Comparing Multiple Predictors of Energy Content in Juvenile Bluefish

James W. Morley [a] , Jeffrey A. Buckel [a] & Thomas E. Lankford Jr. [b]

[a] Center for Marine Sciences and Technology, North Carolina State University, 303 College Circle, Morehead City, North Carolina, 28557, USA

[b] Center for Marine Science, University of North Carolina-Wilmington, 601 South College Road, Wilmington, North Carolina, 28403, USA

Version of record first published: 11 Jul 2012

PLEASE SCROLL DOWN FOR ARTICLE

NOTE

Comparing Multiple Predictors of Energy Content in Juvenile Bluefish

James W. Morley* and Jeffrey A. Buckel

Center for Marine Sciences and Technology, North Carolina State University, 303 College Circle, Morehead City, North Carolina 28557, USA

Thomas E. Lankford Jr.

Center for Marine Science, University of North Carolina–Wilmington, 601 South College Road, Wilmington, North Carolina 28403, USA

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

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

    Two commonly used indicators of energetic status in fish are condition indices and tissue-specific sampling. Condition indices use the length and weight of a fish to determine its relative mass for a given length. The use of condition indices is time- and cost-effective, and condition typically displays a positive relationship with energy density in whole fish (Pangle and Sutton 2005; Rennie and Verdon 2008). However, for some species there is a great deal of variation in energy content for a given value of condition (Jonas et al. 1996; Trudel et al. 2005). Tissue-specific indicators are often more closely related to energy density than condition indices (Plante et al. 2005; Trudel et al. 2005; Kaufman et al. 2007). Some common examples include the energy or lipid density of muscle tissue and the hepatosomatic index, which is the relative mass of the liver to body weight. The best tissue to be used as an energetic indicator often depends on where energy is stored for the species in question (Lambert and Dutil 1997b; Kaufman et al. 2007; Jacobs et al. 2008). Fish primarily store energy as lipid, in the form of triglycerides, and storage depots differ among species (Love 1980; Sheridan 1988). Many species store and utilize lipid

throughout the body (Lizenko et al. 1975; Jorgensen et al. 1997; Slater et al. 2007), while others utilize more specific tissues such as muscle, liver, or mesenteric fat (Love 1980; Sheridan 1988). An important drawback to using tissue-specific indices is that they do not incorporate the relative mass of the fish. For example, it is possible for two fish to appear similar in energetic status based on tissue-specific indicators despite large differences in their relative mass.

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

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

In this study, we examined multiple indicators of energetic status for juvenile bluefish and develop predictive equations for energy content in whole fish. We related whole bluefish energy density to a suite of indicators: the percent dry weight of whole fish (%DW), a condition index, three variations of the hepatosomatic index (HSI), the percent dry weight of muscle tissue (%DW$_{muscle}$), and the energy density of muscle tissue. We also examined the relationship between lipid : protein content and %DW for both whole bluefish and muscle tissue. Last, we

examined differences in body composition between laboratory-held and wild-caught bluefish by comparing the lipid : protein ratio in the muscle tissue of each.

METHODS

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

The energy content of dried tissues and carcasses were determined using proximate composition analysis. Dried bluefish carcasses were homogenized in a food processor, and two 1.5-g subsamples were taken for processing. Dried muscle and liver samples were cut into smaller pieces with dissecting scissors. Neutral lipids were then quantified by Soxhlet extraction with petroleum ether for 4 h; this is an effective technique for estimating triglyceride content (Dobush et al. 1985). The extracted samples were then combusted in a furnace at 450°C for 24 h to determine the mass of the remaining organic material and inorganic ash content. For carcass subsamples, if the lipid content of the two differed by over 6%, a third subsample was processed. This occurred with six bluefish. The proximate composition of the whole carcass was determined by extrapolation using the mean value of the subsamples. To determine the proximate composition of whole fish, data from muscle tissue, liver, and carcass were combined. Carbohydrates are often ignored in proximate analyses due to their low concentrations in fish (Craig 1977; Black and Love 1986; Jonsson et al. 1997), so

we considered the remaining organic material after lipid extraction to be protein. The energy content of tissues and whole fish was calculated using the energy equivalents of lipid (39.54 kJ/g) and protein (23.64 kJ/g) that were shown by Henken et al. (1986) to accurately estimate energy when compared with bomb calorimetry methods.

We examined the relationships between seven different energy indicators and the energy density of whole fish. First, energy density was related to the %DW of whole fish. Next, a condition index was developed. To do this, we combined length and weight data from the 33 bluefish described above with data from 1,112 bluefish from the 2001 and 2002 year-classes. Sampling for the 2001 and 2002 year-classes is described in detail by Morley et al. (2007). Briefly, juvenile bluefish were collected by bottom trawl and hook and line in coastal waters of North Carolina between September 2001 and June 2002 and between September 2002 and June 2003. All weight data were adjusted for gut contents. The data used from the 2001 and 2002 year-classes were within the same length range as the 33 bluefish being used for energy analyses. Least-squares regression was used on log-transformed data to create the following equation:

$$\log_{10}(\text{WW}) = -5.501 + 3.264 \times \log_{10}(\text{FL}),$$
$$r^2 = 0.98, n = 1145 \tag{1}$$

where WW is wet weight and FL is fork length. The residual value of $\log_{10}(\text{WW})$ for the initial 33 bluefish was used as an estimate of condition and was related to estimates of energy density.

We next correlated energy density with three types of HSI:

$$\text{HSI} = (L/\text{WW}) \times 100, \tag{2}$$

where L is the liver wet weight, dry weight, or energy content for the three types of this index.

Last, we examined the relationships between the energy density of whole fish and both %DW$_{\text{muscle}}$ and the energy density of muscle tissue. These two relationships appeared to be nonlinear, we used four nonlinear functions that appeared to fit the data well. First, a linear model was fit:

$$\text{ED} = \beta_0 + \beta_1 \times M, \tag{3}$$

where ED is the energy density of whole fish, β_0 is the intercept, β_1 is the slope, and M is either %DW$_{\text{muscle}}$ or the energy density of muscle tissue. An asymptotic curve was fit with an added parameter for the intercept:

$$\text{ED} = \beta_0 + (\text{ED}_{\max} \times M)/(k + M), \tag{4}$$

where ED$_{\max}$ is the asymptote and k is the level of M at which ED is one-half of the asymptotic value. A sigmoidal curve was

fit, namely,

$$\text{ED} = (\text{ED}_{\max} \cdot M^q)/\left(k^q + M^q\right), \tag{5}$$

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

$$\text{ED} = \beta_0 + \beta_1 \times M \quad \text{for } M \leq \alpha$$
$$\text{ED} = \beta_0 + \beta_1 \times M + \beta_2(M - \alpha) \quad \text{for } M > \alpha \tag{6}$$

where β_1 is the slope of the first segment, β_2 is the difference in slope between the first and second segments, and α is the breakpoint. To determine the most suitable model using muscle tissue, we calculated AIC$_c$ values for each function as

$$\text{AIC}_c = [n \cdot \log_e(\text{RSS}/n) + 2 \cdot K]$$
$$+ [2 \cdot K(K + 1)/(n - K - 1)], \tag{7}$$

where n is sample size, RSS is the residual sum of squares, and K is the number of estimated parameters including the error term (Burnham and Anderson 2002). This is a modified version of the Akaike information criterion with an adjustment for small sample sizes. For each model we also calculated ΔAIC_c values (the difference between the AIC$_c$ of a given model and that of the best model) and the AIC$_c$ weight (w; the probability that a given model is the best one) (Burnham and Anderson 2002).

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

To determine whether our results are applicable to laboratory-held fish, we compared the lipid : protein content in the muscle tissue of laboratory bluefish with that of the wild-caught fish described above. The laboratory bluefish were part of a winter-feeding and growth experiment (J. W. Morley, unpublished data). Briefly, juvenile bluefish ($n = 36$) ranging in fork length from 136 to 170 mm were captured by beach seine in late November 2006. Fish were held in 540-L flow-through tanks at ambient temperatures and fed once daily to satiation. On January 30, bluefish were randomly assigned to six different tanks and each tank was gradually adjusted to one of three temperatures: 9.5, 12.0, and 14.5°C. Bluefish were then fed twice daily to satiation until March 1 when the experiment was ended and fish were sacrificed. The whole-fish energy content was not

determined for these samples, but the procedure for the analysis of muscle tissue was identical to that in the present study.

RESULTS AND DISCUSSION

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

Our condition index was significantly related with energy density, but it explained relatively little variation ($r^2 = 0.33$, $n = 33$, $P < 0.001$; Figure 1). For a given value of condition, energy density varied by up to a factor of two. Other studies have also found that condition explains little of the variation in energy content (Jonas et al. 1996; Trudel et al. 2005). Condition often relates poorly to energy because of the amount of variation in water content (Sutton et al. 2000; Plante et al. 2005; Rennie and Verdon 2008) and lipid content (Copeland and Carline 2004;

$$y = 5.911 + 32.720 \cdot x$$

FIGURE 1. Energy density of whole bluefish in relation to condition. Condition is expressed in terms of the residuals from a regression of $\log_{10}$(wet mass) on $\log_{10}$(fork length) with data from multiple year-classes of bluefish ($n = 1,145$).

Trudel et al. 2005; Rennie and Verdon 2008) for a given value of condition. Further, the relationship between condition and energy or proximate composition can vary seasonally (Neumann and Murphy 1992; Pangle and Sutton 2005; Trudel et al. 2005) and among different populations (Copeland and Carline 2004; Kaufman et al. 2007). The use of condition will often depend on what amount of variation is considered acceptable. However, for many studies that conduct lethal sampling, validating an alternative using tissue-specific indicators may increase the accuracy of conclusions about energetic status.

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

Based on r^2 values and residual patterns, both energetic status indicators using muscle tissue were better predictors of the energy density in whole fish than condition or HSI (Figure 3). Based on $\Delta \mathrm{AIC}_c$ scores, the piecewise, sigmoid, and linear functions using %DW$_{muscle}$ as the predictor were good candidate models for estimating the energy density of whole fish (Table 1). Of these candidate models, we chose to proceed with the piecewise function because it had the highest model weight (w). In the piecewise model, %DW$_{muscle}$ is linearly related to the energy density in whole fish up to a breakpoint value of 26.4% (Figure 3A). Beyond this breakpoint, energy increased more slowly with further increases in %DW$_{muscle}$. The relationship between energy density in muscle tissue and that in whole fish was also best explained with a nonlinear model (Table 1; Figure 3B). However, our results revealed that incurring the additional laboratory costs required to determine the energy density in muscle tissue does not result in a more accurate predictor of total energy content. Muscle tissue has been shown to be an effective indicator for other species. Kaufman et al. (2007) found the lipid content of walleye muscle tissue to be more closely related to somatic proximate composition than HSI. Jacobs et al. (2008) found the proximate composition of both the abdominal wall and whole fillets to be effective at predicting energy content in whole
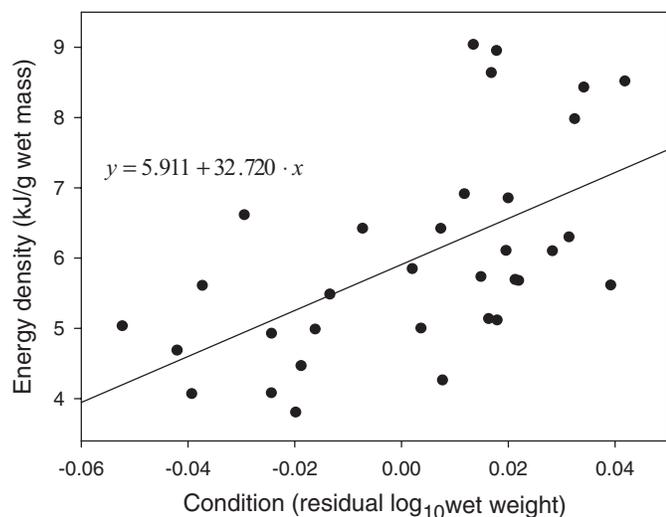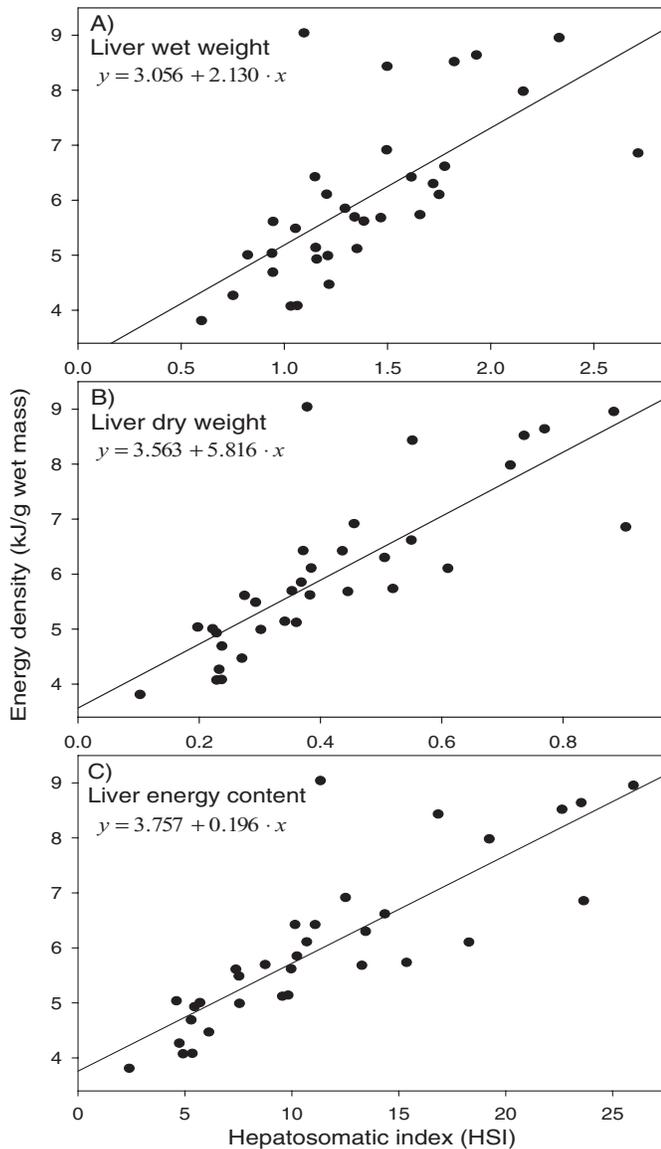
FIGURE 2. Energy density of whole bluefish in relation to hepatosomatic indexes based on **(A)** liver wet mass, **(B)** liver dry mass, and **(C)** liver energy content.

FIGURE 3. Energy density of whole bluefish in relation to **(A)** percent dry weight of white muscle tissue fit with piecewise regression and **(B)** energy density of white muscle tissue fit with a sigmoidal function. The functions fit to the data were chosen based on the lowest AIC_c scores (Table 1).

striped bass *Morone saxatilis*. Further, the ability to use water content in muscle tissue to estimate energy content in whole fish has been shown for winter flounder *Pseudopleuronectes americanus* (Plante et al. 2005), coho salmon *Oncorhynchus kisutch*, and Chinook salmon *O. tshawytscha* (Trudel et al. 2005).

There is an important distinction to be made between using tissue-specific indicators as a proxy for energetic status and using predictive equations derived from indicators to determine energy content in whole fish. We advocate the second approach for two reasons. First, theoretically, two fish of the same length and energy density can vary greatly in mass. In this situation, a study that only reports energy density values, or that uses an energetic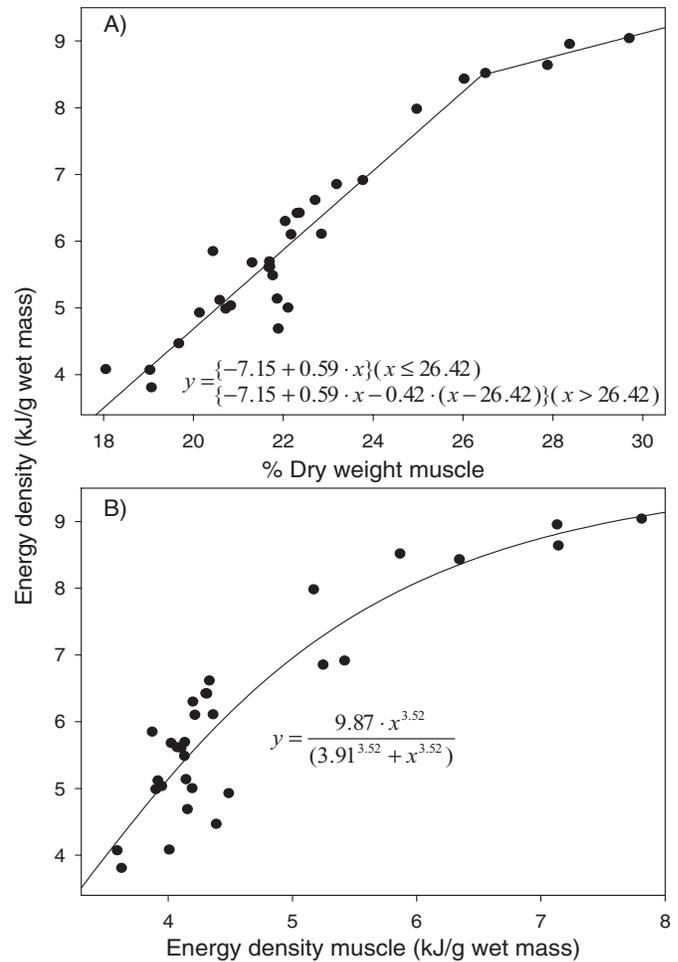 indicator, will not realize the difference in energetic status between these two fish. For example, Einen et al. (1998) found the proximate composition of Atlantic salmon to remain fairly stable when starved, while body mass declined. Simpkins et al. (2003) found HSI to remain stable in rainbow trout *O. mykiss* starved for over 100 d, despite substantial loss of body lipid and protein. The second reason deals with tissue-specific indicators that are nonlinearly related to the energy content of whole fish, as we have shown with muscle tissue in bluefish. In this situation, changes in the indicator will not be equally proportional to changes in energetic status. Therefore, for juvenile bluefish we recommend using the predictive equation to convert values of $\%DW_{muscle}$ to energy density (Table 1) and then multiplying by the wet weight of the fish to determine total energy content.

The reason for the nonlinear relationship between the energy density of whole fish and muscle tissue (Figure 3B) is due to the nonuniform way bluefish store lipid throughout the body. When whole fish are examined, the lipid : protein ratio increases

TABLE 1. Models of energy density (kJ/g wet mass) for whole bluefish (ED) using either the percent dry weight in muscle tissue (%DW$_M$) or the energy density of muscle tissue (ED$_M$) as a predictor variable ($n = 31$). The means (SDs) of the two predictors were as follows: %DW$_M$ = 22.51 (2.76), and ED$_M$ = 4.67 (1.09). Abbreviations are as follows: K = the number of parameters, RSS = the residual sum of squares, AIC$_c$ = the Akaike information criterion corrected for small sample size, ΔAIC$_c$ = the mean AIC$_c$ difference, and w = the mean AIC$_c$ weight.

Function	Equation		K	r^2	RSS	AIC$_c$	ΔAIC$_c$	w
	Percent dry weight muscle (%DW$_M$)							
Linear	$ED = -5.45 + 0.51 \cdot \%DW_M$		3	0.91	6.05	−43.75	1.53	0.18
Asymptotic	$ED = -16.51 + (48.59 \times \%DW_M)/(25.75 + \%DW_M)$		4	0.91	5.69	−43.00	2.28	0.12
Sigmoid	$ED = (12.40 \cdot \%DW_M^{4.15})/(22.56^{4.15} + \%DW_M^{4.15})$		4	0.92	5.37	−44.82	0.46	0.31
Piecewise[a]	$ED = -7.15 + 0.59 \cdot \%DW_M$	for $\%DW_M \leq 26.42$						
	$= -7.15 + 0.59 \cdot \%DW_M - 0.42 \cdot (\%DW_M - 26.42)$	for $\%DW_M > 26.42$	5	0.93	4.82	−45.28	0	0.39
	Energy density muscle (ED$_M$)							
Linear	$ED = 0.47 + 1.20 \cdot ED_M$		3	0.79	14.19	−17.34	27.94	0.00
Asymptotic	$ED = -42.33 + (56.95 \cdot ED_M)/(0.79 + ED_M)$		4	0.83	11.51	−21.17	24.11	0.00
Sigmoid	$ED = (9.87 \cdot ED_M^{3.52})/(3.91^{3.52} + ED_M^{3.52})$		4	0.83	11.29	−21.77	23.51	0.00
Piecewise	$ED = -2.32 + 1.87 \cdot ED_M$	for $ED_M \leq 5.18$						
	$= -2.32 + 1.87 \cdot ED_M - 1.14 \cdot (ED_M - 5.18)$	for $ED_M > 5.18$	5	0.82	11.61	−18.06	27.22	0.00

[a] The mean square of the regression = 301.21, while the mean square error = 0.179. The estimates of the regression parameters (SEs) are as follows: $\beta_0 = -7.15$ (1.04), $\beta_1 = 0.59$ (0.05), $\beta_2 = -0.42$ (0.19), and $\alpha = 26.42$ (1.08); refer to equation (6) in Methods for parameter definitions.

linearly with %DW ($r^2 = 0.94$, $n = 33$, $P < 0.001$; Figure 4A). However, in muscle tissue we observed a biphasic relationship with lipid : protein content and %DW$_{muscle}$. A piecewise function was used to describe this relationship ($r^2 = 0.89$, $n = 724$, $P < 0.01$; Figure 4B). Below the breakpoint of 23.1%, the lipid : protein ratio in muscle tissue remained near zero and newly deposited tissue is almost entirely protein. As a result of lipids being stored in nonmuscle tissues first, the energy density of the whole fish increases faster than the energy density of muscle (Figure 3B). Once %DW$_{muscle}$ exceeds the threshold of 23.1%, the added organic mass in muscle tissue is mostly lipid and the energy content of muscle increases rapidly (Figure 4B).

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

Juvenile bluefish held in aquaria for 4 months during the winter developed much higher lipid : protein ratios in their muscle
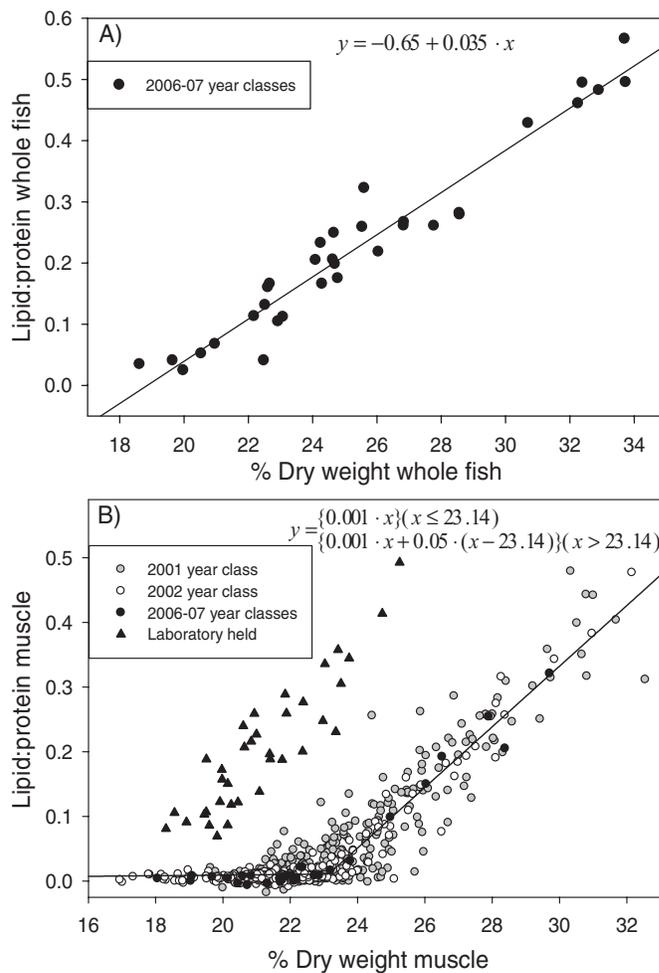

FIGURE 4. Lipid : protein ratios in relation to percent dry weight for **(A)** whole bluefish and **(B)** white muscle tissue of laboratory-held and wild-caught bluefish, with the piecewise regression function fit to wild-caught bluefish data.

tissue than wild bluefish (Figure 4B). Only muscle tissue was examined, so it is uncertain whether lipid content is higher throughout the body of laboratory bluefish. However, we advise against applying our predictive equation to laboratory bluefish because our results suggest that the energy content of whole captive fish would be underestimated. Research on other species suggests that the lack of activity in laboratory fish probably leads to altered body composition. Simpkins et al. (2003) held rainbow trout in the laboratory, and some fish were forced to swim continually. These active trout had lower lipid densities for a given value of %DW than did sedentary fish. Further, the relationship between condition and lipid density differed between active and sedentary fish. Similarly, studies with walleyes and bluegills *Lepomis macrochirus* have shown the relationship between condition and body composition to differ between laboratory and wild fish (Copeland and Carline 2004; Copeland et al. 2010).

Juvenile bluefish recruit along the entire U.S. East Coast, and during the fall and early winter they migrate south to overwinter (Wuenschel et al. 2012). Our sampling area off North Carolina is near the northern limit of the overwintering range of juvenile bluefish (Morley et al. 2007; Wuenschel et al. 2012). By sampling during the migratory period, we were able to collect juveniles that initially recruited over a large geographic area. Further, by sampling between November and April we were able to capture juvenile bluefish across the full range in energetic status (Morley et al. 2007). The %DW$_{muscle}$ values for the present study (2006–2007 year-classes) encompass nearly the entire range that has been observed for juvenile bluefish (Figure 4B). Therefore, the energy storage patterns we described are applicable to the whole population. However, the accuracy of our predictive model for larger bluefish (>310 mm) is uncertain. Previous research examining whole bluefish suggests that the relationship between water content and energy, lipid, and protein content is consistent across size (Hartman and Brandt 1995; Hartman and Margraf 2008). However, it is uncertain whether lipid storage patterns in specific tissues change in larger mature adults. Also, the accuracy of models predicting energy content may vary among populations (Kaufman et al. 2007). Future studies using our predictive models on larger bluefish or non-U.S. populations would benefit from determining whole-fish energy content on a limited sample to validate the model.

We are confident that our predictive equations will perform well with independent data, since the physiology of energy storage in fish appears to be relatively simple. For example, a majority of the variation ($r^2 > 0.9$) in the energy content of fish can be explained using only percent dry weight (Hartman and Brandt 1995; Ciancio et al. 2007). In this study we confirmed that the patterns of energy storage in bluefish are consistent between studies; our relationship of percent dry weight to energy density in whole fish was similar to that of Hartman and Brandt (1995) despite our using different methods to determine energy content. Further, we found similar patterns of lipid storage for bluefish muscle tissue across multiple year-classes (Figure 4B).

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

REFERENCES

Black, D., and R. M. Love. 1986. The sequential mobilisation and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. Journal of Comparative Physiology B 156:469–479.

Brown, M. L., and B. R. Murphy. 1991. Relationship of relative weight (W_r) to proximate composition of juvenile striped bass and hybrid striped bass. Transactions of the American Fisheries Society 120:509–518.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodal inference: a practical information-theoretic approach, 2nd edition. Springer, New York.

Ciancio, J. E., M. A. Pascual, and D. A. Beauchamp. 2007. Energy density of Patagonian aquatic organisms and empirical predictions based on water content. Transactions of the American Fisheries Society 136:1415–1422.

Copeland, T., and R. F. Carline. 2004. Relationship of lipid content to size and condition in walleye fingerlings from natural and aquaculture environments. North American Journal of Aquaculture 66:237–342.

Copeland, T., B. R. Murphy, and J. J. Ney. 2010. The effects of feeding history and environment on condition, body composition and growth of bluegills *Lepomis macrochirus*. Journal of Fish Biology 76:538–555.

Craig, J. F. 1977. The body composition of adult perch, *Perca fluviatilis* in Windermere, with reference to seasonal changes and reproduction. Journal of Animal Ecology 46:617–632.

Dhieb, K., M. Ghorbel, O. Jarboui, and A. Bouain. 2006. Interactions between reproduction and fisheries in bluefish, *Pomatomus saltatrix* (Pomatomidae), from Gulf of Gabes (Tunisia). Cybium 30:355–364.

Dobush, G. R., C. D. Ankney, and D. G. Krementz. 1985. The effect of apparatus, extraction time, and solvent type on lipid extractions of snow geese. Canadian Journal of Zoology 63:1917–1920.

Einen, O., B. Waagan, and M. S. Thomassen. 1998. Starvation prior to slaughter in Atlantic salmon (*Salmo salar*) I. Effects on weight loss, body shape, slaughter- and fillet-yield, proximate and fatty acid composition. Aquaculture 166:85–104.

Elliott, J. M. 1976. Body composition of brown trout (*Salmo trutta* L.) in relation to temperature and ration size. Journal of Animal Ecology 45:273–289.

Graves, J. E., J. R. McDowell, A. M. Beardsley, and D. R. Scoles. 1992. Stock structure of the bluefish *Pomatomus saltatrix* along the mid-Atlantic coast. U.S. National Marine Fisheries Service Fishery Bulletin 90:703–710.

Hartman, K. J., and S. B. Brandt. 1995. Estimating energy density of fish. Transactions of the American Fisheries Society 124:347–355.

Hartman, K. J., and F. J. Margraf. 2008. Common relationships among proximate composition components in fishes. Journal of Fish Biology 73:2352–2360.

Henken, A. M., H. Lucas, P. A. T. Tijssen, and M. A. M. Machiels. 1986. A comparison between methods used to determine the energy content of feed, fish and faeces samples. Aquaculture 58:195–201.

Jacobs, J. M., M. R. Rhodes, B. Martin, D. McIntosh, W. F. Van Heukelem, and R. M. Harrell. 2008. Tissue-specific sampling for the estimation of striped bass whole-body proximate composition. Transactions of the American Fisheries Society 137:386–392.

Jonas, J. L., C. E. Kraft, and T. L. Margenau. 1996. Assessment of seasonal changes in energy density and condition in age-0 and age-1 muskellunge. Transactions of the American Fisheries society 125:203–210.

Jonsson, N., B. Jonsson, and L. P. Hansen. 1997. Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon *Salmo salar*. Journal of Animal Ecology 66:425–436.

Jorgensen, E. H., S. J. S. Johansen, and M. Jobling. 1997. Seasonal patterns of growth, lipid deposition and lipid depletion in anadromous Arctic charr. Journal of Fish Biology 51:312–316.

Juanes, F., J. Hare, and A. G. Miskiewicz. 1996. Comparing early life history strategies of *Pomatomus saltatrix*: a global approach. Marine and Freshwater Research 47:365–379.

Kaufman, S. D., T. A. Johnston, W. C. Leggett, M. D. Moles, J. M. Casselman, and A. I. Schulte-Hostedde. 2007. Relationships between body condition indices and proximate composition in adult walleyes. Transactions of the American Fisheries Society 136:1566–1576.

Lambert, Y., and J. D. Dutil. 1997a. Condition and energy reserves of Atlantic cod (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock. Canadian Journal of Fisheries and Aquatic Sciences 54:2388–2400.

Lambert, Y., and J. D. Dutil. 1997b. Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus morhua*)? Canadian Journal of Fisheries and Aquatic Sciences 54(Supplement 1):104–112.

Lantry, B. F., and R. O'Gorman. 2007. Drying temperature effects on fish dry mass measurements. Journal of Great Lakes Research 33:606–616.

Lizenko, Y. I., V. S. Sidorov, and O. I. Potapova. 1975. Seasonal variations in the lipid composition of the tissues and organs of the large cisco (*Coregonus albula*) in Karelian Lakes. Journal of Ichthyology 15:465–472.

Love, R. M. 1980. The chemical biology of fishes, volume 2. Academic Press, London.

Morley, J. W., J. A. Buckel, and T. E. Lankford. 2007. Winter energy storage dynamics and cohort structure of young-of-the-year bluefish *Pomatomus saltatrix* off North Carolina. Marine Ecology Progress Series 334:273–286.

Nanton, D. A., A. Vegusdal, A. M. Bencze Røra, B. Ruyter, G. Baeverfjord, and B. E. Torstensen. 2007. Muscle lipid storage pattern, composition, and adipocyte distribution in different parts of Atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil. Aquaculture 265:230–243.

Neumann, R. M., and B. R. Murphy. 1992. Seasonal relationships of relative weight to body composition in white crappie, *Pomoxis annularis* Rafinesque. Aquaculture and Fisheries Management 23:243–251.

Pangle, K. L., and T. M. Sutton. 2005. Temporal changes in the relationship between condition indices and proximate composition of juvenile *Coregonus artedi*. Journal of Fish Biology 66:1060–1072.

Plante, S., C. Audet, Y. Lambert, and J. D. L. Noue. 2005. Alternative methods for measuring energy content in winter flounder. North American Journal of Fisheries Management 25:1–6.

Rennie, M. D., and R. Verdon. 2008. Development and evaluation of condition indices for the lake whitefish. North American Journal of Fisheries Management 28:1270–1293.

Sheridan, M. A. 1988. Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. Comparative Biochemistry and Physiology 90B:679–690.

Simpkins, D. G., W. A. Hubert, C. Martinez Del Rio, and D. C. Rule. 2003. Physiological responses of juvenile rainbow trout to fasting and swimming activity: effects on body composition and condition indices. Transactions of the American Fisheries Society 132:576–589.

Slater, J. J., T. E. Lankford, and J. A. Buckel. 2007. Overwintering ability of young-of-the-year bluefish *Pomatomus saltatrix*: effect of ration and cohort of origin on survival. Marine Ecology Progress Series 339:259–269.

Sutton, S. G., T. P. Bult, and R. L. Haedrich. 2000. Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. Transactions of the American Fisheries Society 129:527–538.

Trudel, M., S. Tucker, J. F. T. Morris, D. A. Higgs, and D. W. Welch. 2005. Indicators of energetic status in juvenile coho salmon and Chinook salmon. North American Journal of Fisheries Management 25:374–390.

Wiedenmann, J., and T. E. Essington. 2006. Density-dependent overwinter survival in young-of-year bluefish (*Pomatomus saltatrix*)? A new approach for assessing stage-structured survival. Canadian Journal of Fisheries and Aquatic Sciences 63:1934–1943.

Wuenschel M. J., K. W. Able, J. A. Buckel, J. W. Morley, T. Lankford, A. C. Branson, D. O. Conover, D. Drisco, A. Jordaan, K. Dunton, D. H. Secor, R. J. Woodland, F. Juanes, and D. Stormer. 2012. Recruitment patterns and habitat use of young-of-the-year bluefish along the United States east coast: insights from coordinated coastwide sampling. Reviews in Fisheries Science 20:80–102.