

2015

**Condition of Juvenile Fishes in Estuarine Nursery Areas:
Measuring Performance and Assessing Temporal and Spatial
Dynamics with Multiple Indices.**

Ryan W. Schloesser
College of William and Mary - Virginia Institute of Marine Science

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<https://dx.doi.org/doi:10.25773/v5-7qtv-bk08>

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**Condition of Juvenile Fishes in Estuarine Nursery Areas: Measuring Performance and
Assessing Temporal and Spatial Dynamics with Multiple Indices**

A Dissertation

Presented to

**The Faculty of the School of Marine Science
The College of William and Mary in Virginia**

**In Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy**

by

Ryan W. Schloesser

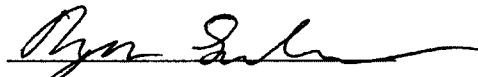
2015

APPROVAL SHEET


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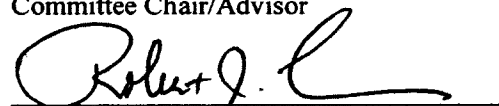
the requirements for the degree of


Doctor of Philosophy



Ryan W. Schloesser


Approved, by the Committee, April 2015


Mary C. Fabrizio, Ph.D.
Committee Chair/Advisor


Robert J. Latour, Ph.D.


Richard W. Brill, Ph.D.


Wolfgang K. Vogelbein, Ph.D.


Jeffrey A. Buckel, Ph.D.
North Carolina State University
Morehead City, NC

This Ph.D. is dedicated to my new son James and loving wife Jess, you've made every day spent completing my dissertation worth it.

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ACKNOWLEDGMENTS

This dissertation could not have been completed without the assistance of numerous individuals. I would first like to express my gratitude to Dr. Mary C. Fabrizio, who was always willing to provide the guidance I needed to conduct my research, and I appreciate her constructive comments and patience throughout my research and writing. I would also like to thank the other members of my committee: Drs. Robert J. Latour, Richard W. Brill, Wolfgang K. Vogelbein, and Jeffrey A. Buckel for their insights and help with improving the quality of my research. I also thank Drs. Eric Hilton, Tracey Sutton, and Mike Unger for providing the equipment that I needed for this work.

The research would not have been possible without funding from the Virginia Sea Grant and Virginia Marine Resources Commission, as well as numerous VIMS grants and awards.

I could have never collected the large number of specimens used in this research alone, so I am appreciative to all the people that assisted with my field work and specimen collection. In particular, I'd like to thank the past and present employees of the VIMS Juvenile Fish Trawl Survey and the Juvenile Striped Bass Seine Survey, notably: Hank Brooks, Aimee Comer, Chris Davis, Jennifer Greaney, Voight Hogge, Emily Loose, Wendy Lowery, Leonard Machut, Ryan Norris, Troy Tuckey, and Virginia Zakrzewski. I'd also like to thank the past and present students of the Fabrizio lab: Mark Henderson, Ben Marcek, Vaskar Nepal, Lauren Nys, and Branson Williams for all their helpful discussions, and assistance with field work. A special thanks to Lauren Nys for the many hours she spent helping me process specimens, even after long days in the field.

Finally, I would like to thank all my friends and family members for their support, they made my time at VIMS truly enjoyable.

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AUTHOR'S NOTE

The chapters in this dissertation were written as manuscripts for publication. Therefore, these chapters were written in the third person to reflect the contributions of my co-authors, and use different citation and reporting styles (e.g., for common names of species) to reflect journal guidelines. The citations for the chapters are:

Chapter 1

Schloesser, R.W., and M.C. Fabrizio. Submitted. The utility of condition indices as surrogates of energy density and lipid content in three juvenile fish species. *Journal of Fish Biology*.

Chapter 2

Schloesser, R.W., and M.C. Fabrizio. Accepted. Relationships among proximate components and energy density of juvenile Atlantic estuarine fishes. *Transactions of the American Fisheries Society*.

Chapter 3

Schloesser, R.W., and M.C. Fabrizio. Submitted. Temporal dynamics of condition for estuarine fishes in their nursery habitats. *Marine Ecology Progress Series*.

Chapter 4

Schloesser, R.W., and M.C. Fabrizio. In preparation. Nursery habitat quality assessed by the condition of juvenile fishes: not all estuarine areas are equal. *Marine and Coastal Fisheries*.

Chapter 5

Schloesser, R.W., and M.C. Fabrizio. In preparation. Does condition mediate thermal stress of juvenile fishes during winter residence in temperate estuaries? *Journal of Experimental Marine Biology and Ecology*.

ABSTRACT

The focus of this dissertation is to investigate relationships among indices of fish condition and examine temporal and spatial dynamics in condition of juvenile summer flounder, striped bass, and Atlantic croaker from Virginia estuaries. In the first two chapters, I compare multiple direct (energy density and proximate components) and indirect (length-based indices, hepatosomatic index, and relative subdermal lipids) approaches for assessing fish health to identify suitable means of estimating the lipid and energy content of juvenile fishes. Because indirect approaches can rapidly estimate the condition of juvenile fishes, these indices were then used to assess spatial and temporal variability in the condition of these species in the third and fourth chapters. Finally, in the fifth chapter I describe the influence of condition on mediating thermal stress of juvenile fishes during winter. Length-based indices, subdermal lipid estimates, and water content of whole fish can accurately assess lipid and energy content for juveniles of these species. Temporal patterns in length-based condition indices, the hepatosomatic index (HSI), and relative estimates of subdermal lipid content revealed energy-storage strategies used by juveniles to prepare for physiologically stressful events: the onset of winter and migration to the continental shelf. Notably, mean subdermal lipid reserves increased in preparation for migration of juvenile Atlantic croaker and summer flounder, whereas mean HSI values increased for all three species during winter. Spatial patterns in condition identified habitats that produced well-conditioned individuals which were energetically prepared for these physiologically stressful events. Prior to migration, Atlantic croaker were in high condition in locations with a low density of conspecifics, whereas summer flounder captured in coastal lagoons of the eastern shore of Virginia exhibited high condition. The mouth of the Rappahannock River and upestuary regions of the York River supported striped bass in high condition during the winter. Regardless of species, individuals that reside in Chesapeake Bay during winter are expected to decline in condition due to low water temperatures and reduced feeding opportunities. Yet, variability in physiological tolerances and energy utilization strategies among species differentially influenced their resistance to thermal stress, such that Atlantic croaker have a high probability of mortality in prolonged winters whereas striped bass do not. Mortality of summer flounder is strongly impacted by a blood flagellate infection common during winter. My findings suggest that the condition of individual fish can be used to understand energy allocation strategies of juvenile fishes and identify areas that may represent essential habitats that support individuals in healthier condition.

Condition of Juvenile Fishes in Estuarine Nursery Areas

INTRODUCTION

Estuaries provide important nursery areas for many species, yet the dynamic environment in temperate estuaries results in nursery habitats whose suitability varies across temporal and spatial scales. Due to variability in habitat quality, habitat-specific productivity has proved elusive, and survival benefits associated with particular habitats remain largely unexplored (Beck et al. 2001). Temperate estuaries in coastal Virginia encompass diverse habitat types and exhibit a wide range of environmental conditions, presenting an ideal system for investigating temporal and spatial variability in habitat quality. My dissertation examines the suitability of estuarine nursery habitats in Virginia based on the temporal and spatial dynamics in the condition of juvenile fishes.

Fish condition is an integrative metric of environmental stress, biological interactions (e.g., diet and predation), and fish activity; as such, juvenile fish condition may be used as a measure of nursery habitat quality (Stevenson and Woods 2006). High quality habitats are expected to support well-conditioned individuals, whereas suboptimal environments would produce individuals in poor condition. Because condition may influence survival (Wicker and Johnson 1987; Thompson et al. 1991; Brodersen et al. 2008; Evans et al. 2014), high quality nursery areas should provide an increased contribution to overall cohort production. Indeed, the influence of fish condition on year-class strength and recruitment variability was a motivating factor for my dissertation research. The condition of individual fishes has the potential to play a critical role in recruitment variability due to differential survival rates for poor- versus well-conditioned individuals. Rather than focusing on average individuals, our understanding of

recruitment may be enhanced by focusing on the unique characteristics (e.g., condition) of individuals with an increased probability of survival (Crowder et al. 1992).

In temperate estuaries of Virginia, juvenile fishes must be energetically prepared to survive migration or, if they remain in estuaries, to survive winter conditions. However, the energy-storage strategies used by juvenile fishes to prepare for these physiological challenges are unknown, and variability in energetic preparedness among years and habitats has not been investigated. Mortality during winter and migration may be a selective process acting on poor-condition juvenile fishes because of the extra energy required to support metabolic processes during these physiological challenges (Sogard 1997; Sutton and Ney 2001; Rios et al. 2011). Juvenile summer flounder, Atlantic croaker, and striped bass have life-history strategies with varying degrees of estuarine residency during winter, and indices of condition may be particularly helpful for assessing their energetic preparedness for the onset of winter or offshore migrations. As water temperatures decrease during the fall, juvenile summer flounder and Atlantic croaker typically migrate offshore to the continental shelf (Kraus and Musick 2001; Miller et al. 2003); yet a portion of the summer flounder population may overwinter in Chesapeake Bay. Juvenile striped bass also reside in Chesapeake Bay and its tributaries during winter. Individuals that reside in temperate estuaries during winter months likely endure food deprivation, which will have detrimental effects on metabolism, growth, disease resistance, and ultimately, condition and survival (Barton et al. 2002); all of which may be exacerbated by low temperatures. For example, during winter, juvenile striped bass can lose up to 21% of their total body energy and 50% of their lipid reserves (Hurst and Conover 2003). The energy required to withstand winter conditions is likely to

be species-specific, but the influence of fish condition on cold tolerance and winter survival has not been compared among species.

No single standard exists for measuring condition of fishes, and suitable condition indices may vary among species. Condition in fishes is often assessed via lipid or energy content, because lipids are high-energy compounds that are important for survival during periods of food scarcity and environmental stress. Fish in good condition have a greater lipid content (i.e., higher energy content) than fish in poor condition. However, the standard approaches used to measure energy and lipid content (e.g., bomb calorimetry and proximate composition) are time-consuming and costly. Rapid approaches that provide equivalent information are needed to be able to assess the condition of a large number of juvenile fishes and examine habitat quality over large spatial scales.

In this dissertation, I measure the performance of multiple condition indices and assess temporal and spatial dynamics in condition of juvenile fishes in Virginia estuaries. In chapters 1 & 2, I compare relationships among indirect and direct approaches used to assess fish condition. Indirect approaches include: length-based condition indices, the hepatosomatic index, subdermal lipid estimates from a commercial fish fatmeter (Distell, Old Levenseat, Fauldhouse, West Lothian EH47 9AD, Scotland, UK), and water content of fish tissues; direct approaches include energy content from bomb calorimetry, and protein and lipid contents from proximate composition analysis. In Chapter 3, I describe temporal variability in juvenile fish condition by examining inter-annual differences in condition of multiple year classes, and describe the intra-annual dynamics of condition to reveal energy allocation strategies of juvenile fishes. In Chapter 4, I identify high quality nursery habitats by assessing spatial variability in fish condition among regions in the

Chesapeake Bay, the coastal lagoons of Virginia's eastern shore, and the James, York, and Rappahannock River estuaries. Environmental conditions that typify high quality nursery habitats for these species are also examined. Finally, in Chapter 5, I examine the effect of condition on an individual's response to the food deprivation and cold temperatures typically encountered during winter in Chesapeake Bay. The consequences of variation in condition on cold tolerance and ability to withstand food deprivation may be examined to provide a better understanding of the factors that affect survival during a fish's first year of life (Bertram et al. 1997).

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CHAPTER 1

**The Utility of Condition Indices as Surrogates of Energy Density and Lipid Content in
Three Juvenile Fish Species.**

Abstract

Although the condition of individual fishes influences fitness and survival at all life stages, previous studies have focused mainly on how to measure condition of adults. Fish condition may be assessed by measuring either energy density (kJ g^{-1}) using bomb calorimetry or lipid content (g g^{-1}) using proximate composition analysis. Both, however, can be time consuming and cost-prohibitive. To identify a rapid means of estimating fish condition in juvenile summer flounder *Paralichthys dentatus*, striped bass *Morone saxatilis*, and Atlantic croaker *Micropogonias undulatus*, several indices (length-based, hepatosomatic, and relative lipid readings from the Distell fish fatmeter) were compared with energy density and lipid content measured directly in the same individuals. Fulton's K was significantly related to energy and lipid content of juvenile *P. dentatus* and *M. saxatilis*; relative condition (K_n) was also useful for juvenile *M. saxatilis*. The fatmeter suitably predicted energy and lipid content of *M. undulatus*. Because no single index was best for assessing condition of juveniles for all three species, it is recommended that selection of condition indices should be tailored to the life stage and species of interest.

Introduction

Condition is often used to assess the general health and nutritional status of individual fish and fish populations (Bolger and Connolly, 1989; Goede and Barton, 1990; Brown and Murphy, 2004). Indices of condition have been associated with growth (Adams and McLean, 1985; Gabelhouse, 1991) and swimming performance (Kolok, 1992; Martinez *et al.*, 2003), among other measures of fitness. These associations are based on the premise that the condition of an individual is correlated with its energy or lipid stores, yet this assumption is not always tested.

Energy and lipid content of an individual is also related to its survival. In both laboratory and wild settings, lipid content has been associated with improved survival of larval (Lochmann *et al.*, 1995; Booth and Alquezar, 2002) and juvenile fishes (Gardiner and Geddes, 1980; Thompson *et al.*, 1991; Booth and Hixon, 1999; Simpkins *et al.*, 2003). Additionally, sufficient energy stores (i.e., lipids) are needed to fuel long-distance migrations when feeding activity may be reduced or suspended (Jonsson *et al.*, 1997; Rios *et al.*, 2011). Stressful environmental conditions negatively influence individual fish (e.g., via metabolic rates), and over time, ultimately dictate the physiological state of the individual (Fry, 1971; Neill *et al.*, 1994). Therefore, fish condition and survival routinely vary among individuals, reflecting their exposure to different environmental and feeding histories. Individuals in relatively high condition may represent a small segment of the population, but because they have an increased probability of survival, these individuals may ultimately drive population-level effects (Crowder *et al.*, 1992; Stevenson and Woods, 2006).

Appropriate measures of fish condition (i.e., energy and lipid content) must be identified to investigate population-level effects, such as survival. Direct means of measuring energy and lipid content include bomb calorimetry and proximate composition analysis (hereafter referred to as direct condition indices). Proximate composition analysis provides an estimate of the proportion of water, lipid, organic (e.g., protein and carbohydrate), and inorganic (e.g., elemental oxides) compounds in the tissues of an individual fish (Stansby, 1976; Shearer, 1994). Bomb calorimetry directly estimates energy density of individuals by measuring the amount of heat released by combustion of dried tissue (Cummins and Wuycheck, 1971; Glover *et al.*, 2010). Proximate composition analysis and bomb calorimetry are time consuming and lethal methods that are usually cost-prohibitive when applied to large numbers of individuals. Thus, these methods have limited utility in large-scale or long-term studies of populations.

Alternative, indirect indices of fish condition can be applied more rapidly and at lower cost to estimate energy and lipid content; some are non-lethal. Indirect indices are often based on easily measured features (e.g., morphometrics) which may reflect energy or lipid content, although justification for use of these indices is often lacking (Stevenson and Woods, 2006). Many indirect indices of fish condition may be broadly categorized as either length-based, organosomatic, or based on tissue properties. Length-based indices include Fulton's *K* and relative condition factor (Anderson and Neumann, 1996; Froese, 2006). Fulton's *K* is a measure of individual fish robustness because greater body mass at a given length corresponds to better condition. Because Fulton's *K* is relatively simple to estimate, it provides a rapid assessment of relative "well-being" (Ricker, 1975), however, this index may or may not reflect proximate constituents. For example, the proportion of

water in fish may increase during starvation to maintain body shape, and therefore, individuals may have similar K values despite differences in energy density (Gardiner and Geddes, 1980). Additionally, K may be correlated with body size for some species (Cone, 1989; Sutton *et al.*, 2000), and therefore, it should be measured using fish of similar length (Bolger and Connolly, 1989; Anderson and Neumann, 1996). Relative condition factor (K_n) can compensate for size-dependent changes in growth (Le Cren, 1951); however, K_n can be applied only to populations that share the same allometric growth relationship (Anderson and Neuman, 1996).

Organosomatic indices use the mass of the liver, stomach, intestines, gonads, spleen, kidney, or other viscera and express condition as the proportion of mass attributable to a particular organ. The hepatosomatic index (I_H) is believed to be a good measure of condition because many fish species store lipids in their liver (Jensen, 1979; Lambert and Dutil, 1997), it is sensitive to feeding history (Heidinger and Crawford, 1977; Foster *et al.*, 1993), and has been shown to increase prior to emigration (Stallings *et al.*, 2010). However, in species that do not use the liver as a significant storage organ, the hepatosomatic index may be poorly correlated with whole-fish lipid content (Peters *et al.*, 2007).

Indirect condition indices may also be based on tissue properties. The transmission of low-power microwaves through tissues may be used to estimate lipid content using the Distell fish fatmeter (hereafter, "fatmeter"; Kent, 1990). The fatmeter has been used to estimate somatic lipid content and gross somatic energy density in a field setting (Crossin and Hinch, 2005). For many species, fatmeter readings are correlated with lipid content measured by proximate composition analysis, but they can

also reflect total dry body mass, water mass, and energy density (Table I). The majority of these relationships have been developed for adult fishes only, and the species investigated with the fatmeter likely accumulate large lipid stores as adults. The utility of this technique may differ among species with different body distributions of proteins and lipids, but this has not been thoroughly studied to date.

The objective of this study was to determine how well various condition indices predict energy density and lipid content in juveniles (< 1 year old) of three mid-Atlantic estuarine fishes: summer flounder *Paralichthys dentatus* (L. 1766), striped bass *Morone saxatilis* (Walbaum 1792), and Atlantic croaker *Micropogonias undulatus* (L. 1766). These species support important fisheries and have unique life-history strategies that may influence the distribution and role of lipids as energy stores. For example, lipid content increases prior to winter in *M. saxatilis* (Hurst and Conover, 2003), and storage of energy (via lipids or proteins) likely occurs before the off-shore migrations in *P. dentatus* and *M. undulatus* (McKeown, 1984). To identify indirect condition indices that can rapidly estimate energy and lipid content of a large number of juvenile fishes for these species, several length-based indices, the hepatosomatic index, and relative lipid readings from the fatmeter were compared to total body energy and lipid content from the same individuals (determined by proximate composition analysis and bomb calorimetry, respectively). Only one other study was found that has explicitly examined lipid content of juvenile fishes with the fatmeter (Hanson *et al.*, 2010), but this was for salmonids. Collection of samples from temporally and spatially restricted domains may bias condition indices when characterizing condition of individuals outside that domain (Pope and Kruse, 2007), therefore, we sampled individual fish from multiple Virginia estuaries

throughout the year. A laboratory study was conducted to identify appropriate fatmeter protocols and to examine the effect of gut fullness on fatmeter readings. Additionally, because a low percentage of wild specimens may be in poor condition due to starvation (Selleslagh and Amara, 2013), feeding regimes were employed to increase the observed range of energy and lipid content for *P. dentatus* and *M. saxatilis*. The range of observed energy and lipid content for wild caught *M. undulatus* was acceptably broad.

Materials and Methods

Field Collections

All animal collection, handling, care, and experimental protocols complied with relevant laws of the United States and were approved by the College of William and Mary's Institutional Animal Care and Use Committee. All individuals were euthanized in an ice slurry as recommended by Blessing et al. (2010) and described in the approved IACUC protocols.

Juvenile *M. saxatilis*, *P. dentatus*, and *M. undulatus* were sampled monthly between August 2010 and August 2012 from Virginia's estuaries (Fig. 1). In addition, fish were collected from August 2010 to August 2013 to develop length-mass models. Most samples were obtained according to a random stratified design using a 9.14-m otter trawl following sampling protocols described by Tuckey and Fabrizio (2013).

Additionally, *P. dentatus* were collected twice monthly from known nursery areas in Mobjack Bay and the coastal lagoons of the Virginia Eastern Shore using a 4.88-m otter trawl deployed from a small vessel. Monthly length thresholds were used to identify juvenile fish (i.e., age-0 fish; Tuckey and Fabrizio, 2013), but analyses focused on

individuals that were large enough to sample appropriately with the fatmeter. In this study, juvenile *P. dentatus* between 156 and 290 mm total length (T_L , $n = 73$), *M. saxatilis* between 117 and 200 mm fork length (F_L , $n = 66$), and *M. undulatus* between 117 and 220 mm T_L ($n = 72$) were examined. After capture, individuals were immediately euthanized in an ice slurry and returned to the lab where they were measured for length, body mass, and relative lipid content using the fatmeter (model FFM-992). For each specimen, length was recorded to the nearest millimeter and mass was measured with a Mettler Toledo XA204 balance (accurate to 0.1 mg); fish were then frozen for later processing (hepatosomatic and direct condition indices).

Laboratory Feeding Tests

Live *P. dentatus* and *M. saxatilis* were collected in October 2010 and maintained in recirculating (300 L) aquaria containing filtered York River water at 12-17° C and salinity of ~ 24. Fish were offered Zeigler finfish G prepared diets (either 0.25 % or 3 % body mass, three times weekly). To identify preferred fatmeter placements and assess the influence of gut fullness on fatmeter readings, lipid contents were measured with the instrument on various locations on the body before and after feeding during weekly examinations from December 2010 to February 2011. Individuals that fed (evidenced by a distended gut) were used to investigate the influence of gut fullness on fatmeter readings. Individuals that refused to feed (identified by a continuous decline in condition) were used to identify appropriate fatmeter measurement locations and establish relationships between fatmeter measurements and direct condition indices. Individuals that fed were not used in condition index comparisons because differences in proximate

constituents (and lipid storage sites) between laboratory and wild specimens may potentially reduce the predictive relationships among indirect condition indices and energy or lipid content.

Condition Indices

Length-based indices, the hepatosomatic index, and the fatmeter were evaluated for their ability to reflect lipid content and energy density of wild-caught juvenile *P. dentatus*, *M. saxatilis*, and *M. undulatus* (Table II). Preliminary testing was used to identify protocols necessary for assessing the suitability of the fatmeter for these species. Established protocols helped minimize the influence of fatmeter placement or gut fullness on the predictive ability of the approach. Because large amounts of fat may be stored beneath the skin around the lateral line, and because fat content often decreases from head to tail (Stansby, 1976), a location immediately behind the operculum and parallel to the lateral line was selected as the site from which to estimate lipid content with the fatmeter. Preliminary observations indicated that this location yielded more consistent results among consecutive readings and allowed maximum contact between the fatmeter sensor and the specimen. Because *P. dentatus* store excess lipids in the fin ray musculature (Gaylord *et al.*, 2003), this area was evaluated as a potential measuring site; however, consistent readings were not obtained at this site with the fatmeter, and this site was eliminated from further consideration. Individual fish were measured with the fatmeter four times on each side of the body. The side of the fish measured with the fatmeter that best correlated with energy and lipid content was identified and used in remaining fatmeter assessments. Accuracy of the fatmeter varies, and depends on lipid

content of the fish: accuracy is reported as $\pm 1.5\%$ when lipid content is between 2-15 %; $\pm 2.5\%$ when lipid content is between 16-30 %; and $\pm 4\%$ when lipid content is $> 31\%$ (Anonymous, 2010). Fatmeter accuracy was confirmed by recording seven repeated measures of fatmeter lipid content from nine *M. undulatus* held on ice over a period of 5 hrs; mean (\pm S.E.) fatmeter lipid readings varied no more than $3.4 \pm 0.8\%$.

Fulton's condition index (K) was calculated using:

$$K = W * L^{-3} * 10^N$$

where W = wet body mass (g), L = length (mm), and N = an integer to bring the value of K near 1 (Ricker, 1975). Relative condition factor (K_n) was calculated using:

$$K_n = W * (aL^b)^{-1}$$

where a and b are constants describing the allometric relationship for fish in a given population (Le Cren, 1951). Constants were estimated using nonlinear least-squares via the Nlin procedure in SAS 9.3 (SAS Institute, Cary NC; Table III). The residuals from these nonlinear regressions (hereafter, length-mass residuals) were also assessed for their ability to reflect lipid content (proximate composition) and energy density (bomb calorimetry) (e.g., Sutton *et al.*, 2000).

For all individuals, the liver was extracted in a manner that minimized loss of body fluids (this was necessary to preserve the accuracy of proximate composition analysis). Liver mass was measured to calculate the hepatosomatic index (I_H) using:

$$I_H = W_{\text{liver}} * W^{-1} * 100$$

where W_{liver} = wet mass of liver (g) and W is as before (Jensen, 1979; Lambert and Dutil, 1997).

After liver extraction, gut contents were removed in preparation for bomb calorimetry and proximate composition analysis. Whole fish were homogenized in a blender and dried at 55° C for four to seven days. Dried tissues were pulverized with a mortar and pestle, and partitioned into two samples: one for bomb calorimetry and the other for proximate composition analysis. Dried tissue samples were combusted in a Parr 6300 bomb calorimeter to determine energy density in calories per gram of dry fish tissue, which was then converted to energy density per gram wet body mass (kJ g^{-1}). The bomb calorimeter was calibrated using a benzoic acid standard, and the calibration was checked every ten measurements (mean difference (\pm S.E.) = $0.035 \pm 0.035 \text{ kJ g}^{-1}$, *t*-test, $t_{26} = 1.0$, $P = 0.32$, corresponds to a 0.13 % error). Tissues for proximate composition analysis (to assess lipid content) were shipped to the Department of Zoology, Southern Illinois University, a laboratory certified in performing these analyses. More specifically, lipid content was determined using a modified Folch extraction technique (chloroform/methanol mixture was used as the solvent) and is presented as percent wet body mass.

Statistical Analyses

The relationships among indirect and direct condition indices were examined with general linear models. Because individuals of many fish species show an increase in lipid content, and thus energy content, with body size (Brown and Murphy, 2004; Wuenschel *et al.*, 2006), fish length was included as a potential predictor of lipid and energy content. The indirect indices and fish length were considered fixed effects. Interaction plots suggested that the relationship between direct and indirect condition indices varied by

fish size, therefore, interactions between length and the indirect condition indices were also included in predictive models. Models were fit with the Mixed procedure in SAS 9.3 using restricted maximum likelihood (REML) for parameter estimation. Because some of the predictors exhibited collinearity, all predictors were centered to remove these effects (Table IV; Quinn and Keough, 2002). Energy and lipid content data were log-transformed to improve normality and homogeneity of variance, necessary assumptions of these linear models. Fatmeter readings were log-transformed because this transformation provides the best linear relationship with lipid content (Colt and Shearer, 2001; Crossin and Hinch, 2005; Hanson *et al.*, 2010). Model selection within and among indirect condition indices was determined using Akaike's information criterion corrected for small sample size (AIC_c ; Burnham and Anderson, 2002). When selected models included multiple predictors, partial regressions were used to examine the relationship between indirect and direct condition indices (Quinn and Keough, 2002). The proportion of the total variation in energy and lipid content explained by the indirect condition indices was determined using a REML-based estimate of the coefficient of determination (r^2_{REML}) as discussed in Singer (1998).

Results

The midline of the body was a feasible measurement site with the fatmeter for small juvenile fishes of all three species. At this location, the left side of *M. saxatilis*, and an average of readings from both sides of *M. undulatus* provided the highest correlation with energy and lipid content (Table V). Results are reported from fatmeter readings on the non-eyed side of *P. dentatus*, which are similar to those using an average of fatmeter

readings from both sides (Table V). Gut fullness had no significant effect on fatmeter readings. The mean difference (\pm S.E.) of fatmeter readings on individuals before and after feeding was 0.12 ± 0.08 relative lipid units for *P. dentatus* (paired *t*-test, $t_{32} = -1.48$, $P = 0.15$), and 0.29 ± 0.47 relative lipid units for *M. saxatilis* (paired *t*-test, $t_{20} = 0.62$, $P = 0.54$).

Energy and lipid content could be reliably estimated by a number of indirect metrics, but these varied depending on species, and not all indirect condition indices were suitable. Fulton's *K* provided a suitable means of estimating energy and lipid content for *P. dentatus* and *M. saxatilis*, and for *M. saxatilis*, K_n also adequately estimated energy density (by convention, AIC_c values within two units suggest a similar ability to predict the response; Burnham and Anderson, 2002) (Fig. 2(a), (b)). Relative lipid readings from the fatmeter were reliable predictors of energy and lipid content for juvenile *M. undulatus* when length and the interaction between length and the fatmeter were included as predictors (Table VI(c); Fig. 2(c)). Two indices were unsuitable for assessing energy and lipid content of juvenile fishes for these species: residuals from the length-mass regression, and the hepatosomatic index. For ease in comparing our results with previously published data, coefficients of determination (r^2) are provided for all condition index comparisons (Table V). REML-based correlations (r^2_{REML} , Table VI) from predictive models were lower than coefficients of determination (r^2 from Table V), unless other predictors (e.g., length) were also incorporated in the model.

Even though juvenile fish were from a relatively small size range, length remained an important predictor of energy and lipid content for some of the indirect condition indices. This was particularly so for *P. dentatus* and *M. undulatus*; length did

not improve predictions of energy or lipid content for *M. saxatilis*, regardless of the condition index investigated. Predictions of *P. dentatus* lipid content improved when K_n and the length-mass residuals were adjusted for the effects of length. Similarly, estimates of energy density were improved when length was incorporated in the predictive model with K_n . To estimate lipid content of *M. undulatus*, length was included in all models except the models using Fulton's K or the fatmeter, and the length-indirect index interaction was included in the model predicting lipid with length-mass residuals. Estimating energy density of *M. undulatus* from K_n and length-mass residuals required the inclusion of fish length in the model, whereas the prediction of energy content with the fatmeter was best for models that incorporated length and the interaction between length and the fatmeter.

Several models, particularly for *M. saxatilis*, explained limited amounts (< 40 %) of the total variability in energy and lipid content (Table VI), which may be due in part to inclusion of samples from broad temporal and spatial domains. Individuals from a limited temporal sampling period or from sites in a relatively small portion of the sampling area showed reduced variability in condition (potentially due to similar environmental histories), and analyses on a subset of the specimens resulted in improved correlations among direct and indirect condition indices. For example, the correlation (r^2) between Fulton's K and log-transformed lipid content for *P. dentatus* collected in November and December only ($n = 15$) was high (0.80). Similarly, the observed correlation between K_n and log-transformed energy content was higher (0.51) for *M. saxatilis* from the laboratory and the Rappahannock River ($n = 33$). Nevertheless, predictive relationships among

condition indices were developed to be applied to the temporal and spatial ranges of interest despite lower correlations.

Discussion

Results from this study suggest that the energy and lipid storage strategies used by juvenile fishes may ultimately influence the utility of condition indices for predicting energy or lipid content. General linear models identified suitable indirect condition indices for estimating condition of juvenile *P. dentatus*, *M. saxatilis*, and *M. undulatus*, albeit the selected index varied by species.

Energy and lipid content were reliably estimated from Fulton's *K* for *M. saxatilis* and *P. dentatus*. Fulton's *K* has been identified as a suitable estimator of condition for juvenile fishes, with correlations (r^2) ranging from 0.55 to 0.8 for *K* and lipid or energy content (Costopoulos and Fonds, 1989; Pangle and Sutton, 2005; Hanson *et al.*, 2012), but *K* was poorly related to lipid or energy content for other species ($r^2 < 0.25$; Trudel *et al.*, 2005; Peters *et al.*, 2007; Davidson and Marshall, 2010). Fulton's *K* is useful for examining changes in condition among seasons (Heincke, 1908 from Froese, 2006); specimens collected throughout the year (as done here) would exhibit such seasonal changes and provide sufficient contrast in the data to permit description of the relationship between *K* and energy and lipid content.

The appropriateness of Fulton's *K* as a measure of energy or lipid content has been questioned because it may be correlated with body size in species exhibiting allometric growth. Interestingly, even though *P. dentatus* and *M. saxatilis* exhibit allometric growth ($b \pm CI$ was 3.36 ± 0.01 and 3.19 ± 0.03 , respectively; Table III), *K* and

fish length were not significantly related for these species. Similarly, Gilliers *et al.* (2004) failed to detect relationships between length-based estimates of condition and fish length among flatfishes. Furthermore, although individuals of many fish species show an increase in lipid content with body size, the use of Fulton's K to predict energy or lipid content did not require adjustments for size. Length may not have been necessary to predict energy and lipid content due to the examination of fish from a restricted size range (i.e., juvenile fish). Alternatively, because K is not completely independent of length, the effect of size on energy-storage capacity may be reasonably reflected in this index.

Relationships among length-based and direct condition indices may or may not be consistent among species, particularly when considering length-mass relationships may vary among seasons, populations, or years (Froese, 2006). Simple length-based indices (e.g., K , relative condition) were reliable surrogates of body proximate composition for adult walleye *Sander vitreus* (Mitchill 1818) (Kaufman *et al.*, 2007). However, for juvenile Atlantic salmon *Salmo salar* L. 1758, K_n correlated well with lipid content, but K did not (Sutton *et al.* 2000). Here, K_n and Fulton's K for juvenile *M. saxatilis* were equally suited to describe energy density. Because relative condition accounts for changes in body mass with length due to body shape (Le Cren, 1951), this metric is suitable for comparing individuals that share the same allometric growth pattern. Thus, K_n may be preferable for estimating energy density of juvenile *M. saxatilis* from Virginia estuaries because the allometric length-mass relationship did not differ among groups of fish sampled from multiple locations. Although predictive models with K_n did not require

adjustments for size with *M. saxatilis*, because K_n is size-independent, length improved model predictions for *P. dentatus* and *M. undulatus* with this indirect index.

The utility of the fatmeter for estimating direct condition indices appears to be related to the degree of sub-dermal lipid storage, which may be limited for juvenile fishes that typically allocate energy to growth. The fatmeter appropriately estimated condition for *M. undulatus*, which exhibited a wide range of lipid content. However, results suggest that the fatmeter may have limited utility for predicting whole body energy and lipid content for juvenile *P. dentatus* and *M. saxatilis* due to a limited range of sub-dermal lipid stores. Indeed, *P. dentatus* store excess lipids in the finray musculature (Gaylord *et al.*, 2003), and *M. saxatilis* store lipids primarily in the viscera (Jacobs *et al.*, 2008). Because the instrument is designed to measure lipid-storage under the skin, gut contents and other visceral lipid stores are outside the measurement area and do not influence fatmeter readings from juvenile fishes. These findings are therefore consistent with a previous study that found no difference in mean fatmeter lipid measurements between adults measured when intact or after the gut was removed (Davidson and Marshall, 2010). Examination of juvenile fishes excluded maturing individuals that may exhibit lower fatmeter readings (and lower hepatosomatic index values) because fish undergoing maturation tend to allocate energy reserves to gonads (Perrin and Sibly, 1993). Differences in fatmeter readings between the two sides of the fish, such as those observed for *M. saxatilis* may reflect differential storage of lipids or operator-induced variability due to orientation and/or degree of contact.

Studies comparing condition indices often use laboratory-held individuals to extend the range of observed energy or lipid content. However, differences in proximate

constituents have been documented between wild and laboratory-held individuals, potentially hampering the development of predictive relationships. Differences include the quantity and composition of fatty acids (Alasalvar *et al.*, 2002), temporal patterns in lipid content (Thompson *et al.*, 1991), and variations in lipid:protein ratios in muscle tissue (McDonald *et al.*, 1998; Morley *et al.*, 2012). Here, elevated hepatosomatic index values were observed despite low values of *K* in laboratory specimens that fed (similar to those observed in *Gadus morhua* L. 1758, Grant *et al.*, 1998). These observations indicate that laboratory-held fish may also increase lipid storage in the liver despite limited growth in body mass, potentially leading to different tissue-specific distributions of proximate components (and thus energy-storage products) compared with their wild conspecifics.

Relationships between energy and lipid content and indirect approaches based on tissue properties (such as the fatmeter) are rarely developed for juvenile fishes and the few studies reported in the literature often used laboratory-held specimens. For juvenile *Oncorhynchus mykiss* (Walbaum 1792), a poor correlation was found between lipid from proximate composition and fatmeter estimates when investigating hatchery fish (Hanson *et al.*, 2010); however, the source (i.e., hatchery or wild fish) and size (i.e., juvenile or adult) of fish used to calibrate the fatmeter in the study is unknown, and may have influenced the results. The differences in composition among wild and laboratory-held specimens are likely to be pronounced when fish are fed to satiation due to differences in nutritional value of food resources and higher activity levels of wild fish. However, if laboratory-held individuals are deprived of food, body composition may be similar to wild specimens that do not feed successfully. Because comparisons of individuals from

wild and aquaculture settings may not be physiologically meaningful (Copeland and Carline, 2004), applying condition indices developed with laboratory fishes to wild populations should be avoided unless it can be shown that the composition and distribution of proximate components is similar among wild and laboratory-held fish.

Residuals from length-mass regression and the hepatosomatic index were unsuitable surrogates for energy and lipid content, but the reasons varied. The validity of residuals from a length-mass regression for estimating condition has been questioned (Garcia-Berthou, 2001; Green, 2001; but see Schulte-Hostedde *et al.*, 2005). Regression residuals are less commonly used to investigate fish condition, and this may be due to the inability to meet key assumptions of the length-mass model (e.g., residuals are independent of body size). In contrast, the hepatosomatic index is commonly used to estimate fish condition, but was not a good predictor of energy or lipid content of juvenile fishes here. The liver may not be a primary storage organ for lipids in juveniles (Litvin *et al.*, 2011) due in part to energy allocation strategies of juvenile fishes. Allocating energy to growth may result in better survival (Hurst and Conover, 2003), so juvenile fish may not store lipids in the liver, resulting in a weak relationship between the hepatosomatic index and energy or lipid content for this life-history stage.

Juvenile fishes may also store energy as constituents other than lipids, and this may result in weak relationships among indirect and direct condition indices. For example, fishes that store energy as protein (instead of lipid) would not only exhibit lower lipid content, but also lower energy density because protein contains less energy by mass than lipid. Under these circumstances, establishing a strong predictive relationship is difficult due to the low and potentially limited range of observed values (Weisberg,

2014). Protein is, in fact, used as an energy reserve in some species (e.g., *Oncorhynchus nerka* (Walbaum 1792), Mommensen, 2004). Some flatfishes use stored protein to supplement energy needs during adverse circumstances (e.g., food deprivation, Jobling, 1980; Maddock and Burton, 1994); juvenile *P. dentatus* may also use this strategy. Furthermore, flatfishes generally have a relatively low fat content (often ~ 1 %) and relatively higher protein content (~ 20 %) (Stansby, 1976). Because most flatfishes do not regularly migrate over large spatial areas, Stansby (1976) hypothesized a reduced need to store fat in muscle tissue. Indeed, compared with *M. saxatilis* and *M. undulatus*, juvenile *P. dentatus* store a higher percentage of total energy as proteins (Schloesser and Fabrizio, unpublished data).

The distribution of proximate constituents, notably lipid, throughout the body may influence the utility of some indirect techniques for assessing energy or lipid content and depends on the species examined. Length-based measures of condition seem appropriate for species or life stages that have minimal lipid stores or that rely on protein as an additional energy source (e.g., *P. dentatus*). Relative lipid measurements from the fatmeter are appropriate if sub-dermal lipids are a significant storage depot or if those stores change proportionately with other storage strategies (e.g., *M. undulatus*). Ultimately, only a comparison of indirect indices to energy or lipid content can determine if an indirect index will be useful for assessing condition for a given species and life stage. When making comparisons among direct and indirect condition indices, researchers should consider the appropriateness of using laboratory or wild specimens, and identify temporal and spatial ranges from which samples should be collected to ensure comparisons among condition indices are appropriate for the question of interest.

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Table I. Correlation (r^2) between estimates of lipid content from the fatmeter and lipid content from proximate composition analysis, dry body mass, water mass, and energy density for various species. Correlations were determined with a variety of approaches including the use of aquaculture, wild, or wild-caught then experimentally manipulated individuals. The use of whole fish or muscle fillets for fatmeter readings and lipid content also varied among studies.

Scientific name	Common name	Correlation (r^2)				Citation
		Lipid	Dry mass	Water mass	Energy	
<i>Anguilla anguilla</i>	American eel		0.71			Klefoth <i>et al.</i> , 2013
<i>Cyprinus carpio</i>	Common carp	0.38				Kocour <i>et al.</i> , 2007
			0.68			Klefoth <i>et al.</i> , 2013
<i>Clupea harengus</i>	Atlantic herring	0.71				Vogt <i>et al.</i> , 2002
		0.41 - 0.94				Nielsen <i>et al.</i> , 2005
		0.5 - 0.65				Davidson and Marshall, 2010
		0.64				McPherson <i>et al.</i> , 2011
<i>Dicentrarchus labrax</i>	European seabass	0.72				Saillant <i>et al.</i> , 2009
<i>Latrix lineata</i>	Striped trumpeter	0.66				Brandsen <i>et al.</i> , 2007
<i>Maccullochella peelii peelii</i>	Murray cod				0.84	Whiterod, 2010
<i>Oncorhynchus keta</i>	Chum salmon	0.71				Kaga <i>et al.</i> , 2009
<i>Oncorhynchus mykiss</i>	Rainbow trout	0.48 - 0.58				Dourin <i>et al.</i> , 1998
		0.1		0.83		Hanson <i>et al.</i> , 2010
		0.58	0.48 - 0.58			Caldwell <i>et al.</i> , 2013
<i>Oncorhynchus nerka</i>	Sockeye salmon	0.82 - 0.93			0.84 - 0.94	Crossin and Hinch, 2005
<i>Pangasianodon hypophthalmus</i>	River catfish	0.73				Van Sang <i>et al.</i> , 2009
<i>Salmo salar</i>	Atlantic salmon	0.3 - 0.87				Hendry and Beall, 2004

Table II. Number of juvenile specimens collected from Virginia estuaries (Fig. 1) during winter (January-March), spring (April-June), summer (July-September), and fall (October-December) between 2010 and 2012 that were included in condition comparison analyses.

Species	2010		2011				2012			Total
	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	
<i>Paralichthys dentatus</i>	6	25	3	18	7	2	3	5	4	73
<i>Morone saxatilis</i>	0	17	22	6	0	0	21	0	0	66
<i>Micropogonias undulatus</i>	4	16	0	17	17	2	2	12	2	72

Table III. Population specific length-mass relationships for juvenile *Paralichthys dentatus*, *Morone saxatilis*, and *Micropogonias undulatus* determined by nonlinear least-squares regression from fish collected between August 2010 and August 2013 from Virginia estuaries. Parameter estimates were used to calculate length-mass residuals for index comparison, and are provided with confidence intervals (\pm CI). Sample size is given by n . Mass was measured in grams. Fork length is denoted by F_L and total length by T_L .

Species	Model	n
<i>Paralichthys dentatus</i>	Weight = $1.30 \times 10^{-6} (\pm 0.10 \times 10^{-6}) T_L^{3.3588 (\pm 0.0135)}$	2291
<i>Morone saxatilis</i>	Weight = $4.59 \times 10^{-6} (\pm 0.77 \times 10^{-6}) F_L^{3.1931 (\pm 0.0328)}$	624
<i>Micropogonias undulatus</i>	Weight = $1.65 \times 10^{-6} (\pm 0.16 \times 10^{-6}) T_L^{3.3715 (\pm 0.0192)}$	3798

Table IV. Mean value for each indirect condition index (K_n for relative condition, $L-M$ for length-mass, and I_H for the hepatosomatic index). Due to collinearity among predictors, these values were subtracted from individual observations of condition to establish relationships between indirect condition indices and energy and lipid content.

	<i>Paralichthys dentatus</i>	<i>Morone saxatilis</i>	<i>Micropogonias undulatus</i>
Fulton's K	0.9005	1.1689	1.0526
K_n	0.9986	0.9772	0.9699
$L-M$ Residuals	-2.2900	-1.0301	-1.7151
I_H	0.7878	1.8764	1.2559
Fatmeter	0.8264	3.3098	3.2095

Table V. Correlation (r^2) among indirect condition indices (K_n for relative condition, $L-M$ for length-mass, and I_H for the hepatosomatic index) and log-transformed energy (kJ g^{-1} wet body mass) and lipid content (% wet body mass) for juvenile *Paralichthys dentatus* ($n = 73$), *Morone saxatilis* ($n = 66$), and *Micropogonias undulatus* ($n = 72$). Fatmeter readings were taken from two sides of the individual (Fatmeter_{Mean} represents the average) and log-transformed to identify the “best” location for assessing fatmeter lipid content. An asterisk (*) denotes the fatmeter location used for index comparisons. Specimens were collected between August 2010 and August 2012 from Virginia’s estuaries (Fig. 1). All correlations were significant ($P < 0.01$) unless indicated by footnotes.

Species	Indirect condition index	Lipid	Energy
<i>Paralichthys dentatus</i>	Fulton's K	0.39	0.46
	K_n	0.19	0.32
	$L-M$ Residuals	0.27	0.38
	I_H	0.26	0.45
	Fatmeter _{Eyed}	0.39	0.33
	Fatmeter _{Non-eyed} *	0.40	0.44
	Fatmeter _{Mean}	0.41	0.41
<i>Morone saxatilis</i>	Fulton's K	0.30	0.31
	K_n	0.25	0.32
	$L-M$ Residuals	0.23	0.29
	I_H	0.21	0.33
	Fatmeter _{Left} *	0.22	0.12
	Fatmeter _{Right}	0.07 ^a	0.02 ^b
	Fatmeter _{Mean}	0.14	0.06 ^b
<i>Micropogonias undulatus</i>	Fulton's K	0.53	0.48
	K_n	0.22	0.23
	$L-M$ Residuals	0.08 ^a	0.08 ^a
	I_H	0.27	0.31
	Fatmeter _{Left}	0.64	0.58
	Fatmeter _{Right}	0.66	0.61
	Fatmeter _{Mean} *	0.68	0.62

^a $0.01 \leq P < 0.05$

^b $P > 0.05$

Table VI. General linear models used to predict energy (kJ g^{-1} wet body mass) and lipid content (% wet body mass) with individual indirect condition indices (K_n for relative condition, I_H for the hepatosomatic index, $L-M$ for length-mass, and L_T for total length). The lowest Akaike Information Criterion value (corrected for small sample size, AIC_c) was used for model selection (selected model denoted by *). The difference between the AIC_c value for each model and the selected model is denoted by ΔAIC_c . Parameter estimates are provided with confidence intervals (\pm CI). The proportion of the total variation in energy and lipid content explained by the indirect condition indices was determined using a REML-based estimate of the coefficient of determination (r^2_{REML}).

(a) *Paralichthys dentatus*

Direct	Indirect	Model	AIC _c	ΔAIC _c	r ² _{REML}
Energy	Fulton's K*	$\ln(\text{Energy}) = 1.4114 (\pm 0.0230) + 0.8297 (\pm 0.2113) K$	-121.6	0.0	0.46
	K _n	$\ln(\text{Energy}) = 1.4114 (\pm 0.0233) + 0.7331 (\pm 0.1947) K_n + 0.0015 (\pm 0.0007) L_T$	-106.6	15.0	0.44
	L-M Residuals	$\ln(\text{Energy}) = 1.4114 (\pm 0.0246) + 0.0055 (\pm 0.0016) \text{Residuals}$	-102.0	19.6	0.38
	I _H	$\ln(\text{Energy}) = 1.4114 (\pm 0.0233) + 0.2126 (\pm 0.0557) I_H$	-117.1	4.5	0.44
	Fatmeter	$\ln(\text{Energy}) = 1.4114 (\pm 0.0235) + 0.0643 (\pm 0.0172) \ln(\text{Fatmeter})$	-113.3	8.3	0.43
Lipid	Fulton's K*	$\ln(\text{Lipid}) = 0.4748 (\pm 0.1036) + 3.1936 (\pm 0.9533) K$	92.3	0.0	0.38
	K _n	$\ln(\text{Lipid}) = 0.4748 (\pm 0.1012) + 2.7232 (\pm 0.8477) K_n + 0.0083 (\pm 0.0032) L_T$	99.4	7.1	0.41
	L-M Residuals	$\ln(\text{Lipid}) = 0.4748 (\pm 0.0985) + 0.0235 (\pm 0.0068) \text{Residuals} + 0.0073 (\pm 0.0030) L_T$	105.2	12.9	0.44
	I _H	$\ln(\text{Lipid}) = 0.4748 (\pm 0.1136) + 0.6830 (\pm 0.2721) I_H$	108.2	15.9	0.25
	Fatmeter	$\ln(\text{Lipid}) = 0.4748 (\pm 0.1025) + 0.2583 (\pm 0.0752) \ln(\text{Fatmeter})$	95.9	3.6	0.39

(b) *Morone saxatilis*

Direct	Indirect	Model	AIC _c	ΔAIC _c	r ² _{REML}
Energy	Fulton's K*	$\ln(\text{Energy}) = 1.6055 (\pm 0.0276) + 0.9973 (\pm 0.3688) K$	-93.0	0.7	0.30
	K _n *	$\ln(\text{Energy}) = 1.6055 (\pm 0.0275) + 1.1798 (\pm 0.432) K_n$	-93.7	0.0	0.31
	L-M Residuals	$\ln(\text{Energy}) = 1.6055 (\pm 0.0281) + 0.0267 (\pm 0.0105) \text{Residuals}$	-83.4	10.3	0.28
	I _H	$\ln(\text{Energy}) = 1.6055 (\pm 0.0273) + 0.1187 (\pm 0.0424) I_H$	-90.1	3.6	0.32
	Fatmeter	$\ln(\text{Energy}) = 1.6055 (\pm 0.0313) + 0.1853 (\pm 0.0634) \ln(\text{Fatmeter})$	-74.5	19.2	0.10
Lipid	Fulton's K*	$\ln(\text{Lipid}) = 1.2970 (\pm 0.0824) + 2.8906 (\pm 1.1010) K$	47.0	0.0	0.29
	K _n	$\ln(\text{Lipid}) = 1.2970 (\pm 0.0856) + 3.0726 (\pm 1.3432) K_n$	51.5	4.5	0.23
	L-M Residuals	$\ln(\text{Lipid}) = 1.2970 (\pm 0.0866) + 0.0702 (\pm 0.0324) \text{Residuals}$	60.6	13.6	0.22
	I _H	$\ln(\text{Lipid}) = 1.2970 (\pm 0.0875) + 0.2822 (\pm 0.1358) I_H$	58.9	11.9	0.20
	Fatmeter	$\ln(\text{Lipid}) = 1.2970 (\pm 0.0870) + 0.7524 (\pm 0.3522) \ln(\text{Fatmeter})$	56.3	9.3	0.21

(c) *Micropogonias undulatus*

Direct	Indirect	Model	AIC _c	ΔAIC _c	r ² _{REML}
Energy	Fulton's K	$\ln(\text{Energy}) = 1.5252 (\pm 0.0360) + 1.8114 (\pm 0.4488) K$	-58.4	26.6	0.47
	K _n	$\ln(\text{Energy}) = 1.5252 (\pm 0.0356) + 1.8575 (\pm 0.5577) K_n + 0.0050 (\pm 0.0016) L_T$	-49.2	35.8	0.49
	L-M Residuals	$\ln(\text{Energy}) = 1.5252 (\pm 0.0385) + 0.0330 (\pm 0.0126) \text{Residuals} + 0.0058 (\pm 0.0019) L_T$	-30.5	54.5	0.40
	I _H	$\ln(\text{Energy}) = 1.5252 (\pm 0.0414) + 0.1963 (\pm 0.0694) I_H$	-34.7	50.3	0.30
	Fatmeter*	$\ln(\text{Energy}) = 1.4773 (\pm 0.0291) + 0.4895 (\pm 0.0764) \ln(\text{Fatmeter}) - 0.0014 (\pm 0.0014) L_T + 0.0096 (\pm 0.0030) \ln(\text{Fatmeter}) * L_T$	-85.0	0.0	0.75
Lipid	Fulton's K	$\ln(\text{Lipid}) = 1.1721 (\pm 0.1034) + 5.7279 (\pm 1.2875) K$	89.2	22.6	0.52
	K _n	$\ln(\text{Lipid}) = 1.1721 (\pm 0.1021) + 5.6297 (\pm 1.6010) K_n + 0.0163 (\pm 0.0046) L_T$	96.3	29.7	0.53
	L-M Residuals	$\ln(\text{Lipid}) = 1.0987 (\pm 0.1070) + 0.1389 (\pm 0.0374) \text{Residuals} + 0.0192 (\pm 0.0048) L_T - 0.0026 (\pm 0.0013) \text{Residuals} * L_T$	113.2	46.6	0.55
	I _H	$\ln(\text{Lipid}) = 1.1721 (\pm 0.1194) + 0.4396 (\pm 0.2100) I_H + 0.0097 (\pm 0.0056) L_T$	122.3	55.7	0.36
	Fatmeter*	$\ln(\text{Lipid}) = 1.1721 (\pm 0.0859) + 1.2035 (\pm 0.1990) \ln(\text{Fatmeter})$	66.6	0.0	0.67

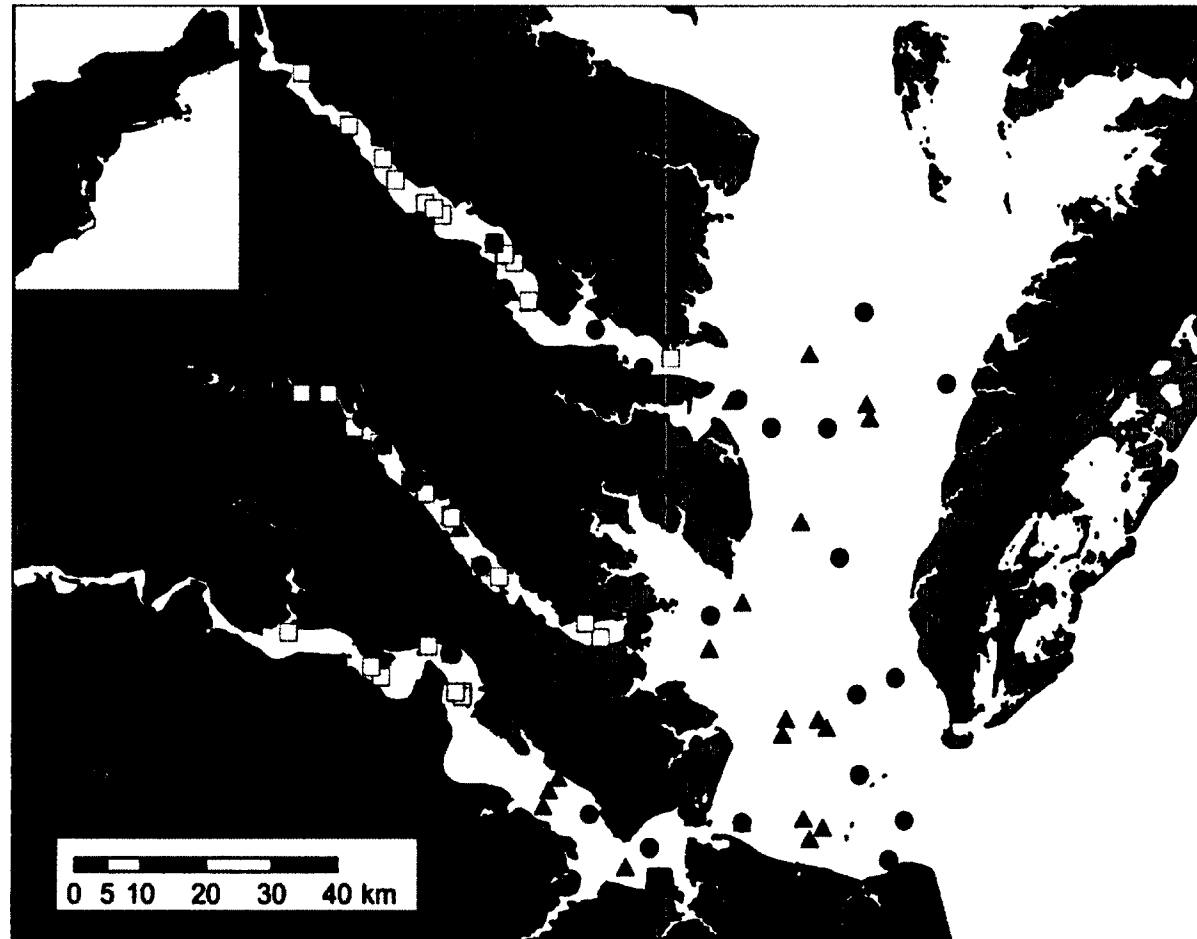


Figure 1. Spatial extent of sampling in Virginia estuaries used to collect *Paralichthys dentatus* (circle, $n = 73$), *Morone saxatilis* (square, $n = 66$), and *Micropogonias undulatus* (triangle, $n = 72$) for comparing condition indices.

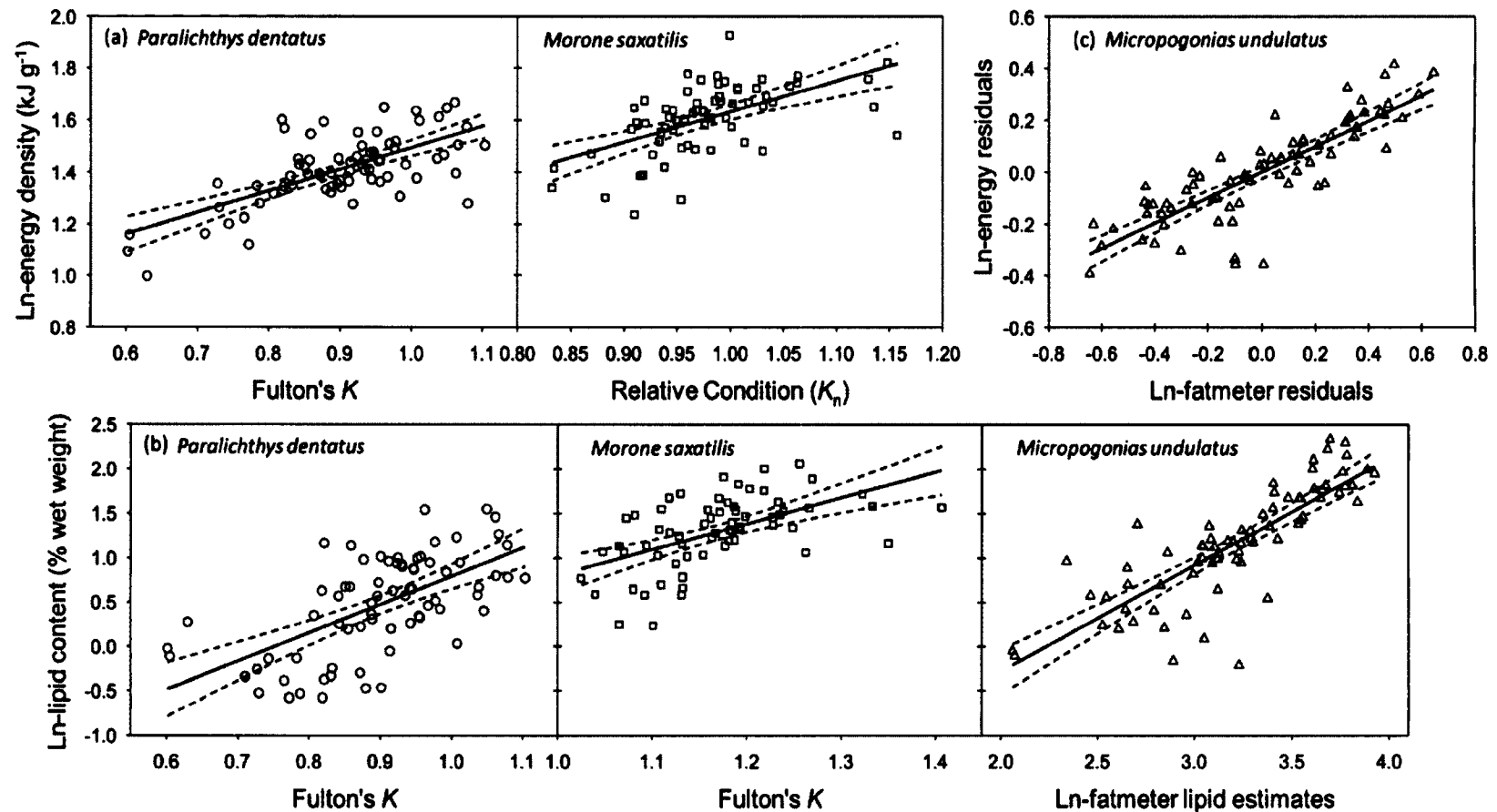


Figure 2. Linear regressions (solid lines) of energy density (a) and the “selected” indirect indices of condition for *Paralichthys dentatus* and *Morone saxatilis*. Regressions of lipid content and the “selected” indirect indices for all three species are shown in panel (b). Panel (c) depicts the partial regression plots used to assess the relationship between fatmeter lipid readings and energy density for *Micropogonias undulatus* (Quinn and Keough, 2002). The indices shown are those selected from the general linear models using the lowest Akaike’s Information Criterion (corrected for small sample size, AIC_c). Dashed lines denote 95% confidence intervals.

CHAPTER 2

Relationships among Proximate Components and Energy Density of Juvenile Atlantic Estuarine Fishes

Abstract

Energy density of juvenile fishes is needed to inform food web and bioenergetic models. Several approaches may be used to estimate energy density, and although these approaches are expected to yield similar estimates, the accuracy of such estimates has not been assessed. Estuarine fishes have unique life-history strategies that influence the water, protein, lipid, and ash composition of tissues. Because proximate components vary among species, preferred approaches for estimating energy density from these components may also differ. We investigated the suitability of five approaches to estimate energy density measured by bomb calorimetry from water content for juvenile Summer Flounder, Striped Bass, and Atlantic Croaker. Approaches were based on: (1) relationships between water and percent composition, (2) relationships between water and the mass of proximate components, (3) water content alone (by percentage and by mass), (4) percent water and fish mass, and (5) published relationships between energy density and percent dry mass. The mean predicted energy densities from alternate approaches were generally within 10% of mean energy densities measured by bomb calorimetry (4.21 kJ/g to 4.94 kJ/g). For Summer Flounder and Atlantic Croaker, regressions based on percent water from whole fish provided estimates of energy density closest to those from bomb calorimetry, and including fish mass improved the accuracy of energy density estimates for Striped Bass. Energy density calculated from percent composition overestimated measured energy density, even with conservative lipid-to-energy conversion factors; this was most pronounced for individuals with energy densities less than 5 kJ/g. Because lipid-to-energy conversion factors may not be temporally stable or spatially invariant, further research is needed on the energy content of lipid classes in fishes. The types of lipids used for energy storage likely vary among life stages and species, and extrapolating approaches to different size-classes or species groups may bias estimates of energy content.

Introduction

Models of energy flow in aquatic ecosystems can be used to investigate the effect of disturbances on fish populations, such as the impact of fishery removals or invasive species (Christensen and Walters 2004; Chesapeake Bay Fisheries Ecosystem Advisory Panel 2006). These models are also critical for understanding species interactions and are particularly useful for exploring trophic linkages and informing ecosystem-based management (Link 2002). Yet, accurate energy density data are necessary to model energy flow realistically (Wuenschel et al. 2006). The flow of energy among trophic levels is modulated by predator-prey interactions, which must also be understood to model growth, survival, and reproduction properly with bioenergetics models (Breck 2008). Indeed, growth rates estimated from bioenergetics models are sensitive to the energy density of prey resources (Mazur et al. 2007). However, both prey availability and the nutritional quality of prey resources are seasonally variable (Adams et al. 1982; Rand et al. 1994). Moreover, energy density varies by species and the nutritional history of an individual (Shearer 1994). In particular, energy content of juvenile fishes reflects changes in trophic linkages and productivity in the lower food web of aquatic ecosystems (Pothoven and Fahnenstiel 2014). Thus, fundamental energy estimates for juvenile fishes are needed to understand energy flow among trophic levels and inform the bioenergetics and food web models needed to implement ecosystem-based management in coastal systems (e.g., Chesapeake Bay, Latour et al. 2003; Lipcius and Latour 2006).

Energy content of fishes differs through ontogeny as individuals shift their energy allocation from growth to storage (e.g., in preparation for reproduction or winter conditions; Wuenschel et al. 2006). Energy allocated to storage typically results in an

increase in lipid content with size (Groves 1970). Such changes in energy and lipid storage strategies suggest that life-stage specific energy data are necessary to model energy flows in an ecosystem. For example, a bioenergetics model for adult Walleye Pollock *Gadus chalcogramma* could not describe energetics of juveniles (Ciannelli et al. 1998); this was likely due to changes in rates of anabolism and catabolism that occur as fish grow (Post 1990; Post and Lee 1996). Unfortunately, patterns of energy allocation among early life stages of fishes remain poorly understood (Fonseca and Cabral 2007).

To assess energy storage during early life, energy content may be measured directly by bomb calorimetry or calculated from proximate components (i.e., proteins and lipids) by assuming an energy equivalent for each component. Bomb calorimetry directly estimates energy density by measuring the amount of heat released through combustion of dried tissue (Cummins and Wuycheck 1971; Glover et al. 2010). Proximate composition analysis measures the proportion of protein, lipid, carbohydrate, water and ash in tissues (Stansby 1976; Shearer 1994). Percent water is typically measured gravimetrically by drying, and ash is measured gravimetrically after combustion of dried tissues, but neither contribute to energy content. Protein content is assessed by quantifying total nitrogen, measuring individual amino acids, or using assays and spectrophotometry (Ninfa et al. 2010). Lipids can be extracted from tissues using a variety of techniques (e.g., Folch or Soxhlet extraction) and solvents (e.g., chloroform-methanol, petroleum ether, or hexane). Carbohydrates comprise a small fraction (< 6%) of fish tissue and are typically neither measured nor reported (Craig et al. 1978; Costopoulos and Fonds 1989). Proximate components may be used to estimate energy content indirectly using the following conversion factors: 23.6 kJ/g for proteins and

between 34.7 and 39.5 kJ/g for lipids (Brett and Groves 1979; Henken et al. 1986; Brett 1995; Breck 2008). Differences in the estimated energy density of lipids are due to variation in lipid composition among species and life stages, as well as the lipid extraction technique and solvents utilized, which can lead to differences in lipid composition between the fish and the extracted samples.

Because the time and costs associated with measuring energy content (by calorimetry or proximate composition) are substantial, alternate means of estimating energy and lipid content have been considered (e.g., percent dry mass or percent water). Percent dry mass has a strong positive linear relationship with percent lipid and energy density both within and across species (Hartman and Brandt 1995). Percent water in muscle tissue is often inversely related to percent lipid (Shearer 1994), and has been proposed as a surrogate for the more costly methods that directly measure lipid or energy content (Plante et al. 2005; Peters et al. 2007). Moreover, water content in muscle may be representative of that in the entire specimen, and therefore, may be a useful estimator of energy and lipid content as well (Jacobs et al. 2008; Morley et al. 2012).

Proximate components are often reported as percentages (e.g., percent water, percent lipid, etc.) to permit comparison of fish composition independent of size. Although percent dry mass and percent lipid can be highly variable among conspecifics, both measures often increase with body mass and length for many fish species (Brown and Murphy 2004; Wuenschel et al. 2006). Thus, fish size is an important covariate and failure to consider this variable in models that estimate protein and lipid content can lead to erroneous conclusions about factors influencing proximate composition (Shearer 1994). Interestingly, although fish mass is commonly recorded, this information is not

always considered when inferring proximate composition or energy density of conspecifics. Models that describe relationships among the mass of proximate components yield strong linear relationships between water mass and the mass of lipids, proteins, and ash across a size range of one or more orders of magnitude (Groves 1970; Breck 2008; Breck 2014). However, it is unknown if these relationships suitably estimate energy and lipid content for individuals from a restricted size range or particular life stage (e.g., juveniles).

Here, we examined the relationship among proximate components and energy density of juvenile Summer Flounder *Paralichthys dentatus*, Striped Bass *Morone saxatilis*, and Atlantic Croaker *Micropogonias undulatus*. These species, along with White Perch *Morone americana* and Spot *Leiostomus xanthurus*, comprise > 75% of the demersal fish community biomass of Chesapeake Bay (Buchheister et al. 2013), and are ecologically important in Chesapeake Bay food webs (Lipcius and Latour 2006). Summer Flounder, Striped Bass, and Atlantic Croaker also have different life-history and feeding strategies that likely influence their proximate composition and energy content. Our objective was to identify simplified approaches to estimate energy and lipid content of juvenile fishes from other proximate components, and investigate the influence of proximate composition on the appropriateness of these approaches. Simple approaches could facilitate analysis of large numbers of fish for the bioenergetic and ecosystem models needed to implement ecosystem-based management. We examined five approaches to estimate energy density measured by bomb calorimetry from water content. Energy density was: (1) calculated from proximate components after estimating percent lipid and protein with percent water, (2) calculated from proximate components

after estimating the mass of lipid and protein with the mass of water, (3) estimated using a linear regression of energy density versus water content (by percentage and by mass), (4) estimated using a linear regression of energy density versus percent water and fish mass (Breck 2008), and (5) estimated from relationships between energy content and percent dry mass from Hartman and Brandt (1995).

Methods

All fish capture, handling, and euthanasia procedures were approved by the College of William and Mary Institutional Animal Care and Use Committee (protocol #IACUC-2010-07-20-6788-mcfabr) and followed all applicable state and federal regulations.

Field collections

Juvenile Summer Flounder ($n = 79$), Striped Bass ($n = 79$), and Atlantic Croaker ($n = 72$) were sampled monthly between August 2010 and August 2012 from lower Chesapeake Bay, the James, York, and Rappahannock Rivers in Virginia, and in coastal lagoons of the Virginia Eastern Shore (Figure 1). Sampling was conducted throughout the year to ensure estimates of energy density reflect seasonal changes in proximate components, but most Summer Flounder were collected from May to July, Striped Bass from December to March, and Atlantic Croaker from June to October. The majority of Summer Flounder (75%), Striped Bass (91%), and Atlantic Croaker (86%) were collected from waters 2-28 m deep according to a random stratified design using a 9.14-m otter trawl; sampling protocols followed those described by Tuckey and Fabrizio (2014). The

remaining Summer Flounder and Atlantic Croaker were obtained from shallow-water (< 6 m) habitats in Mobjack Bay and in coastal lagoons using a 4.88-m otter trawl deployed from a small vessel, and the remaining Striped Bass were collected from shallow-water tributary habitats using a 30.5-m seine. After capture, individuals were euthanized in an ice slurry (Blessing et al. 2010), immediately placed in plastic bags to prevent loss of water mass, and returned to the laboratory where fish mass was measured to 0.1 mg (using a Mettler Toledo scale model XA204) and length to the nearest millimeter. Monthly length thresholds were used to identify juvenile fish (i.e., age-0 fish; Tuckey and Fabrizio 2014), and juveniles were individually frozen for later processing (bomb calorimetry and proximate composition analysis).

Bomb calorimetry and proximate composition analysis

Tissue samples were prepared for bomb calorimetry and proximate composition analysis (water, lipid, protein, and ash content) by homogenizing whole fish in a blender and drying the tissue at 55° C. Prior to homogenization, a small muscle plug (~ 0.1 to 0.3 g) was removed from the dorsal side of a subset of specimens ($n = 44$ for Summer Flounder, $n = 42$ for Striped Bass, and $n = 39$ for Atlantic Croaker); the plug was weighed (to the nearest 0.1 mg) and dried at 55° C. Percent water (Water%) of the entire fish and the muscle plug was determined gravimetrically using:

$$\text{Water\%} = 100 [(M_{\text{wet}} - M_{\text{dry}}) / M_{\text{wet}}] \quad (1)$$

where M_{wet} = wet mass (g) and M_{dry} = dry mass (g). Dried tissues (including the muscle plug) were further homogenized with a mortar and pestle, and partitioned for bomb calorimetry and proximate composition analysis. Energy density per unit dry mass (E_{dry} ,

cal/g_{dry mass}) was determined by combusting two replicates of dried tissues in a Parr 6300 bomb calorimeter. If the two replicates differed by more than 3% of their average, a third replicate was analyzed and averaged with the nearest replicate. The bomb calorimeter was calibrated using a benzoic acid standard, and the calibration was checked every ten measurements. No significant difference (*t*-test: $t_{26} = 1.0$, $P = 0.32$) was detected between the expected mean and measured mean energy density of the standard (mean \pm SE difference: 0.035 ± 0.035 kJ/g, or about 0.13% error). Energy density per unit wet mass (E_{wet} , kJ/g_{wet mass}) was subsequently calculated using:

$$E_{\text{wet}} = 4.184 \times 10^{-5} [E_{\text{dry}} * (1 - \text{Water\%})] \quad (2)$$

Proximate components were analyzed by a certified laboratory (Department of Zoology, Southern Illinois University). Percent ash (inorganic elements) was determined gravimetrically by combusting dried tissue in a muffle furnace for four hours at 650° C. Percent protein was determined using a protein analyzer (FP-528; Leco Corporation, St. Joseph, Michigan), and percent lipid using a modified Folch extraction technique with a chloroform/methanol mixture as the solvent. We followed the usual practice of disregarding carbohydrates. Energy density was estimated from protein and lipid content using conversion factors of 23.6 kJ/g for proteins (Craig et al. 1978; Henken et al. 1986) and two commonly cited values for lipids, 36.2 and 39.5 kJ/g (Craig et al. 1978; Brett and Groves 1979).

Statistical analyses

Independent general linear models (GLMs) were developed to compare approaches to estimate energy density for each species. Linear relationships were

examined between energy density measured by bomb calorimetry and estimates of energy density based on relationships between water and percent composition, relationships between water and the mass of proximate components, water content alone, percent water and fish mass (Breck 2008), and relationships from Hartman and Brandt (1995). Hereafter, the use of “content” will refer to proximate components in terms of both percent and mass. Predictions of energy density based on these approaches were developed using water content estimates from whole fish and from a muscle plug. The relationship between percent water estimates from the whole fish and from a muscle plug was examined by linear regression using the GLM procedure in SAS 9.3 (SAS Institute, Inc., Cary, NC). To help interpret the utility of each approach for the three species, differences in mean percent composition of lipids, proteins, and ash were examined using analysis of variance (ANOVA) with the GLM procedure in SAS. The Tukey-Kramer method was used to perform multiple comparison tests with an experiment-wise α of 0.05.

All data, either in measured units or log-transformed units, used to estimate energy density and proximate components with linear models met the assumption of homogeneous variances. For Summer Flounder and Atlantic Croaker, the distributions of the response (i.e., energy density, lipid content) and residuals were occasionally skewed, however, samples sizes were sufficient that regression parameter estimates were robust to the assumption of normality (Quinn and Keough 2002).

Lipid conversion factors

To examine the effect of using different values for lipid-energy conversion factors (36.2 and 39.5 kJ/g), we applied linear regressions using the GLM procedure in SAS. The linear models included energy density measured by bomb calorimetry as the response and estimates of energy density from percent composition of protein and lipid (the ratio of component mass to fish mass) as predictors. For each conversion factor, we tested the null hypothesis of no difference between mean energy density measured by bomb calorimetry and mean energy density estimated by percent composition by examining the confidence intervals around parameter estimates for each linear regression; under the null hypothesis we expect the intercept to equal zero and the slope to equal one.

Relationships between water and proximate components (percent composition and mass of each component)

Linear models to estimate proximate components from water content were developed using the percent composition and the mass of each component following Breck (2014) (Figure 2). The percentages of proteins, lipids, and ash (from whole fish) measured by proximate composition analysis were estimated by percent water (from whole fish and a muscle plug) using linear regression (hereafter, “percent-composition regressions”; GLM procedure in SAS; Figure 2 A). Quality control revealed that one Summer Flounder and one Atlantic Croaker had erroneous percent lipid estimates from Folch extraction; percent lipid estimates from Soxhlet extraction were substituted for those individuals.

To calculate the mass of individual components, percent compositions were first normalized to 100% to account for measurement error (Breck 2014); this strategy

assumes error is proportional across the components. Normalizing also ensured that 100% of the total mass of the fish was accounted for by the proximate components even though carbohydrates were disregarded. Component masses were then log-transformed to meet assumptions of homogeneity of variance. Water mass was used in linear models to estimate lipid, protein, and ash mass (hereafter, “component-mass regressions”; Figure 2 B). As an alternative means of assessing lipid mass, we subtracted water mass, the estimated protein mass, and the estimated ash mass from the total mass to derive an indirect estimate of lipid mass (Groves 1970). We tested the null hypothesis that component masses increased proportionally with water mass by examining the confidence intervals around parameter estimates from the component-mass regressions; under the null hypothesis, the intercept equals zero and the slope equals one. Failure to reject the null hypothesis implies an isometric relationship between proximate components and water (Breck 2014). The entire process was repeated using water mass of whole fish estimated from percent water in a muscle plug.

Estimates of lipid and protein content from the percent-composition and component-mass regressions (estimated directly or indirectly, by using water content from the whole fish or an estimate of water content from a muscle plug) were independently compared with “true” lipid and protein measurements from proximate composition analysis by average deviation. Average deviation was calculated as:

$$\text{Average deviation} = \frac{\sum_i^n (\text{Estimated}_i - \text{Measured}_i)}{n} \quad (3)$$

where i represents each fish and n represents the sample size. Models with the smallest average deviation were considered “best” and were used to calculate energy density (Figure 2).

Water content

We used linear models to estimate the energy density of individual fish from four measures of water content (percent water and water mass from a muscle plug or from the whole fish). For these analyses, half the individuals were randomly selected to develop the relationship between measures of water content and energy density from bomb calorimetry. Parameter estimates from the fitted relationship were obtained from the GLM procedure in SAS and used to estimate the energy density of the remaining individuals. To ensure that energy estimates from these approaches were not biased by a particular selection of individuals, a bootstrap procedure was used to repeat the selection and model fitting process 100 times. Estimates of energy density for each individual from each model were then compared with energy density measured by bomb calorimetry via average deviation using equation (3). The approach that resulted in the smallest average deviation (averaged across all 100 bootstrap models) was identified as the preferred approach for estimating energy density from water content. Final parameter estimates describing the relationships between water content and energy density for each species, as well as the strength of those relationships (r^2), were determined by model averaging.

Percent water and fish mass

To predict energy density with percent water and fish mass, we used the same approach described for estimating energy density with water content. We randomly selected half of the individuals to fit the linear model described by Breck (2008):

$$E_{\text{wet}} = \mu + \alpha \times \text{Water\%} + \beta \times \log(M_{\text{wet}}) \quad (4)$$

where E_{wet} and M_{wet} are as before, and $water\%$ was determined from whole fish and from a muscle plug. A bootstrap procedure was used to repeat the selection and model fitting process 100 times, and the parameter estimates (μ , α , β) from each model fit were used to estimate energy density of the remaining individuals. As before, the deviation between measured and estimated energy densities, final parameter estimates, and r^2 values were determined by averaging across all models.

Percent dry mass using relationships from Hartman and Brandt (1995)

Published relationships between percent dry mass and energy density are available for multiple taxonomic levels; a predictive relationship for all species combined is also available (Hartman and Brandt 1995). However, adult individuals were used to develop these relationships, thus potentially limiting their application to juveniles. In addition to estimating energy density with the all-species model, we also calculated energy densities for Summer Flounder using the published parameter estimates at the level of order, for Striped Bass at the level of order and species, and for Atlantic Croaker at the level of order and family. Calculations were made using percent dry mass estimated from whole fish and from the muscle plugs.

Energy density comparisons

We determined the best approach for estimating energy density by calculating average deviations between energy-density estimates from all approaches and energy density measured by bomb calorimetry using equation (3). Energy estimates from the following approaches were considered: relationships between water and proximate

components using (1) percent-composition regressions, (2) component-mass regressions, (3) water content, (4) percent water and fish mass, and (5) percent dry mass using relationships from Hartman and Brandt (1995).

Results

We observed species-specific differences in proximate composition among juvenile Summer Flounder, Striped Bass, and Atlantic Croaker (Figure 3). Juvenile Summer Flounder ranged from 116 to 290 mm total length (TL), Striped Bass from 84 to 200 mm fork length (FL), and Atlantic Croaker from 106 to 220 mm TL. Although the range of sizes varied for each species, our samples represented individuals of similar age (< 1 year). For a given size, the maximum observed percent lipid was lowest in Summer Flounder (Figure 4); mean percent lipid and mean percent ash were significantly lower for Summer Flounder than for the other two species (ANOVA for lipid: $F_{2, 227} = 31.60$, $P < 0.01$; ANOVA for ash: $F_{2, 227} = 31.42$, $P < 0.01$). Atlantic Croaker had significantly lower mean percent protein than the other species (ANOVA: $F_{2, 227} = 44.22$, $P < 0.01$). The three species differed in mean percent water (Summer Flounder > Atlantic Croaker > Striped Bass; ANOVA: $F_{2, 279} = 52.86$, $P < 0.01$).

Percent water in the muscle plug and whole fish were significantly correlated for Striped Bass ($r^2 = 0.79$, $n = 42$, $P < 0.01$) and Atlantic Croaker ($r^2 = 0.88$, $n = 39$, $P < 0.01$). Parameter estimates (\pm 95% CI) relating whole-fish percent water to that of a muscle plug are:

$$\text{Water}_{\text{Striped Bass}} = -52.709 (\pm 21.338) + 1.621 (\pm 0.269) \times \text{Water}_{\text{plug}} \quad (5)$$

$$\text{Water}_{\text{Atlantic Croaker}} = 17.592 (\pm 7.305) + 0.750 (\pm 0.093) \times \text{Water}_{\text{plug}} \quad (6)$$

Although percent water in the plug was significantly correlated with percent water in whole Summer Flounder, the strength of the correlation was low ($r^2 = 0.13$, $n = 44$, $P = 0.02$).

Relationships between Water and Proximate Components (Percent Composition and Mass of each Component)

We found significant differences between energy density measured by bomb calorimetry and energy density estimated from percent composition of individual fish. Proximate components and energy content are reported relative to wet mass because these are the units used for bioenergetics modeling (Hartman and Brandt 1995). For all species, energy density calculated from proximate components overestimated measured mean energy density whether using low (36.2 kJ/g) or high (39.5 kJ/g) lipid-energy conversion factors. Overestimation was most apparent for individuals with energy densities less than 5 kJ/g (Figure 5); measured and calculated energy densities are similar for individuals with energy densities ≥ 5.5 kJ/g. To permit further comparison of approaches we hereafter report results using the low lipid-energy conversion factor because the resulting estimates of energy density were more similar to direct measures from bomb calorimetry (Table 1). Although energy estimates calculated from proximate composition were biased high, the bias was consistent across the range of energy densities observed for juvenile Summer Flounder. The bias was also smaller than confidence intervals around the relationship between estimates of energy density from percent composition and measures of energy density from bomb calorimetry. As a result, the slope and intercept for Summer Flounder were not significantly different from one

and zero, respectively, indicating that these approaches provide similar levels of uncertainty (Table 2, Figure 5). For Striped Bass and Atlantic Croaker, however, the slope and intercept were significantly different from one and zero (Table 2, Figure 5) because estimates of energy density from proximate composition overestimated measured energy density only at low energy values. Therefore, estimates of energy density from proximate composition are different from measurements of energy density from bomb calorimetry for these two species.

For all three species, we found a strong linear relationship ($r^2 \geq 0.96$) between whole-body water mass and protein mass, as well as between whole-body water mass and ash mass ($r^2 \geq 0.94$); slightly lower r^2 values were observed when using water mass from a muscle plug. The strength of the relationship between water mass and lipid mass varied among species (Table 3, Figure 6). Based on confidence intervals, the relationship between water mass and proximate component mass was not consistently isometric among components (Table 3). For all three species, parameter estimates were similar between regressions using whole-body water content and water content of a muscle plug for both percent-composition and component-mass regressions (with the exception of Summer Flounder percent lipid; Table 3), which was due in part to the large uncertainty of parameter estimates from the model relating proximate components with water content of a muscle plug. Unlike the component-mass relationships, the strength of the linear relationships between percent water and percent composition of proteins, lipids, and ash was weak to moderate ($r^2 \leq 0.6$, Table 3, Figure 6), except for the relationship with lipids for Atlantic Croaker.

Based on the smallest average deviation, whole fish water content best estimated protein and lipid content for Summer Flounder. Energy densities calculated from these estimates of proximate composition differed from mean energy density measured by bomb calorimetry (4.21 kJ/g) by 0.24 kJ/g (by percent) and 0.38 kJ/g (by mass) (Table 4). For Striped Bass, water content of the muscle plug well estimated protein content, and the preferred approach for estimating lipid mass of juvenile Striped Bass was to calculate it indirectly from whole fish water mass and associated regressions. The preferred approaches used to estimate proximate composition of Striped Bass resulted in calculated energy densities that differed by 0.18 kJ/g (by percent) and 0.22 kJ/g (by mass) from mean energy density measured by bomb calorimetry (4.94 kJ/g) (Table 4). Finally, water content from a muscle plug suitably estimated protein and lipid content for Atlantic Croaker, with the exception of lipid mass, for which indirect calculations from water content of a plug was preferred. The preferred approaches resulted in estimated energy densities that differed by 0.28 kJ/g (by percent) and 0.24 kJ/g (by mass) from mean energy density measured by bomb calorimetry (4.68 kJ/g) (Table 4).

Water Content

Regardless of species, energy density of juvenile fishes measured by bomb calorimetry can be reliably estimated from percent water of the whole fish (parameter estimates \pm 95% CI) using:

$$\text{Energy}_{\text{Summer Flounder}} = 25.903 (\pm 3.581) - 0.278 (\pm 0.046) \times \text{Water}\% \quad (7)$$

$$\text{Energy}_{\text{Striped Bass}} = 36.595 (\pm 3.909) - 0.418 (\pm 0.052) \times \text{Water}\% \quad (8)$$

$$\text{Energy}_{\text{Atlantic Croaker}} = 34.790 (\pm 2.237) - 0.394 (\pm 0.029) \times \text{Water}\% \quad (9)$$

These relationships were significantly correlated ($r^2_{\text{Summer Flounder}} = 0.80, n = 79, P < 0.01$; $r^2_{\text{Striped Bass}} = 0.88, n = 79, P < 0.01$; and $r^2_{\text{Atlantic Croaker}} = 0.96, n = 72, P < 0.01$), and resulted in smaller average deviations than relationships involving water content from a muscle plug (which had smaller sample sizes) or water mass of the whole fish. The average deviations were: 0.14 kJ/g from a mean of 4.21 kJ/g for Summer Flounder, 0.18 kJ/g from 4.94 kJ/g for Striped Bass, and 0.18 kJ/g from 4.68 kJ/g for Atlantic Croaker (Table 4).

Percent Water and Fish Mass

Estimates of energy density for juvenile Striped Bass based on percent water from whole fish are improved by including fish mass. However, fish mass had no effect on estimates of energy density for the size range of Summer Flounder and Atlantic Croaker investigated, or when using percent water from a muscle plug to estimate energy density. Energy density of juvenile Striped Bass was estimated using:

$$\begin{aligned} \text{Energy}_{\text{Striped Bass}} = & 34.307 (\pm 3.623) - 0.397 (\pm 0.046) \times \text{Water}\% \\ & + 0.211 (\pm 0.119) \times \log(M_{\text{wet}}) \end{aligned} \quad (10)$$

This relationship resulted in an average deviation of 0.16 kJ/g from 4.94 kJ/g (Table 4), and a significant correlation of 0.91 ($n = 79, P < 0.01$).

Percent Dry Mass using Relationships from Hartman and Brandt (1995)

Estimates of energy density derived from the published models of Hartman and Brandt (1995) were most suitable at the lowest taxonomic level available for these species: order for Summer Flounder (when using percent dry mass from whole fish),

species for Striped Bass (when using percent dry mass from a muscle plug), and family for Atlantic Croaker (when using percent dry mass from a muscle plug) (Table 4). With the preferred source of dry mass measurements (plug or whole fish), estimated energy densities from relationships at these taxonomic levels were within 0.4 kJ/g of mean energy densities (4.21 kJ/g to 4.94 kJ/g) estimated by bomb calorimetry for juveniles of these species.

Discussion

The energy density of juvenile Summer Flounder, Striped Bass, and Atlantic Croaker can be accurately estimated from percent water of the whole fish, and estimates of energy density for Striped Bass can be further improved by accounting for fish mass. Strong relationships were found between protein mass and water mass and between ash mass and water mass; these relationships may also be used to assess lipid mass accurately for species that exhibit high variability in lipid content. However, caution should be taken when estimating energy density from proximate components because estimates may be upwardly biased for juveniles with energy densities < 5 kJ/g. The energy density of individuals with a relatively greater proportion of low-energy lipids (e.g., phospholipids and highly unsaturated lipids) may not be accurately estimated with commonly used lipid-to-energy conversion factors (e.g., 36.2 kJ/g and 39.5 kJ/g).

Juvenile Summer Flounder, Striped Bass, and Atlantic Croaker exhibited increases in energy density with size, which reflects the increasing proportion of lipids in fishes as they grow. This pattern is consistent with previously reported changes in lipid content in juvenile Shortbelly Rockfish *Sebastes jordani* and Weakfish *Cynoscion regalis*

(Norton et al. 2001; Litvin 2011). Larger individuals typically consume larger prey that may have higher energy and lipid contents, and as a result, changes in diet through ontogeny may increase the lipid content of an individual (Anthony et al. 2000; Wuenschel et al. 2006). The low proportion of lipids observed in Summer Flounder suggests that proteins or other components are also used for energy storage; this strategy has been observed in another flatfish, the Winter Flounder *Pleuronectes americanus* (Maddock and Burton 1994). Alternatively, energy storage may not be a critical life function for juvenile Summer Flounder. Indeed, simple calculations revealed that only 15% of the total body energy of juvenile Summer Flounder was attributed to lipids. Many flatfishes exhibit relatively low lipid contents, even as adults (Stansby 1976). Conversely, Striped Bass and Atlantic Croaker had a higher proportion of lipids for a given size, and lipids constituted a greater proportion of the total body energy (~ 26% of total energy).

The degree of total body energy associated with lipid storage may determine the appropriateness of a given analytical approach for estimating lipid content. The accuracy of lipid mass estimates derived from water mass was low for species that exhibited high variation in lipid content. For example, lipid content among similarly sized individual Atlantic Croaker was highly variable, resulting in a high average deviation between measured lipid mass and that estimated from water mass. Conversely, Summer Flounder lipid mass was less variable, and resulted in a low average deviation. Although we found that direct estimation of lipid mass from total body water mass was a reasonable approach for Summer Flounder, we do not recommend this approach for juvenile Striped Bass and Atlantic Croaker. Both species exhibited high variability in lipid mass among individuals, and as a result, lipid mass was best estimated indirectly by subtracting estimates of

protein and ash mass from measured dry mass. This indirect approach accurately estimates lipid content for larval to adult Rainbow Trout *Oncorhynchus mykiss*, Bluegill *Lepomis macrochirus*, Common Carp *Cyprinus carpio*, Lake Trout *Salvelinus namaycush*, and Coho Salmon *Oncorhynchus kisutch* (Breck 2014). One drawback of the indirect method is that measurement error in the other proximate components may bias estimates of lipid mass (as detailed in Breck 2008, 2014). In particular, because lipids are high-energy compounds, inaccurate estimates of lipid content will lead to biased estimates of energy density. In fact, using the indirect approach, we observed negative estimates of lipid mass for some individuals of all three species. Similar observations were reported by Breck (2014) who ascribed these biases to overestimation of ash content of larval fishes. Because our samples did not include larval fishes, it appears that the subtraction approach may yield negative lipid estimates for individuals whose mass is near the low end of the size range examined.

The strong relationship between whole-fish and muscle-plug water content for Striped Bass and Atlantic Croaker suggests that whole-fish water content can be estimated from the water content of a muscle plug for these species. Therefore, lipid content (mass or percentage) may be estimated from water content of a muscle plug as well. Jacobs et al. (2008) found that percent water from the belly muscle is linearly related to total body water for age-1 Striped Bass (215-290 mm; $r^2 = 0.82$), and we observed similar correlations between percent water in the muscle plug and the whole body for Striped Bass ($r^2 = 0.79$). However, estimates of lipid content based on whole fish water content outperformed estimates based on muscle plug water content for juvenile Striped Bass; this may be due to the high degree of lipid storage in the liver

during winter (when the majority of Striped Bass were collected in this study; *pers. obs.*). Water content from a muscle plug can be used to estimate lipid content reliably for Atlantic Croaker by either percent-composition or component-mass regressions. Yet, uncertainty in characterizing the water content of Summer Flounder using a muscle plug suggests lipid estimates should only be inferred from whole fish water content.

Water mass exhibited high correlations with protein mass and ash mass for the three species we studied, supporting a physicochemical link among these components (Breck 2014). Furthermore, the strong relationship between whole-body water and protein content did not differ among species. High positive correlations between water mass and protein mass have also been observed for larval to adult Bluegill ($r^2 = 0.999$), Common Carp ($r^2 = 0.999$), and Largemouth Bass *Micropterus salmoides* ($r^2 = 0.993$) (Breck 2008), as well as three salmon species (Sockeye Salmon *Oncorhynchus nerka*, Pink Salmon *O. gorbuscha*, and Chinook Salmon *O. tshawytscha*; $r^2 > 0.95$; Groves 1970). Conversely, we observed weak relationships between percent water and protein ($r^2 < 0.3$) and between percent water and ash ($r^2 < 0.35$, except for percent water from a muscle plug for Atlantic Croaker). The poor ability to estimate percent protein introduced error into calculations of energy density from proximate components, and resulted in estimates of energy density from percent composition regressions that were less accurate than those estimated directly from percent water.

The regression slopes between protein and water mass, and ash and water mass were approximately one for the three species examined, suggesting an isometric relationship between these components for the size range of fish investigated here. These relationships are expected to be allometric for a larger range of fish mass (Breck 2008,

2014). If individuals contain only structural lipids, lipid mass would also increase proportionally with the other proximate components. However, the use of lipids for energy storage results in a regression slope between lipid and water mass that deviates from one. As a result, a species that stores a greater proportion of energy as lipids (such as Atlantic Croaker) would exhibit a higher slope for the regression of lipid mass on water mass. We suggest that this slope indicates the importance of lipids as energy-storage compounds for a species or life-history stage, which further supports the lower reliance on lipid-based energy reserves for juvenile Summer Flounder relative to the other species.

Our observations suggest that lipid-to-energy conversion factors may not be temporally stable or spatially invariant. Energy density estimates obtained from proximate composition analysis (using percent composition) significantly overestimated energy density estimates from bomb calorimetry for the same fish when 39.5 kJ/g is used to convert lipid content to energy. Thus, we do not recommend use of this conversion factor for juvenile fishes. Brett and Groves (1979) suggest using 36.2 kJ/g because fishes have highly unsaturated fatty acids which have a lower overall energy density than saturated fatty acids. However, we found that the use of the 36.2 kJ/g conversion factor also overestimated energy content relative to estimates obtained directly from bomb calorimetry, particularly for individuals with an energy density < 5 kJ/g. We interpret this to indicate that small juvenile fishes contain a higher proportion of unsaturated triglycerides and phospholipids with energy densities < 36.2 kJ/g and, as individuals grow and feed on more energy-dense prey resources, the proportion of phospholipids declines and there is a transition to storage of unsaturated triglycerides with an energy

density closer to 36.2 kJ/g. It is possible that juvenile fishes in our study area consumed prey that were consistently lower in the types of unsaturated triglycerides than are consumed elsewhere. However, this is unlikely because of the diversity of prey resources in Chesapeake Bay and because of the similarity of invertebrate prey available to juvenile fishes throughout mid-Atlantic estuaries (Llanos et al. 2002).

Inconsistencies between calculated and direct estimates of energy content suggest that a single lipid-to-energy conversion factor may not be appropriate for all life stages or for all species. Not surprisingly, previous studies provide inconsistent guidance on the appropriate lipid-energy conversion factors. Some studies document differences between estimates of energy from bomb calorimetry and those derived from proximate composition (Craig 1977; Breck 2008). Estimates of energy content from proximate components (using 39.5 kJ/g) are 7.2% higher than energy estimates from bomb calorimetry for European Perch *Perca fluviatilis* (Craig 1977). Similarly, estimates of energy density based on fish mass and proximate composition estimated from percent water are higher than measures of energy density for Common Carp using 36.4 kJ/g (Breck 2008). These studies contrast with others that report similar energy estimates using bomb calorimetry and proximate composition. For example, gross energy content estimated by bomb calorimetry and proximate composition analysis are similar using 39.5 kJ/g as the conversion factor for African catfish *Clarias gariepinus* (Henken 1986), and using 35.5 kJ/g for European Perch (Craig et al. 1978). In the latter study, conversion factors were directly determined from extracted lipids. Interestingly, differences in observed and estimated energy densities for Common Carp are minimized using a conversion factor of 35.6 kJ/g, similar to Craig et al. (1978) (Breck 2008). Upwardly

biased estimates of energy content resulting from the application of high lipid-energy conversion factors suggest that lipid composition varies among species and through ontogeny (Tocher 2010). Thus, the energy content of the lipid fraction may need to be measured directly to estimate energy density reliably from proximate components (Craig et al. 1978).

In summary, we demonstrate that some alternate approaches to estimate energy density may be useful for juvenile stages of Summer Flounder, Striped Bass, and Atlantic Croaker. Ultimately, if the objective is to estimate energy content (versus lipid content) of juveniles for these species, regressions based on percent water of the whole fish outperformed all other approaches examined. As expected, using lipid and protein content to estimate energy content requires additional calculations and assumptions that introduce bias. Even though we used similar methods to Hartman and Brandt (1995), our estimates of energy density from water content were likely more accurate because Hartman and Brandt (1995) did not consider body mass when estimating energy density; as we demonstrated, such consideration may improve estimates of energy density for certain species (e.g., Striped Bass) or size ranges. Furthermore, Hartman and Brandt (1995) derived parameter estimates from adult specimens that appear to overestimate energy density of juvenile fishes. Overestimates of energy density may also result from the application of inappropriate lipid-energy conversion factors, i.e., conversion factors of 36.2 kJ/g or higher are unsuitable for individuals with energy densities < 5 kJ/g. Estimates of energy content are preferable when developed specifically for the species and life stage of interest, because extrapolating methods to different size-classes, life stages, or habitats is likely to bias estimates of energy content (Trudel et al. 2005).

Accurate estimation of energy density for juvenile fishes based on the approaches examined here will allow for more realistic models of energy flow among trophic levels and improved estimation of vital rates in bioenergetics models.

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Table 1. Mean difference between energy estimates from bomb calorimetry and proximate composition analysis for juvenile Summer Flounder ($n = 79$), Striped Bass ($n = 79$), Atlantic Croaker ($n = 72$), and across all species ($n = 230$). All differences are negative and significantly different from zero ($P < 0.05$), suggesting significant overestimation of energy density by proximate composition analyses.

Species	Conversion factor	Difference	<i>t</i>	<i>P</i>
Summer Flounder	36.2 kJ/g	-0.205	-12.31	< 0.01
	39.5 kJ/g	-0.267	-16.07	< 0.01
Striped Bass	36.2 kJ/g	-0.054	-2.11	0.04
	39.5 kJ/g	-0.174	-7.14	< 0.01
Atlantic Croaker	36.2 kJ/g	-0.105	-4.19	0.01
	39.5 kJ/g	-0.231	-10.11	< 0.01
All	36.2 kJ/g	-0.121	-8.90	< 0.01
	39.5 kJ/g	-0.224	-17.75	< 0.01

Table 2. Species-specific relationships (\pm 95% CI) between energy estimates from bomb calorimetry (BC) and proximate composition analysis (PCA) for juvenile Summer Flounder ($n = 79$), Striped Bass ($n = 79$), and Atlantic Croaker ($n = 72$). "All" represents the relationship pooled across species. A lipid-energy conversion factor of 36.2 kJ/g was used to calculate energy content from PCA. All models are significant as determined by the F -ratio ($P < 0.05$).

Species	Model	F	r^2
Summer Flounder	$\text{energy}_{\text{BC}} = -0.270 (\pm 0.402) + 1.015 (\pm 0.091) \times \text{energy}_{\text{PCA}}$	494.5	0.87
Striped Bass	$\text{energy}_{\text{BC}} = -0.706 (\pm 0.447) + 1.131 (\pm 0.089) \times \text{energy}_{\text{PCA}}$	638.9	0.89
Atlantic Croaker	$\text{energy}_{\text{BC}} = -0.471 (\pm 0.252) + 1.077 (\pm 0.052) \times \text{energy}_{\text{PCA}}$	1,718.4	0.96
All	$\text{energy}_{\text{BC}} = -0.620 (\pm 0.176) + 1.106 (\pm 0.037) \times \text{energy}_{\text{PCA}}$	3,482.8	0.94

Table 3. Parameter estimates (95% CI) from linear models for estimating proximate components (protein, lipid, and ash) from water content via percent-composition regressions and component-mass regressions for juvenile Summer Flounder, Striped Bass, and Atlantic Croaker from Virginia estuaries. The mean difference between measured proximate components and estimated values by regressions is denoted by average deviation (Avg. dev.); indirect estimation is denoted by "Ind.". Bold values identify the preferred approaches to estimate proximate components.

Parameter	Summer Flounder		Striped Bass		Atlantic Croaker	
	Whole fish	Plug	Whole fish	Plug	Whole fish	Plug
Percent-composition regressions						
Protein						
Intercept	45.303 (11.534)	25.253 (10.845)	28.894 (8.678)	53.063 (20.015)	29.654 (7.638)	17.926 (7.065)
Slope	-0.378 (0.148)	-0.120 (0.135)	-0.176 (0.115)	-0.477 (0.252)	-0.200 (0.010)	-0.051 (0.090)
r ²	0.25	0.07	0.11	0.27	0.19	0.03
Avg. dev.	0.674	0.883	0.602	0.478	0.798	0.627
Lipid						
Intercept	38.742 (9.562)	4.430 (8.861)	65.002 (11.457)	103.161 (34.095)	69.247 (6.532)	63.223 (5.978)
Slope	-0.473 (0.123)	-0.029 (0.111)	-0.811 (0.151)	-1.250 (0.429)	-0.857 (0.086)	-0.753 (0.076)
r ²	0.43	0.01	0.60	0.46	0.85	0.92
Avg. dev.	0.531	0.682	0.780	0.870	0.657	0.539
Ash						
Intercept	11.553 (6.594)	7.677 (5.255)	-8.473 (6.581)	-12.407 (18.773)	-4.450 (3.141)	-4.653 (2.625)
Slope	-0.101 (0.085)	-0.048 (0.066)	0.167 (0.087)	0.209 (0.236)	0.115 (0.095)	0.116 (0.033)
r ²	0.07	0.05	0.16	0.07	0.31	0.57
Avg. dev.	0.383	0.400	0.442	0.472	0.350	0.242
Component-mass regressions						
Protein						
Intercept	-1.599 (0.080)	-1.643 (0.221)	-1.493 (0.072)	-1.608 (0.152)	-1.624 (0.156)	-1.978 (0.238)
Slope	1.000 (0.020)	1.002 (0.050)	0.971 (0.023)	0.985 (0.045)	0.986 (0.045)	1.068 (0.064)
r ²	0.99	0.97	0.99	0.98	0.96	0.97
Avg. dev.	0.967	1.242	0.253	0.246	0.472	0.400
Lipid						
Intercept	-5.299 (0.431)	-4.542 (1.083)	-4.773 (0.508)	-4.221 (1.085)	-5.168 (0.906)	-7.279 (1.475)
Slope	1.368 (0.106)	1.187 (0.246)	1.535 (0.163)	1.342 (0.323)	1.584 (0.263)	2.145 (0.400)
r ²	0.90	0.69	0.82	0.64	0.67	0.76
Avg. dev.	0.661	0.838	0.400	0.469	0.849	0.821
Ind. avg. dev.	1.102	1.379	0.285	0.342	0.491	0.432
Ash						
Intercept	-3.195 (0.152)	-3.029 (0.398)	-2.812 (0.175)	-3.033 (0.409)	-2.972 (0.162)	-2.644 (0.238)
Slope	1.034 (0.037)	0.996 (0.090)	0.969 (0.056)	1.026 (0.122)	1.031 (0.047)	0.937 (0.065)
r ²	0.98	0.92	0.94	0.88	0.96	0.96
Avg. dev.	0.411	0.530	0.160	0.201	0.169	0.143

Table 4. Average deviation (Avg. dev.) between energy density measured by bomb calorimetry and alternative approaches to estimate energy density from percent-composition regressions, component-mass regressions, water content (by percent and by mass), and published relationships using percent dry mass (Hartman and Brandt 1995). Bold values identify the preferred approaches to estimate energy density, and values in italics signify that estimates of energy density based on water content in a muscle plug were more accurate than those based on water content from whole fish. For percent-composition and component-mass regressions, energy density estimates were derived from water content sources identified by bold in Table 3; for example, energy density of Striped Bass estimated by component-mass regressions used protein mass estimated from water in a muscle plug and lipid mass estimated indirectly based on water mass from whole fish. SD refers to the standard deviation of the deviations, and sample size is given by *n*.

Approach	Summer Flounder			Striped Bass			Atlantic Croaker		
	Avg. dev.	SD	<i>n</i>	Avg. dev.	SD	<i>n</i>	Avg. dev.	SD	<i>n</i>
Percent-composition regressions	0.243	0.125	79	0.178	0.139	42	0.275	0.213	39
Component-mass regressions	0.379	0.240	79	0.218	0.194	42	0.236	0.173	39
Percent water	0.144	0.121	79	0.175	0.164	79	0.182	0.126	72
Water mass	0.285	0.233	79	<i>0.530</i>	0.373	42	<i>0.630</i>	0.449	39
Percent water and fish mass	0.144	0.120	79	0.164	0.139	79	0.182	0.131	72
Hartman and Brandt (1995)	0.284	0.143	79	<i>0.361</i>	0.242	42	<i>0.244</i>	0.196	39

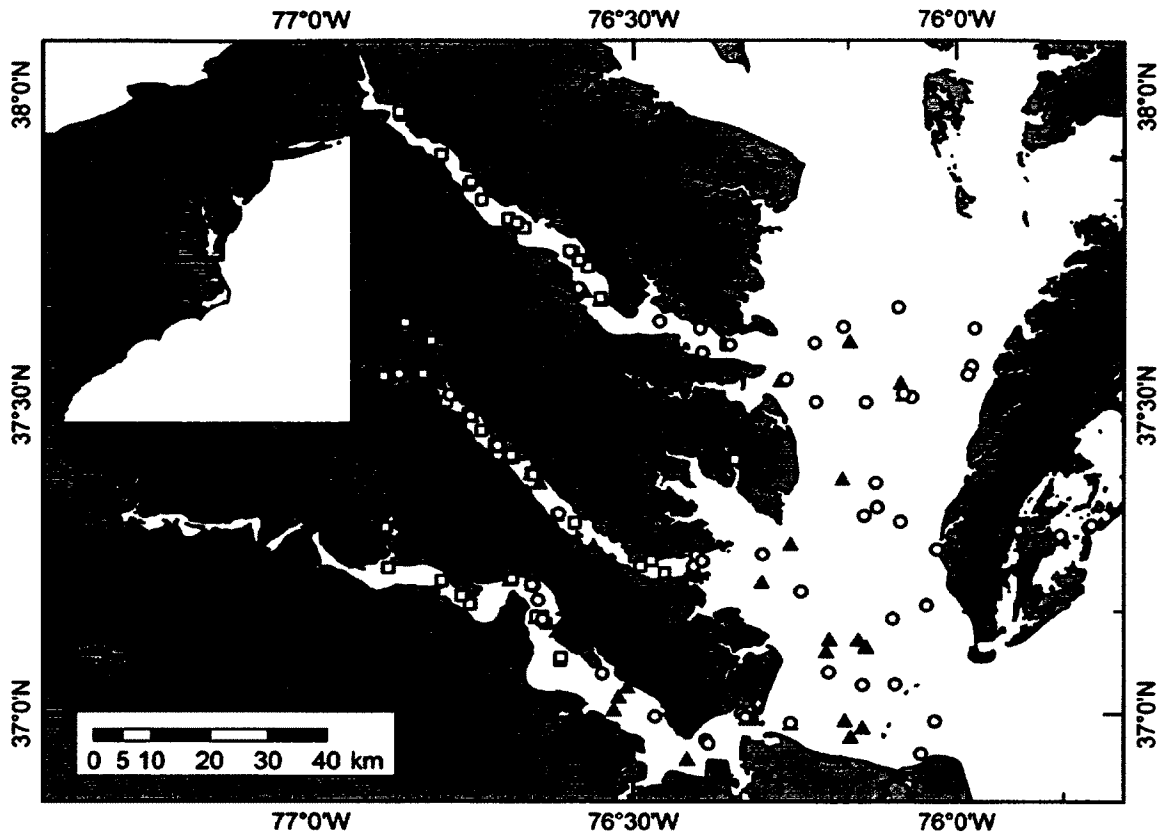


Figure 1. Sampling locations in Virginia for Summer Flounder (grey circle), Striped Bass (open square), and Atlantic Croaker (filled triangle) used in the analysis of proximate components and energy density.

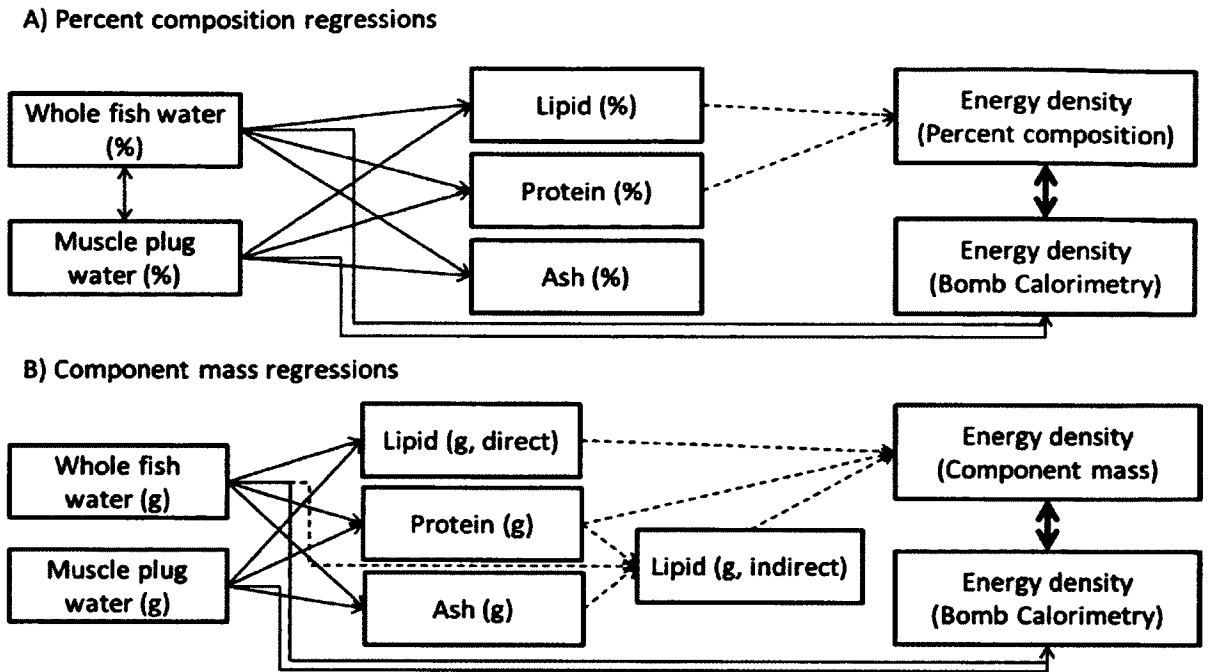


Figure 2. Flow chart diagramming the approaches used to develop estimates of energy density from proximate components using regressions based on percent composition (A) and component mass (B). All estimates were compared with energy density measured directly by bomb calorimetry. Solid lines indicate a linear regression was used to estimate proximate components or energy density from water content, dashed lines indicate that estimates were calculated, and bold lines indicate comparisons of energy density estimates from multiple alternate approaches (two for percent composition regressions and four for component mass regressions).

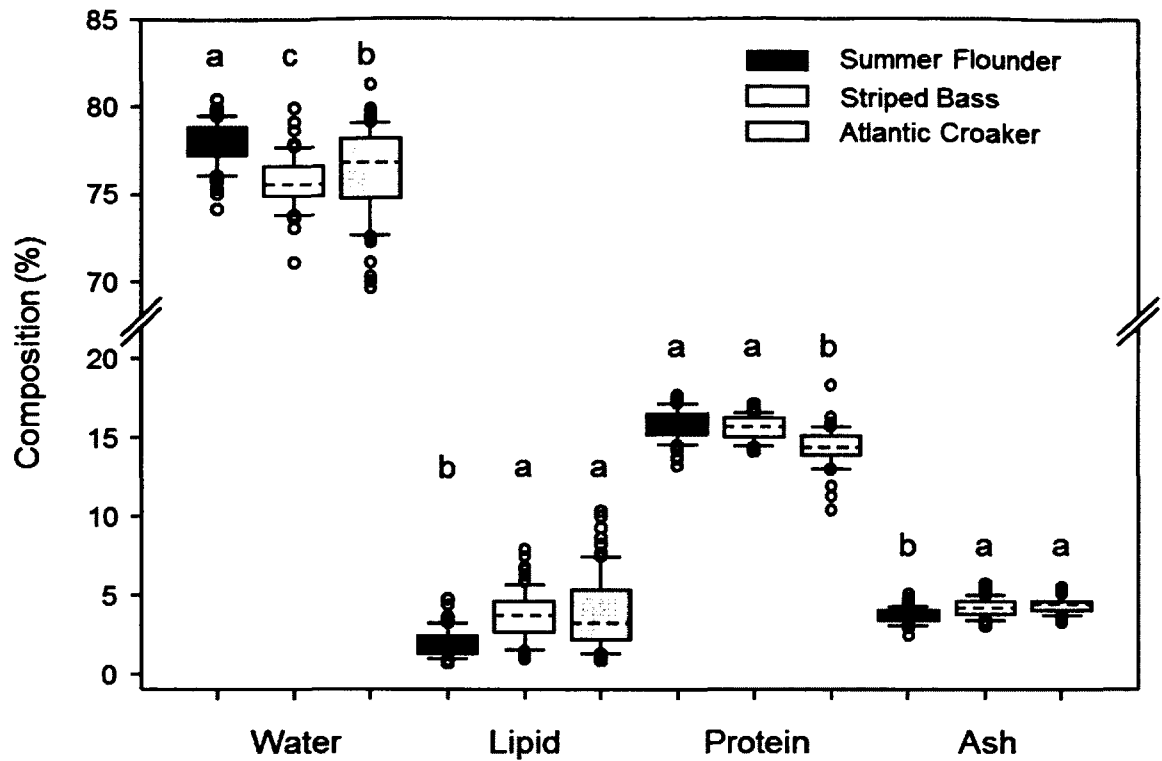


Figure 3. Proximate composition of Summer Flounder ($n = 79$, dark grey), Striped Bass ($n = 79$, white), and Atlantic Croaker ($n = 72$, light grey) from Virginia estuaries. Dashed lines represent median values; boxes denote 25th and 75th percentiles; error bars denote 10th and 90th percentiles; open circles represent values outside the 10th and 90th percentiles. Letters indicate significantly different composition means among species determined by linear models at $\alpha = 0.05$.

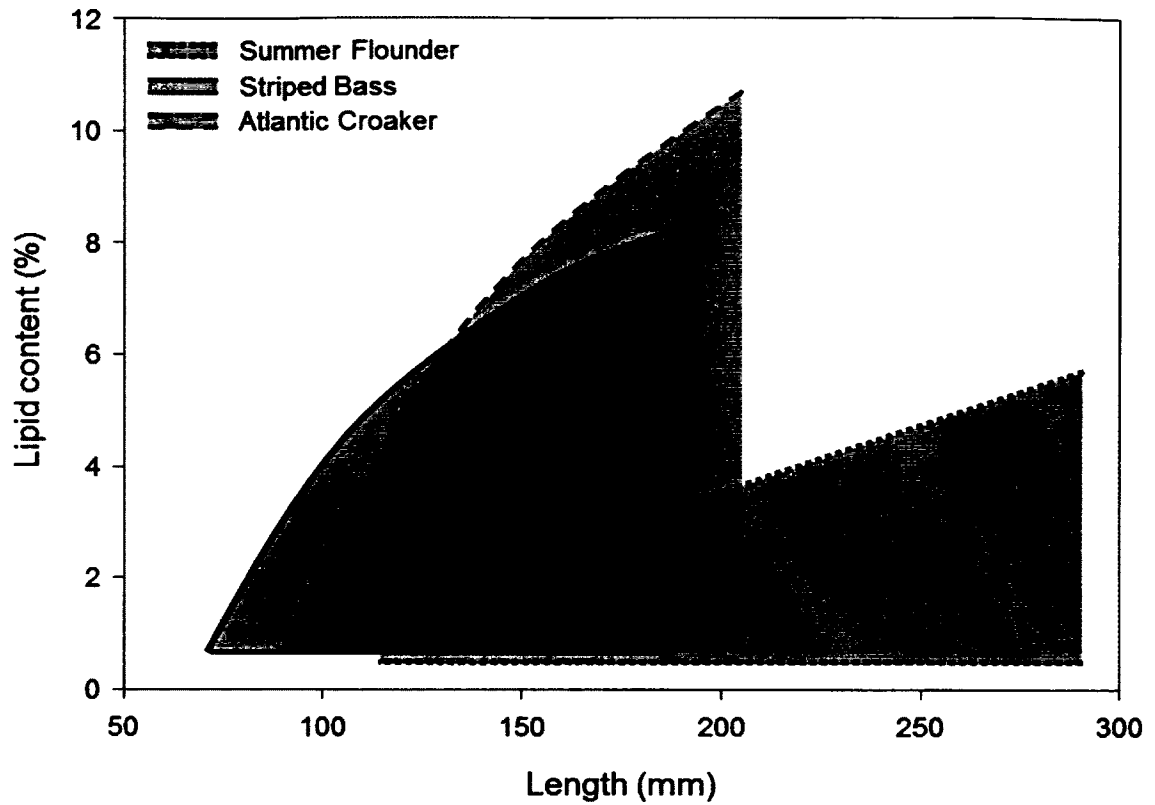


Figure 4. Range in proportion of lipids ($g_{\text{lipid}} / g_{\text{wet mass}}$) for Summer Flounder (dotted line), Striped Bass (solid line), and Atlantic Croaker (dashed line) over the size ranges investigated. Lines were drawn to encompass the percent lipid values observed in this study.

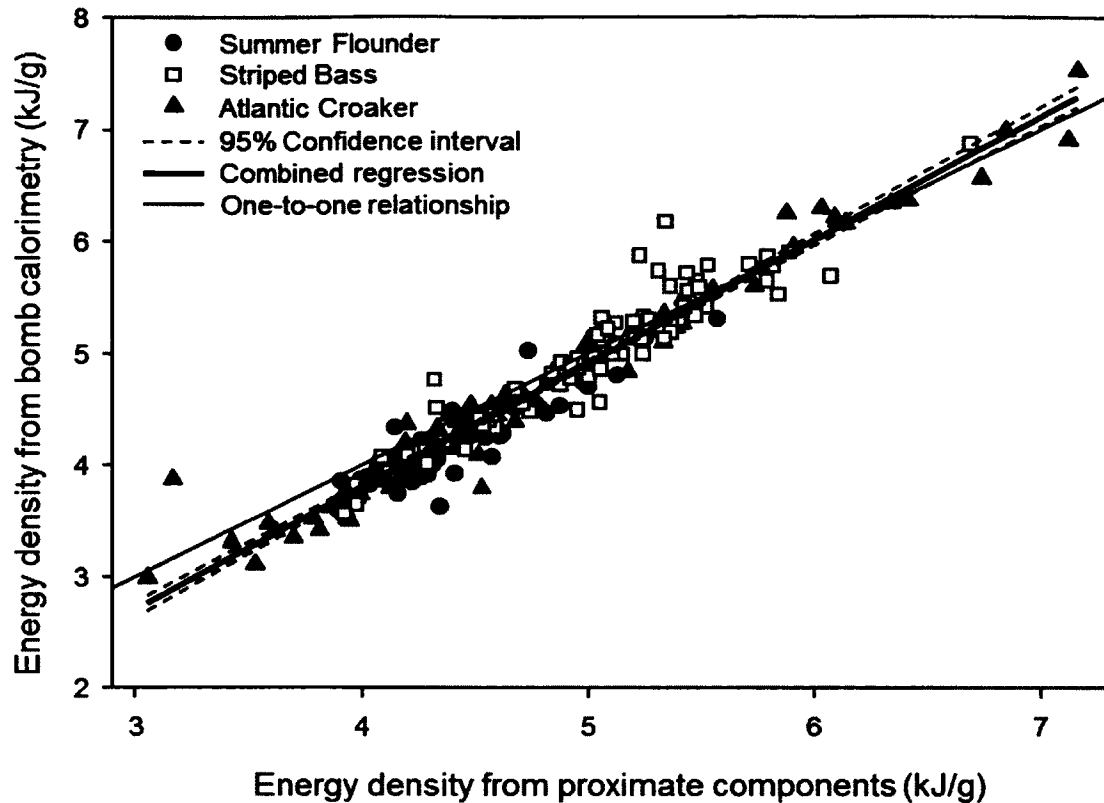


Figure 5. Relationship between energy density estimates from bomb calorimetry and energy density calculated from proximate composition analysis (percent composition) using 23.6 kJ/g for protein and 36.2 kJ/g for lipid. The combined regression shows the relationship pooled across species. Pooled ($n = 230$) and species-specific (Summer Flounder, $n = 79$; Striped Bass, $n = 79$; Atlantic Croaker, $n = 72$) relationships are presented in Table 2.

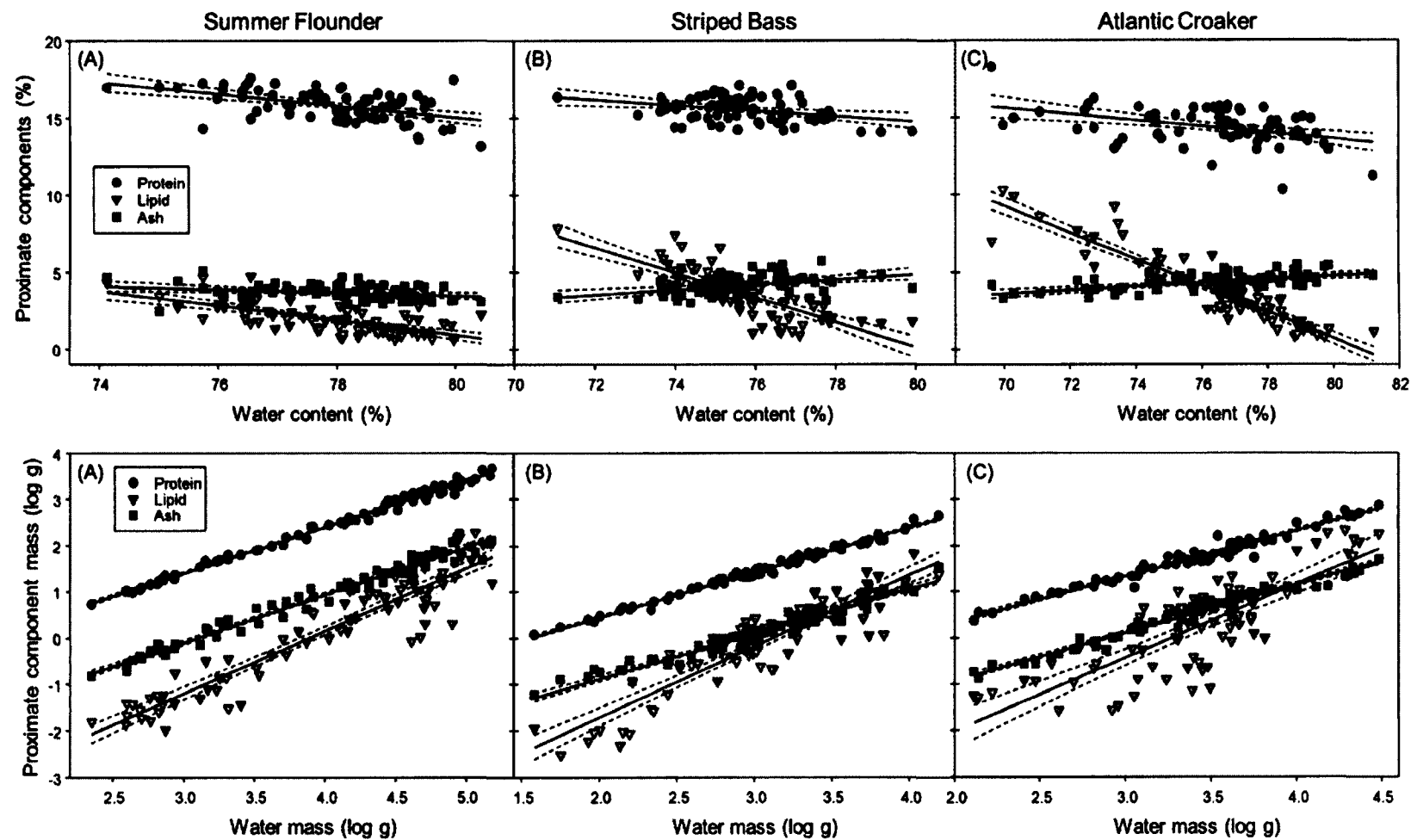


Figure 6. Relationship between proximate components and water expressed as percent (top panels) and mass (bottom panels) determined by linear regression (solid line) for whole (A) Summer Flounder ($n = 79$), (B) Striped Bass ($n = 79$), and (C) Atlantic Croaker ($n = 72$) from Virginia estuaries. Dashed lines denote 95% confidence intervals.

CHAPTER 3

Temporal Dynamics of Condition for Estuarine Fishes in their Nursery Habitats

Abstract

Recruitment is a key process that sustains marine fish stocks, but it is highly variable among years. The condition of individuals in a year class may contribute to recruitment variability due to differential survival of poor- and well-conditioned fish. Additionally, intra-annual dynamics of condition indices reveal energy allocation strategies that maximize survival during physiologically challenging processes (e.g., migration or winter temperatures below thermal tolerances). We examined intra-annual patterns in condition indices for juveniles of three species collected from estuarine nursery areas. Condition of juvenile summer flounder ($n = 1,771$), Atlantic croaker ($n = 3,911$), and striped bass ($n = 874$) was assessed monthly for multiple year classes using length-based indices, the hepatosomatic index (HSI), and relative lipid estimates from the Distell fish fatmeter. Mean condition varied among year classes for all species, and year-adjusted patterns differed depending on the index and species considered. In the fall, juvenile summer flounder and Atlantic croaker exhibited dissimilar patterns in condition as measured by Fulton's K and subdermal lipids, which suggests these species have different energy allocation strategies prior to migration. Partial migrations exhibited by these populations may be due to differences in mean condition among years, which are influenced by inter-annual changes in environmental conditions and density-dependent effects. All species that remained in the estuary during winter showed increased HSI values, suggesting a typical strategy for winter survival among these temperate species. Our understanding of recruitment variability may be improved by assessing year-specific differences in mean condition and intra-annual patterns in energy allocation strategies.

INTRODUCTION

The condition of individual recruits may play a critical role in recruitment variability due to differential survival of poor- versus well-conditioned individuals. Here, we refer to recruitment as the survival of juveniles to the adult stage. Survival of fishes is notably influenced by condition during adverse environmental or physiological challenges when individuals experience increased stress (e.g., long migrations, or physiological extremes). For example, lipid content is associated with improved survival during periods of reduced feeding activity, as seen in both short-term (<1 month) studies on larvae (Lochmann et al. 1995; Booth & Alquezar 2002) and long term (>4 months) studies on juveniles (Thompson et al. 1991; Simpkins et al. 2003). Feeding history and nutritional insufficiency (e.g., low lipid content) have also been inferred to influence patterns of survival in wild populations (Newsome & Leduc 1975; Gardiner & Geddes 1980; Wicker & Johnson 1987). Furthermore, survival to adulthood is lower for juvenile steelhead *Oncorhynchus mykiss* in poor condition as denoted by body injuries, fin damage, or external signs of disease (Evans et al. 2014). However, the use of condition indices to explain variation in year-class strength, as measured by the abundance of young-of-the-year fish, has not been explored.

Recruitment is one of the key processes ensuring the sustainability of marine fish stocks. Yet, recruitment has proved difficult to estimate because interannual variations in year-class strength are common even when spawning stock biomass is relatively stable (Anderson 1988); this also results in a poor relationship between year-class strength and stock size (Maunder 2012). Strong year classes depend on the appropriate convergence of spawning-stock characteristics (e.g., age structure and biomass) and environmental

factors (Neill et al. 1994; Cardinale & Arrhenius 2000). Environmental factors influencing year-class strength include the physicochemical conditions throughout the first year of life (Houde 2009), food availability (Martino & Houde 2010), habitat quality (Gibson 1994), and inter-specific competition and predation rates (Sogard 1997; Walters & Kitchell 2001). Environmental factors interact to affect the metabolism of an individual, and the physiological state of the animal may thus represent the compounding of those external effects over time (Neill et al. 1994). For example, an individual that resides in a suboptimal environment may have increased metabolic costs to maintain homeostasis, and the longer it resides in that environment, the more physiological stress it would endure. As a result, the influence of external factors may be manifested in the condition of individual fish, such that optimal environmental factors and biological interactions will lead to production of juvenile fish in good condition. Ideal environmental conditions maximize growth and survival as well (Gibson 1994), potentially leading to increased recruitment.

Our understanding of recruitment may be enhanced by focusing on unique characteristics of individuals capable of surviving adverse environmental influences (Crowder et al. 1992; Miller 2007). When the condition of individuals differs substantially, population-level impacts may be derived from atypical individuals with an increased probability of survival (Crowder et al. 1992). Indeed, due to the importance of energy reserves for survival, measures of larval fish condition may be used to improve the utility of abundance data to forecast year-class strength from these early stages (O'Connell 1980; Frank & McRuer 1989). The low correlation between larval abundance and year-class strength may potentially arise from use of abundance data uncorrected for

the proportion of larvae in poor condition (Frank & McRuer 1989). Similarly, the relationship between juvenile abundance and recruitment may be improved by accounting for the condition of juveniles.

Here, we investigate temporal dynamics of fish condition for summer flounder *Paralichthys dentatus*, Atlantic croaker *Micropogonias undulatus*, and striped bass *Morone saxatilis* while they occupy estuarine nursery areas of Virginia. Virginia estuaries exhibit high variability in environmental conditions, with temperatures ranging from 0-30°C, freshwater to polyhaline habitats, and intermittent hypoxia in deep waters; these factors are likely to influence condition over time and among years. Furthermore, summer flounder, Atlantic croaker, and striped bass represent disparate families with different life-history strategies (e.g., anadromous vs. shelf spawners) and ecological niches (e.g., feeding guilds), which may shape how individuals respond to environmental variability (Houde 1997).

We examined patterns in condition relative to processes that challenge juveniles of these species (e.g., offshore migrations or the onset of winter) by sampling fish throughout the year. In the mid-Atlantic region, as water temperatures decrease during fall (towards the end of their first year of life), juvenile summer flounder and Atlantic croaker move offshore to the continental shelf (Kraus & Musick 2001; Miller et al. 2003). Individuals in poor condition may suffer higher mortality rates during migration (Brodersen et al. 2008); therefore, they must build energy reserves prior to long-distance movements (Rios et al. 2011). However, contingents of the mid-Atlantic populations of these species also reside in Chesapeake Bay during winter. Unlike summer flounder and Atlantic croaker, striped bass reside in tributaries of Chesapeake Bay during their first

year of life. Winter mortality of juvenile striped bass has been demonstrated in mid-Atlantic estuaries (Hurst & Conover 1998), suggesting that energy reserves are important for these individuals to survive the winter. In Chesapeake Bay, winter severity can explain the relationship between age-0 abundance of striped bass and recruitment to the fishery at ages 3-5 ($r^2=0.87$, $P<0.05$; Martino 2008). Preparation for migration and winter conditions may differ among years and species, and these differences may be revealed by the temporal dynamics of condition for juvenile summer flounder, Atlantic croaker, and striped bass.

Because no single index is likely to reflect all changes in body composition accurately (Brown & Murphy 2004), we employed multiple approaches to describe and assess temporal dynamics in condition: two length-based indices, the hepatosomatic index (HSI), and relative estimates of subdermal lipid from the Distell fish fatmeter (hereafter, "fatmeter"). Length-based indices, such as Fulton's condition factor (K ; Ricker 1975) and relative condition (K_n ; Le Cren 1951), assume greater body mass at a given length corresponds to better condition (Anderson & Neumann 1996; Froese 2006). The hepatosomatic index refers to the ratio of liver mass to body mass, and it is assumed to be a measure of condition because many fishes store lipids in their liver (Jensen 1979; Adams & McLean 1985; Lambert & Dutil 1997). Likewise, subdermal lipid content is assumed to be a measure of condition, and the fatmeter measures subdermal lipid content in a non-destructive manner using low-power microwaves (Kent 1990). It has been used previously to estimate somatic lipid content and gross somatic energy density in a field setting (Crossin & Hinch 2005).

Our objective is to relate temporal patterns in each of the three condition indices to the life-history strategies of juvenile summer flounder, Atlantic croaker, and striped bass. We assume that condition indices that are elevated prior to or during physiologically stressful events reflect preparedness for those events, and high mean values for the condition indices identify year classes with individuals that are more likely to survive and therefore, to recruit to the adult populations.

MATERIALS AND METHODS

All fish capture, handling, and euthanasia procedures were approved by the College of William & Mary Institutional Animal Care and Use Committee (protocol #IACUC-2010-07-20-6788-mcfabr) and followed all applicable state regulations and federal guidelines.

Field collections

Juvenile summer flounder ($n = 1,771$), Atlantic croaker ($n = 3,911$), and striped bass ($n = 874$) were sampled monthly between Nov 2010 and June 2014 from Virginia's coastal waters (Fig. 1). Most samples were collected using a 9.14-m otter trawl in the Chesapeake Bay and the James, York, and Rappahannock Rivers, following sampling protocols described by Tuckey & Fabrizio (2014). Additionally, summer flounder and Atlantic croaker were collected in Mobjack Bay and the coastal lagoons of the Virginia Eastern Shore (hereafter, "Oyster, VA") using a 4.88-m otter trawl. The number of conspecifics collected at each station was recorded, and used to calculate local density (individuals per m^2). Temperature ($^{\circ}C$), salinity (psu), and dissolved oxygen ($mg L^{-1}$)

were measured at depth (m) at each sampling location. For Oyster, VA, environmental conditions varied widely with tidal cycles and therefore, temperature, salinity, and dissolved oxygen were averaged over the daily sampling period. Following capture, individuals were immediately euthanized by immersion in an ice slurry and returned to the laboratory for condition measurements.

Condition measurements

In the laboratory, individuals were measured for length, subdermal lipids, body mass, and liver mass. Length was recorded to the nearest millimeter. We examined condition indices for juvenile (i.e., age-0 fish) summer flounder between 156 and 290 mm total length (TL), Atlantic croaker between 117 and 220 mm TL, and striped bass between 117 and 200 mm fork length (FL). Individuals were considered juveniles if they were smaller than monthly length thresholds described by Tuckey & Fabrizio (2014).

Only fish large enough to sample appropriately with the fatmeter were considered in this analysis (R. Schloesser, *pers. obs.*). Fatmeter (model FFM-992) measurements were taken from the midline of the body behind the operculum. At this location, four measurements were taken from the non-eyed side of summer flounder ($n = 1,770$), both sides of Atlantic croaker ($n = 3,884$), and the left side of striped bass ($n = 874$); readings from these locations exhibit high correlation with energy content as measured by bomb calorimetry (Schloesser & Fabrizio, *in review*). Measurements were averaged to assess relative subdermal lipid content of individual fish. Accuracy of the fatmeter varies and depends on lipid content of the sample: accuracy is $\pm 1.5\%$ when lipid content is

between 2-15 %; ± 2.5 % when lipid content is between 16-30 %; and ± 4 % when lipid content is > 31 % (Anonymous 2010).

Body and liver mass were measured with a Mettler Toledo XA204 balance (accurate to 0.1 mg). Because some individuals were kept alive for other studies and because some livers were degraded and could not be weighed accurately, the hepatosomatic index was calculated for 1,674 summer flounder, 3,559 Atlantic croaker, and 776 striped bass using:

$$\text{HSI} = W_{\text{liver}} * W^{-1} * 100 \quad (1)$$

where W_{liver} = wet mass of liver (g) and W = wet body mass (g) (Jensen 1979; Lambert & Dutil 1997).

Fulton's condition index (K) was calculated for all individuals using:

$$K = W * L^{-3} * 10^N \quad (2)$$

where W is as before, L = length (mm), and N = an integer to bring the value of K near 1 (Ricker 1975). Relative condition factor (K_n) was calculated for all individuals using:

$$K_n = W * (aL^b)^{-1} \quad (3)$$

where a and b are constants describing the allometric relationship for fish in a given population (Le Cren 1951). Constants were estimated using nonlinear least-squares modeling via the Nlin procedure in SAS (version 9.3, SAS Institute, Cary NC; Schloesser & Fabrizio, *in review*). The length-based indices that best represent energy content as measured by bomb calorimetry for these species are Fulton's condition factor (K) for summer flounder and Atlantic croaker, and relative condition (K_n) for striped bass (Schloesser & Fabrizio, *in review*); these length-based indices will therefore be the focus of our analyses.

Intra-annual patterns in condition

We depicted intra-annual patterns in condition of juvenile fish that reside in Virginia estuaries using generalized additive models (GAMs). For each species, the GAM of condition adjusted for the effect of year class was:

$$Y_{ij} = \mu + \alpha_i + g(\text{day}) + \varepsilon_{ij} \quad (4)$$

where Y_{ij} = the condition of the j^{th} individual from the i^{th} year class as measured by length-based indices, HSI, or the fatmeter;

μ = the intercept, representing the overall mean condition of individuals;

α_i = the effect of the i^{th} year class;

$g(\text{day})$ = nonparametric smoothing function for ordinal day;

ε_{ij} = the random unexplained error, assumed to be independent and normally distributed.

The GAM was fit using the GAM function in R (R Core Team 2014). The degrees of freedom associated with fitting the smoothing function (k) was chosen by identifying local minima in generalized cross validation (gcv) scores (k was constrained to less than 10 to minimize over-fitting). Analyses of condition focused on biological years beginning in June for summer flounder, May for Atlantic croaker, and October for striped bass.

Inter-annual patterns in condition and environmental effects

We used a general linear mixed model approach to analyze the effect of year class and environmental factors on mean condition of juvenile fishes as measured by length-based indices (K or K_n), HSI, and the fatmeter. To assess the effect of environmental

conditions, we included the following random factors in the model: salinity, dissolved oxygen, and depth at the station where the individual was collected; local density of conspecifics was included as a fixed factor. The interaction between year class and local density was included in some of the models (HSI and subdermal lipid for summer flounder; Fulton's K and subdermal lipid for Atlantic croaker) because interaction plots suggested the presence of an interaction. Environmental factors were centered to reduce collinearity, however, the collinearity between water temperature and month could not be removed through centering or standardizing these data (tolerance values remained < 0.10). Because month is a proxy for multiple environmental effects including temperature (e.g., prey availability), we retained month in the models and excluded water temperature. By accounting for these random factors and interactions, we were able to assess factors of interest (month and year class) and identify temporal patterns in condition indices while holding environmental effects constant. Because we collected specimens from a random stratified sampling design, any spatial bias in the temporal patterns of condition should be minimized. For summer flounder and Atlantic croaker, the distributions of subdermal lipid readings and model residuals were skewed, as were those for striped bass HSI values. Subdermal lipid readings were square-root transformed and HSI values were log-transformed because these transformations most suitably met the linear model assumption of homogeneous variances.

Model building followed the approach of Littell et al. (2006) and Zuur et al. (2007). First, we identified the appropriate variance-covariance structure, and then we assessed the need to include interactions. Finally, we identified whether year class, month, and local density helped explain patterns in condition indices. Models were fit

using the MIXED procedure in SAS 9.3. We modeled heterogeneity in the variances among year classes with the group option of the repeated statement in the MIXED procedure. We specified the Kenward-Roger method for calculating degrees of freedom due to the presence of multiple random effects (Kenward & Roger 1997). For each species and condition index, a preliminary model was constructed that included all random environmental variables (except for water temperature); both simple variance components and year-class-specific variance components were considered for preliminary models. We identified the best fitting model with REML-based estimates of Akaike's information criterion corrected for small sample size (AIC_c ; Burnham & Anderson 2002). Next, the interaction between year class and local density was included as a fixed factor if it reduced AIC_c , and the combination of year class, month, local density, and interaction that minimized AIC_c was determined using maximum likelihood (ML) estimation. The model describing juvenile fish condition was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma + \delta + \rho + \kappa + (\alpha\gamma)_i + \epsilon_{ijk} \quad (5)$$

where Y_{ijk} = the condition of the k^{th} individual from the i^{th} year class and the j^{th} month as measured by length-based indices, HSI, or the fatmeter;

μ = the intercept, representing the overall mean condition of all individuals;

α_i = the effect of the i^{th} year class;

β_j = the effect of the j^{th} month;

γ = the effect of local density;

δ = the random effect of salinity (mean = 0, variance = σ_δ^2);

ρ = the random effect of dissolved oxygen (mean = 0, variance = σ_ρ^2);

κ = the random effect of depth (mean = 0, variance = σ_κ^2);

$(\alpha\gamma)_i$ = the effect of the interaction between year class and local density; and ϵ_{ijk} = the random unexplained error, assumed to be independent and normally distributed.

Because models within two AIC_c units have a similar ability to estimate the expected response (Burnham & Anderson 2002), we selected the simplest model within two AIC_c units of the lowest value as the final model. Final model parameters were estimated using REML. Estimates of least squares means of condition indices for each year class were compared using Tukey's honestly significant difference test, which adjusts the significance level due to multiple comparisons. For some indices, a significant interaction between year class and local density was detected, suggesting the effect of local density (i.e., fish captured in the same tow) on condition indices varied among years. Therefore, we report differences in least square means among year classes (t tests) because these model-based means are appropriately adjusted for the changing effect of density on condition each year. The indices without a significant interaction include: K for summer flounder, HSI for Atlantic croaker, and all striped bass condition indices; for these indices we report the significance of main effects (density and year class) directly (F tests).

Contrast statements in the general linear mixed models were used to examine differences in mean condition among seasons and interpret intra-annual patterns in mean condition. Generally, seasons were identified as consecutive two- or three-month periods that corresponded with a physiologically meaningful life-history event. For summer flounder and Atlantic croaker, binning occurred around the month of peak migration (November and October, respectively), and for striped bass, binning occurred around

January to permit examination of changes in condition among different water temperature regimes. Therefore, summer flounder mean condition was compared among four periods: summer (July-August), the period prior to peak migration (September-October), the period after peak migration (December-January), and winter (February-March).

Similarly, Atlantic croaker were examined during summer (June-July), prior to peak migration (August-September), after peak migration (November-December), and during winter (January-March). Finally, striped bass were examined during the declining temperatures of early winter (November-December), the low temperatures of late winter (February-March), and the warming temperatures of spring (April-June). Significance in all analyses was assessed at $\alpha = 0.05$.

RESULTS

Intra-annual patterns in condition

Fish condition varied by the index used to measure condition, the time of year, and by species (Fig. 2). In general, summer flounder mean K and HSI values declined through the summer, and were significantly less in the period prior to migration than during any other season investigated (Fig. 2, Table 1). Mean subdermal lipid stores increased temporarily in the fall, notably in October, perhaps in preparation for migration. On average, individuals that remained in Chesapeake Bay after the peak migration period had significantly lower mean subdermal lipids than what was observed prior to peak migration (Fig. 3). Conversely, mean K and HSI were significantly higher for individuals that resided in Chesapeake Bay after peak migration than for those collected prior to November. After peak migration, mean K declined and mean HSI

increased significantly for individuals captured in Chesapeake Bay during winter. Many of these individuals had HSI values greater than 1.5 units, levels not observed prior to peak migration (Fig. 3).

Juvenile Atlantic croaker exhibited seasonal patterns in Fulton's K and subdermal lipids that were opposite to those observed in summer flounder (Fig. 2, Table 1). Mean values of these indices increased during the summer and were highest prior to the time when juvenile Atlantic croaker typically leave Chesapeake Bay. Fulton's K and subdermal lipids were significantly lower for individuals residing in the bay during winter than during other times of the year. Mean HSI values exhibited a pattern similar to that in summer flounder, with mean values lower during the pre-migration period (< 2 units for most individuals, Fig. 3), and significantly higher during the post-migration and winter seasons.

Striped bass exhibited high mean HSI values during winter (Fig. 2), and mean HSI values were significantly higher during late winter compared with early winter (Table 1). Mean HSI values declined to significantly lower levels in spring as fish resumed somatic growth. Mean subdermal lipid content was also significantly lower during the spring than during early or late winter, potentially due to energy being allocated to growth. Patterns in mean K_n were less apparent than for the other indices; relative condition was variable over the time period investigated, and no significant differences in mean relative condition were detected among seasons.

Inter-annual patterns in condition and environmental effects

Environmental factors influence mean condition of juvenile fish, but effects vary by species, time, and with the manner in which condition is expressed. A few patterns were discernable for those environmental effects that explained a significant amount of variability in condition. Juvenile summer flounder and striped bass collected in higher salinity waters were typically in better mean condition than conspecifics from lower salinity habitats, whereas Atlantic croaker in lower salinities exhibited higher mean condition. Individuals of the 2011 year class of striped bass that resided in deeper waters were in better mean condition than those from shallow areas, but we observed the opposite in the 2010 and 2013 year classes. Dissolved oxygen generally had a positive effect on mean condition of summer flounder in the 2011 and 2013 year classes, but a negative effect on the 2012 year class.

For juvenile summer flounder, mean values of Fulton's K varied significantly among year classes ($F_{2,1714} = 177.5$, $P < 0.01$), and were highest in 2011 and lowest in 2012 (Fig. 4). Regardless of year, local density at the sampling site had a significant positive effect on K ($\gamma = 0.77$, $F_{1739} = 37.6$, $P < 0.01$), indicating that individuals exhibiting high condition were found in aggregations. Patterns in summer flounder subdermal lipids were influenced by the significant interaction between local density and year class ($F_{2,1748} = 5.4$, $P < 0.01$). Mean subdermal lipids were higher for the 2013 year class than the 2011 ($t_{1664} = 3.4$, $P < 0.01$) and 2012 ($t_{1733} = 11.5$, $P < 0.01$) year classes, and higher for the 2011 than for the 2012 year class ($t_{1584} = 3.9$, $P < 0.01$; Fig. 4). We examined year-class specific models to further investigate the effect of local density; local density had a significant negative effect on subdermal lipids for the 2012 year class ($t_{836} = -4.6$, $P < 0.01$), but no effect of local density was detected for the 2011 or 2013

year class. Although the year class-density interaction was not statistically significant for HSI ($F_{2,1408} = 2.5$, $P = 0.08$) and no differences were detected in mean HSI among year classes (Fig. 4), the data suggest that a weak interaction may be present. As before, we examined the effect of the interaction with year-specific models. Local density had a significant positive effect on HSI for the 2012 year class ($t_{1119} = 4.9$, $P < 0.01$), but had no effect for the 2011 and 2013 year classes.

In general, the effects of year class and local density on mean condition of Atlantic croaker exhibited a pattern unlike that observed for summer flounder (Fig. 4). No effect of local density was detected on HSI. Mean HSI varied significantly among year classes ($F_{2,1828} = 14.0$, $P < 0.01$), with the 2013 year class having higher mean HSI values than the 2012 year class ($t_{2949} = 5.3$, $P < 0.01$). A significant interaction was detected between year class and local density for K ($F_{2,1213} = 5.6$, $P < 0.01$), however, no significant effects of local density on K were detected in the year-specific models; this may be due to smaller sample sizes in the year-specific model for the 2011 year class. Fulton's K was significantly higher in 2012 than in 2013 ($t_{2760} = 7.1$, $P < 0.01$; Fig. 4). A significant interaction was also detected between year class and local density for subdermal lipids ($F_{2,1509} = 17.2$, $P < 0.01$). Year-specific models indicated that local density had a significant positive effect on subdermal lipids in 2012 ($t_{2527} = 2.3$, $P = 0.02$), but no effect in other years. Mean subdermal lipids were higher for the 2013 year class than for the 2011 and 2012 year classes (2011: $t_{208} = 3.9$, $P < 0.01$; 2012: $t_{3770} = 14.4$, $P < 0.01$; Fig. 4).

Condition indices for juvenile striped bass also varied by year class and local density at the sampling site. Mean values for all three indices were significantly different

among year classes (K_n : $F_{3,12} = 61.4$, $P < 0.01$; HSI: $F_{3,183} = 28.1$, $P < 0.01$; subdermal lipids: $F_{3,335} = 82.8$, $P < 0.01$; Fig. 4). Mean relative condition was higher in 2011 than in 2010 ($t_{69} = 4.5$, $P < 0.01$), and mean K_n in 2010 and 2011 was higher than in 2013 (2010 vs 2013: $t_{103} = 3.3$, $P = 0.04$; 2011 vs 2013: $t_{855} = 14.2$, $P < 0.01$). Due to the low number of individuals collected in 2012, mean relative condition of the 2012 year class was not significantly different from any other year class. Similarly, mean HSI was significantly higher in 2011 than in 2013 ($t_{194} = 6.7$, $P < 0.01$), but neither was different from the 2012 year class. The 2010 year class exhibited significantly lower mean HSI values than all other year classes (2011: $t_{442} = 8.6$, $P < 0.01$; 2012: $t_{110} = 3.4$, $P < 0.01$; 2013: $t_{483} = 4.1$, $P < 0.01$). Mean subdermal lipids were significantly higher in 2010 and 2011 than in 2012 ($t_{671} = 5.6$, $P < 0.01$; $t_{702} = 5.6$, $P < 0.01$) and 2013 ($t_{477} = 11.4$, $P < 0.01$; $t_{233} = 12.8$, $P < 0.01$). A significant negative effect of local density on condition was detected for K_n ($F_{1,773} = 5.8$, $P = 0.02$) and subdermal lipids ($F_{1,507} = 24.6$, $P < 0.01$) across all year classes, suggesting density-dependent effects on condition of juvenile striped bass.

DISCUSSION

Seasonal patterns in mean relative mass (K and K_n), HSI, and subdermal lipids were apparent for juvenile summer flounder, Atlantic croaker, and striped bass, but the dynamics of these indices were not congruent. The dissimilar patterns suggest that multiple condition indices can provide a more complete understanding of the energy allocation strategy of fish during the time they are resident in estuarine nursery areas. For example, contingents of the mid-Atlantic populations of summer flounder and Atlantic croaker may migrate to the continental shelf in the fall, or may reside in Chesapeake Bay

during winter. We propose that this partial migration may be partly explained by differences in energy allocation of individual fish. Furthermore, dynamics among condition indices reflect the life-history strategies used by these species (i.e., migration or residence during winter). Energy allocation strategies may also differ among years, as evidenced by variations among condition indices and year classes, and each index provides a different indicator of energy allocation over time.

The life-history strategy of summer flounder and Atlantic croaker is typified by juveniles moving out of estuarine waters prior to the onset of winter. Despite this common strategy, these species display surprisingly different energy allocation patterns. Atlantic croaker dramatically increase their subdermal lipid stores and relative mass throughout the spring and summer in preparation for egress that occurs around September and October (Miller & Able 2002); muscle energy and body mass are high prior to egress for other migratory species as well (e.g., bluefish *Pomatomus saltatrix*, Morley et al. 2007). Individuals in better condition may be more likely to undertake and survive migration (Brodersen et al. 2008). Because subdermal lipid reserves are preferentially used to support migration in some species (Leonard and McCormick 1999), this index may be useful for assessing preparedness for migration. Conversely, patterns in the condition of summer flounder suggest a decline in mean relative mass, low mean HSI values, and only a minor increase in mean subdermal lipid prior to emigration (around October, Desfosse 1995). Juvenile summer flounder may not store fuel for migration in the liver or in subdermal tissues, but energy may come from alternative tissues, or these fish may use protein as a source of energy for migration. However, if summer flounder use protein to fuel migrations, then length-based indices are expected to increase prior to

migration. Instead, we observed a decline in robustness as evidenced by a greater increase in length than in mass prior to emigration. If, however, HSI and subdermal lipids are indicators of preparedness, then a portion of the juvenile summer flounder may not be well prepared to undertake a long-distance migration. These fish may not move long distances or may remain in Chesapeake Bay during winter. Because summer flounder and Atlantic croaker have the potential to mature at the end of their first year of life (6-40% of summer flounder, Morse 1981; > 85% of Atlantic croaker, Barbieri et al. 1994), preparedness for migration may have consequences for population productivity. Individuals that moved out of Chesapeake Bay were not available to our sampling gear for condition assessment and thus, no direct comparison was made between out-migrants and winter residents. A comparison of residents and migrants is necessary to confirm our hypothesis that individuals that emigrate in winter are in better condition than conspecifics that remain in nursery habitats.

The importance of local density on mean condition also differed for summer flounder and Atlantic croaker. Due to the similarity of life-history strategies of these species, and based on previous work (Wood & Austin 2009), we would expect similar patterns in the annual abundances of juveniles for these species. For the same reasons, we expect similar patterns in mean condition, and thus, a similar influence of density on mean condition for summer flounder and Atlantic croaker. Although these species exhibited similar patterns in abundance (e.g., high relative abundance of both species in 2012, Tuckey & Fabrizio 2014), patterns in mean condition were dissimilar between these species, as was the influence of salinity and density. For Atlantic croaker, the significant interaction between local density and year class on subdermal lipid reserves

was driven by a positive influence of local density in 2012, suggesting individuals may have aggregated or catchability was high at times when mean condition was high. Yet, for summer flounder, there was a negative influence of local density on subdermal lipids in the same year. Indeed, Atlantic croaker sampled in 2012 had high mean subdermal lipid measurements and mean K values, whereas these condition indices were low for summer flounder in 2012.

A greater number of individual summer flounder from the 2012 year class resided in Chesapeake Bay during winter, compared with observations from 24 other year classes (Tuckey & Fabrizio, *unpubl. results*). We suspect that the 2012 year class comprised a greater proportion of individuals that failed to build sufficient energy reserves in terms of subdermal lipid and protein stores (denoted by low K values). Indeed, a larger number of individuals with low subdermal lipids were observed in winter of 2012 than in any other year (Fig. 3). Several explanations are consistent with the observed elevated mean HSI values for individuals that reside in Chesapeake Bay during winter. Liver lipids could be increasing over winter as individuals remobilize lipids to the liver. Alternatively, HSI could increase due to a decline in body mass (via reduced protein and/or water mass) while liver mass is conserved. Finally, mortality of individuals with low HSI values would also increase the average HSI observed in winter (e.g., the lack of summer flounder with HSI values <1 after December, Fig. 3). All of these explanations may also act in combination. We do not expect that high HSI values during winter were due to individuals feeding, because the majority of individuals held in a laboratory and deprived of food for up to 14 weeks displayed HSI values between 1 and 2 units (mean = 1.53 units; R. Schloesser, *pers. obs.*). Regardless, a prominent shift occurred in energy-storage

patterns for all year classes before and after the migration period: a greater proportion of individuals exhibited high subdermal lipid readings prior to the migration period, while a greater proportion exhibited high HSI values after the migration period. We hypothesize that summer flounder in Chesapeake Bay exhibit condition-dependent partial migration, a phenomenon also observed in cyprinids, whereby healthier fish migrate in higher proportion and earlier in the season than conspecifics in sub-optimal condition (Brodersen et al. 2008). Partial migration may decrease mortality risks of migrants, for example, by avoiding unsuitable environmental conditions or reducing the risk of predation (Brodersen et al. 2008).

Similar to what we observed for summer flounder and Atlantic croaker, juvenile striped bass that remained in Chesapeake Bay during winter had elevated mean HSI values, which on average peaked in March. Others also observed peaks in the average lipid and energy content of striped bass as late as March (Hurst et al. 2000). However, some winters may result in a major energy deficit, and juvenile striped bass can lose up to 21% of their total body energy and 50% of their total body lipid reserves to meet energy demands, notably if pre-winter energy content is high (Hurst & Conover 2003). A large proportion of these stored lipids are expected to be used to support basic metabolic processes and maintain homeostasis (Hurst 2007). Energy deficits combined with prey scarcity and other stressors associated with winter conditions (e.g., near-lethal temperatures, depressed osmoregulatory ability, and disease) may lead to increased mortality of individuals with insufficient lipid stores (Hurst et al. 2000).

Interannual differences in condition were apparent for juvenile summer flounder, Atlantic croaker, and striped bass, with the potential to influence survival and year class

strength. Indices of relative abundance of juvenile fish from fisheries-independent surveys are used to assess year-class strength, and ideally are measured after the cohort has experienced the majority of its early-life mortality (Neill et al. 1994). For many species, 90% of the mortality occurs between egg fertilization and first feeding (Houde 2009). High mortality during the juvenile stage (e.g., during migration or winter) can lead to a poor relationship between larval abundance and abundance of the cohort at a later stage (Stephens et al. 1986). As a result, larval-stage dynamics do not fully account for year-class success or failure (Houde 1997). Factors affecting survival of post-larval stages are important for determining the strength of year classes (Sissenwine 1984), though post-larval mortality is difficult to measure. However, condition is likely associated with post-larval (juvenile) mortality, and as seen here, simple condition indices (e.g., Fulton's K) can be used to assess seasonal changes in the energy reserves of juvenile fishes. Assessments of energy content during critical periods in the life cycle are essential for understanding the population dynamics of coastal species (Rosa et al. 2010). If condition indices can be related to increased likelihood of survival during migration or during winter, then estimates of juvenile abundance could be adjusted by the proportion of individuals that exceed a given condition index threshold. By accounting for individual variability in condition, abundance indices may be refined to reflect year-class strength more accurately. Such refined indices are of benefit to resource management agencies that use these indices in stock assessments to estimate future production of the stock.

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Table 1. Differences in condition among seasons examined with contrast statements in the general linear mixed models. The estimates and a standard errors (SE) are based on differences in least-square means among seasons identified in the contrast statement such that, for example, a negative estimate for the summer vs pre-migration contrast indicates lower condition in summer. Degrees of freedom for the t-tests are denoted by DF, and significance is denoted by P. Fulton's K was measured for summer flounder and Atlantic croaker, and K_n was measured for striped bass.

Contrast statement	Length-based indices (K or K_n)					Hepatosomatic index					Subdermal lipids				
	Estimate	SE	DF	t	P	Estimate	SE	DF	t	P	Estimate	SE	DF	t	P
Summer Flounder															
summer vs pre-migration	0.0437	0.0044	1734	9.84	<0.01	0.1675	0.0236	718	7.09	<0.01	-0.0439	0.0548	1328	-0.80	0.42
summer vs post-migration	-0.0047	0.0086	1402	-0.55	0.58	-0.3393	0.0590	27	-5.75	<0.01	0.1889	0.1271	244	1.49	0.14
summer vs winter	0.0121	0.0092	1376	1.32	0.19	-0.7166	0.0602	33	-11.90	<0.01	0.0658	0.1328	249	0.50	0.62
pre- vs post-migration	-0.0484	0.0071	1517	-6.80	<0.01	-0.5068	0.0473	30	-10.72	<0.01	0.2328	0.1019	307	2.28	0.02
pre-migration vs winter	-0.0316	0.0078	1489	-4.05	<0.01	-0.8841	0.0487	41	-18.14	<0.01	0.1097	0.1083	320	1.01	0.31
post-migration vs winter	0.0169	0.0073	1752	2.31	0.02	-0.3772	0.0334	1652	-11.29	<0.01	-0.1231	0.0832	1748	-1.48	0.14
Atlantic Croaker															
summer vs pre-migration	-0.0781	0.0035	473	-22.35	<0.01	-0.1408	0.0145	536	-9.71	<0.01	-0.6026	0.0309	3846	-19.50	<0.01
summer vs post-migration	0.0112	0.0114	1349	0.98	0.33	-0.8372	0.0517	358	-16.20	<0.01	0.4427	0.0990	1273	4.47	<0.01
summer vs winter	0.1160	0.0186	1359	6.25	<0.01	-0.7509	0.0826	329	-9.09	<0.01	0.9202	0.1785	164	5.16	<0.01
pre- vs post-migration	0.0893	0.0116	1071	7.72	<0.01	-0.6965	0.0528	286	-13.19	<0.01	1.0453	0.0995	1304	10.50	<0.01
pre-migration vs winter	0.1941	0.0188	1277	10.33	<0.01	-0.6101	0.0836	322	-7.30	<0.01	1.5229	0.1787	172	8.52	<0.01
post-migration vs winter	0.1048	0.0181	3161	5.79	<0.01	0.0863	0.0803	1836	1.07	0.28	0.4775	0.1763	819	2.71	<0.01
Striped Bass															
early vs late winter	0.0017	0.0072	779	0.24	0.81	-0.1287	0.0398	268	-3.23	<0.01	-0.1166	0.5660	468	-0.21	0.84
early winter vs spring	0.0021	0.0107	850	0.19	0.85	0.1453	0.0608	86	2.39	0.02	3.9892	0.8446	561	4.72	<0.01
late winter vs spring	0.0004	0.0099	818	0.04	0.97	0.2740	0.0754	23	3.63	<0.01	4.1059	0.9200	183	4.46	<0.01



Fig. 1. Sampling locations in Virginia used to collect Summer Flounder (square), Atlantic Croaker (circle), and Striped Bass (diamond) for analysis of temporal patterns in condition.

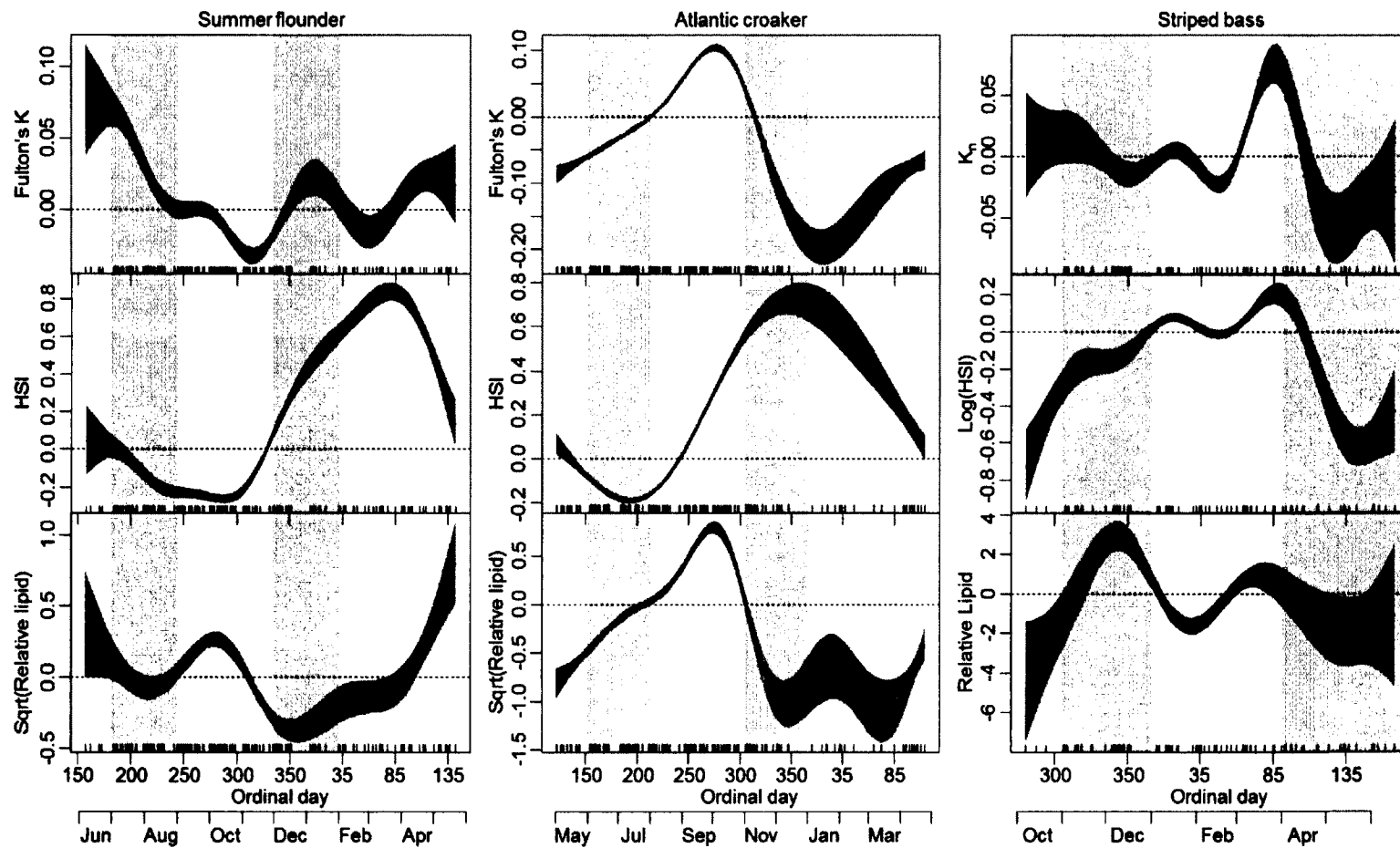


Fig. 2. Temporal dynamics of condition measured by length-based indices (Fulton's K or relative condition (K_n)), the hepatosomatic index (HSI), and relative subdermal lipids measured by the fatmeter for juvenile summer flounder (n 's = 1,771, 1,674, and 1,770), Atlantic croaker (n 's = 3,911, 3,559, and 3,884), and striped bass (n 's = 874, 776, and 874). Hash marks along the x-axis indicate the observed values. The solid line represents the smoothing function \pm two standard errors (dark grey shading) fitted by a generalized additive model. The lighter grey bars denote seasons analyzed with contrast statements (Table 1). The dashed lines denote the mean condition for each yearclass, and therefore, the y-axis represents a deviation from that mean.

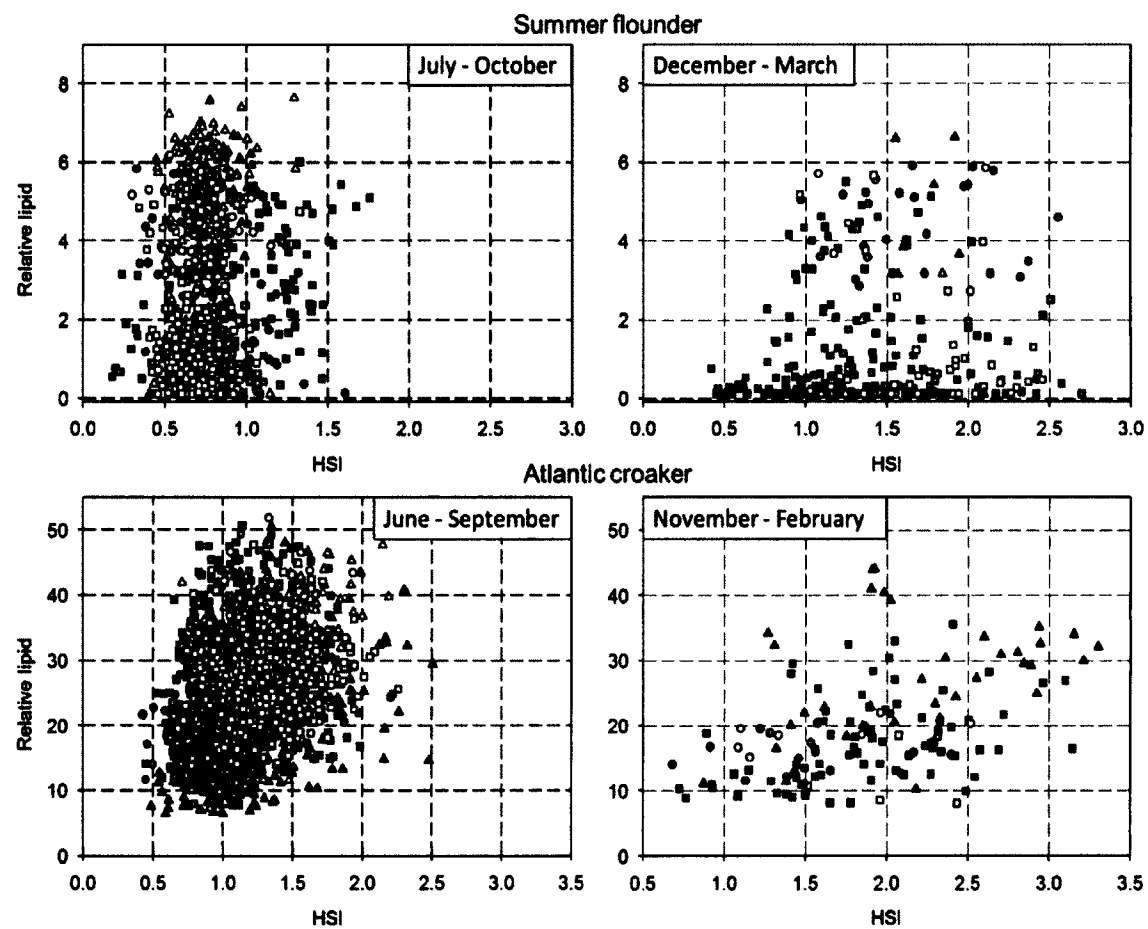


Fig. 3. Relative subdermal lipid storage and hepatosomatic index (HSI) values for individual summer flounder and Atlantic croaker prior to peak migration (July to October and June to September, respectively, shaded black to white) and after peak migration (December-March and November-February, shaded black to white). Symbols indicate year classes: 2011 (circle), 2012 (square), 2013 (triangle). Gridlines aid in highlighting the prominent shift in the proportion of summer flounder above 1.5 HSI units and Atlantic croaker above 2 units in the post-migration period, as well as a general decrease in subdermal lipid storage.

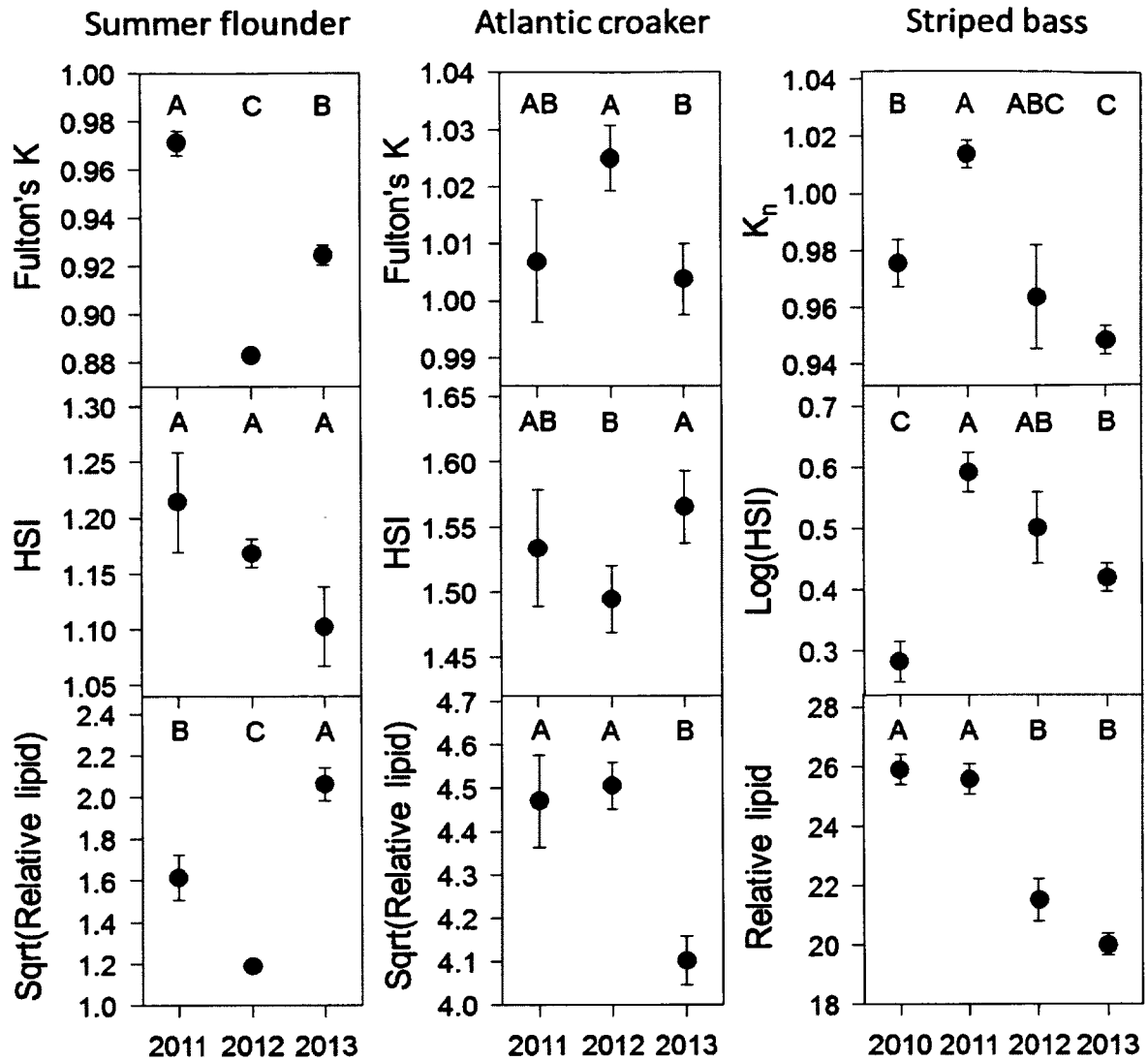


Fig. 4. Mean condition (\pm standard error) for year classes of juvenile summer flounder, Atlantic croaker, and striped bass adjusted for density and environmental effects in a general linear mixed model. Condition was measured by length-based indices (Fulton's K or relative condition (K_n)), the hepatosomatic index (HSI), and subdermal lipid measured by the fatmeter. Letters indicate significantly different condition indices among year classes determined by linear models at $\alpha = 0.05$.

CHAPTER 4

**Nursery Habitat Quality Assessed by the Condition of Juvenile Fishes: Not All Estuarine
Areas are Equal**

Abstract

High quality nursery habitats are expected to contribute disproportionately to the adult population, but the benefits associated with particular habitats or environmental characteristics remain unknown. Well-conditioned individuals are more likely to survive physiologically stressful events and recruit to the adult population. We use spatially-explicit models to examine patterns in condition of juvenile Atlantic Croaker, Summer Flounder, and Striped Bass among nursery areas in coastal Virginia. Condition was assessed using three indices: length-based indices, the hepatosomatic index (HSI), and subdermal lipid estimates. Density of conspecifics, environmental factors (i.e., water temperature, dissolved oxygen, salinity, and depth), and region of capture influenced fish condition, suggesting differential suitability of habitats (kilometers apart) for these estuarine species. Density generally had a stronger influence on mean condition than environmental factors. As expected, mean condition of juvenile Atlantic Croaker and Striped Bass was highest in habitats that had low density of conspecifics, but density had a positive effect on condition of Summer Flounder. With the effects of environmental factors and density held constant, adjusted mean condition indices were high for Summer Flounder in coastal lagoons during the fall, suggesting these areas represent high value, essential nursery habitat for this species. Patterns in adjusted mean condition indices indicated that upestuary habitats provided a positive influence on condition of juvenile Atlantic Croaker between June and September. No patterns in adjusted mean condition were detected among regions for Striped Bass during winter and spring. Differences in condition among nursery areas for these species suggest not all habitats equally prepare individuals for survival, and assessing patterns in condition may further our understanding of habitat quality and productivity.

Introduction

Fisheries management may be improved by considering relevant ecosystem components, particularly habitat quality, as this may impact production and recovery of fish populations through effects on recruitment (Link 2002; Pikitch et al. 2004). Quality nursery habitats are expected to contribute disproportionately to the adult population by supporting increased densities, better survival, faster growth, and successful movement of recruits to adult habitats (Beck et al. 2001). The suitability of habitats for growth and survival varies among nursery areas due to differences in anthropogenic impacts, hydrological conditions, predation, and prey availability (Mittelbach 1986; Gilliers et al. 2006; Searcy et al. 2007; Amara et al. 2007, 2009). Indeed, the ecosystem-based management strategy for Chesapeake Bay recognized the need to assess the suitability of nursery habitats in terms of water quality (e.g., salinity, temperature, and dissolved oxygen), physical characteristics (e.g., substrate and habitat diversity), and prey resources (Chesapeake Bay Fisheries Ecosystem Advisory Panel 2006).

Although the contribution of individual habitats to the overall abundance of fish is one of the key criteria for defining essential fish habitat (Essential Fish Habitat Final Rule 2002), estimation of habitat-specific productivity has proved elusive (Beck et al. 2001). Fish condition is an integrative metric of environmental stress, ecological interactions (e.g., diet and predation), and fish activity; thus, juvenile fish condition may be used as a measure of nursery habitat quality (Stevenson and Woods 2006). Furthermore, because condition may influence survival (Wicker and Johnson 1987; Thompson et al. 1991; Brodersen et al. 2008; Evans et al. 2014), habitats that support well-conditioned juveniles may contribute disproportionately to the adult stock. Variable biotic and abiotic

conditions affect the suitability of nursery habitats for juvenile fishes and produce individuals of varying condition; relationships among fish condition and biotic and abiotic factors may therefore be used to infer differential productivity of habitats.

Nursery areas in coastal Virginia encompass diverse habitat types and exhibit a wide range of environmental conditions that may influence habitat quality and productivity, including hydrodynamics, depth, extent of vegetated habitat, and water quality (e.g., temperature and salinity). We expect that variations in physicochemical characteristics and conspecific density among estuaries in coastal Virginia will alter their value as nursery habitats. As a result, estuarine habitats in Virginia may not be equally suitable as nurseries for juvenile fishes. Here, we infer habitat quality by examining geographic patterns in condition indices for juvenile fishes that utilize Virginia estuaries as nursery areas, focusing on three common species: Atlantic Croaker *Micropogonias undulatus*, Summer Flounder *Paralichthys dentatus*, and Striped Bass *Morone saxatilis*. Our objectives are to identify estuarine habitats in coastal Virginia that are associated with production of juveniles in high mean condition, and determine biotic and abiotic drivers of spatial patterns in condition for these species.

Nursery habitat preferences of Atlantic Croaker, Summer Flounder, and Striped Bass reflect physiological tolerances, food requirements, and availability of refuges from predation; these factors shape the spatial distribution of individuals within estuaries. Juvenile Atlantic Croaker occupy diverse estuarine habitats and tolerate a wide range of temperatures (0-36°C); as they grow, they occupy areas with higher salinities (0-15 psu in spring and 6-26 psu in summer; Diaz and Onuf 1985). Temperature, dissolved oxygen, depth, and salinity influence the habitats occupied by juvenile Summer Flounder in

temperate estuaries (Sackett et al. 2008), and temperature is thought to be one of the key environmental factors influencing habitat quality for juvenile flatfish (Gibson 1994). For juvenile Striped Bass, winter temperature, salinity, and conspecific density are known to influence the suitability of nursery habitats (Hurst and Conover 2002; Martino and Houde 2012). Habitats with suboptimal environmental conditions may lead to individuals in poor condition.

Variation in nursery habitat quality may influence survival of juvenile fishes because individuals will vary in condition, and therefore, exhibit differences in energetic preparation for migration or winter. In the mid-Atlantic region, juvenile fishes approaching age 1 must be energetically prepared for survival during physiologically demanding activities such as winter or migration from coastal estuaries. Juvenile Summer Flounder and Atlantic Croaker move offshore to the continental shelf towards the end of their first year of life (Kraus and Musick 2001; Miller et al. 2003). Although not widely observed in fishes, individuals in low condition are likely to suffer high mortality rates during migration (Brodersen et al. 2008). Conversely, Striped Bass reside in Virginia estuaries during their first year of life, and may suffer mortality during winter if fish are not energetically prepared, for example, through accumulation of lipids. This phenomenon has been observed for juvenile Striped Bass in the Hudson River estuary (Hurst et al. 2000; Hurst and Conover 2002), but has not been examined for juvenile Striped Bass in Chesapeake Bay. Variation in nursery habitat quality may influence survival of juvenile fishes because individuals may vary in condition, and therefore, exhibit differences in energetic preparation for migration or winter.

Multiple indicators of condition have been established for juvenile fish, each a measure of energy allocation to somatic growth or lipid storage. Because multiple condition indices can provide a more complete understanding of energy allocation among tissues (Chapter 3), we used multiple indices to assess fish condition comprehensively and to infer habitat quality. Indices were selected to permit evaluation of energy-storage strategies used by fish and include the hepatosomatic index (HSI), relative estimates of subdermal lipid from the Distell fish fatmeter (hereafter, fatmeter), and length-based indices. The hepatosomatic index reflects lipid storage in the liver (Jensen 1979; Lambert and Dutil 1997). The fatmeter uses low-power microwaves to measure subdermal lipid content (Kent 1990), reflecting energy storage in muscle tissues. Length-based indices, such as Fulton's condition factor (K; Ricker 1975) and relative condition (K_n ; Le Cren 1951), assess accumulation of mass at a given length (Anderson and Neumann 1996; Froese 2006), and are expected to reflect energy allocated to structural proteins in addition to structural and storage lipids. These metrics positively correlate with energy density in the whole fish, and have been used to assess condition in juvenile Atlantic Croaker, Summer Flounder, and Striped Bass (Chapter 1; Chapter 3).

Although use of multiple condition indices provides a more comprehensive assessment of fish condition, variation in habitat quality may not be detected if sample sizes are small (< 30 individuals per area; e.g., Gilliers et al. 2004; Gilliers et al. 2006). High natural variability in condition indices among individuals limits the detectability of significant differences among nursery areas, and therefore large sample sizes (> 30 fish per area) are needed to describe geographic patterns in condition. Furthermore, condition varies during the first year of life of these species (Chapter 3), so sampling needs to be

temporally intense as well. The intense sampling necessary to examine variation in habitat quality raises concern about independent replicates. Individuals within a localized area (sampling location) are exposed to similar environmental conditions and are likely to have been feeding on similar prey; therefore, the observed condition of individuals collected from a given sampling location may not be independent. As a result, high sample sizes from a limited spatial area can result in biased estimates of condition of the population of juvenile fish and biased estimates of the factors influencing individual fish condition. Spatial autocorrelation among samples from multiple locations must be addressed to assess appropriately the effects of environmental and biological factors on mean fish condition. Simultaneous autoregressive (SAR) models address the effect of spatial autocorrelation by describing patterns in the measured response from nearby locations that are likely to have similar environmental properties. Models that account for spatial correlations, including conditional autoregressive models, have been used to describe species distribution and abundance (Fabrizio et al. 2000; Dormann et al. 2007; Kissling and Carl 2008; Elith and Leathwick 2009), but have not been widely used to describe geographic patterns in population traits. Here, we apply spatial models to examine geographic heterogeneity in fish condition and to infer variation in habitat quality and productivity among estuaries in Virginia; specifically, we use SAR models to assess the effect of environmental factors, conspecific density, and region of capture on mean condition of juvenile Atlantic Croaker, Summer Flounder, and Striped Bass.

Methods

All fish capture, handling, and euthanasia procedures were approved by the College of William & Mary Institutional Animal Care and Use Committee (protocol #IACUC-2010-07-20-6788-mcfabr), and were consistent with all applicable state and federal regulations and guidelines.

Field collections

Juvenile Atlantic Croaker ($n = 2,987$), Summer Flounder ($n = 974$), and Striped Bass ($n = 866$) were sampled between November 2010 and June 2014 from Virginia's coastal waters during months when long-distance movements of fish were expected to be minimal. We collected juvenile Atlantic Croaker between June and September because Atlantic Croaker migrate out of temperate estuaries from September to October (Miller and Able 2002); here, October is considered the month of peak migration from Chesapeake Bay. Similarly, Summer Flounder were collected between July and October because the peak of Summer Flounder migration from Virginia estuaries occurs around November (Desfosse 1995; Henderson 2012; Capossela et al. 2013). Juvenile Striped Bass typically do not undertake coastal migrations, but remain within their natal river system throughout the first year of life (Massmann and Pacheco 1961; Williams 2010); we therefore collected juvenile Striped Bass from November to June, which is the latter half of their residency in nursery areas.

Most samples were collected using a 9.14-m otter trawl in the Chesapeake Bay and estuaries of the James, York, and Rappahannock Rivers (Figure 1); collections followed sampling protocols described in Tuckey and Fabrizio (2014). Additionally, Atlantic Croaker and Summer Flounder were collected in Mobjack Bay and the coastal

lagoons of the Virginia Eastern Shore (near Oyster, VA) using a 4.88-m otter trawl because these habitats are used as nursery areas for these species. The majority of Summer Flounder and Striped Bass were immediately euthanized in an ice slurry and returned to the laboratory for condition measurements, but some individuals were kept alive for other studies (Chapter 5). Due to high abundances of Atlantic Croaker, no more than 10 individuals were typically euthanized per trawl sample. The total number of conspecifics collected in each tow was recorded. Area swept was calculated using the average net width and distance traveled during a 5-min tow, and was therefore constant for each gear. Catch numbers and area swept were used to calculate conspecific density at each sampling site (individuals m^{-2}). Temperature ($^{\circ}C$), salinity (psu), and dissolved oxygen ($mg L^{-1}$) were measured near the bottom at each sampling site; tow depth was recorded in meters. Because of the relatively small area and high tidal flushing, most of the environmental variability at Oyster, VA, was associated with tidal cycles (not spatial differences) and therefore, daily mean temperature, salinity, and dissolved oxygen at this location were used in condition analyses.

Condition measurements

Upon return to the laboratory, fish length was recorded to the nearest millimeter. We considered only those individuals that were large enough to sample appropriately with the fatmeter (R. Schloesser, *pers. obs.*), but small enough to be considered juvenile fish (i.e., age-0 fish; Tuckey and Fabrizio 2014). Therefore, condition was assessed for juvenile Atlantic Croaker between 117 and 214 mm total length (TL), Summer Flounder

between 156 and 289 mm TL, and Striped Bass between 117 and 200 mm fork length (FL).

Relative subdermal lipid content of individual fish was measured with the fatmeter (model FFM-992) along the midline of the body and behind the operculum. At this location, four measurements from the non-eyed side of 973 Summer Flounder, four measurements from the left side of 866 Striped Bass, and an average of four readings from each side of 2,960 Atlantic Croaker (8 readings total) were used to estimate relative subdermal lipid content. These locations best represent energy and lipid content for these species (Chapter 1). Accuracy of the fatmeter varies depending on lipid content of the fish (Anonymous 2010): accuracy is reported as $\pm 1.5\%$ for lipid content between 2-15 %; $\pm 2.5\%$ for lipid content between 16-30 %; and $\pm 4\%$ for lipid content $> 31\%$.

Condition indices based on mass were calculated from estimates of body and liver mass (wet mass) measured with a Mettler Toledo XA204 balance (accurate to 0.1 mg). Because some individuals were kept alive for other studies (Chapter 4), and because some livers could not be accurately weighed due to tissue degeneration, the hepatosomatic index was calculated for 918 Summer Flounder, 770 Striped Bass, and 2,670 Atlantic Croaker using:

$$\text{HSI} = W_{\text{liver}} * W^{-1} * 100 \quad (1)$$

where W_{liver} = wet liver mass (g) and W = wet body mass (g) (Jensen 1979; Lambert and Dutil 1997).

Because Fulton's condition factor (K) best represented energy content for Summer Flounder and Atlantic Croaker, and relative condition (K_n) best represented

energy content for Striped Bass (Chapter 1), we used the following equations to calculate K and K_n for these species:

$$K = W * L^{-3} * 10^N \quad (2)$$

where W is as before, L = length (mm; TL for Atlantic Croaker and Summer Flounder; FL for Striped Bass), and N = an integer to bring the value of K near 1 (Ricker 1975); and:

$$K_n = W * (aL^b)^{-1} \quad (3)$$

where W and L are as before, and a and b are constants describing the allometric relationship for fish in a given population (Le Cren 1951). Constants were estimated using nonlinear least-squares estimation via the Nlin procedure in SAS (version 9.3, SAS Institute, Cary NC; Chapter 1).

Spatial analysis

To examine patterns in condition of juvenile Atlantic Croaker, Summer Flounder, and Striped Bass among nursery areas in coastal Virginia, we used autoregressive models that allowed us to model the spatial autocorrelation in mean fish condition among nearby sampling sites. The SAR models estimate the mean condition of juveniles at each sampling site as a linear function of the environmental predictors at each site and the condition of individuals from neighboring sites; spatial correlation among neighboring sites is addressed by assessing spatially-dependent error. We also examined conditional autoregressive models, which estimate mean fish condition at a given site conditional on measures of the response at neighboring sites. However, model selection criteria suggested that SAR models provided a better description of spatial patterns in fish

condition. Furthermore, we also considered non-spatial models that do not account for the effect of neighboring sites on observed mean condition (i.e., ignore spatial correlations) because we hypothesized that high variation in condition among individuals may exceed variation among sampling sites and regions.

We assessed patterns in mean condition of juvenile fish for four regions in each of the James, York, and Rappahannock River estuaries, and six regions in the lower Chesapeake Bay. Mobjack Bay and the coastal lagoons near Oyster, VA, are smaller systems that encompassed a single region each (Figure 1). Because our regions encompassed relatively large areas (25,200 to 66,200 ha per region in Chesapeake Bay, and 2,400 to 29,400 ha per region in the tributaries), the likelihood of mixing of fish from neighboring regions was reduced. Application of spatial models requires the assumption of population closure, which was reasonable given the scale of our regions and life-history characteristics of the species we examined. In particular, samples were collected during months when long-distance movements of fish were expected to be minimal (Miller and Able 2002; Williams 2010; Henderson et al. 2014).

To ensure condition estimates for juveniles from a given region were not biased by collection date, we removed inter- and intra-annual trends in fish condition. To detrend individual condition indices, we applied simple linear models with year and month as categorical predictors. For these models, the distributions of some indices were non-normal and residuals were highly skewed (Summer Flounder and Atlantic Croaker subdermal lipids, and Striped Bass HSI values). Subdermal lipid readings were square-root transformed and HSI values were log-transformed because these transformations most suitably met the linear model assumption of homogeneous variances. Individual fish

data were centered after transformation by subtracting the annual and monthly condition means determined from the appropriate linear model. Because individuals collected in the same tow occupied a similar environment and were not strictly independent samples, we calculated mean centered condition indices at each sampling site. These time-adjusted (i.e., detrended) mean condition indices were used in remaining analyses.

We developed non-spatial and SAR models to analyze the effects of region, environmental factors, and conspecific density on mean condition of juvenile fishes as measured by length-based indices (K or K_n), HSI, or relative subdermal lipid estimates. Concerning environmental factors, we investigated the fixed effects of site-specific salinity, dissolved oxygen, and depth, as well as the fixed effect of “region”, which represented the cumulative effect of environmental factors that were not explicitly specified in the model. In particular, “region” was used to represent variations in prey availability and substrate type, which were not measured but have been linked to variation in energy and lipid storage in fishes (Adams et al. 1982; Lloret and Planes 2003).

Spatial autocorrelation in condition was modeled using a variance-covariance matrix based on spatial weights to describe the spatial relationship of fish condition among neighboring sampling sites. A variety of neighborhood distances and neighbor weighting schemes were considered for modeling spatial weights. We considered neighbor distances ranging from 0 to 25 km at approximately 1.11 km intervals (corresponding with 0.01 degrees of latitude) and three weighting schemes: binary weighting (sites are either included or excluded as neighbors), standardized weighting (covariances are scaled by the number of neighbors for each site), and variance-stabilized

weighting (a method that balances multiple weighting schemes; see Tiefelsdorf et al. 1999). These weighting schemes were applied using inverse distance weighting and equal weighting among neighbors. To identify the appropriate neighbor distances and weighting schemes for each species-condition index combination, we used both Akaike Information Criteria (AIC) and minimum residual spatial autocorrelation (minRSA), as suggested by Kissling and Carl (2008). Specifically, we first calculated the minRSA to determine a range of neighbor distances that reduced the spatially-dependent error; this resulted in multiple models for each combination of species, condition index, and weighting scheme. Next, we selected the model with the combination of neighbor distance and weighting scheme that resulted in the lowest AIC value (Table 1).

We built multiple SAR and non-spatial models that incorporated combinations of environmental factors and conspecific density to describe patterns in condition indices for each species (Kissling and Carl 2008; Plant 2012). The SAR model for each species-index combination that included density and environmental factors was considered the global model (3 species \times 3 indices = 9 global models):

$$Y_{ij} = \mu + \alpha_i + \beta_1(\text{Density})_j + \beta_2(\text{Sal})_j + \beta_3(\text{dO}_2)_j + \beta_4(\text{T})_j + \beta_5(\text{Depth})_j + \lambda W_{u_{ij}} + \varepsilon_{ij} \quad (4)$$

where Y_{ij} = the average, time-adjusted mean condition of individuals from sampling site j in region i as measured by length-based indices, HSI, or subdermal lipid estimates;

μ = the intercept, representing the overall mean condition of individuals from all regions;

α_i = the relative effect of region i when density and environmental factors are held constant;

β_{1-5} = the partial regression coefficients for local density, salinity (Sal), dissolved oxygen (dO_2), temperature (T), and depth at each site j ;

λ = the spatial autoregression coefficient;

W = the spatial weights matrix identified in Table 1;

u_{ij} = the spatially dependent error at site j in region i ; and

ε_{ij} = the spatially independent and normally distributed residual error for site j in region i .

The global model was reduced to a non-spatial model by allowing $u_{ij} = 0$. We examined ~30 reduced models for each species-index combination to identify the combination of density, environmental, and spatial effects that best explained patterns in mean condition using AIC. The final model was the “best” model as determined by AIC, or a simpler model within two AIC units of the “best” model. Because models within two AIC units have a similar ability to estimate the expected response (Burnham and Anderson 2002), we selected the simpler model to improve interpretability. Non-spatial models were fit to the data using the `gls` function, and SAR models were fit using the `spautolm` function in R (`spdep` package; R Core Team 2014).

Results

Not surprisingly, the mean temporally-detrended condition indices varied among estuarine regions in Virginia, and the observed geographic patterns were unique to each species. In general, juvenile Atlantic Croaker from all regions exhibited similar region-

specific patterns in mean condition as estimated by mean K, HSI, and subdermal lipid values in the period prior to offshore migration. Condition indices were relatively high for individuals in Chesapeake Bay (except the southwest region), and were relatively low for Atlantic Croaker from the mouth and lower regions of the James River estuary (Figure 2 A-C). For Summer Flounder, observed spatial patterns in condition were index-specific. The lower James River estuary and northeast Chesapeake Bay region supported juvenile Summer Flounder with relatively high mean K and subdermal lipids in the months prior to migration (Figure 3 A, C). Summer Flounder also had relatively high mean K and HSI values in the middle of Chesapeake Bay, Mobjack Bay, and in the coastal lagoons (Figure 3 A, B). No spatial autocorrelation was detected for mean subdermal lipids in Striped Bass. Yet, we did observe that mean K_n , HSI, and subdermal lipids were relatively high for Striped Bass sampled from the mouth of the Rappahannock River estuary between November and June (Figure 4 A-C). Mean K_n and HSI of Striped Bass were high throughout much of the York River estuary, and both indices were relatively low throughout much of the James River estuary. Environmental conditions, conspecific density, and region of capture were identified as important factors driving these observed patterns in condition indices for all three species.

Environmental and density effects

Environmental influences on mean fish condition were generally less important than the effect of conspecific density on mean condition (Table 2). Environmental influences also varied among species and are therefore explored separately for each species. For Atlantic Croaker, density had a significant negative effect on mean

condition; individuals in high condition were observed in areas with low local densities (~1-4 individuals per 100 m², such as the upper Chesapeake Bay, Figure 2). Water temperature, dissolved oxygen, and salinity had significant positive influences on mean K and subdermal lipid values for juvenile Atlantic Croaker, suggesting that mean condition was high for individuals associated with warmer, saltier, and more oxygen-enriched waters. In general, temperature and dissolved oxygen increased from the Chesapeake Bay main stem to upestry regions (regional means: ~22 to 27°C, and from 4.4 to 6.7 mg L⁻¹), implying upestry regions may support juvenile Atlantic Croaker in high condition. However, salinity increased from upestry regions to the Chesapeake Bay main stem and coastal lagoons (regional means: 3 to 31 psu), therefore, low salinities in upestry regions appear to counteract the positive influence of temperature and dissolved oxygen. On average, mean values of HSI were high for individual Atlantic Croaker that used habitats with cool temperatures and high salinities, such as those observed in the Chesapeake Bay main stem. Depth also had a positive influence on mean subdermal lipid content of juvenile Atlantic Croaker, implying that individuals with high subdermal lipids resided in deeper waters (~13 m) of the Chesapeake Bay main stem and river mouths.

For Summer Flounder, mean K and HSI increased with local density (Table 2), suggesting that some regions of the sampled estuaries support high densities of well-conditioned Summer Flounder. This observation may be the result of the high mean K and HSI values observed among juvenile Summer Flounder from coastal lagoons, where densities were higher than those observed in Chesapeake Bay (Figure 3 A, B, D). Juvenile Summer Flounder utilize warm (~19-26°C), deep (> 8.5 m), saline (~25-31 psu) habitats (Sackett et al. 2008), and in general, we found that depth had a positive effect on

condition, salinity had a negative effect on condition, and the effect of temperature on condition of juvenile Summer Flounder depended on the index examined. Deep waters, such as those at the mouths of the estuaries (regional means: 9-12.8 m), supported individuals with high K and subdermal lipids (Table 2). More robust (high K) Summer Flounder with high subdermal lipids were associated with low salinity waters such as those in upestry regions (regional means: 12-17 psu). Warm environments, such as those in the Chesapeake Bay main stem (regional means: 22-24°C), supported juvenile Summer Flounder with high mean K and HSI, but low mean subdermal lipid content (Table 2).

Few significant environmental effects were detected for Striped Bass (Table 2). Juvenile Striped Bass with high K_n values were collected in deeper habitats (up to 15.4 m) and where conspecific density was low (Figure 4 A, D). A negative effect on mean HSI values was detected for fish occupying high salinity waters, such as those associated with the downestuary regions of the York River estuary (16 psu), where oxygen concentrations were lower than other regions examined ($\sim 9 \text{ mg L}^{-1}$); this likely resulted in the negative effect of dissolved oxygen. A positive effect on mean HSI values was detected for juvenile Striped Bass occupying cool waters, such as the upestry regions of the York River estuary (5-7°C). However, the influence of these environmental factors was weaker than the effect of region on mean HSI values.

Regional patterns in condition

Regional patterns in mean adjusted fish condition were evident for Atlantic Croaker and Summer Flounder, but were less apparent for Striped Bass. We attribute the

variation in these adjusted means (i.e., means adjusted for temporal patterns, local density, temperature, salinity, dissolved oxygen, and depth) to spatial variation in biotic and abiotic factors not explicitly included in the model. Such factors include prey availability, substrate type, predator abundance, and habitat complexity. Due to the manner in which spatial models were parameterized, adjusted means represent the deviation from the adjusted mean condition of individuals from the mouth of the James River estuary (Atlantic Croaker and Summer Flounder) or the mouth of the Rappahannock River (Striped Bass; no individuals were collected from the mouth of the James River estuary).

Adjusted mean condition indices (\pm 95% confidence intervals) of juvenile Atlantic Croaker from the mouth of the James River estuary prior to migration were: $K = 0.92 \pm 0.02$, $HSI = 0.67 \pm 0.06$, and $(\text{subdermal lipid})^{-0.5} = 3.70 \pm 0.18$. Relative to this region, a pattern emerged of relatively high adjusted mean condition in upestuary regions for juvenile Atlantic Croaker, regardless of the condition index examined (Figure 5). In the James, York, and Rappahannock River estuaries, the adjusted mean condition declined from upestuary regions to the mouth, and this decline was most pronounced in the James River estuary (Figure 5). In contrast, mean adjusted condition indices were similar among regions throughout the Chesapeake Bay main stem and coastal estuaries.

For juvenile Summer Flounder, the adjusted mean condition indices (\pm 95% confidence intervals) at the mouth of the James River estuary prior to migration were: mean $K = 0.88 \pm 0.02$, $\log(HSI) = 0.60 \pm 0.05$, and $(\text{subdermal lipid})^{-0.5} = 1.81 \pm 0.17$. Relative to this region, adjusted mean condition was highest in the coastal lagoons for all condition indices examined (Figure 6), therefore, habitats in coastal Virginia are of high

quality for juvenile Summer Flounder. In Chesapeake Bay, adjusted mean values of Fulton's K and subdermal lipids generally increased from upestuary regions toward the mouth of the rivers and into Chesapeake Bay proper, particularly for the Rappahannock and York River estuaries. No patterns were apparent in mean adjusted K and subdermal lipid estimates for Summer Flounder from the James River estuary and lower Chesapeake Bay regions (Figure 6), suggesting similarity in the effect of environmental conditions not measured in this study (e.g., prey availability and other features associated with high quality habitats). Similarly, no spatial patterns in mean adjusted HSI values were readily apparent for fish from Chesapeake Bay and the tidal tributaries (Figure 6). However, we observed high values of mean adjusted HSI for Summer Flounder from the coastal lagoons.

The effects of region of capture on mean condition indices of juvenile Striped Bass were similar after adjusting for environmental conditions (Figure 7). The adjusted mean condition indices (\pm 95% confidence intervals) for Striped Bass in the mouth of the Rappahannock River estuary during winter were: $K_n = 1.04 \pm 0.04$, $\log(\text{HSI}) = 1.66 \pm 0.20$, and subdermal lipid = 35.64 ± 6.56 . The data suggest that the head of the Rappahannock, York, and James River estuaries had a negative effect on mean HSI values in Striped Bass relative to the mouth of the Rappahannock River estuary (Figure 7), and that mean condition increased towards the mouths of these tributaries. However, variability was too high to permit detection of statistically-significant spatial patterns in mean HSI.

Discussion

Juvenile Atlantic Croaker, Summer Flounder, and Striped Bass displayed large variations in condition among estuaries in Virginia. Habitats that support individuals in high condition (e.g., Chesapeake Bay for Atlantic Croaker, coastal lagoons for Summer Flounder, and the mouth of the Rappahannock River for Striped Bass) may represent high value, essential habitats for these species. Habitats that supported juveniles in high condition were not necessarily continuous in space, and this was likely due to complex interactions among biotic and abiotic factors, some of which we were not able to measure. Local conspecific density typically had a larger effect on mean condition of fish, even though environmental characteristics (e.g., temperature, salinity, dissolved oxygen, and depth) directly influenced the condition of individuals in these areas. Spatial patterns in regional effects on juvenile fish condition were also apparent, and these reflect regional habitat characteristics not explicitly considered in the models (e.g., prey availability, substrate type, predator abundance, and habitat complexity). Ultimately, the spatial variation in mean condition of fish suggests that not all estuarine habitats equally prepare juveniles for physiologically stressful events such as offshore migrations (Atlantic Croaker and Summer Flounder) or survival during winter (Striped Bass).

Spatial models, such as the simultaneous autoregressive (SAR) models applied here, account for the lack of independence among fish from neighboring sites. With the exception of Striped Bass subdermal lipids, SAR models better explained patterns in condition than non-spatial models which ignore spatial correlations among observed condition indices. Because individuals from neighboring areas are exposed to similar physical, chemical, and biological conditions, spatial autocorrelation was addressed to permit identification of the potential effects of density-dependent and density-

independent factors on fish condition. The distances over which condition indices exhibited correlations differed by species and index, such that spatial autocorrelation was evident over a larger range of distances for condition indices for Summer Flounder, but over similar distances for condition indices for Atlantic Croaker. Differences in these critical distances among species and indices may result from movement of individuals among sites (but within regions) during the sampling period, variability in the abundance, distribution, and composition of prey resources, and the differential response of condition indices to changes in feeding rate and especially starvation (“latency”; Ferron and Leggett, 1994).

Assessment of habitat value for estuarine fishes requires consideration of multiple indices and large numbers of fish because energy allocation strategies may vary among individual juveniles (e.g., energy may be allocated to growth or storage; Post and Parkinson 2001; Sogard and Spencer 2004). The three species we examined represent two distinct life-history strategies and demonstrate variation in the manner in which energy is allocated among tissues in preparation for migration and winter. Because Atlantic Croaker and the majority of Summer Flounder typically migrate to the continental shelf before the onset of winter, the subdermal lipid index may best reflect habitat suitability because fish use these to provide energy for long-distance migration (Leonard and McCormick 1999; Chapter 3). Conversely, HSI may be used to assess the condition of juvenile Striped Bass during winter (Chapter 3). Inconsistent spatial patterns among condition indices, such as those observed for Summer Flounder and Striped Bass, suggest individuals residing in different habitats may use different energy-storage strategies prior to migration or winter. Variation in energy-storage strategies may occur

among contingents of a population due to differences in behavior among individuals (e.g., partial migration; Kerr and Secor 2009). Indeed, because Summer Flounder exhibit condition-dependent partial migration (Chapter 3), habitat quality may affect population-level processes by influencing energy allocation and behavioral strategies of individual fish.

The spatial distribution of individuals in high or low condition may provide insight on biotic and abiotic factors that influence habitat quality in temperate estuaries. We sampled estuaries throughout Virginia that are occupied by juvenile Atlantic Croaker, Summer Flounder, and Striped Bass to maximize the contrast in density and environmental factors among sampling sites, and thus to allow for detection of these effects. Conspecific density significantly affected spatial patterns in condition indices for all species-index combinations except Summer Flounder subdermal lipid and Striped Bass HSI and subdermal lipid. Moreover, the effect of conspecific density on mean condition surpassed that of all environmental parameters considered. Environmental factors considered in the models (i.e., temperature, salinity, dissolved oxygen, and depth), and those represented by the factor “region” were only of secondary importance. We note that many studies that examine spatial variation in condition, growth, or survival focus primarily on the influence of environmental factors. Yet we found that environmental factors were not as effective in describing variation in condition as was local density. Future field-based studies would benefit from a focus on density-dependent effects on fish condition and habitat quality assessment. Furthermore, laboratory experiments to examine the influence of environment on condition and vital rates must consider the

effect of fish density to permit inferences from laboratory-based observations to wild populations.

For Atlantic Croaker, similar spatial patterns among condition indices suggest that the energy allocation strategy of this species is invariant throughout Virginia estuaries. However, differences in mean condition among regions suggests that not all habitats are equal, and individuals occupying different habitats may be differentially prepared for migration (Litvin et al. 2014). For example, juvenile Atlantic Croaker in the northernmost regions of Chesapeake Bay that we sampled exhibited high mean condition indices, and thus, may be well prepared to migrate offshore. The major factor driving high condition of juvenile Atlantic Croaker in the upper Chesapeake Bay was conspecific density, and this factor exerted a negative effect on condition. Intraspecific competition resulting from high densities decreases individual food consumption, and leads to reduced growth in fish (Post et al., 1999; Amundsen 2007; Searcy et al. 2007). Similarly, reduced food consumption leads to reduced protein and lipid stores (Bar 2014), and is expected to result in low values of K, HSI, and subdermal lipids. We observed high densities of Atlantic Croaker in the lower James River estuary and southwest region of Chesapeake Bay, areas which contain individuals in poor condition that may be less prepared for migration. As a result, these habitats in southern Virginia may represent nursery areas of low recruitment to the adult population despite the high density of juveniles. We suggest that the spatial scale of sampling should be expanded to encompass the full range of the species to compare habitat quality of Virginia estuaries with that of other temperate estuaries along the eastern US coast.

We observed juvenile Atlantic Croaker with high condition indices from habitats that supported low densities of this species, and these habitats were generally warmer, more saline, and had higher dissolved oxygen levels than other habitats examined. High dissolved oxygen levels permit higher metabolic scope, such that individuals residing in high oxygen habitats are able to allocate a greater amount of energy to other fitness-related activities (e.g., energy storage; Claireaux et al. 2000; Craig and Crowder 2005). Alternatively, dissolved oxygen may reflect other factors, such as primary production (Odum 1956; Kemp and Boynton 1980), that positively influence condition of Atlantic Croaker. Warm temperatures (~24°C) promote rapid growth of juvenile Atlantic Croaker (Searcy et al. 2007), and as seen here, warm temperatures may also promote high condition. In the laboratory, small juveniles (10-20 mm) grow more slowly in high salinity water (20 psu) than low salinity water (5 psu) (Peterson et al. 1999), yet our field-based observations indicated that wild juveniles > 100 mm TL exhibited high condition in areas of high salinity (up to 30 psu). The inconsistent relationships between energy allocation (growth or storage) and environmental factors (temperature and salinity) suggest that complex interactions among environmental factors may ultimately affect energy allocation strategies and thus, spatial patterns in condition indices.

With the effect of density and environmental factors held constant among regions, adjusted mean condition (as measured by all condition indices examined) of juvenile Atlantic Croaker appears to decline from upestuary regions to the eastern side of Chesapeake Bay. This pattern suggests that upestuary habitats provide resources (e.g., high prey abundance and refuge from predation) that result in high mean condition. Juvenile Atlantic Croaker in upestuary habitats may be exposed to energy-rich prey

resources that are less available in downstream regions, or reduced predation pressure in these regions may allow for more frequent feeding activity. Low predation stress (and other stressors) may also result in individuals that incur low metabolic costs and therefore have more energy to commit to storage or growth. This gradient in condition of Atlantic Croaker was discernable in the York and Rappahannock River estuaries, but was most evident in the James River estuary. The rate of freshwater discharge in the James River is four to seven times higher than in the York and Rappahannock Rivers, and this difference in discharge rates affects the fish community structure within these estuaries (Tuckey and Fabrizio 2013). The observed pattern in Atlantic Croaker condition suggests that the environmental gradient in the James River estuary also structures predator and prey communities in a manner that has a strong positive effect on mean condition of juvenile Atlantic Croaker in upestuary habitats.

For Summer Flounder, spatial patterns among condition indices suggest that energy allocation strategies during the months prior to offshore migration are inconsistent among regions, implying that preparedness for migration varies spatially as well. For example, juvenile Summer Flounder in the downestuary regions of the Rappahannock River had lower mean subdermal lipid and K values, but higher mean HSI values relative to most other regions. Assuming that subdermal lipids reflect preparedness for migration and HSI reflects preparedness for winter (Chapter 3), the observed patterns in mean condition of individuals from the lower Rappahannock River estuary indicate that this contingent of the population may be more likely to remain in the estuary during winter. Indeed, in other species that exhibit partial migration, the resident contingent exhibited lower consumption and growth rates than their migratory counterparts (e.g., White Perch

Morone americana, Kerr and Secor 2009). We propose that habitats that support Summer Flounder with low mean K and subdermal lipid values may be low quality nurseries for this species, and contingents utilizing these habitats may consist of individuals that are more likely to reside in the estuary during winter than to participate in offshore migrations. Conversely, mean subdermal lipid estimates were generally higher for regions on the eastern side of the Chesapeake Bay than for regions on the western side or in the tidal tributaries, suggesting these individuals are well prepared for migration.

Virginia's coastal lagoons support juvenile Summer Flounder in relatively high mean condition. These habitats also support high densities of Summer Flounder (Wyanski 1990) and are, therefore, important high-quality nursery areas. Similar to Atlantic Croaker, the effect of conspecific density on Summer Flounder condition was greater than that of the environmental factors we considered. But unlike Atlantic Croaker, Summer Flounder exhibited a positive influence of density on mean K and HSI values (i.e., locations such as the coastal lagoons that support dense aggregations of juvenile Summer Flounder also supported fish in relatively high mean condition). A positive influence of density has been observed in a group-living fish species that inhabit artificial reefs (Yeager et al. 2014); factors such as an improved ability to locate resources or increased feeding time due to reduced predation risks are thought to account for the observed effects of density on condition. In our study, the positive effect of density may also be attributed to individuals aggregating in areas with abundant prey resources, such as mysid shrimp, which are important prey for juvenile Summer Flounder in Virginia (Latour et al. 2008; Buchheister and Latour 2011). Unfortunately, little information is available for the spatial distribution and relative abundance of mysid shrimp. This makes

it difficult to associate this important prey resource with high-quality habitats that support well-conditioned Summer Flounder.

Our observation of high densities of juvenile Summer Flounder in coastal lagoons is consistent with other studies that suggest the distribution of juvenile Summer Flounder is influenced by salinity, such that individuals preferentially occupy high salinity marsh habitats (Packer and Hoff 1999; Sackett et al. 2008). Yet, we observed a negative influence of salinity on mean subdermal lipid and K values for this species. We suspect the negative effect of salinity is attributed to the high condition of Summer Flounder in the low salinity, upestuary regions of the tributaries (regional means ~ 12 psu). Lab-based (Malloy and Targett 1991) and field-based (Nys 2014) studies suggest no significant effect of salinity (10-30 psu) on feeding and growth of juvenile Summer Flounder. Condition indices for Summer Flounder may be more sensitive to osmoregulatory stress than growth, thus the effect of salinity detected in our study may not be detected had we examined growth. Alternatively, salinity may be correlated with other beneficial factors that were not examined (e.g., high prey availability and reduced predator abundance).

Few factors investigated here described a significant amount of variation in mean condition of juvenile Striped Bass in Virginia estuaries during winter and spring. No significant effects of environmental characteristics were detected on subdermal lipid content, which was invariant among sampling regions. Although depth affected mean K_n , the negative effect of density was much greater than the effect of depth. Similar to Atlantic Croaker, intraspecific competition resulting from high densities is expected to decrease food consumption, and thus, condition. When depth and density were held constant, no interpretable patterns in adjusted mean K_n were observed among regions.

Therefore, we propose that neither K_n nor subdermal lipids are suitable condition indices to assess habitat quality for juvenile Striped Bass in Virginia estuaries from November to June.

The hepatosomatic index may be a reliable indicator of habitat quality for Striped Bass because it reflects preparedness for winter (Chapter 3). However, environmental factors had relatively weak effects on mean HSI compared with the effect of region of capture, and estimates of regional effects exhibited high variability. Large confidence intervals around estimates of mean adjusted condition suggest that additional abiotic and biotic factors (that were not included in the models) are needed to describe variability in condition among Striped Bass from neighboring sampling sites. Alternatively, a low proportion of the sites that we sampled exhibited high densities of Striped Bass, and thus, our study did not fully characterize areas with high density (> 2 individuals per 100 m^2). As a result, the relationship between mean condition and local density may have been poorly described for this species.

Our results show that multiple condition indices, when considered simultaneously, may be used to infer quality of nursery habitats in temperate estuaries, and that density-dependent effects have a stronger impact on mean fish condition than do environmental effects. Spatial patterns in juvenile fish condition were especially apparent for Atlantic Croaker and Summer Flounder, and the strong influence of conspecific density on condition of these species suggests that the value of habitats depended on the degree to which individuals aggregate. Furthermore, environmental conditions in Chesapeake Bay exhibit decadal oscillations (Austin 2002), such that the temporal stability of nursery-habitat quality may not be constant. Environmental changes and

fluctuations in annual productivity (i.e., density) of fish stocks are expected to influence the quality of nursery habitats, but the rate at which habitat quality may change is unknown. Nursery habitats in estuaries of Virginia may not be equally favorable for recruits, and assessing patterns in juvenile fish condition will further our understanding of habitat quality and fish productivity.

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Table 1. Neighborhood distances and weighting styles used to develop spatial weights matrices among sampling sites. Distances were measured in kilometers. Binary weighting refers to sites being included as neighbors or not, and variance stabilization balances multiple weighting schemes (see Tiefelsdorf et al. 1999). No distance or style is specified for Striped Bass subdermal lipid because a non-spatial model best described condition measured by this index.

Condition index	Atlantic Croaker		Summer Flounder		Striped Bass	
	Distance	Style	Distance	Style	Distance	Style
Length-based	6.66	Binary	6.66	Binary	13.32	Variance stabilization
Hepatosomatic	7.77	Binary	1.11	Binary	8.88	Variance stabilization
Subdermal lipid	6.66	Binary	16.66	Variance stabilization	NA	NA

Table 2. Environmental effects on the mean condition of juvenile fishes as measured by length based indices (Fulton's K or K_n), the hepatosomatic index (HSI), and relative subdermal lipids. All estimates were significant ($P < 0.05$) unless indicated by footnotes. Environmental factors that did not explain variation in the condition indices are denoted by NS.

	Atlantic Croaker			Summer Flounder			Striped Bass		
	K	HSI	Lipid	K	HSI	Lipid	K_n	HSI	Lipid
Water temperature ($^{\circ}$ C)	0.007 (0.003)	-0.035 (0.010)	0.113 (0.029)	0.008 (0.002)	0.034 (0.006)	-0.079 (0.023)	NS NS	-0.072 (0.015)	NS NS
Dissolved oxygen (mg / L)	0.011 (0.006)	NS NS	0.117 (0.055)	NS NS	0.014* (0.02)	NS NS	-0.005* (0.006)	-0.067 (0.047)	NS NS
Salinity (ppt)	0.016 (0.003)	0.019 (0.010)	0.132 (0.028)	-0.015 (0.003)	NS NS	-0.128 (0.038)	NS NS	-0.023 (0.013)	NS NS
Depth (m)	NS NS	NS NS	0.026 (0.018)	0.003 (0.002)	NS NS	0.027 (0.021)	0.006 (0.004)	NS NS	NS NS
Density (individuals / m^3)	-0.214 (0.084)	-0.499 (0.260)	-0.871 (0.722)	2.731 (1.684)	10.963 (4.561)	NS NS	-1.258 (1.242)	NS NS	-115.9* (131.1)

* $P > 0.05$

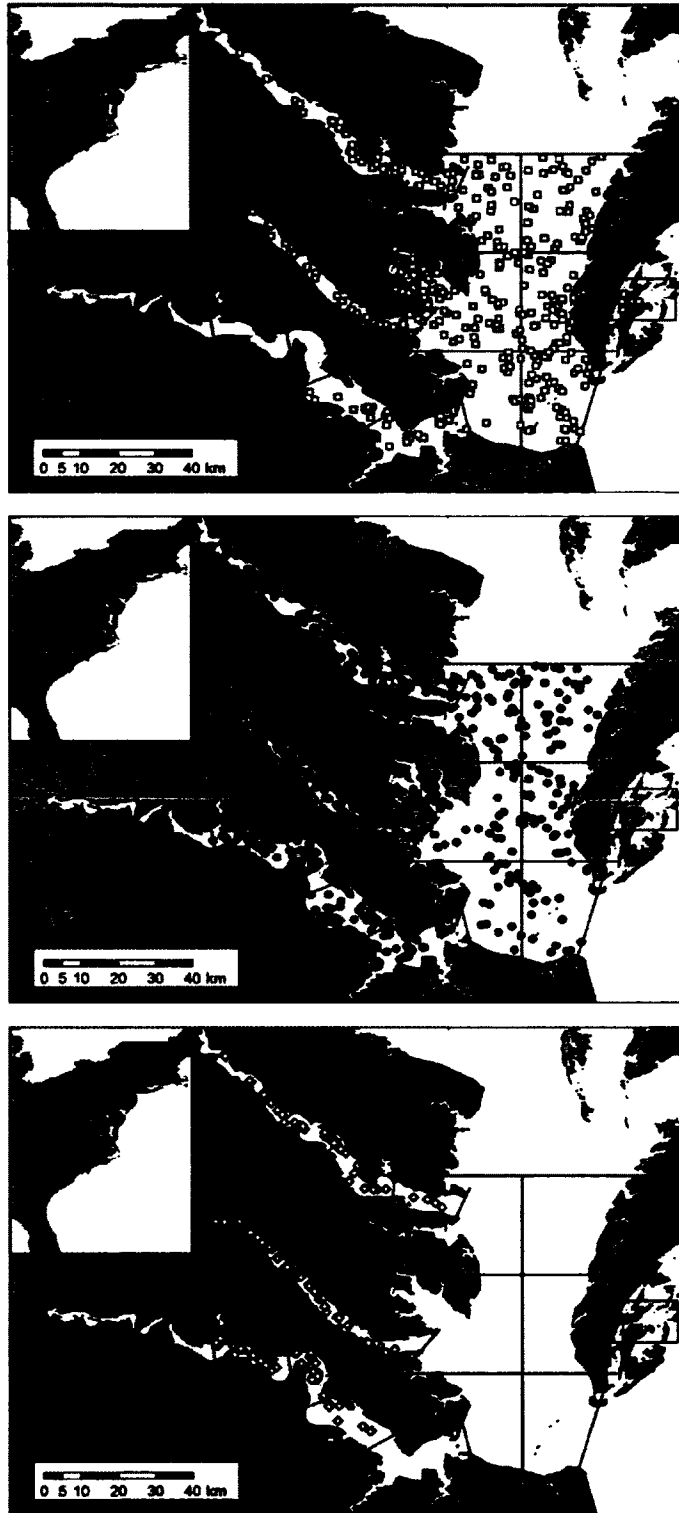


Figure 1. Sampling locations in Virginia used to collect Summer Flounder (square; $n = 484$), Atlantic Croaker (circle; $n = 650$), and Striped Bass (diamond; $n = 211$) for analysis of geographic patterns in condition. Solid lines denote region borders.

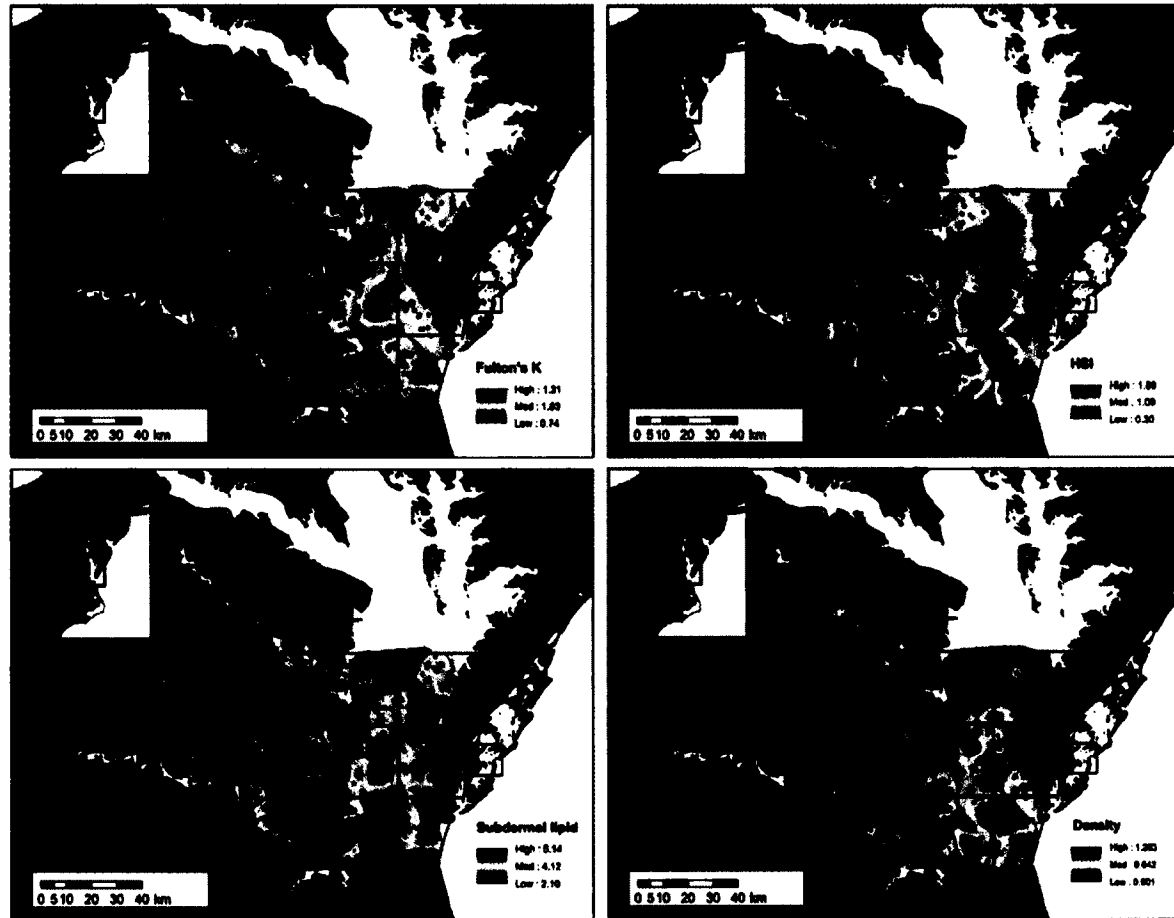


Figure 2. Geographic patterns in condition of juvenile Atlantic Croaker from June to September (2011-2013). Condition was measured by A) Fulton's condition factor, B) the hepatosomatic index (HSI), and C) subdermal lipid estimates from the fatmeter. Red denotes high mean condition, and blue denotes low mean condition. Panel D shows patterns in density (individuals/m²) of Atlantic Croaker from sampled sites (circles). Images were made with inverse distance weighting in ArcGIS, and interpolated over neighborhood distances determined by the "best" spatial weights matrices (Table 1; a distance of 0.7 was used for density) and a power of 1.5. Solid lines denote spatial region borders.

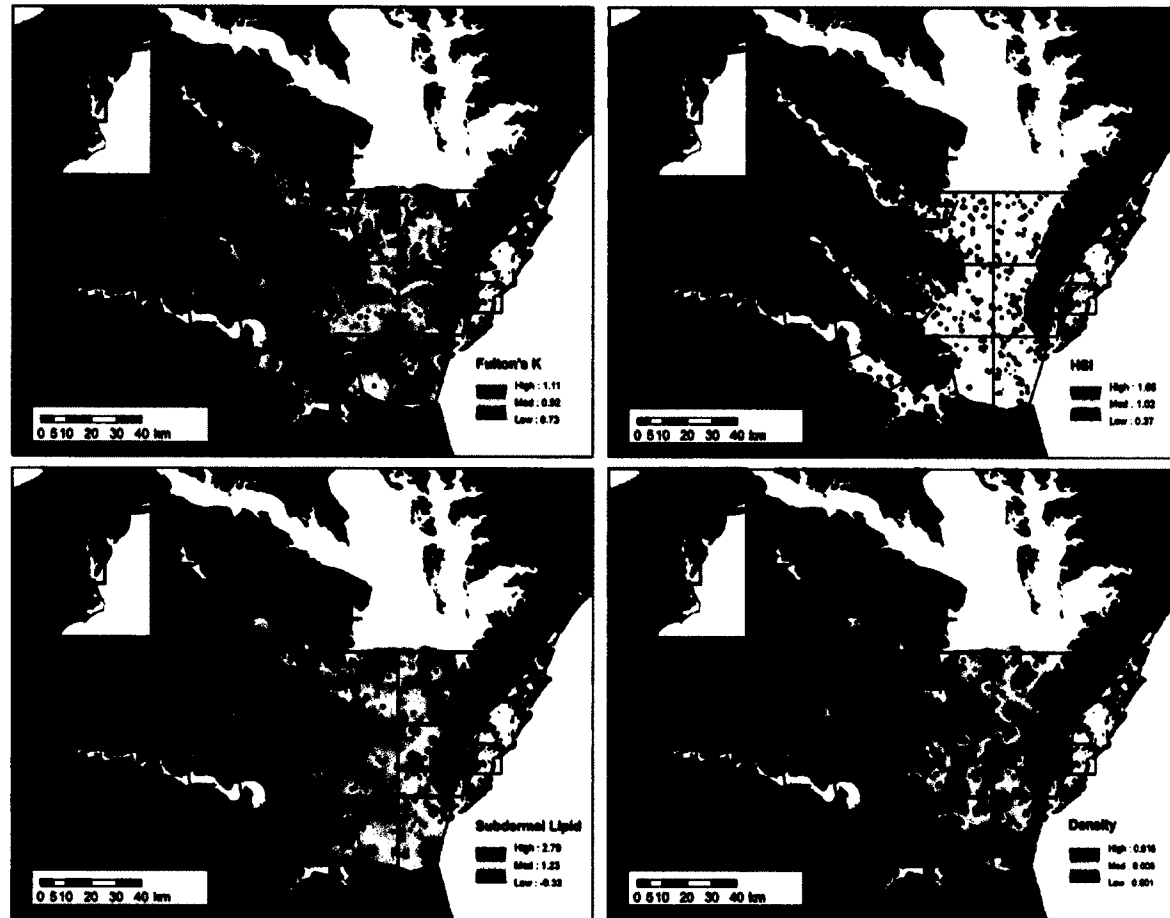


Figure 3. Geographic patterns in mean condition of juvenile Summer Flounder from July to October (2011-2013). Condition was measured by A) Fulton's K, B) the hepatosomatic index (HSI), and C) subdermal lipid estimates from the fatmeter. Red denotes high mean condition, and blue denotes low mean condition. Panel D shows patterns in density (individuals/m²) of Summer Flounder from sampled sites (circles). Images were made with inverse distance weighting in ArcGIS, and interpolated over neighborhood distances determined by the "best" spatial weights matrices (Table 1; a distance of 0.7 was used for density) and a power of 1.5. Solid lines denote spatial region borders.

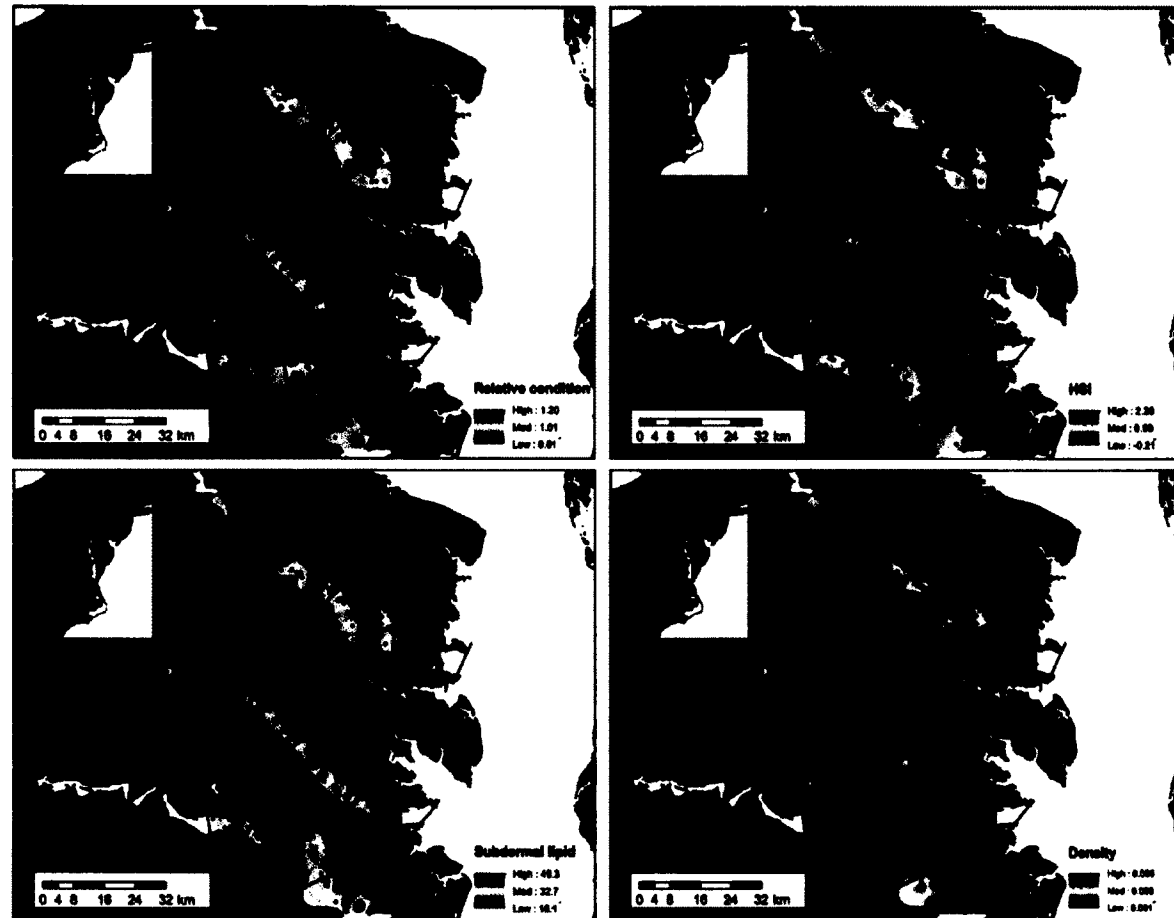


Figure 4. Geographic patterns in mean condition of juvenile Striped Bass from November to June (2010-2013). Condition was measured by A) relative condition, B) the hepatosomatic index (HSI), and C) subdermal lipid estimates from the fatmeter. Red denotes high mean condition, and blue denotes low mean condition. Panel D shows patterns in density (individuals/m²) of Striped Bass from sampled sites (circles). Images were made with inverse distance weighting in ArcGIS, and interpolated over neighborhood distances determined by the “best” spatial weights matrices (Table 1; a distance of 0.7 was used for density) and a power of 1.5. Solid lines denote spatial region borders.

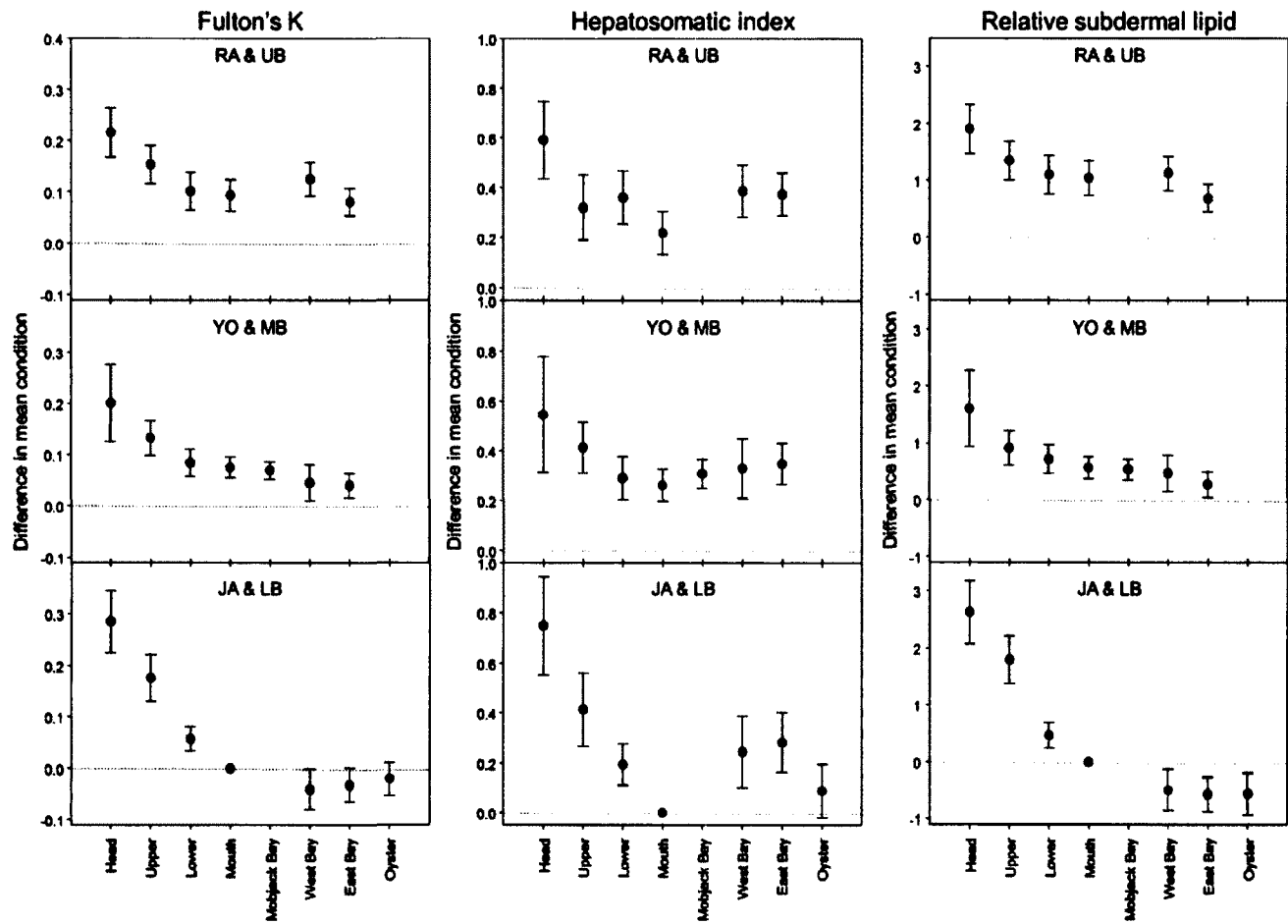


Figure 5. The effect of region on mean condition of juvenile Atlantic Croaker, expressed as the difference in mean condition (\pm 95% confidence interval) from the mouth of the James River estuary (dotted lines), the base region used in the model; environmental and density effects were held constant. Regions on the x-axis are organized from west to east, starting at most upestuary region, and the panels are organized from north to south: RA & UB = Rappahannock River and upper Chesapeake Bay, YO & MB = York River and middle Chesapeake Bay, JA & LB = James River and lower Chesapeake Bay.

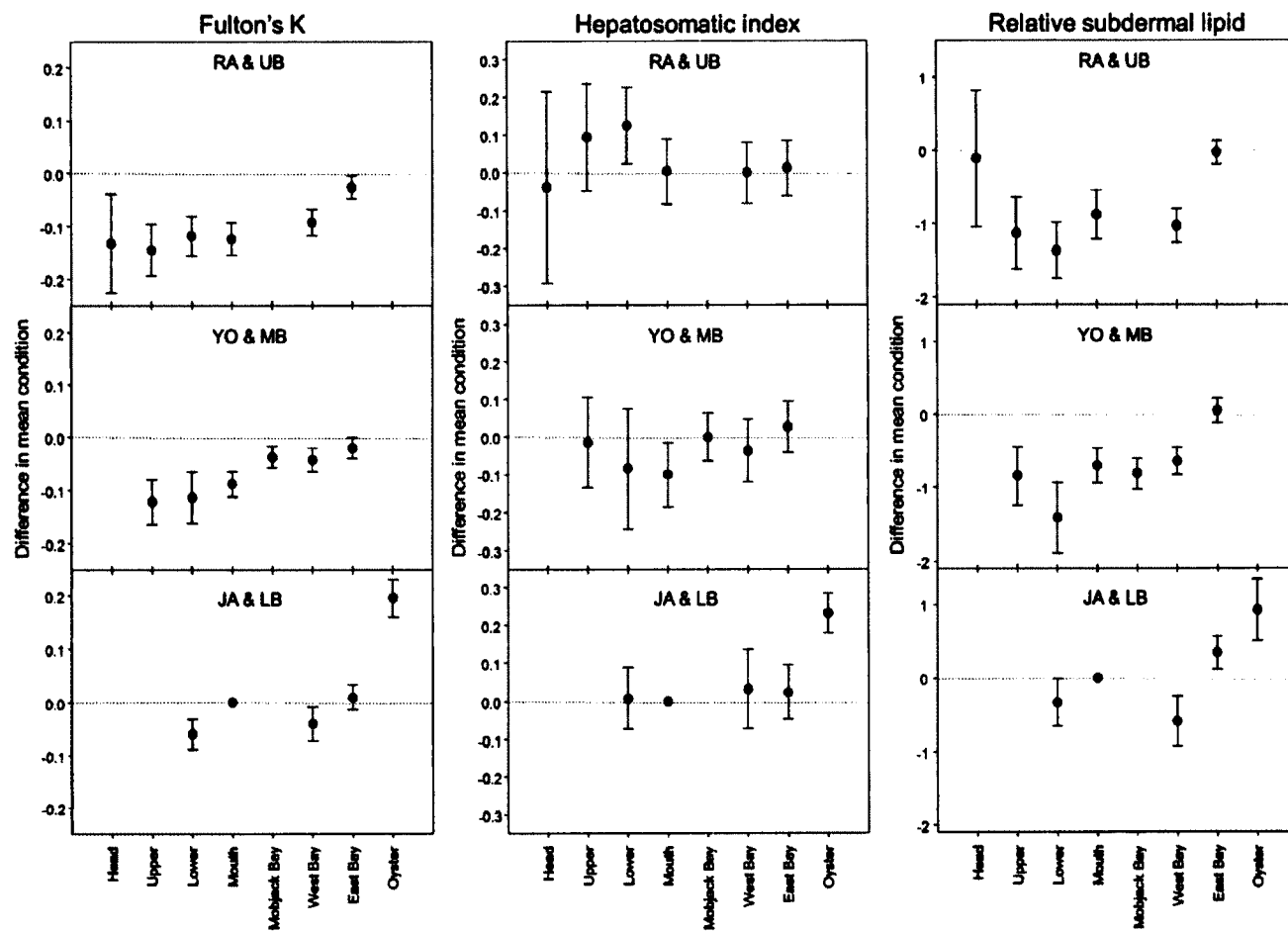


Figure 6. The effect of region on mean condition of juvenile Summer Flounder, expressed as the difference in mean condition (\pm 95% confidence interval) from the mouth of the James River estuary (dotted lines), the base region used in the model; environmental and density effects were held constant. Regions on the x-axis are organized from west to east, starting at most upestuary region, and the panels are organized from north to south: RA & UB = Rappahannock River and upper Chesapeake Bay, YO & MB = York River and middle Chesapeake Bay, JA & LB = James River and lower Chesapeake Bay. No Summer Flounder were collected at the head of the York River estuary, or in the head and upper region of the James River estuary.

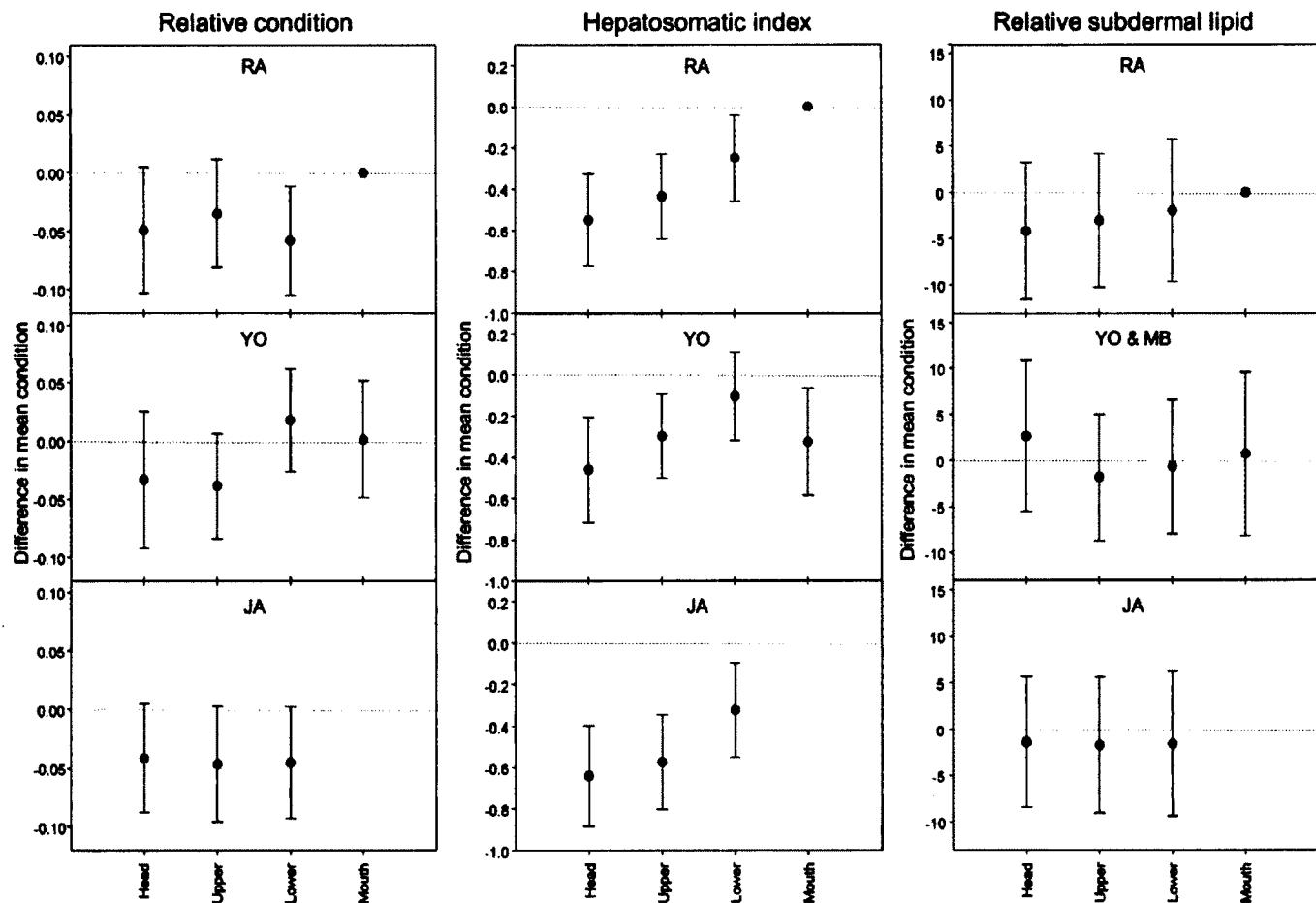


Figure 7. The effect of region on mean condition of juvenile Striped Bass, expressed as the difference in mean condition (\pm 95% confidence interval) from the mouth of the Rappahannock River estuary (dotted lines), the base region used in the model; environmental and density effects were held constant. Regions on the x-axis is organized from west to east, starting at most upestuary region, and the panels are organized from north to south: RA = Rappahannock River, YO = York River, JA = James River. No Striped Bass were collected in the mouth of the James River estuary.

CHAPTER 5

**Does Condition Mediate Thermal Stress of Juvenile Fishes during Winter Residence in
Temperate Estuaries?**

Abstract

For some fishes, unfavorable conditions during winter may result in the mortality of juveniles residing in temperate estuaries, and individuals in poor condition may be particularly susceptible to mortality. To determine the role of condition in mediating mortality associated with prey scarcity and thermal stress, we experimentally simulated winter conditions (e.g., reduced feeding opportunities and cold temperatures) that juvenile Atlantic croaker, summer flounder, and striped bass experience in temperate estuaries. Fish condition was measured weekly using length-based indices and non-lethal subdermal lipid estimates. Stress was induced by weekly, acute temperature declines for up to 17 weeks, and stress was assessed from observed behavioral responses, namely, poor swimming and the inability to maintain orientation. As expected, mean condition of Atlantic croaker and striped bass declined rapidly due to reduced feeding and thermal stress. For summer flounder, mean Fulton's condition factor remained constant for several weeks before declining and mean subdermal lipid content increased during the winter trial period. However, weekly changes in condition only influenced thermal stress for striped bass, and striped bass that survived the onset of winter had a low probability of exhibiting stress. The probability that an individual Atlantic croaker exhibited thermal stress increased with duration of exposure to simulated winter conditions, and summer flounder that had a blood flagellate *Trypanoplasma bullocki* infection were more likely to exhibit stress than conspecifics that were not infected. Contrary to expectations, individual Atlantic croaker and summer flounder with high subdermal lipids entering winter were more likely to exhibit thermal stress, which suggests these species were energetically prepared for migration, and not for residency in temperate estuaries during winter. Species-specific responses to winter conditions and thermal stress reflect different physiological tolerances and life-history strategies.

Introduction

Mortality during winter can be an important process acting on the juvenile stage of some temperate fishes and a key factor in understanding annual recruitment variability for temperate species (Sogard 1997; Sutton and Ney 2001). Winter mortality may be due to predation, starvation, disease, or the inability to maintain internal homeostasis in the face of changing environmental conditions (e.g., temperature, salinity, etc.), or a combination of these factors (Hurst et al. 2000; Hurst 2007; Slater et al. 2007). To maintain homeostasis during winter, temperate species use lipid stores when coping with prey scarcity and reduced feeding opportunities (Hurst 2007). Lipid content also mediates cold temperature tolerance of fish, such that cold tolerance increases with high concentrations of long-chain highly unsaturated fatty acids (Craig et al. 1995; Kelly and Kohler 1999). Individuals with sufficient lipid reserves to meet their metabolic demands may exhibit lower mortality when exposed to winter conditions than individuals with insufficient lipid reserves (Henderson et al. 1988; Thompson et al. 1991). Indeed, mortality risk may be best predicted by lipid content determined by proximate composition analysis (Simpkins et al. 2003), but this requires sacrificing individuals. Unfortunately, the ability of nonlethal condition indices (that reflect lipid content) to assess mortality risk of individual fish exposed to cold temperature and reduced feeding opportunities has not been thoroughly examined for estuarine species.

When prey scarcity and reduced feeding opportunities persist for an extended period of time, fish may exhibit rapid mass loss and depleted energy reserves (Bar 2014). Because of this, energetic preparedness influences winter mortality of individuals, particularly at the northern extent of their range (Hurst 2007). Cohorts of juvenile fish

that are able to maximize energy storage prior to the onset of winter have a higher survival advantage than those that maximize somatic growth (Post and Parkinson 2001). Similarly, early lipid reserve formation is critical for survival in higher latitudes, where growing seasons are short and winters may be severe (Conover et al. 1997; Hurst and Conover 2003). For example, early-spawned sand smelt (*Atherina presbyter*) develop large fat reserves, whereas later-spawned, smaller individuals have insufficient fat reserves to survive normal winters (Henderson et al. 1988). Similarly, small Colorado pikeminnow (*Ptychocheilus lucius*) have particularly low survival when they are deprived of food during winter due to depressed lipid and energy reserves (Thompson et al. 1991).

The amount of stored energy required to withstand winter conditions is likely to vary by species, particularly for species with different life-history strategies. Juvenile summer flounder (*Paralichthys dentatus*), Atlantic croaker (*Micropogonias undulatus*), and striped bass (*Morone saxatilis*) have life-history strategies with varying degrees of residency in temperate estuaries during winter. Post-larval Atlantic croaker reside in mid-Atlantic coastal estuaries during winter, but the majority emigrate to the shelf prior to winter at the end of their first year of life (i.e., as juveniles). The influence of winter conditions on survival of post-larval Atlantic croaker has been investigated (Lankford and Targett 2001; Hare and Able 2007), but the influence of winter conditions on survival of juvenile Atlantic croaker has received little attention. During winter, all juvenile (age-0) striped bass reside in coastal estuaries like Chesapeake Bay. Juvenile summer flounder are facultative winter residents of coastal estuaries (Chapter 3); estuarine residents likely have insufficient subdermal lipid reserves to migrate to the continental shelf (Chapter 3). Individuals that reside in Chesapeake Bay during winter

must be energetically prepared to endure food deprivation and low temperatures, because exposure to these environmental stressors can have detrimental effects on metabolism, growth, disease resistance, and ultimately survival (Barton et al. 2002). For example, temperatures below 5°C greatly increase the mortality rate of post-larval Atlantic croaker (Lankford and Targett 2001); temperatures below 2-3°C increase the mortality rate of juvenile summer flounder (Malloy and Targett 1991); and temperatures below 2°C increase the mortality rate of juvenile striped bass (Hurst and Conover 2002). Chesapeake Bay waters may exhibit temperatures as low as 1-2°C during winter, and average less than 5°C throughout January and February (Cronin et al. 2003). Fish condition may influence the ability of individuals to endure physiologically stressful conditions during winter. By examining the survival consequences of variation in condition among individuals, we aim to provide a better understanding of factors that affect survival during a fish's first year of life (Bertram et al. 1997).

We experimentally simulated the cold temperatures and reduced feeding opportunities during winter to examine the effect of winter conditions and changes in energy reserves on the risk of thermal stress for juvenile fishes. Acute thermal stress was elicited by weekly, short-duration (5 hr) declines in temperature ($< 1^{\circ}\text{C hr}^{-1}$). We used non-lethal length-based indices and estimates of subdermal lipid content, which approximate lipid and energy content of whole fish (Chapter 1), to measure the condition of fish exposed to winter conditions for up to 17 weeks. Individuals that are exposed to cold temperatures for long time periods without feeding likely endure chronic stress. These indices appropriately assess declines in condition of individual fish because length-

based condition indices and the subdermal lipid layer decrease during starvation (Maddock and Burton 1994).

Methods

All fish capture, handling, and euthanasia procedures were approved by the College of William and Mary Institutional Animal Care and Use Committee (protocol #IACUC-2010-07-20-6788-mcfabr) and followed all applicable state and federal guidelines and regulations.

We collected 44 juvenile summer flounder and 40 juvenile Atlantic croaker from the Virginia portion of Chesapeake Bay, and 40 juvenile striped bass from the York and Rappahannock River estuaries with a 9.14-m otter trawl (Figure 1). Atlantic croaker were collected on 25 Oct 2012, summer flounder on 26 Nov 2012 and 27 Nov 2012, and striped bass on 10 Dec 2013 and 12 Dec 2013. Individuals were returned to the laboratory and measured for relative subdermal lipid content with the Distell fish fatmeter (model FFM-992). Fatmeter measurements were taken from the midline of the body behind the opercle. For each individual, we used four measurements from the left side of striped bass, four measurements from the non-eyed side of summer flounder, and an average of four readings from each side of Atlantic croaker (8 readings total) to assess relative subdermal lipid content of an individual fish. For these species, measurements from these locations are highly correlated with energy content as measured by bomb calorimetry (Chapter 1). Accuracy of the fatmeter varies among measurements and depends on lipid content of the fish: accuracy is reported as $\pm 1.5\%$ for lipid content between 2-15 %; \pm

2.5 % for lipid content between 16-30 %; and ± 4 % for lipid content > 31 %

(Anonymous 2010).

Length was measured to the nearest millimeter (total length for Atlantic croaker and summer flounder, fork length for striped bass), and wet mass was measured to the nearest 0.1 mg with a Mettler Toledo XA204 balance. We examined Atlantic croaker between 150 and 202 mm, summer flounder between 143 and 297 mm, and striped bass between 119 and 180 mm. The length-based indices that suitably represent energy content as measured by bomb calorimetry for these species are Fulton's condition factor (K) for summer flounder and Atlantic croaker, and relative condition (K_n) for striped bass (Chapter 1). Therefore, Fulton's condition index (K) was calculated for summer flounder and Atlantic croaker using:

$$K = W * L^{-3} * 10^N \quad (1)$$

where W = mass (g), L = length at capture (mm), and N = an integer to bring the value of K near 1 (Ricker 1975). Relative condition factor (K_n) was calculated for striped bass using:

$$K_n = W * (aL^b)^{-1} \quad (2)$$

where a and b are constants describing the allometric relationship for fish in a given population (Le Cren 1951). Constants for equation (2) were estimated using nonlinear least-squares modeling with the Nlin procedure in SAS (version 9.3, SAS Institute, Cary NC; Chapter 1).

Cold-tolerance trials

After capture, individuals were fin clipped for identification and maintained in recirculating (300 L) aquaria at ambient York River salinity (24 to 28 psu for Atlantic croaker and summer flounder, 18 to 20 psu for striped bass); individuals were held for a minimum of one-week before cold-tolerance trials began. To mimic winter conditions in the field, we adjusted temperatures weekly to reflect the weekly average winter temperature of waters from which individuals were observed during winter months from 2004 to 2014 (M. Fabrizio, *unpublished data*; Figure 2). Therefore, temperature declined weekly in aquaria. Temperatures were held constant once weekly average water temperatures reached 5°C; this temperature was chosen because it is above the lethal temperature for these species, but below the temperature at which feeding and growth occurred (6°C for Atlantic croaker, Johnson 1978; 6°C for summer flounder, Malloy and Targett 1991; 7°C for striped bass, Wawronowicz and Lewis 1979). Food was withheld from half the individuals to simulate reduced feeding opportunities. The remainder were offered food twice weekly, but most individuals did not feed. The lack of feeding was confirmed by modeling the influence of food presence or absence on changes in condition and probability of stress.

Relative subdermal lipid estimates and mass were measured on all individuals the day prior to each trial. Length was not measured because caudal fin tissue was cannibalized by conspecifics; thus, weekly length-based indices of condition were calculated using length at capture and mass on the day prior to the trial. External injuries were also noted weekly, particularly for summer flounder, which exhibited symptoms of infection with a blood flagellate *Trypanoplasma bullocki* common to this species during winter (Burreson 1982). Trials commenced on 6 Nov 2012 for Atlantic croaker, 7 Dec

2012 for summer flounder, and 17 December 2013 for striped bass. We employed weekly short-duration trials because exposure to high-frequency cold events was expected to result in observable thermal stress responses (Anderson and Scharf 2014). Weekly intervals also allowed for measurable changes in condition as well as recovery from stress among trials.

Before each cold-tolerance trial, water temperature was slowly decreased from ambient to a set temperature ($\pm 0.5^{\circ}\text{C}$) at which the trial began: 5°C for Atlantic croaker, 4°C for summer flounder, and 3.5°C for striped bass. Preliminary trials indicated that these starting temperatures were required for individuals to exhibit a stress response during a trial. Because acclimation temperature plays a large role in cold tolerance (Beitinger and Bennett 2000), the magnitude of the difference between ambient temperature and water temperature at the start of the trial was recorded (hereafter referred to as Δ_{temp}). At the start of each trial, temperatures were set to decline rapidly during a 5 hour period. The rate of decline started at $\sim 1^{\circ}\text{C hr}^{-1}$, as described in Pangle et al. (2005), and thereafter, the rate of decline varied due to differences in ambient water temperature (Figure 3). Because temperature declined most rapidly during the first hour of the trial, we calculated T_{slope} , or the average rate of decline during the first hour. Because we wanted to test individuals repeatedly, we used a non-lethal endpoint that is associated with mortality to assess thermal stress (Davis 2010). During each trial, individuals were observed at 15-minute intervals for signs of reflex impairment, as evidenced by an inability to maintain appropriate orientation, reduced swimming ability, fin erection, or mouth gaping (Davis 2010). Individuals exhibiting two or more of these reflex impairments were considered stressed (Davis 2010), and removed from the trial. Because

flounder are relatively sedentary and remain motionless on the bottom of the tank, reflex impairment was assessed by gently flipping individuals onto their eyed side every 30 minutes. An individual that did not orient itself within 10 seconds after flipping was considered stressed and removed from the trial. All fish removed from the trial were placed in individual aquaria and gradually warmed to ambient temperature.

Individuals unable to recover from thermal stress (after being warmed to ambient temperature) were likely to succumb to mortality and were humanely euthanized by immersion in an ice slurry. Condition of euthanized individuals was assessed by length-based indices, subdermal lipid estimates, and the hepatosomatic index (HSI). HSI was calculated using:

$$\text{HSI} = W_{\text{liver}} * W^{-1} * 100 \quad (3)$$

where W_{liver} = wet mass of liver (g) and W = wet body mass (g) (Jensen 1979).

Individuals that survived until the termination of trials were euthanized and their final condition measured as before. The weekly trials for Atlantic croaker were terminated on 5 Feb 2013, when remaining individuals succumbed to thermal stress. Weekly trials were terminated on 3 April 2014 for striped bass and on 28 Mar 2013 for summer flounder, when ambient York River water temperatures exceeded 10°C, indicating increasing temperature and spring conditions.

Statistical analyses

Condition dynamics during winter

We used linear models with repeated measures to describe the decline in condition of juvenile Atlantic croaker, summer flounder, and striped bass held in the lab

and exposed to declining temperatures. To compare changes in condition among species, we modeled mean condition using time (week) since commencement of trials rather than calendar date. Because small individuals may exhaust lipid reserves faster than larger individuals, we included length at capture in the model (Johnson and Evans 1990; Biro et al. 2004). Subdermal lipid estimates for Atlantic croaker were log-transformed to meet the linear model assumption of homogeneous variances. To model the correlation between consecutive condition assessments from the same individuals, we considered multiple variance-covariance structures (e.g., compound symmetry, auto-regressive, banded Toeplitz, and unstructured), and selected the model with the lowest Akaike information criterion corrected for small samples (AIC_c; Burnham and Anderson 2002). An auto-regressive variance-covariance matrix was selected for all models except for declines in subdermal lipids in striped bass, for which a compound symmetry matrix was selected. An auto-regressive variance-covariance structure suggests that estimates of condition which are made closer in time are more highly correlated than estimates made farther apart in time, whereas compound symmetry suggests that estimates of condition are equally correlated regardless of when condition was measured.

The repeated-measures model used to describe the decline in observed condition in juvenile fishes was:

$$Y_{ij} = \mu + \alpha_i + \beta + \delta + \gamma + \rho + \varepsilon_{ij} \quad (3)$$

where Y_{ij} = the condition of individual j in week i as measured by length-based indices or the fatmeter;

μ = the intercept, representing the overall mean condition of all individuals;

α_i = the fixed effect of week i (time since commencement of trials);

β = the fixed effect of condition at capture;
 δ = the fixed effect of length at capture;
 γ = the fixed effect the presence of food;
 ρ = the fixed effect the effect of the presence of infection symptoms (for summer flounder only);
 ε_{ij} = the random unexplained error for individual j in week i , assumed to be independent and normally distributed.

Models for repeated measures of condition were implemented with the Mixed procedure in SAS (version 9.3, SAS Institute, Cary NC) using the Kenward-Roger method for calculating degrees of freedom (Kenward and Roger 1997). The final model used to explain declines in condition was selected based on minimum AIC_c. Models within two AIC_c units are expected to provide similar estimates of the response (Burnham and Anderson 2002), so the simplest model (i.e., the model with the fewest factors) within two AIC_c units of the lowest value was selected as the final model. A spline was fit to the least-squares means estimates from the final model to depict declines in condition through time.

Thermal stress induced by winter conditions

We used logistic regression with repeated measures to assess the weekly mean probability of observing a thermal stress response induced by winter conditions for juvenile summer flounder, Atlantic croaker, and striped bass. We hypothesized that stress was associated with the amount of time an individual was exposed to cold temperatures, the presence or absence of a thermal stress response in the previous trial, the presence or

absence of food, temperature patterns during each trial (Δ_{temp} and T_{slope}), and the condition of an individual at capture and at the time of the trial; these factors were included as fixed effects in the logistic models. Length at capture was not included in the model because declines in condition (equation 3) were better assessed by condition at capture. Separate logistic models were used to assess the effect of the different condition indices (length-based indices and relative subdermal lipid storage) on stress. For Atlantic croaker, the collinearity between time (week) and Δ_{temp} could not be removed through centering or standardizing these data (tolerance values remained < 0.10), therefore we retained time and excluded Δ_{temp} from the model. For summer flounder and striped bass, interaction plots suggested that the effect of condition at the time of the trial depended on Δ_{temp} , thus, this interaction was included in models for these species. At week 3, some summer flounder were observed with visible wounds on their ventral surface, and these individuals later developed ascites due to a flagellate infection (Burreson 1982). For this species, the presence or absence of symptoms of infection (wounds, ascites) was included as a predictor in the model; this factor replaced the prior stress factor because these factors were not independent.

We used a repeated-measures design and considered several variance-covariance structures to model the correlation between successive measurements of condition (e.g., auto-regressive, banded toeplitz, compound symmetry, and independent). The independent structure was used to describe the relationship among repeated measures from individual fish because this structure yielded the lowest value of the quasilielihood independence criterion (QIC; Pan 2001). We modeled the probability of exhibiting a stress response for each trial week using the logistic regression:

$$Y_{ij} = \mu + \alpha_i + \gamma + \Delta_i + \delta_i + \beta + \rho + \kappa_i + (\kappa^* \Delta)_i + \varepsilon_{ij} \quad (4)$$

where Y_{ij} = the probability of observing a stress response for individual j in trial week i ;

μ = the intercept, representing the mean probability of observing a stress response;

α_i = the effect of the i^{th} week of exposure to cold temperatures;

γ = the effect of the presence of food;

Δ_i = the effect of Δ_{temp} in week i ;

δ_i = the effect of T_{slope} in week i ;

β = the effect of condition at capture (length-based indices or subdermal lipids);

ρ = the effect of the presence of a stress response in the prior week (Atlantic croaker and striped bass), or the effect of the presence of infection symptoms (summer flounder);

κ_i = the effect of length-based condition indices or subdermal lipids measured at the time of trial i ;

$(\kappa^* \Delta)_i$ = the effect of the interaction between Δ_{temp} and weekly condition measures;

ε_{ij} = the random unexplained error, assumed to be independent and binomially distributed.

For this model, we specified a binomial distribution and a logit link function to fit generalized estimating equations using the Genmod procedure in SAS (Pan 2001; Quinn and Keough 2002). Final models were selected using QIC_u for variable selection because QIC_u approximates QIC when the generalized estimating equations are correctly specified (Pan 2001). Models within two units of the minimum QIC_u were expected to

have a similar ability to estimate the response, so the simplest model within two QIC_u units of the lowest value was selected as the final model.

Results

Condition dynamics during winter

As expected, fish mass and subdermal lipids declined throughout the experiment for Atlantic croaker and striped bass, but mean condition indices for summer flounder exhibited unique patterns. Regardless of species, no differences in weekly mean condition were detected among unfed and fed individuals that were withheld food or provided food. Declines in mean K and subdermal lipids of Atlantic croaker were best described by condition at capture (Fulton's K: $\beta = 1.02$, $t = 13.35$, $P < 0.01$; subdermal lipids: $\beta = 0.03$, $t = 18.48$, $P < 0.01$). Based on these indices, mean condition declined rapidly in the first few weeks and remained relatively stable thereafter (Figure 4, 5). For individuals that survived until the end of the trials, mean condition declined again around week 12.

Mean K_n and subdermal lipids for striped bass also declined rapidly at the beginning of the trials. After four weeks, declines in mean K_n were more gradual (Figure 8), and subdermal lipids remained relatively constant after six weeks (Figure 9). Similar to Atlantic croaker, K_n and subdermal lipids were higher on average for striped bass in relatively high condition at capture (K_n : $\beta = 0.78$, $t = 6.01$, $P < 0.01$; subdermal lipid: $\beta = 0.17$, $t = 2.41$, $P = 0.02$).

Declines in mean Fulton's K for summer flounder were influenced by K and length at capture, as well as the presence of infection symptoms. Mean K for summer

flounder remained relatively stable for the first eight weeks, before declining rapidly (Figure 6). Larger individuals and individuals in better condition (higher K) at capture had significantly higher mean K values each week (K: $\beta = 0.70$, $t = 6.76$, $P < 0.01$; length: $\delta = 0.0005$, $t = 3.19$, $P < 0.01$). Individuals that exhibited symptoms of flagellate infection had significantly higher mean K values than individuals that did not exhibit symptoms ($\rho = 0.02$, $t = 3.92$, $P < 0.01$). Surprisingly, mean subdermal lipid estimates steadily increased in juvenile summer flounder over the duration of the study (Figure 7). On average, weekly mean subdermal lipids estimates were greater for individuals with relatively high lipid estimates at capture ($\beta = 1.17$, $t = 13.27$, $P < 0.01$), but mean subdermal lipids were lower for individuals that were larger at time of capture ($\delta = -0.01$, $t = -3.09$, $P < 0.01$).

Atlantic croaker that succumbed to mortality during cold-tolerance trials had mean HSI values that were similar, regardless of when the individual succumbed (Figure 10). Mean HSI values were significantly lower for individual striped bass and summer flounder that succumbed to mortality near the end of the trial period (Figures 11, 12).

Thermal stress induced by winter conditions

For juvenile Atlantic croaker, subdermal lipid content was a better predictor of thermal stress induced by simulated winter conditions than Fulton's K. Contrary to expectations, the relationship between subdermal lipids and stress response in juvenile Atlantic croaker was such that fish with greater subdermal lipids at capture were, on average, more likely to exhibit thermal stress in response to declining temperatures ($\beta = 0.07$, $\chi^2 = 5.39$, $P = 0.02$). This observation may be counter-intuitive under the

assumption that lipids mitigate mortality of fish exposed to low temperatures, but it suggests that the tissues in which lipids are stored -- liver versus subdermal tissues -- may influence lipid availability. The probability that an individual Atlantic croaker exhibited thermal stress increased significantly through time ($\alpha = 0.48$, $\chi^2 = 29.65$, $P < 0.01$), and individuals that exhibited stress were more likely to do so in the subsequent exposure to declining water temperatures ($\rho = 2.25$, $\chi^2 = 8.21$, $P < 0.01$; Figure 13). The probability of exhibiting thermal stress increased significantly for trials which exhibited a faster rate of temperature decline during the first hour ($\delta = 3.67$, $\chi^2 = 8.61$, $P < 0.01$).

The probability that juvenile summer flounder exhibited thermal stress was relatively constant through time ($\chi^2 = 2.62$, $P = 0.11$; Figure 14), but increased significantly for individuals exhibiting symptoms of flagellate infection ($\rho = 1.48$, $\chi^2 = 9.45$, $P < 0.01$). By the end of this study, 70% of the summer flounder exhibited symptoms of infection. Similar to Atlantic croaker, subdermal lipid content was a better predictor of stress than Fulton's K, and individuals with greater subdermal lipids at capture were more likely to exhibit thermal stress than conspecifics with lower subdermal lipids ($\beta = 0.27$, $\chi^2 = 4.79$, $P = 0.03$). Although AIC_c suggested that models that included Δ_{temp} were better descriptors of the probability of thermal stress for summer flounder, the effect of Δ_{temp} was not different from zero ($\chi^2 = 0.5$, $P = 0.48$); this indicates that the effect of Δ_{temp} on the probability of observing thermal stress could not be accurately assessed, potentially due to similar Δ_{temp} values for several trials.

The probability that striped bass exhibited a stress response during winter declined significantly during the first few weeks that cold temperatures were endured, and remained low thereafter ($\alpha = -0.43$, $\chi^2 = 11.06$, $P < 0.01$; Figure 15). Both weekly

relative condition and relative condition at time of capture influenced the probability of observing thermal stress, but their influences were opposing (weekly K_n : $\kappa = -39.6$, $\chi^2 = 8.67$, $P < 0.01$; K_n at capture: $\beta = 28.3$, $\chi^2 = 5.35$, $P = 0.02$). These results suggest that striped bass that were more likely to exhibit thermal stress had high condition at time of capture, but low condition at the time of the test, implying that condition declined rapidly in these individuals. Similar to summer flounder, AIC_c suggested that Δ_{temp} was an important predictor in the model of thermal stress for striped bass, but the effect of Δ_{temp} was not statistically significant (Δ_{temp} : $\Delta = 0.77$, $\chi^2 = 3.09$, $P = 0.08$).

Discussion

The high probability that Atlantic croaker and flagellate-infected summer flounder exhibited thermal stress in response to simulated winter conditions suggests contingents of these species may have higher rates of mortality if they remain in temperate estuaries during winter. Interestingly, individuals in high condition prior to the onset of winter were more likely to exhibit thermal stress. The majority of juvenile Atlantic croaker residing in estuaries in winter are likely unable to survive winter conditions at the end of their first year of life, particularly if temperatures are $< 4^\circ\text{C}$ and feeding opportunities are reduced for 12 weeks or more. Despite limited feeding, summer flounder maintained high condition for several weeks, suggesting individuals which reside in estuaries during winter may survive if they have not been infected by the blood flagellate *Trypanoplasma bullocki*. However, the high prevalence of infection during winter likely results in high mortality rates (Burreson 1982). Striped bass, a species that utilizes estuarine nursery areas during winter, were more tolerant of winter conditions

and less likely to exhibit thermal stress despite declines in individual condition. Because the probability of exhibiting thermal stress for juvenile Atlantic croaker and summer flounder was not influenced by mean condition each week, we discuss condition dynamics during winter separately from the thermal stress induced by winter conditions.

Condition dynamics during winter

The condition of an individual prior to the onset of winter influences its ability to endure the food deprivation that occurs during winter. The resiliency of fishes to food deprivation is associated with three phases of energy mobilization: (1) short-term (one to four weeks) use of storage lipids and proteins that results in rapid loss of mass, (2) a longer period of slower mass loss associated with the use of storage lipids and conservation of structural proteins, and (3) a period of high mass loss that occurs once a critical lipid threshold is reached and structural proteins are used as the primary energy source (~ 0.7 – 5% lipid, depending on species; Bar 2014). In general, mean condition of fish held in the laboratory declined through time, consistent with the effect of food deprivation. Although we provided access to food, fish were not seen feeding, an observation that is consistent with previously reported lower thermal thresholds for feeding (Johnson 1978; Wawronowicz and Lewis 1979; Malloy and Targett 1991).

Patterns in the observed decline of length-based indices and subdermal lipids in juvenile Atlantic croaker were consistent with phases of energy mobilization during winter (Bar 2014), and we observed declines in condition of juvenile striped bass that were consistent with the first two phases described above. Yet despite declines in mean K and subdermal lipids, mean HSI values of Atlantic croaker that succumbed to mortality

did not differ among weeks, suggesting that liver mass declined proportionally with body mass. Instead of depleting lipids stored in the liver, Atlantic croaker that succumbed to mortality may have preferentially utilized subdermal lipids or lipids stored elsewhere in the body, potentially resulting in a second rapid decline in subdermal lipids. Striped bass did not exhibit a second decline in mean K_n or subdermal lipids, potentially due to the continued mobilization and use of lipids from the liver (as evidenced by a decline in mean HSI among individuals that were euthanized). As a result, simulated winter conditions did not result in striped bass using proteins as a major energy source (phase 3).

The patterns we observed in mean condition indices of summer flounder were not consistent with energy mobilization patterns described by Bar (2014) and observed in Atlantic croaker and striped bass. Despite food deprivation, mean K of juvenile summer flounder was relatively stable for eight weeks. The expected one to four week decline in mass associated with the rapid loss of proteins and lipids was not apparent, suggesting summer flounder may be able to withstand food deprivation longer than other species. Summer flounder are sedentary fish with relatively low metabolic rates (Seibel and Drazen 2007; Capossela et al. 2012), so they can meet energy demands by metabolizing less protein and lipid mass than fish with higher routine metabolic rates. As lipid and protein decrease in flatfish, the relative water content of the muscle increases and total mass and K remain stable (Maddock and Burton 1994). After eight weeks, we observed rapid declines in mean K , likely reflecting loss of protein mass and the water associated with it (Chapter 2; Breck 2014).

Although mean HSI and K declined in summer flounder during winter, mean subdermal lipid content increased. The increase in mean subdermal lipids observed in our

study is consistent with the observed patterns in subdermal lipids for wild summer flounder in winter (Chapter 3) and thus, was not an artefact of fish being in the laboratory. Summer flounder appear to use a unique strategy of energy mobilization during winter, which may be explained by several processes that likely operate in combination: (1) mobilize lipids to subdermal tissues, (2) preferential use of energy stores from the liver, (3) selective mortality, and (4) protein catabolism. First, food-deprived fish may mobilize lipids to subdermal layers. However, in another flatfish (winter flounder, *Pleuronectes americanus*), energy utilization during starvation results in the depletion of the subdermal lipid layer (Maddock and Burton 1994), thus casting doubt on lipid transfer to subdermal tissues as an explanation of increasing subdermal lipids in summer flounder during winter. Second, individuals may preferentially use energy stored in the liver instead of subdermal lipids. The decline in mean HSI observed in the laboratory suggests that lipids and glycogen stored in the liver may be important energy sources during winter (Larsen et al. 2001), however, our work with wild fishes showed that mean HSI increased during winter (Chapter 3). This implies that either wild fish feed during winter, wild fish metabolic rates are lower than those of lab-held fish, summer flounder may selectively deplete either subdermal lipids or energy reserves in the liver depending on environmental circumstances, or that wild fish with low HSI values experience higher mortality than conspecifics with high HSI values. Similarly, mortality of individuals with low subdermal lipids in our study would result in the observed increase in the mean lipid content measured in surviving individuals. Finally, protein may be preferentially catabolized during periods of food deprivation, and thus, the proportion of lipid mass in subdermal tissues would exhibit an increase over time. Cold temperatures

reduce metabolism and fish may preferentially conserve lipids by deriving energy from protein (Bar 2014). The subdermal lipids that are conserved may represent structural and not storage lipids. Ultimately, we suspect that a combination of protein catabolism and selective mortality of low lipid individuals resulted in the observed increase in mean subdermal lipids in our study.

Thermal stress induced by winter conditions

The condition index that best predicts thermal stress in winter differs among species. Subdermal lipid content was a better predictor of thermal stress than Fulton's K for Atlantic croaker and summer flounder, whereas K_n was important for inferring stress in striped bass. Contrary to what was expected, we observed that individuals with high condition indices at time of capture were more likely to exhibit thermal stress than individuals with low condition indices at capture. Thermal stress of juvenile fishes occurred over a wide range of condition index values, suggesting mortality would not have been due to poor condition alone. Mortality of juveniles in wild populations results from multiple processes, starvation being only one possibility (Hurst et al. 2000; Slater et al. 2007).

In some temperate fishes, the relative allocation of energy to lipid storage increases prior to winter (Hurst and Conover 2003), and the composition of those lipids affects cold tolerance (Craig et al. 1995; Kelly and Kohler 1999). An increase in lipid concentrations, particularly polyunsaturated fatty acids (PUFAs), may increase cold tolerance by increasing membrane fluidity and preserving membrane function (Hazel 1995). Although we did not measure PUFAs, our results suggest that Atlantic croaker and

summer flounder with high subdermal lipids at capture were more likely to exhibit thermal stress in winter than conspecifics with low subdermal lipids. This counter-intuitive result may be explained by the tissues used to store lipids. For example, lipids may be allocated to subdermal tissues, to the liver, or both. Accumulation of subdermal lipids appear to be the energy-storage strategy of species that migrate out of coastal estuaries in winter, whereas energy storage in the liver may be preferred by species that overwinter in coastal bays and estuaries (Chapter 3). In our study, individual Atlantic croaker and summer flounder with high subdermal lipids may have allocated fewer lipids for storage in the liver, and therefore, these fish would have low tolerance of cold temperatures and be likely to exhibit stress.

During winter, the probability of observing a stress response increased for Atlantic croaker, decreased for striped bass, and exhibited no significant change for summer flounder. Atlantic croaker may have a lower capacity to withstand low temperature extremes than the two other species examined. Rates of mortality increase for post-larval Atlantic croaker (< 65 mm) that experience winter temperatures below 3°C (Lankford and Targett 2001). In a similar study, individuals < 50 mm lost equilibrium at 1.5°C, and mortality occurred between 0.5 and 1°C (Joseph 1972). In our study, the loss of equilibrium occurred at warmer temperatures (mean temperature ~4°C), possibly due to the additional stress of food limitation, differences in the size of fish examined, protocols for temperature acclimation, and rates of temperature decline. The rate that water temperatures declined during the first hour of the trial significantly affected the probability that Atlantic croaker exhibited signs of thermal stress, even though the rates of temperature decline were similar among trials (< 1°C hr⁻¹) and may

have been sufficiently slow to permit acclimatization (Beitinger et al. 2000). We expect that exposure of an individual to weekly sub-lethal thermal stresses likely had a cumulative effect that culminated in observed stress in trials when temperature declines were higher than average. Once an individual Atlantic croaker exhibited signs of thermal stress in response to an acute drop in temperature, the individual was more likely to do so in the subsequent trial. Ultimately, repeated exposures to low temperatures and the subsequent stress responses may result in Atlantic croaker having a low probability of survival during extended cold periods, regardless of the amount of lipid stored prior to winter at the end of their first year of life.

During winter, juvenile striped bass reside in tributaries of the Chesapeake Bay, so we expected individuals to be well adapted to winter conditions. Indeed, the probability of exhibiting a stress response was low after the first few weeks of simulated winter conditions. Mortality of striped bass observed during the first few weeks may be due in part to the large change in temperature from ambient conditions that was implemented in the early trials. We observed that Δ_{temp} was a significant predictor of thermal stress and Δ_{temp} was high only in the first few weeks of the study. Striped bass that exhibited stress responses in the early weeks were those individuals with low relative condition (K_n). Interestingly, most of the individuals that succumbed to thermal stress also had high K_n values at capture. This suggests that upon exposure to simulated winter conditions in the laboratory, condition declined more rapidly in these striped bass than in individuals which ultimately did not exhibit stress. Similarly, a greater magnitude of energy loss has been documented for striped bass and bluefish (*Pomatomus saltatrix*) with high energy levels prior to winter (Hurst and Conover 2003; Morley et al. 2007).

The rapid decline in energy of well-conditioned individuals may explain why low temperature tolerance of striped bass seems unrelated to its energetic state (Hurst and Conover 2002). Because individuals of varying condition were exposed to the same water temperatures and similarly deprived of food in our study, the observed faster decline in condition of fish with high K_n at time of capture may be associated with behavioral differences (e.g., swimming activity) that results in higher routine metabolic rates in well-conditioned juveniles.

During winter, most of the population of summer flounder, including juveniles, migrates to the continental shelf; although a portion may reside in Chesapeake Bay. Habitat selection (shelf vs bay) may depend on an individual's energy-storage strategy of individuals (Chapter 3). The partial migration strategy of juvenile summer flounder (Chapter 3) suggests that individuals with sufficient energy reserves in the liver may be physiologically capable of withstanding winter conditions in Chesapeake Bay. As a result, the probability of exhibiting thermal stress was low for summer flounder not infected with the trypanosome blood flagellate; this pattern in the probability of thermal stress was more similar to striped bass (a species which resides in estuaries during winter) than to Atlantic croaker (a species which typically emigrates from coastal estuaries). In other studies, mortality rates of juvenile summer flounder during winter depended on the rate of temperature decline, rather than on the final exposure temperature (Malloy and Targett 1994). Yet we found that the rate of temperature decline did not significantly influence the probability of thermal stress in this species.

Thermal stress in winter was more likely to be observed in summer flounder infected with a blood flagellate than in uninfected conspecifics. The infected individuals

in our study picked up the parasite prior to capture because the flagellate is communicable among flounder only in the presence of its leech vector (*Calliobdella vivida*, Burreson 1982). All infected individuals would have expressed symptoms in this study because acute infections develop when water temperatures decline below 15°C (Burreson and Zwerner 1982). The high probability of infected fish exhibiting a stress response was expected because many infected fish die 3-4 months after developing ascites and anemia (Burreson 1982). Furthermore, mortality of infected fish approaches 100% when fish are exposed to temperatures below 5°C (Burreson 1982), indicating winter survival of summer flounder may be low if the infection rate observed for individuals in our study (70%) is representative of the entire population within Chesapeake Bay during winter. However, fish may survive if ascites develops late in winter (e.g., March; Burreson and Zwerner 1984). Here, individuals that expressed symptoms as early as January 23rd survived until temperatures warmed to 10°C. In 10°C waters, fish may produce antibodies and eliminate the flagellate from peripheral circulation (Burreson 1982), but complete elimination of the flagellate does not occur until water temperatures reach 22°C (Sypek and Burreson 1983).

Fish condition may influence winter survival, but the condition indices we examined did not mediate thermal stress as expected. For example, weekly measures of condition did not influence thermal stress of Atlantic croaker or summer flounder. Instead, high subdermal lipids at capture were associated with thermal stress. The link between fish condition and thermal stress during winter may be better understood by focusing on variation in HSI, as juveniles that survive winter in estuarine habitats have high mean HSI values (Chapter 3). Yet, we determined that striped bass are well suited

for winter survival in the temperate estuaries of Virginia, and individuals that survive the onset of winter have a low probability of exhibiting stress and subsequent mortality. Atlantic croaker that reside in estuaries during winter may suffer high rates of mortality due to physiological intolerance of cold temperatures. Winter mortality may also be of particular concern for summer flounder because poor condition individuals reside in estuaries during winter (Chapter 3), where they are susceptible to infection and disease-associated mortality.

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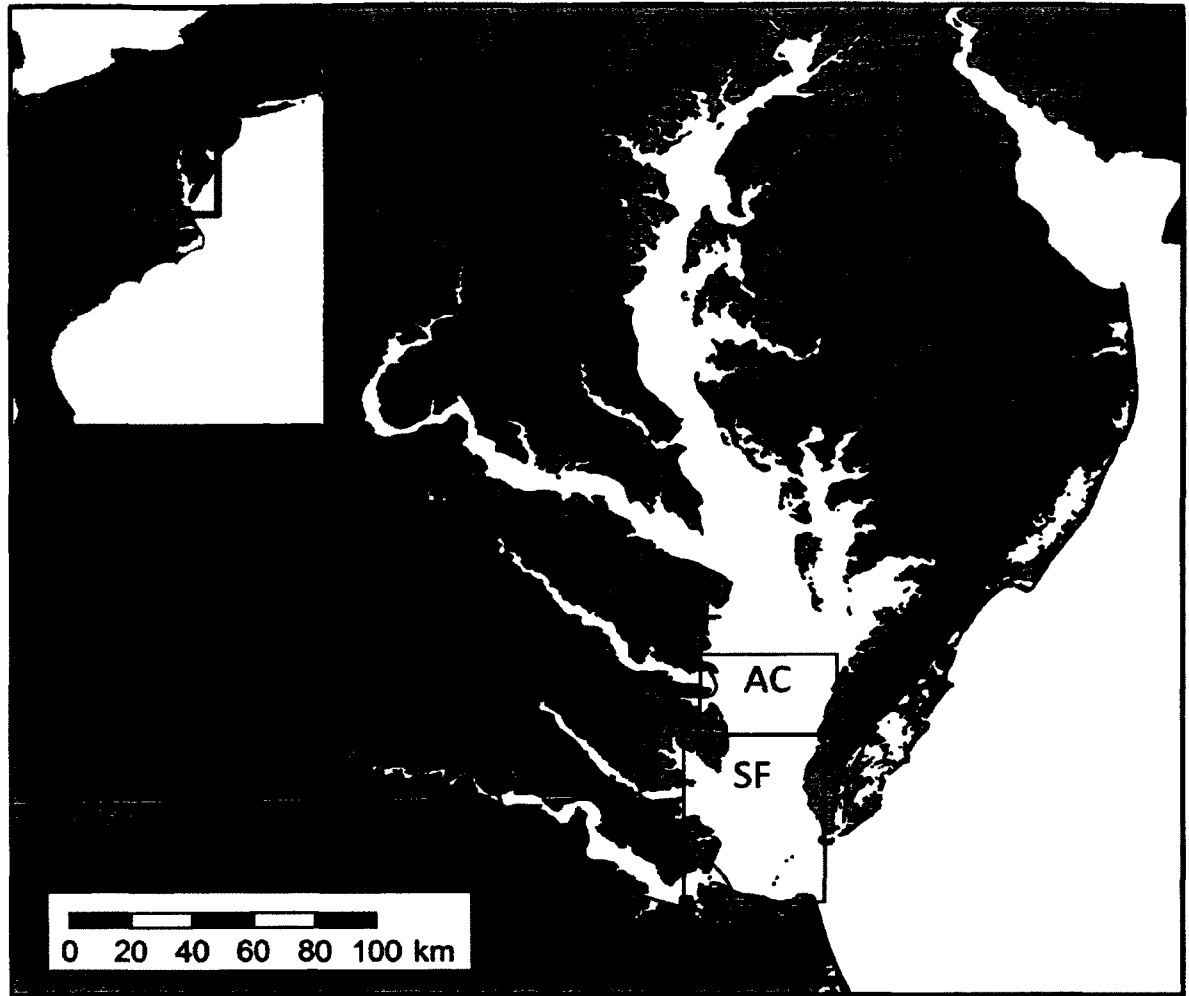


Figure 1. Sampling areas in Chesapeake Bay from which individual Atlantic croaker (AC), summer flounder (SF), and striped bass (SB) were collected for cold-tolerance trials. Atlantic croaker were collected on 25 Oct 2012, summer flounder on 26 Nov 2012 and 27 Nov 2012, and striped bass on 10 Dec 2013 and 12 Dec 2013.

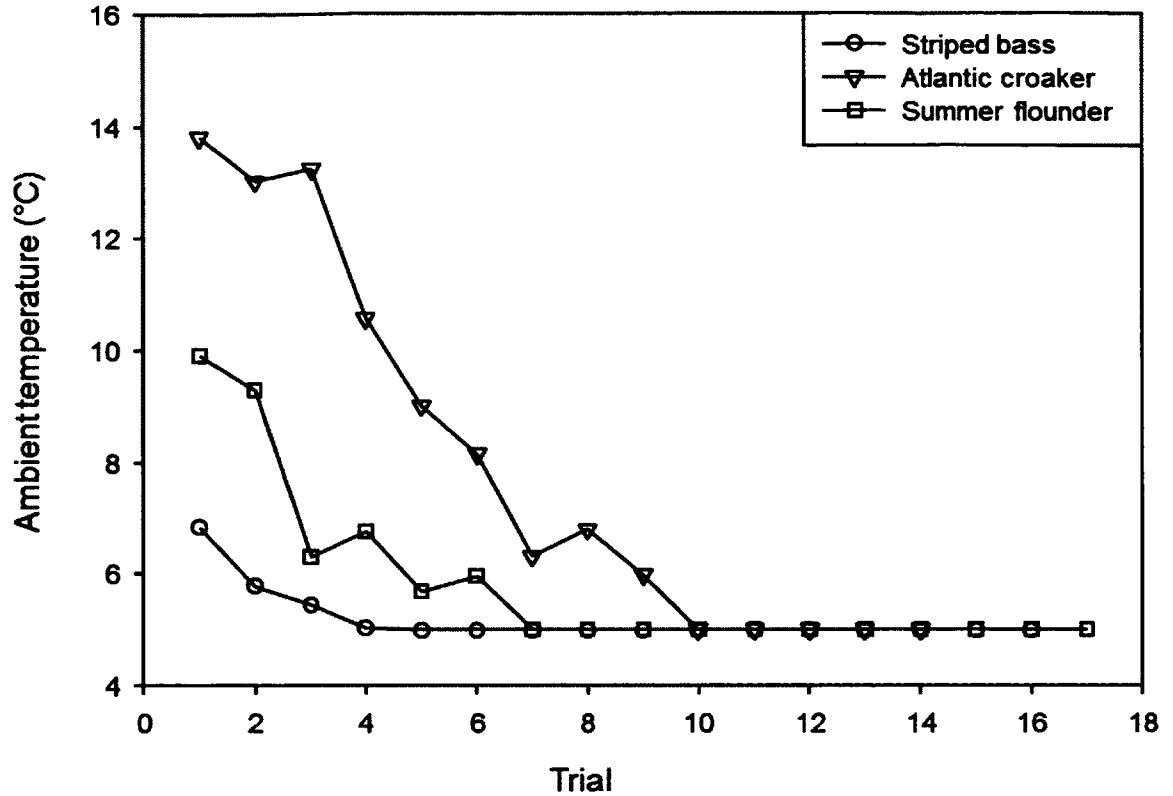


Figure 2. Weekly ambient mean water temperature during cold-tolerance trials. Trials occurred during 14 consecutive weeks between 6 Nov 2012 and 5 Feb 2013 for Atlantic croaker, during 17 consecutive weeks between 7 Dec 2012 and 28 Mar 2013 for summer flounder, and during 16 consecutive weeks between 17 December 2013 and 3 April 2014 for striped bass. All fish were collected from Chesapeake Bay in 2012 or 2013.

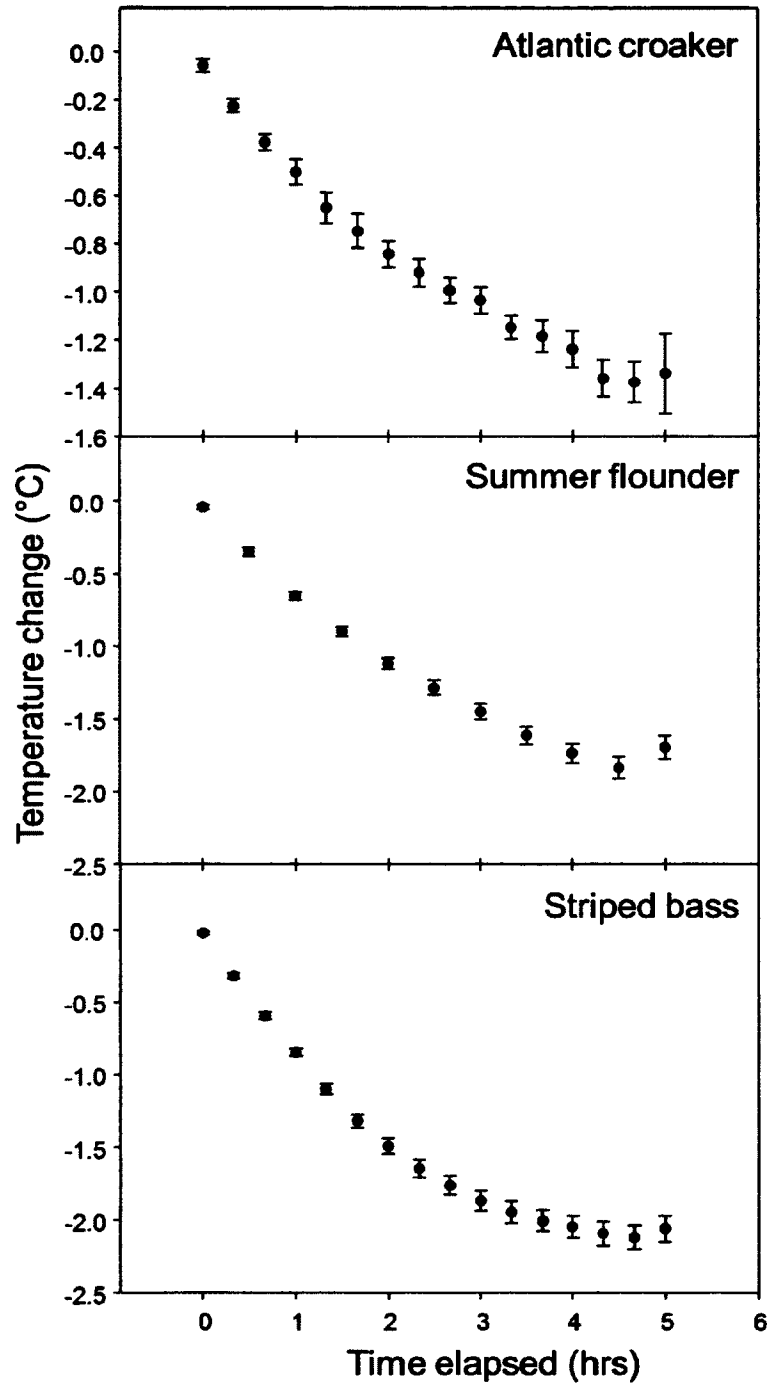


Figure 3. Temperature declines during the first 5 hours of the cold-tolerance trials. Mean temperature is represented by filled circles (\pm one standard error), and mean temperature at 0 hours was $\sim 5^{\circ}\text{C}$ for Atlantic croaker, $\sim 4^{\circ}\text{C}$ for summer flounder, and $\sim 3.5^{\circ}\text{C}$ for striped bass. Means and standard errors were estimated from 14 weekly trials for Atlantic croaker, 17 weekly trials for summer flounder, and 16 weekly trials for striped bass.

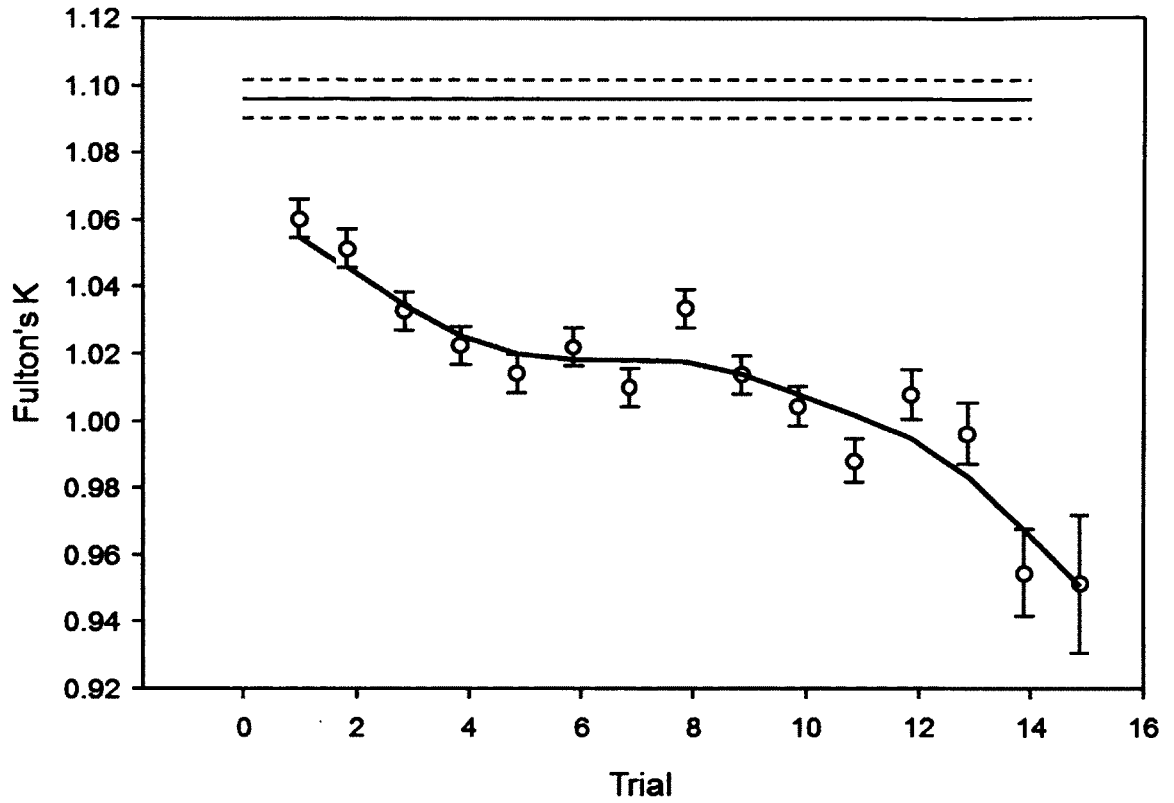


Figure 4. Mean (\pm standard error) Fulton's K for juvenile Atlantic croaker during 14 weekly trials to assess cold tolerance of individual fish. The solid curve is a spline function fit to the weekly data and is provided as a visual aid only. The solid horizontal line indicates the mean condition (\pm one standard error denoted by horizontal dashed lines) of individuals at time of capture.

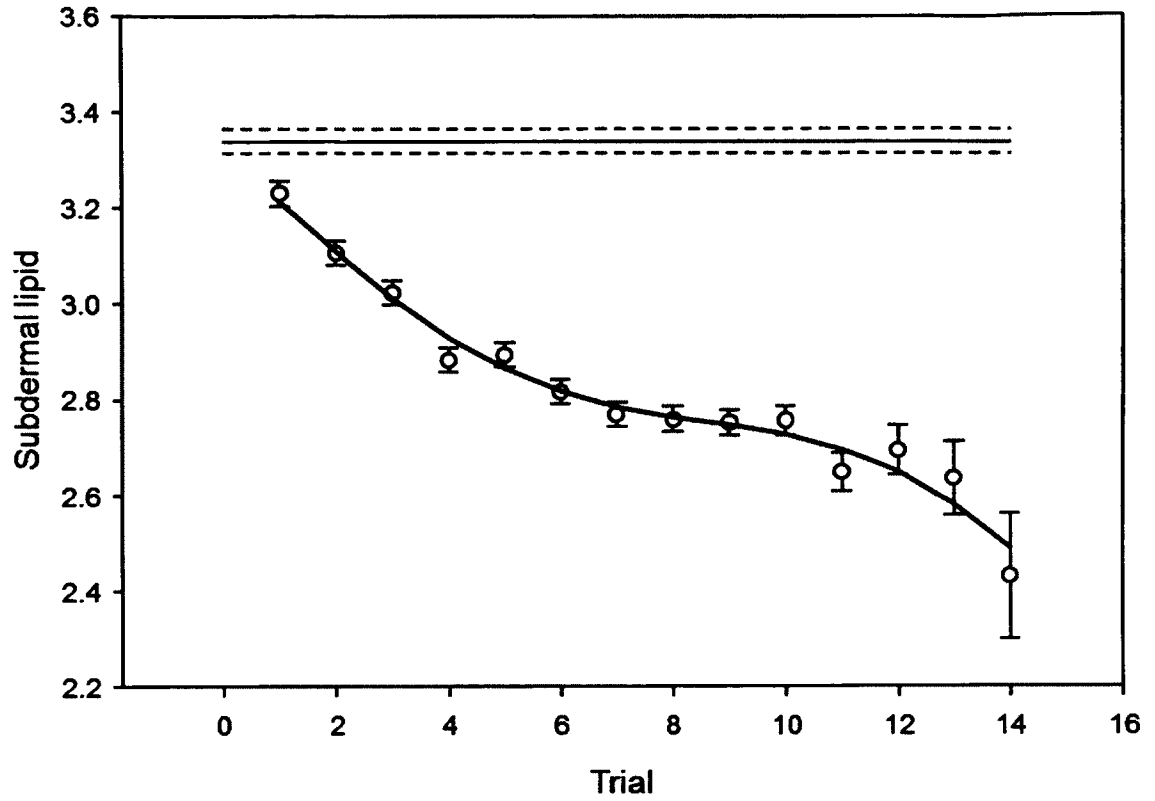


Figure 5. Mean (\pm standard error) subdermal lipid estimates for juvenile Atlantic croaker during 14 weekly trials to assess cold tolerance of individual fish. The solid curve is a spline function fit to the weekly data and is provided as a visual aid only. The solid horizontal line indicates the mean condition (\pm one standard error denoted by horizontal dashed lines) of individuals at time of capture.

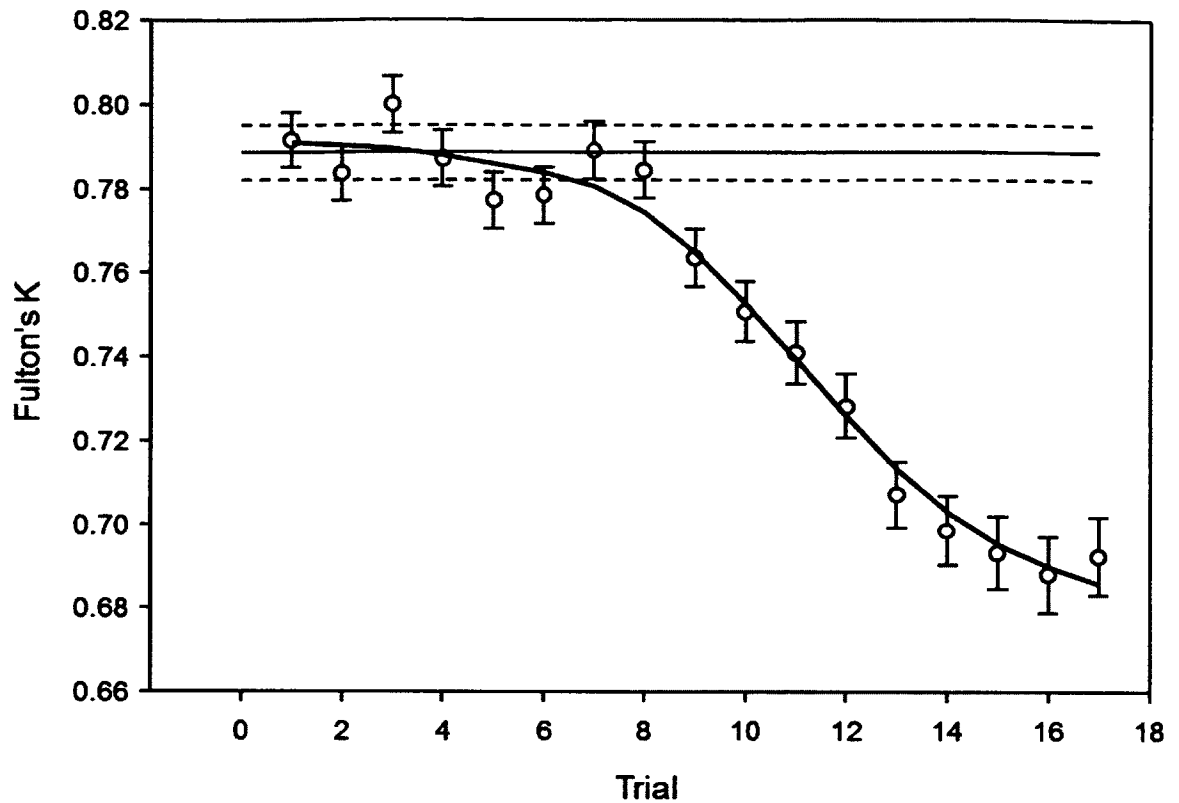


Figure 6. Mean (\pm standard error) Fulton's K for juvenile summer flounder during 17 weekly trials to assess cold tolerance of individual fish. The solid curve is a spline function fit to the weekly data and is provided as a visual aid only. The solid horizontal line indicates the mean condition (\pm one standard error denoted by horizontal dashed lines) of individuals at time of capture.

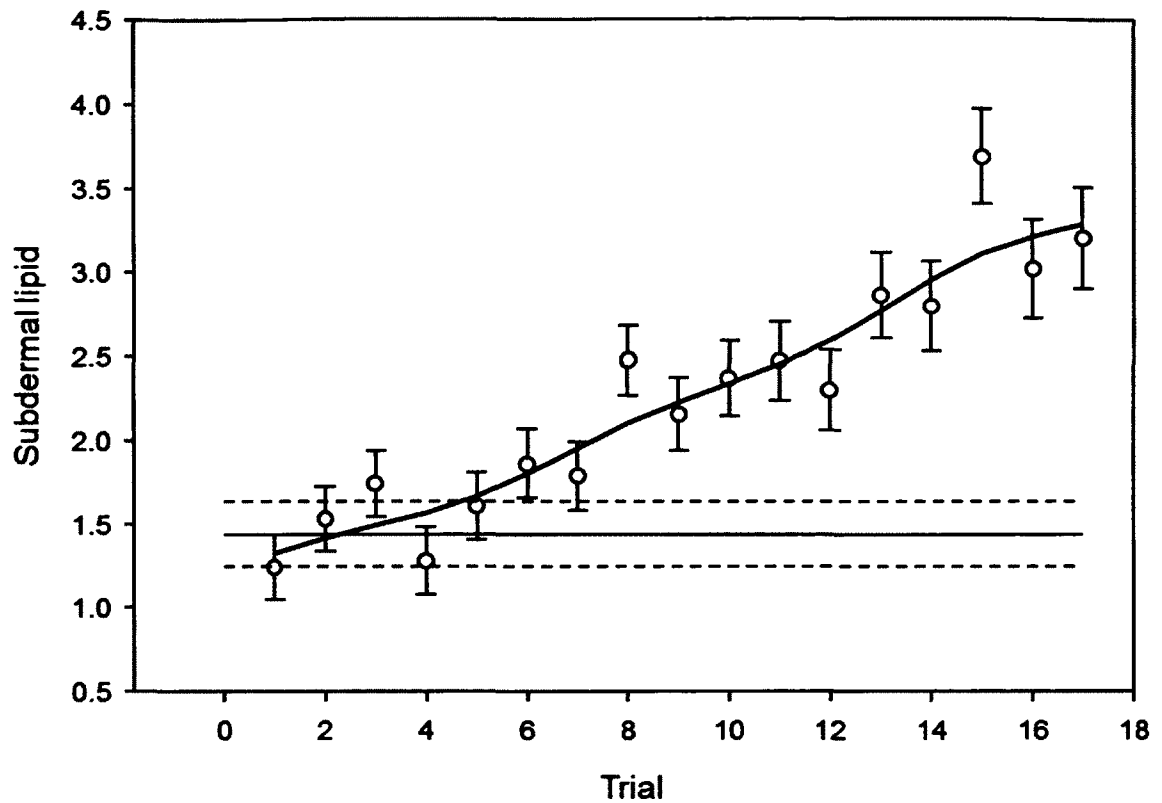


Figure 7. Mean (\pm standard error) subdermal lipid estimates for juvenile summer flounder during 17 weekly trials to assess cold tolerance of individual fish. The solid curve is a spline function fit to the weekly data and is provided as a visual aid only. The solid horizontal line indicates the mean condition (\pm one standard error denoted by horizontal dashed lines) of individuals at time of capture.

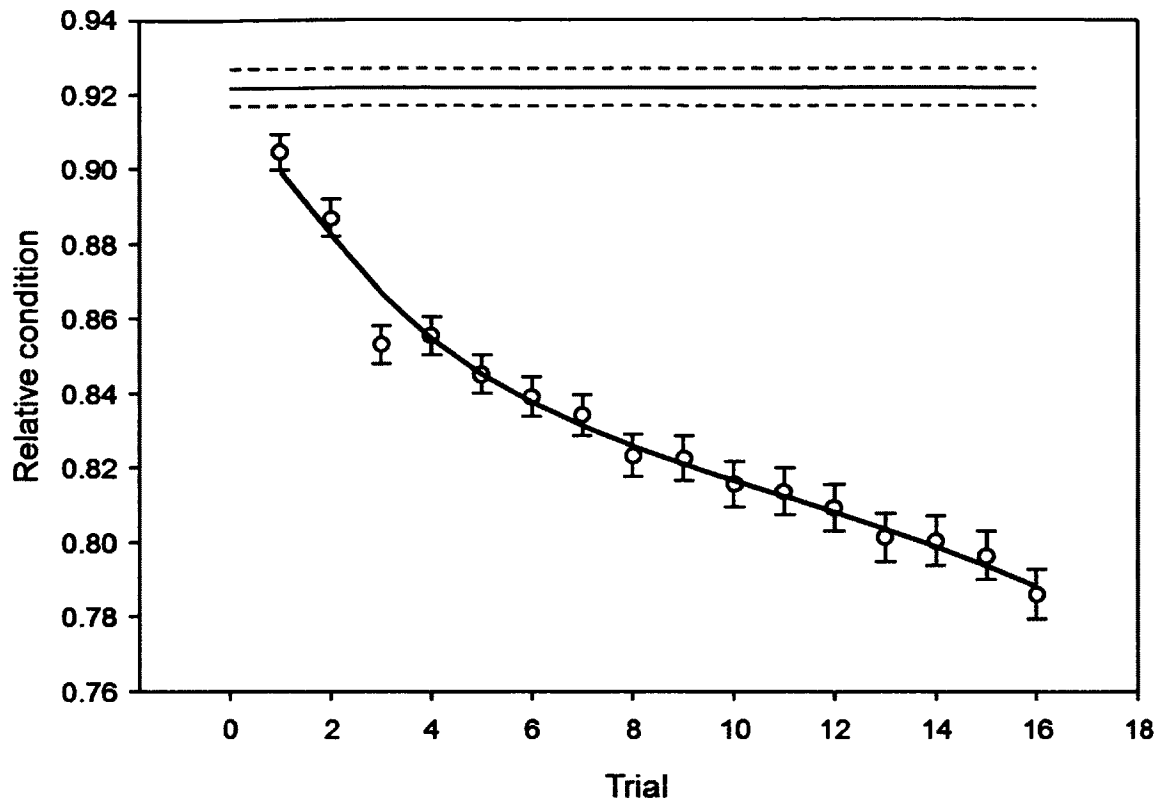


Figure 8. Mean (\pm standard error) relative condition for juvenile striped bass during 16 weekly trials to assess cold tolerance of individual fish. The solid curve is a spline function fit to the weekly data and is provided as a visual aid only. The solid horizontal line indicates the mean condition (\pm one standard error denoted by horizontal dashed lines) of individuals at time of capture.

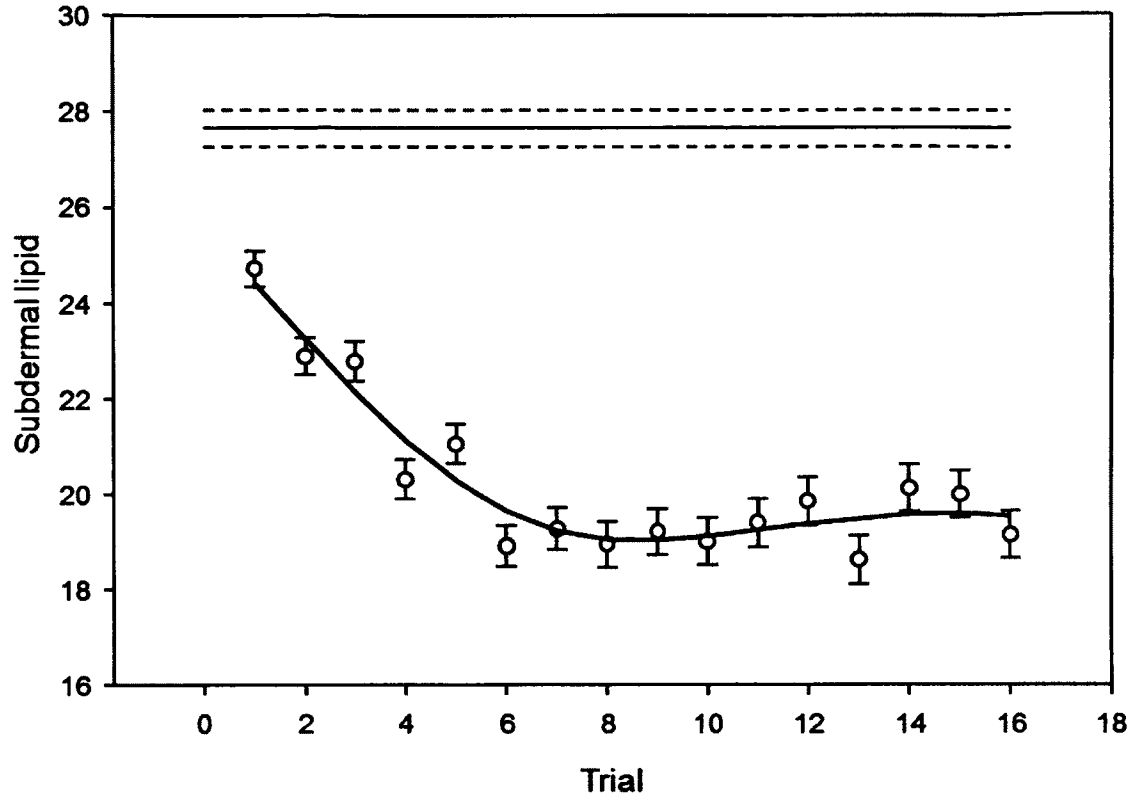


Figure 9. Mean (\pm standard error) subdermal lipid estimates for juvenile striped bass during 16 weekly trials to assess cold tolerance of individual fish. The solid curve is a spline function fit to the weekly data and is provided as a visual aid only. The solid horizontal line indicates the mean condition (\pm one standard error denoted by horizontal dashed lines) of individuals at time of capture.

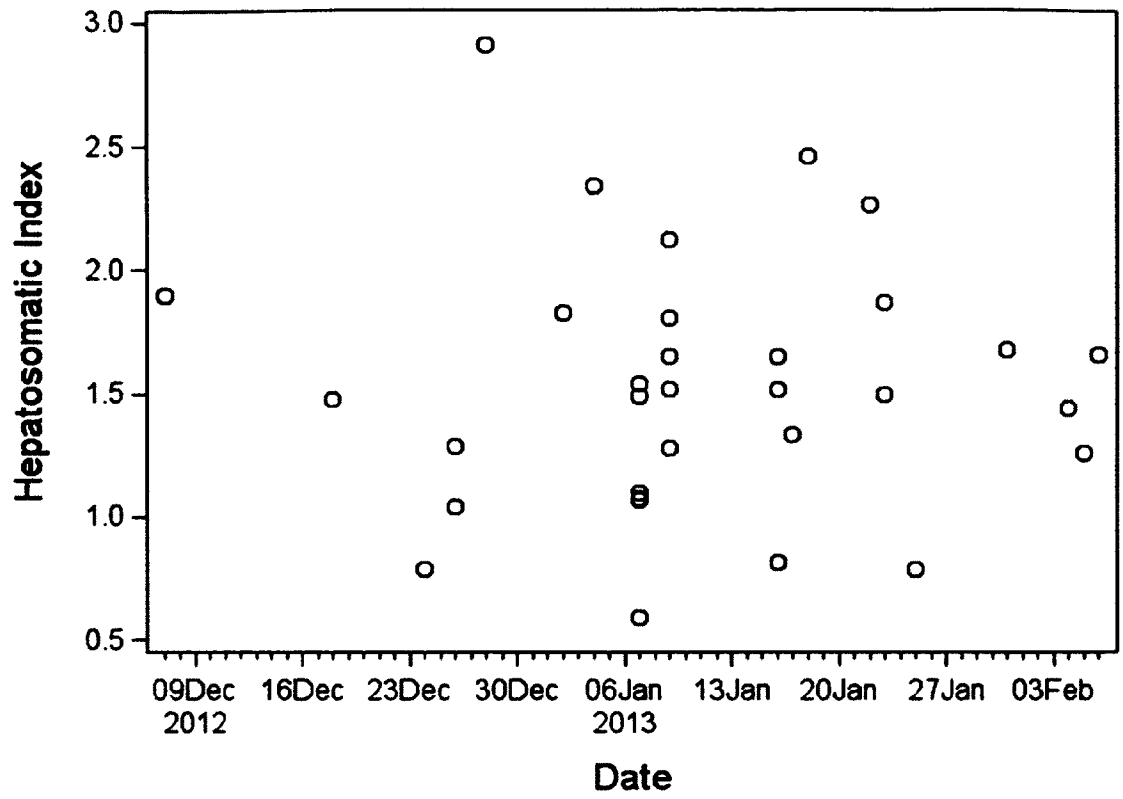


Figure 10. Hepatosomatic index values for individual Atlantic croaker that did not recover from thermal stress and succumbed to mortality (circles).

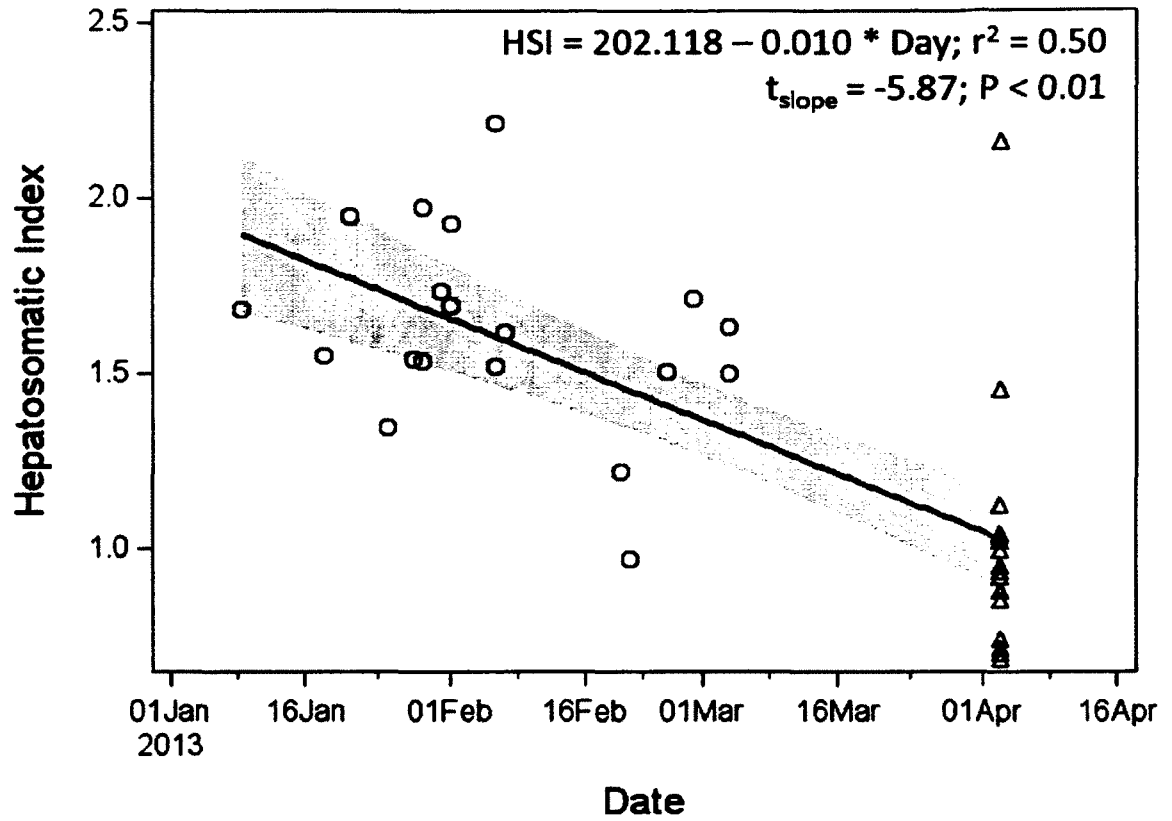


Figure 11. Hepatosomatic index (HSI) values for individual summer flounder that did not recover from thermal stress and succumbed to mortality (circles), and individuals that survived until the end of trials but were euthanized to permit estimation of HSI (triangle). The decline in mean HSI during the study is indicated by the solid line (\pm 95% confidence interval indicated by shading).

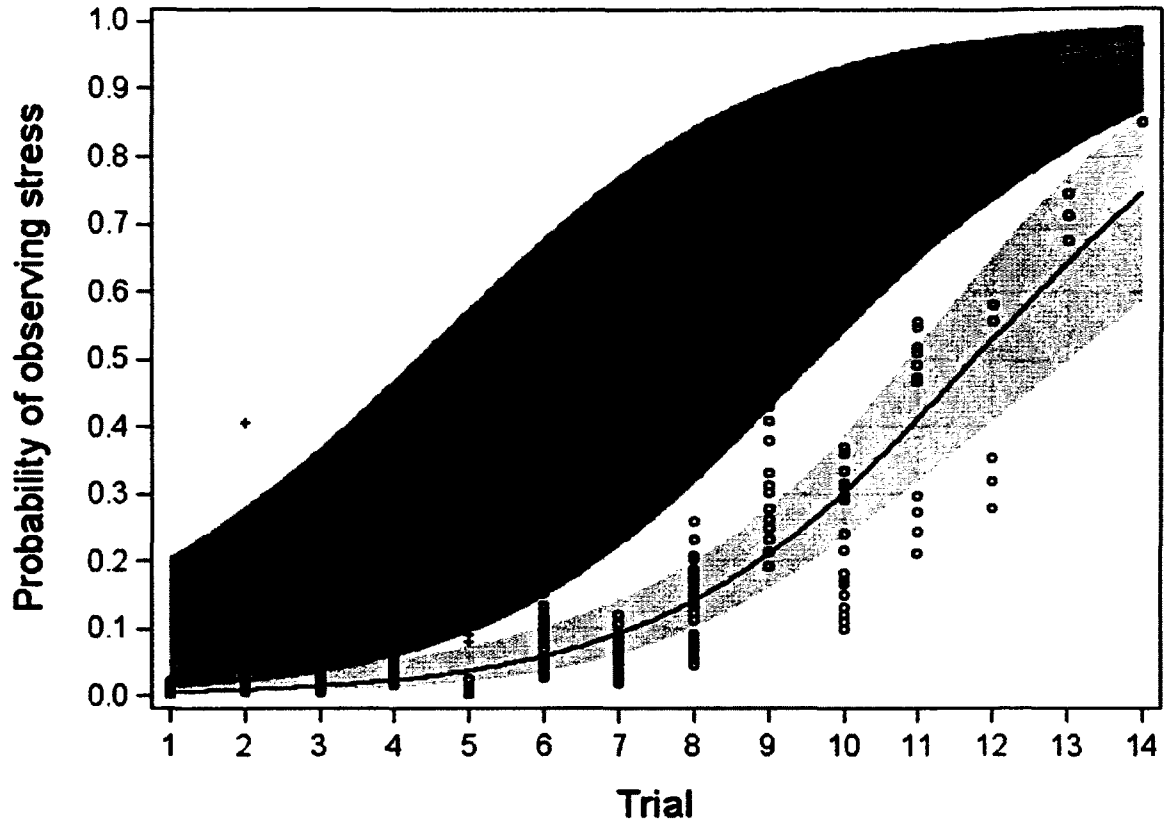


Figure 13. The mean probability of observing thermal stress (\pm 95% confidence intervals indicated by shading) for juvenile Atlantic croaker that exhibited a stress response in the prior trial (dashed line and crosses) and for juveniles that were not stressed in the prior trial (solid line and circles). Sample size in a given trial ranged from 40 individuals in the first trial to two in the 14th trial.

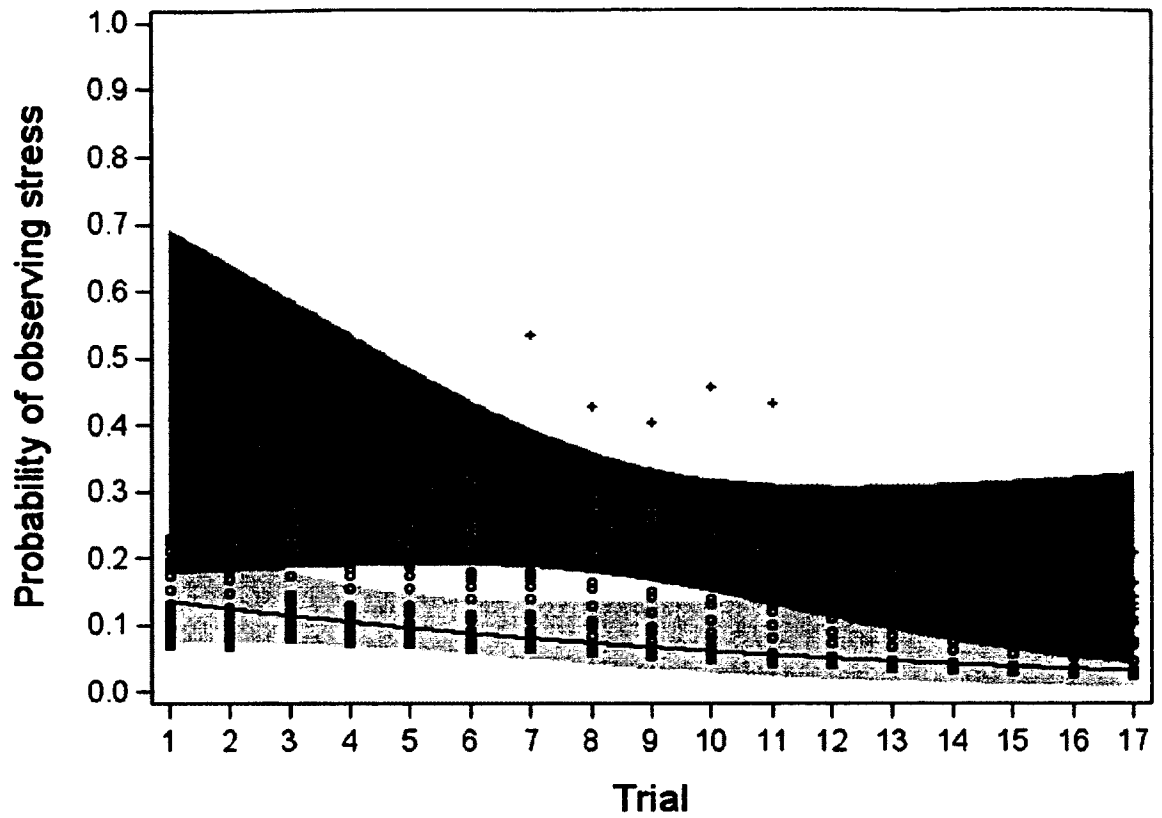


Figure 14. The mean probability of observing thermal stress (\pm 95% confidence intervals indicated by shading) for juvenile summer flounder that were infected by blood flagellates (dashed line and crosses) and for juveniles that were not infected (solid line and circles). Sample size in a given trial ranged from 44 individuals in the first trial to 17 in the 17th trial.

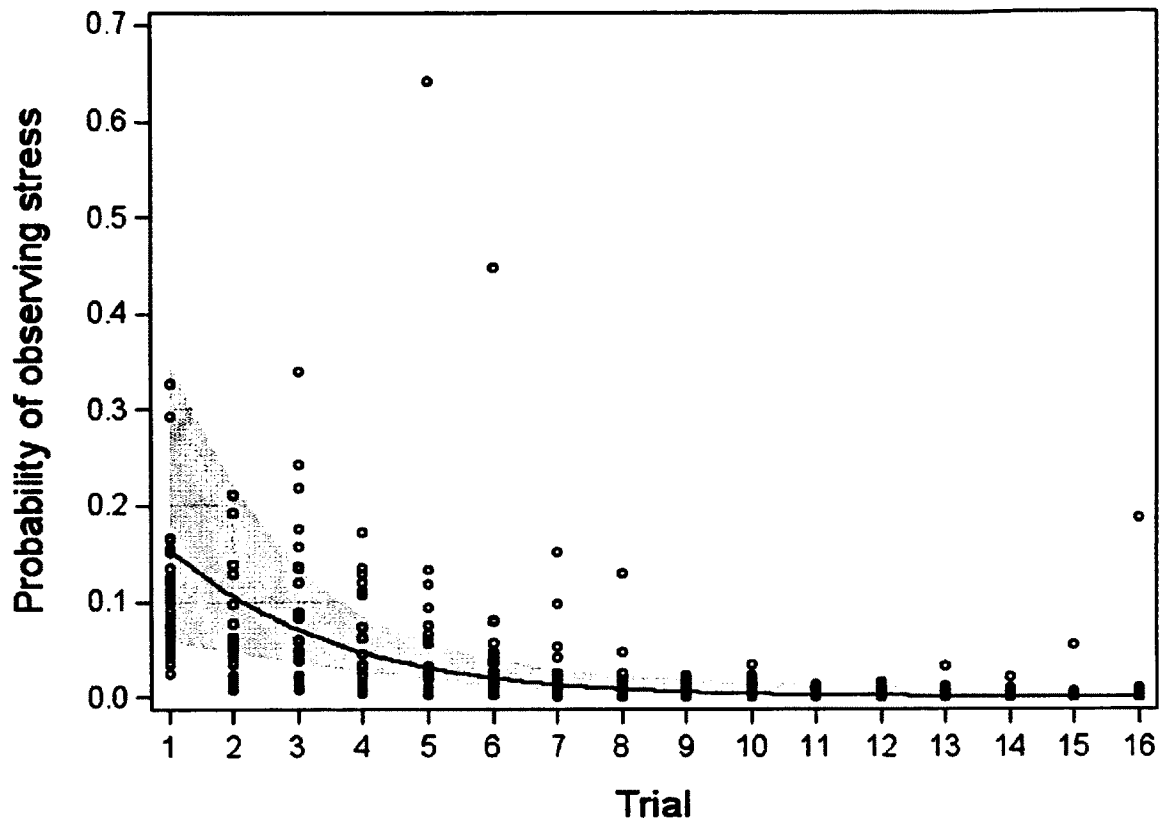


Figure 15. The mean probability of observing thermal stress (\pm 95% confidence interval indicated by shading) for juvenile striped bass among trials. Circles denote the probability of observing stress for each individual. Sample size in a given trial ranged from 40 individuals in the first trial to 20 in the 16th trial.

CONCLUSIONS

Length-based condition indices, the hepatosomatic index, and relative subdermal lipids may be used to assess the energy and lipid content of juvenile Atlantic croaker, summer flounder, and striped bass. However, the suitability of these indices to assess energy and lipid content differed among species, and a single metric of condition will not represent the energy allocation strategies used by juveniles with different life-history strategies. Multiple condition indices and the temporal and spatial context from which juvenile fish condition is measured must be considered to deduce population-level effects of variability in condition.

Temporal and spatial patterns in condition of juvenile fishes may be revealed by intensive sampling of fish at appropriate scales. The condition dynamics revealed by length-based indices, the hepatosomatic index, and subdermal lipids were strongly influenced by the life-history strategies of the species investigated. Temporal patterns in condition indices suggest that the energy-storage strategy used by juvenile Atlantic croaker and summer flounder to prepare for migration to the continental shelf (increased subdermal lipid storage) is different from the strategy used by individuals that survive during winter (lipid storage in the liver). Furthermore, mean condition varies among year classes and among habitats for the three species, indicating that temporal and spatial variability in environmental characteristics influences the ability of juvenile fishes to build and maintain energy reserves. Spatial patterns in juvenile fish condition may be used to identify high quality nursery habitats (i.e., areas that support well-conditioned individuals), and these patterns suggest that the suitability of a given nursery habitat

varies depending on species; for example, coastal lagoons are favorable habitats for juvenile summer flounder, whereas the upper Chesapeake Bay is favorable for juvenile Atlantic croaker. Niche partitioning may reduce competition for limited resources among species, but competition among conspecifics influences the quality of nursery habitats, as evidenced by the significant effect of fish density on juvenile fish condition. Strong density-dependent effects also suggest that the condition of juvenile fish is dependent on the degree to which these species aggregate and on factors that influence aggregation (e.g., prey availability or habitat complexity). The latter may ultimately explain which habitats support well-conditioned juveniles.

Well-conditioned individuals appear better prepared to survive physiologically stressful events imposed by winter conditions in temperate estuaries. Energetic preparedness for winter may help stave off effects of food deprivation, given that the mean condition indices of wild-captured Atlantic croaker, summer flounder, and striped bass decline when fish are experimentally exposed to simulated winter conditions (i.e., food deprivation and acute drops in water temperature). However, the mortalities that occurred among experimental fish appear to be associated with exposure to multiple stressors (e.g., disease and thermal stress) that act in combination with food deprivation. My results imply that winter mortality may not influence population size of juvenile striped bass in Chesapeake Bay, but winter conditions may negatively impact the portion of the Atlantic croaker population that remain in estuarine areas during winter. Juvenile Atlantic croaker have a high probability of mortality during prolonged winters with water temperatures $< 4^{\circ}\text{C}$.

Juvenile summer flounder employ a partial migration strategy. Although the majority of juveniles migrate to the continental shelf at the end of their first year of life, a small portion of the population remains in estuarine areas during winter. Partial migration of the juvenile population implies that year-class strength reflects the fraction of individuals which are energetically prepared for migration and those that are prepared to remain in the estuary during winter. The proportion of juveniles that remain in Chesapeake Bay and associated estuaries during winter may depend on yearly variations in energy storage, which leads to yearly variation in mean condition indices. I found that in years when juvenile summer flounder abundance is high, mean subdermal lipid content is low and mean HSI values are high. This pattern of low subdermal lipids and high HSI values is consistent with condition metrics of individuals observed in Chesapeake Bay during winter. However, preparedness for winter, as indicated by high HSI values, may not guarantee survival of non-migratory summer flounder during winter because mortality is strongly affected by the presence of an infectious flagellate *Trypanoplasma bullocki*. The flagellate is common in summer flounder during winter, and mortality of infected fish approaches 100% when fish are exposed to temperatures below 5°C (Burreson 1982).

Because the condition of individual fish may influence survival (Wicker and Johnson 1987; Thompson et al. 1991; Brodersen et al. 2008; Evans et al. 2014), indirect measures of condition may be used to understand processes that influence recruitment variability. However, more work is needed before measures of condition can be used to refine estimates of year-class strength. To build on the research presented here, I recommend four avenues of investigation:

- (A) confirm differences in energy allocation strategies among resident and migratory contingents of juvenile Atlantic croaker and summer flounder populations by sampling both contingents concurrently,
- (B) assess energetic preparedness of migratory contingents of juvenile Atlantic croaker and summer flounder using behavioral experiments,
- (C) confirm that high mean HSI values for juveniles collected during winter reflect lipid mass
- (D) quantify inter-annual variability in *T. bullocki* infection rates in summer flounder, and
- (E) examine additional factors contributing to regional differences in juvenile fish condition in Chesapeake Bay.

Each of these investigations is described below.

(A) A comparison of hepatosomatic and subdermal lipid indices between resident and migratory fish is needed to confirm that these indices reflect preparedness for migration to the continental shelf (subdermal lipids) and for winter survival of non-migratory individuals (HSI), particularly for juvenile Atlantic croaker and summer flounder. I hypothesize that high HSI values and low subdermal lipids indicate individuals that will reside in temperate estuaries during winter, whereas low HSI values and high subdermal lipids identify individuals that will undertake migration. Here, I did not assess the condition of individuals that had emigrated from Chesapeake Bay and occupied coastal shelf habitats during winter. Condition indices of this contingent must be assessed to evaluate my hypothesis of the role of lipid storage strategy in partial migration. Alternatively, the subdermal lipid content of individuals may be assessed with

the Distell fish fatmeter in the months prior to migration and a tagging study aimed at recapturing individuals during winter (both within and outside of Chesapeake Bay) would also yield estimates of subdermal lipid storage for residents and migrants.

(B) A swimming challenge is needed to assess the endurance of individuals of varying condition and estimate the amount of energy required to migrate from key nursery habitats to overwintering grounds on the continental shelf. If prey availability is limited, not all individuals that attempt to migrate out of Chesapeake Bay are likely to have sufficient energy reserves to reach the continental shelf. The amount of energy required for an individual to survive migration should be assessed to determine the proportion of juvenile Atlantic croaker and summer flounder that undertake migration that have sufficient energy reserves.

(C) Histological examination of the liver is needed to identify the component (lipids, glycogen, or protein) or components contributing to the observed high liver mass during winter. Although increases in liver mass is associated with increases in liver lipid for juveniles of some species (e.g., haddock *Melanogrammus aeglefinus*, Nanton et al. 2001), this needs to be confirmed for juvenile striped bass, summer flounder, and Atlantic croaker.

(D) Infection of juvenile summer flounder by the blood flagellate *T. bullocki* may be high during winter (up to 100%), and infection is fatal once symptoms (e.g., ascites) are expressed (Burreson 1982). Therefore, it is also necessary to determine the proportion of summer flounder that reside in Chesapeake Bay during winter and that are infected with *T. bullocki*. The proportion of juvenile summer flounder exhibiting clinical signs of disease reflects the proportion of individuals that may succumb to mortality during

winter in Chesapeake Bay (Burreson and Zwerner 1984). During years when a large number of juveniles remain in estuaries during winter, the proportion of individuals that are infected could be used to adjust estimates of year class strength for disease-associated mortality of resident summer flounder.

(E) Finally, regional differences in juvenile fish condition suggest that preparedness for migration or survival during the winter may vary among contingents that utilize different habitats (Chesapeake Bay vs coastal shelf). Information on prey availability, predator abundance, substrate type, and habitat complexity at sampling stations were unavailable for this study. The combined influence of these covariates was considered using the factor “region” in the spatial models, but teasing out the contribution of individual covariates to the observed regional effects on mean condition is necessary. In particular, a tool that quantifies the annual production of energy from the prey community that is available to predatory fish has recently been developed (Tableau et al. 2015), and should be considered in future studies of habitat quality.

Year-class strength is highly variable due to the cumulative mortality of juvenile fishes (Sissenwine 1984), and the condition of individual fishes may be used to understand this variability. However, the four avenues of investigation suggested here need to be resolved to allow fisheries managers to assess the effect(s) of condition on juvenile mortality (at least for Atlantic croaker, summer flounder, and striped bass). If differential mortality rates for poor- and well-conditioned individuals within the same year class and among habitats can be quantified, then recruitment indices could be adjusted for the proportion of individuals in poor condition (Frank and McRuer 1989; Crowder et al. 1992). Such refined indices would benefit the resource management

agencies that use these indices within stock assessments to estimate future production. For example, catch limits may be appropriately set to reduce fishing pressure in response to poor-conditioned year classes that may not subsequently recruit to the adult population. Additionally, nursery habitats that consistently support high abundances of well-conditioned juveniles should be a focus of conservation efforts to maintain productivity of summer flounder, Atlantic croaker, and striped bass stocks.

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APPENDIX I

Comparison among Distell Fish Fatmeter Calibrations

During the course of this dissertation, the Distell fish fatmeter required servicing and recalibration to ensure proper performance. To ensure that estimates of subdermal lipids did not differ among calibrations, I compared fatmeter readings on the same individuals among calibrations. In order to do so, individuals were measured with the fatmeter under the original calibration (calibration 1), frozen, and thawed again to be measured with the fatmeter under a different calibration (calibrations 2-4). Fatmeter readings among calibrations were compared by linear regression. If subdermal lipid estimates are similar among calibrations, the regressions are expected to have slopes equal to one. However, to ensure differences in readings were not due to the effect of freezing, individuals were also measured when fresh and thawed under the same calibration. I found no difference in the slope between fatmeter readings for calibrations 1 and 2, calibrations 1 and 3, and the slope of fatmeter readings among fresh and thawed fish (Table 1), suggesting that observed differences among calibrations were due solely to the effect of freezing, and not due to the act of recalibration. Calibration 1 and 4 did differ in slopes (Table 1), but this was because only a few individuals could be examined; those individuals did not represent readings at the high end of observed subdermal lipid estimates, resulting the slope being slightly underestimated.

Table 1. Similarity of Distell fish fatmeter estimates of subdermal lipids for individuals among calibrations and among measurement made on the same individual when fresh and thawed.

	Slope	SE	N
Fresh vs thawed	0.833	0.017	291
Calibration 1 vs 2	0.828	0.034	52
Calibration 1 vs 3	0.749	0.131	68
Calibration 1 vs 4	0.721	0.045	22

APPENDIX II

Comparison among Folch and Soxhlet techniques for assessing lipid content

A subset of dried tissue samples (Summer Flounder, $n = 33$; Striped Bass, $n = 45$; Atlantic Croaker, $n = 24$) was examined for lipid content by Soxhlet extraction using diethyl ether as the solvent. Estimates of percent lipid from Soxhlet extraction were compared to percent lipid determined by Folch extraction to confirm accurate lipid estimates for Chapter 2. Percent lipid data from Folch and Soxhlet extraction techniques were compared using linear regression. For lipid estimate comparisons, five samples were removed from analyses for Striped Bass due to limited tissue quantity, which resulted in high lipid estimates from Soxhlet extraction. Percent lipid data from Folch and Soxhlet extraction were log-transformed for Summer Flounder and Atlantic Croaker to improve normality and homogeneity of variance. Striped Bass lipid data were normally distributed, and therefore, not transformed.

Estimates of percent lipid from Folch and Soxhlet extraction techniques were significantly correlated with each other ($r^2 = 0.68$, $n = 32$, $P < 0.01$ for Summer Flounder; $r^2 = 0.77$, $n = 37$, $P < 0.01$ for Striped Bass; $r^2 = 0.87$, $n = 22$, $P < 0.01$ for Atlantic Croaker). Because Atlantic Croaker had the highest correlation between lipid content measured by Folch and Soxhlet extraction, the two techniques are more consistent for species with relatively high lipid content. Based on confidence intervals, linear models suggest a slope equal to one to describe the relationship between Folch and Soxhlet lipid measurements for Striped Bass and Atlantic Croaker (Table 1). A slope significantly less than one was found for the relationship between Folch and Soxhlet lipid measurements for Summer Flounder, potentially due to a limited range of observed lipid values and high

variability at relatively low lipid values. Intercepts were not significantly different from zero for all species.

Table 1. Relationships between lipid content measured from Folch and Soxhlet extraction techniques for juvenile Summer Flounder ($n = 33$), Striped Bass ($n = 45$), and Atlantic Croaker ($n = 24$).

Species	Model	r^2
Summer Flounder	$\log(\text{lipid}_{\text{Folch}}) = 0.150888 (\pm 0.16469) + 0.73360 (\pm 0.18973) * \log(\text{lipid}_{\text{Soxhlet}})$	0.68
Striped Bass	$\text{lipid}_{\text{Folch}} = -0.25284 (\pm 0.84536) + 0.96421 (\pm 0.17861) * \text{lipid}_{\text{Soxhlet}}$	0.77
Atlantic Croaker	$\log(\text{lipid}_{\text{Folch}}) = -0.22568 (\pm 0.30803) + 1.06737 (\pm 0.19300) * \log(\text{lipid}_{\text{Soxhlet}})$	0.87

VITA

Ryan W. Schloesser

Born in Madison, WI on July 1, 1984. Graduated from Lakeside Lutheran High School, Lake Mills, WI in 2002. Graduated *summa cum laude* from Texas A&M at Galveston with a Bachelor of Science in Marine Biology in 2006. Earned a Master of Science in Wildlife and Fisheries from Texas A&M in 2008. Entered the Doctor of Philosophy program at the Virginia Institute of Marine Science in 2009.