td>Oligoplites saurus</td>
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<td>11/17/05</td>
<td>97</td>
<td>71.92</td>
<td>9.02</td>
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<tr>
<td>Menidia menidia</td>
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<td>70 – 80</td>
<td>75.00</td>
<td>2.40</td>
</tr>
<tr>
<td>Menidia menidia</td>
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<td>50 – 53</td>
<td>73.02</td>
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<tr>
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<td>40 – 70</td>
<td>74.01</td>
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<td>4.00</td>
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<tr>
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<td>69 – 83</td>
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### Table 2.6

Moisture content (MC) and Lipid content (LC) percentages for all prey lipid extractions. N represent number of fish in each extraction. Lengths represents the range of prey lengths in each extraction. Capture date is also listed.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Date</th>
<th>Lengths</th>
<th>MC</th>
<th>LC</th>
</tr>
</thead>
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<tr>
<td>Mugil curema</td>
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<td>58.31</td>
<td>14.39</td>
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<td>119 - 141</td>
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<td>405</td>
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<td>3</td>
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<td>40 – 70</td>
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REFERENCES


