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Author: Hartman, Kyle J.

Source: Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science, 3(1) : 307-316

Published By: American Fisheries Society

URL: <https://doi.org/10.1080/19425120.2011.603961>

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ARTICLE

## Temperature Effects on Bioelectrical Impedance Analysis (BIA) Used to Estimate Dry Weight as a Condition Proxy in Coastal Bluefish

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### Abstract

The highly migratory nature of bluefish *Pomatomus saltatrix* makes comprehensive study of their populations and their potential responses to factors such as competition, habitat degradation, and climate change difficult. Body composition is an important ecological reference point for fish; however, estimating body composition in fish has been limited by analytical and logistical costs. We applied bioelectrical impedance analysis (BIA) to estimate one body composition component (percent dry weight) as a proxy of condition in bluefish. We used a tetra polar Quantum II BIA analyzer and measured electrical properties in the muscles of bluefish at two locations per fish (dorsal and ventral). In total, 96 bluefish ranging from 193 to 875 mm total length were used in model development and testing. On 59 of these fish BIA measures were taken at both 15°C and 27°C. Temperature had a significant negative effect on resistance and reactance. A subsample of these fish was then analyzed for dry weight as a percentage of their whole body weight (PDW), which is a good indicator of condition because it is highly correlated with fat content in fish. The BIA models predicting PDW inclusive of all lengths of bluefish were highly predictive for 15°C (stepwise regression) and 27°C. Regression ( $R^2_{\text{pred}}$ ) values that estimate future predictive power suggest that both models were robust. Strong relationships between PDW and other body composition components, coupled with the BIA models presented here, provide the tools needed to quantitatively assess bluefish body composition across spatial and temporal scales for which assessment was previously impossible.

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The growth of fish is believed to be an integrated measure of well-being that is linked to reproductive success, survival, habitat quality, and competition (Brandt et al. 1992; Roy et al. 2004; Amara et al. 2009; Vehanen et al. 2009). In aquaculture and other applications, such as those employing fish bioenergetics models, growth is often determined by measuring differences in the total weight of fish over time. However, fish are 60–90% water, and they often compensate for loss of fat by replacing it with water, making the use of total weight to measure growth

and condition problematic (Shearer 1994; Breck 2008; Hartman and Margraf 2008). To fully evaluate growth in weight of fish requires knowledge of the percent dry mass of the fish. Dry mass can be measured on an individual by oven drying or by freeze drying but, in addition to being lethal, this process can be cumbersome for large individuals or impossible for rare taxa.

Bioelectrical impedance analysis (BIA) has been used to determine water mass in human subjects since the 1970s and is now widely used in health clubs to assess human body condition.

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Received April 7, 2010; accepted January 25, 2011

Recently, BIA has been developed as a nonlethal method used to estimate wet and dry masses, as well as lipid, protein, and ash masses in several species of fish (Cox and Hartman 2005; Duncan et al. 2007). Cox and Hartman (2005) developed models to estimate composition masses of brook trout *Salvelinus fontinalis* using BIA. Models for cobia *Rachycentron canadum* (Duncan et al. 2007) and Great Lakes fish (Pothoven et al. 2008) have also been developed. These studies in fish failed to consider temperature effects or length bias in their analysis. Cox and Heintz (2009) found a significant effect of temperature upon BIA-derived phase angle in salmonids, but other BIA studies with fish ignored the influence of temperature upon BIA measures. Electrical properties are influenced by temperature, so it must be considered in model development and model application.

Previous studies employing BIA to estimate fish body composition predicted only body mass (Cox and Hartman 2005; Duncan et al. 2007). Estimating mass has been problematic because the length of the electrical circuit (or detector length) is highly correlated with fish length and measures were made at consistent relative locations on each fish. This means that much like BIA use in humans, much of the predictive power is achieved through the relationship between length (or height) and mass (Hofer et al. 1969; Lukaski et al. 1985; Kushner and Schoeller 1986). In theory, fat does not conduct electricity and hence resistance (i.e., the measure of the opposition by a body to the passage of a steady electrical current) is sensitive to the fat levels. Likewise, reactance (i.e., the opposition of a body to alternating DC due to capacitance of inductance) is sensitive to cell volume in an area. Thus, although previous work with BIA in fish primarily estimated body masses, BIA holds the potential to estimate body percent composition, which is less dependent on fish length. However, to date only a study by Pothoven et al. (2008) attempted to estimate lipid percentages in Great Lakes fish, but without success. However, the Pothoven et al. (2008) study was field-based and necessarily lacked the range of lipid levels, or control for temperature effects, that is possible in laboratory studies.

Bluefish *Pomatomus saltatrix* are an ecologically and economically important species along the U.S. Atlantic coast. However, their widespread distribution makes study of population demographics and parameters such as body composition and growth difficult (Salerno et al. 2001). Studies across large spatial scales may identify heterogeneity of body composition or condition that could identify areas of population stress, pollution, or competition. However, such studies are currently limited by our reliance upon measures of condition that are often inaccurate (e.g., total-weight-based measures) or laboratory measures such as proximate composition, which are either logistically or economically limiting (Cox and Hartman 2005). Strong predictive relationships have been found that relate percent dry weight (PDW) to energy content (Hartman and Brandt 1995a) and body composition (percent lipid and protein) in bluefish (Hartman and Margraf 2008), indicating that it could be used as a proxy for

overall fish condition. Therefore, the objective of this study was to evaluate the influence of temperature upon BIA measures and further develop the BIA tools necessary to measure PDW, as a proxy for condition, in coastal bluefish.

## METHODS

We collected 60 bluefish via angling in the Atlantic Ocean off Sandy Hook, New Jersey, in October 2006. These bluefish were transported alive to the National Oceanic and Atmospheric Administration's J. J. Howard Marine Sciences Center, where they were held in water-flow-through tanks. These fish fell into two length-groups: small bluefish ranging from 193 to 267 mm total length (TL) and larger bluefish ranging from 401 to 875 mm TL. This natural gap in fish length distribution roughly corresponded to age-0 (small) and older (large) bluefish (Hartman and Brandt 1995b).

Fish were separated into tanks based on size, and subsequently 32 were fed thawed fish ad libitum daily to achieve high body condition and 28 were fasted (about 1 month for age-0 fish or about 2 months for older fish) to achieve low body condition. Our goal in this study was to obtain bluefish of varying sizes and varying fat levels from which to develop model data sets for BIA analysis. Therefore, feeding regimes were considered of secondary importance to developing bluefish of differing body composition; using these fish we also coincidentally evaluated the influence of temperature upon their BIA measures. Thus, although some fish were fasted and others were fed, these were not true "treatments" in the experimental design but rather were conditions under which bluefish were held to ensure the range of body conditions needed for the study.

We also collected 36 bluefish (198–452 mm TL) in August 2006 in the Patuxent River off Solomons, Maryland. These fish were transported to Chesapeake Biological Laboratory, where they were held in water-flow-through tanks for less than 24 h before their BIAs were measured at ambient water temperatures of 27°C. These Maryland fish were included in model and test data sets for the 27°C models and were assumed to represent fish of intermediate body condition (i.e., neither fasted nor fed ad libitum in their natural environment).

*Bioelectrical impedance measurement.*—We used a tetra polar Quantum II BIA Analyzer (RJL Systems, Clinton Township, Michigan) to measure the electrical properties of the bluefish. The BIA analyzer was equipped with a pair of 28-gauge stainless steel needle electrodes with signal and detector electrodes fixed at 10 mm apart for each electrode (Cox and Hartman 2005). Fish were anesthetized in MS-222 (tricaine methanesulfonate) and placed on their right side on a nonconductive surface. Needle electrodes (5-mm insertion length) were inserted into the fish at consistent locations: dorsally (posterior to the opercula and anterior of the caudal fin with both positioned midway between the lateral line and dorsal midline) and ventrally (posterior of the pelvic fin and anterior of the anal fin near the ventral midline; Figure 1). For both the dorsal and ventral locations we

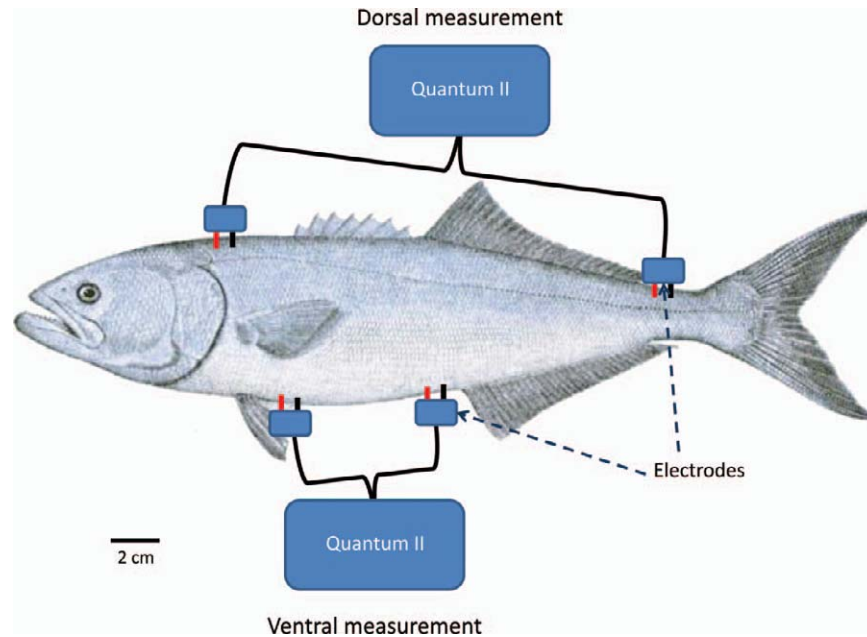


FIGURE 1. Placement of bioelectrical impedance analysis probes on the bluefish. Dorsal measures were located midway between the lateral line and dorsal midline, one probe in vertical alignment with the posterior edge of the opercle and the second midway between the posterior of the second dorsal fin and the anterior edge of the caudal peduncle. Ventral measures were along the ventral midline, one probe immediately posterior to the pelvic fin insertions and the other posterior to the anal vent.

recorded the resistance and reactance and the electrode placement length (or detector length, a measure of the electrical path between electrodes) for each fish. We also recorded total length (mm) and weight (g) of each fish, and each fish was tagged with a passive integrated transponder (PIT) tag to identify it for later BIA measures (in the temperature experiment) or for laboratory measures of dry mass. Once all measures were completed on a fish it was euthanized in an overdose of MS-222, bagged and frozen for later analysis of dry mass. To determine this, PIT tags were removed and fish were filleted to increase surface area for drying, and then the entire fish was dried in an oven at 70°C until a constant dry weight was achieved (range of 3–5 d). Percent dry weight was calculated for each fish: total dry weight as a percentage of total wet weight.

**Temperature experiment.**—To evaluate the influence of temperature on BIA measures in bluefish, we measured the BIAs of PIT-tagged individuals at warm (27°C) and cold (15°C) temperatures. We were only able to control temperatures at J.J. Howard Marine Sciences Center, so only the Sandy Hook fish were used in the temperature experiments.

Prior to our taking BIA measures, 59 bluefish were acclimated to 27°C for a period of 2 weeks. Individuals were then anesthetized in MS-222; PIT-tagged with a unique code; measured for length and weight; and finally both dorsal and ventral measures of resistance, reactance, and detector lengths were determined. Once these measures were completed the fish was immediately placed into another tank and maintained at 15°C

for 24–36 h before it was anesthetized and remeasured for BIA at this lower temperature. Fish were then euthanized in an overdose of MS-222. We assumed that the body composition did not change appreciably between BIA measures over this time and that body composition at the start of the experiment (27°C) was the same as at the end of the experiment (15°C). The resulting repeated measure on each individual was used to evaluate temperature effects on dorsal and ventral BIA measures. A series of independent paired *t*-tests ( $\alpha = 0.05$ ) were used to test for differences in dorsal resistance, dorsal reactance, ventral resistance, ventral reactance, and dorsal and ventral detector lengths measured at 15°C with those at 27°C.

**Model development and validation.**—Bioelectrical impedance analysis measures provide resistance and reactance of the fish from which we calculate additional electrical properties used as candidate predictor variables in the BIA model. These electrical properties include resistance in series, resistance in parallel, capacitance in series, capacitance in parallel, reactance in series, reactance in parallel, and phase angle (Cox and Hartman 2005; Table 1). Resistance and reactance are affected by the length of the circuit (detector length). Therefore, we also calculated standardized impedance measures by dividing resistance and reactance by the detector length and included them as candidate variables in our BIA models (Table 1, E8 and E9, respectively). Stepwise regression was used to determine the best fit model for prediction of percent dry weight. We evaluated variables from electrical properties derived from single

TABLE 1. Electrical variables for AC series and parallel circuits used as candidate predictor variables in bioelectrical impedance analysis models of bluefish percent dry weight. The variables were calculated for both dorsal and ventral measurement locations.

Electrical variable	Abbreviation	Units	Measure or equation
Detector length	DL	mm	Linear measure between electrodes
Resistance in series	$R$	$\Omega$ (ohms)	Measured directly by Quantum II
Reactance in series	$X_c$	$\Omega$	Measured directly by Quantum II
Resistance index	$E1$	$\Omega$	$DL^2/R$
Parallel resistance index	$E2$	$\Omega$	$DL^2/LRp$ , where $LRp = R + (Xc^2/R)$
Reactance index	$E3$	$\Omega$	$DL^2/Xc$
Parallel reactance index	$E4$	$\Omega$	$DL^2/LXcp$ , where $LXcp = Xc + (R^2/Xc)$
Parallel capacitance index	$E5$	pF (picofarads)	$DL^2/LCpf$ , where $LCpf = (\pi \cdot E7)/Xc$
Impedance index	$E6$	$\Omega$	$DL^2/LZ$ , where $LZ = (R^2 + Xc^2)^{0.5}$
Phase angle	$E7$	$^\circ$ (degrees)	$\text{atan}(Xc/R)$
Standardized resistance	$E8$	$\Omega/\text{mm}$	$R/DL$
Standardized reactance	$E9$	$\Omega/\text{mm}$	$Xc/DL$

BIA locations (dorsal or ventral BIA measures) as well as both dorsal and ventral locations in the models.

We also evaluated whether all sizes of bluefish could be included in a single model for each temperature or whether models for discrete sizes were warranted. Although the goal was to develop a single model for bluefish across all lengths, models specific to length-groups of fish could be more accurate in estimating fish PDW because a small fish at 28% PDW could be in higher condition than a large fish at 28% PDW. When we parsed the data set by fish length-groups (small versus large fish), we lacked sufficient sample size to further split the data into model and test data sets for small and large bluefish. Therefore, we used the complete data set ( $N = 60$  at  $15^\circ\text{C}$  and  $N = 95$  at  $27^\circ\text{C}$ ) to develop models for small ( $<400$  mm TL) and large ( $\geq 400$  mm TL) bluefish.

Using the data sets for small and large bluefish at each temperature, we determined the best models to predict the percent dry weight of bluefish by using electrical properties from dorsal-only measures, ventral-only measures, and dorsal and ventral measures simultaneously. Measurement locations or combination of locations were evaluated because a single or multiple measurement location potentially represents a tradeoff between time in handling fish and accuracy in predictions of body composition. By comparing relative model fit and the number of model parameters retained, we evaluated whether models developed using bluefish of all lengths combined performed as well as those based on discrete length-groups. To evaluate the fit of these models for each data set, a leave-one-out validation approach using prediction sum of squares (PRESS) residuals was used (Myers 1990; Rosenberger and Dunham 2005). The PRESS residuals are estimated by leaving a single observation out and calculating a residual by subtracting the observed value from that predicted by a regression model predicted with the remaining observations.

The PRESS residuals were compared with residuals estimated from the overall means model producing an  $R^2$ -like statistic ( $R^2_{\text{pred}}$ ) that indicates the overall predictive performance (Myers 1990).

After determining that a model using all observations ( $N = 60$  at  $15^\circ\text{C}$ , and  $N = 95$  at  $27^\circ\text{C}$ ), which included all lengths of bluefish, performed comparably to BIA models for discrete length-groups, we proceeded with developing and testing a bluefish BIA model at  $15^\circ\text{C}$  and  $27^\circ\text{C}$  using a model and an independent test data set. The observations on 59 Sandy Hook fish were sorted by total length and then every fourth observation was removed for the model data set until the model set contained 41 and the test set included 18 fish at  $15^\circ\text{C}$  and  $27^\circ\text{C}$ . One additional fish was measured at  $15^\circ\text{C}$  only and included in the  $15^\circ\text{C}$  model data set. The Patuxent River fish were all collected at  $27^\circ\text{C}$ , so these observations were randomly assigned to either the  $27^\circ\text{C}$  model ( $N = 28$ ) or  $27^\circ\text{C}$  test ( $N = 8$ ) data sets. Hence, the  $15^\circ\text{C}$  model and test sets contained 42 and 18 observations, respectively, while the  $27^\circ\text{C}$  model and test data sets contained 69 and 26 observations. The test and model sets were similar with respect to the lengths of fish ( $15^\circ\text{C}$ : test = 207–807 mm, model = 193–844 mm;  $27^\circ\text{C}$ : test = 204–807 mm, model = 193–875 mm) and the range of percent dry weights of fish ( $15^\circ\text{C}$ : test = 20.2–40.4%, model = 16.3–40.3%;  $27^\circ\text{C}$ : test = 20.2–40.4%, model = 20.2–40.6%) at each temperature (Figure 2).

Once these  $15^\circ\text{C}$  and  $27^\circ\text{C}$  models were established, we evaluated them using PRESS residuals as above and then conducted a sensitivity analysis by increasing or decreasing the resistance, reactance, and detector length values from the dorsal and ventral locations individually by  $\pm 10\%$  and compared the model predictions of PDW. A measured variable was considered sensitive if varying the input by 10% resulted in more than a 10% change in the predicted PDW (Bartell et al. 1986).

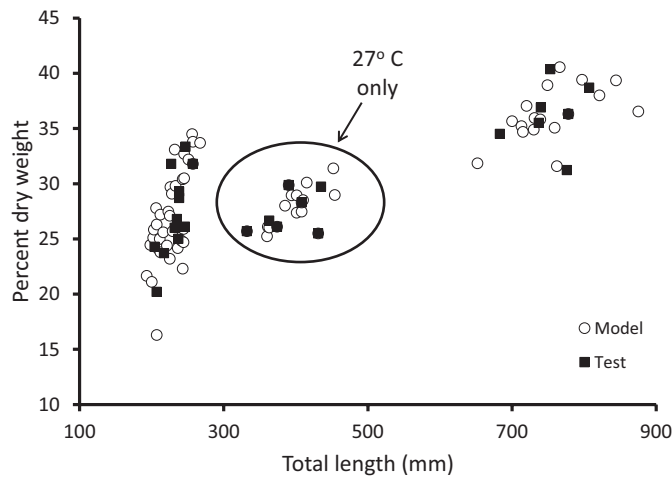


FIGURE 2. Test and model data sets used for validating that bluefish bioelectrical impedance analysis models for percent dry weight (PDW) were similar with respect to the distribution of total lengths and PDW. Data points for fish 300–475 mm in length were only available at 27°C, while those for fish of all other lengths were available at both 15°C and 27°C.

## RESULTS

### Temperature Influence on BIA Measures

Temperature had a significant, negative influence on the resistance and reactance of bluefish tissue (Figure 3). Dorsal resistance, dorsal reactance, ventral resistance, and ventral reactance between 27°C and 15°C for all lengths and between discrete length-groups (small and large) of bluefish were all significantly

different (paired *t*-tests: all  $P < 0.015$ ), although detector length between measures at each temperature were not significant (paired *t*-tests: all  $P > 0.11$  for dorsal and ventral). Across both length-groups of fish, the average dorsal resistance declined 35.8% and ventral resistance declined 20.4% from 15°C to 27°C. Reactance measures declined at lower rates than resistance but were similar between dorsal (−12.7%) and ventral measures (−12.9%) from 15°C to 27°C.

### Fish Size Influence on BIA Models

Models combining all lengths of bluefish were significant ( $P < 0.001$ ) at both temperatures and explained 86% of the variability in the percent dry weight of bluefish at both temperatures (Table 2; Figure 4). At 15°C the model for small bluefish had an additional parameter retained in the model, a similar coefficient of determination (83%), but a lower  $R^2_{\text{pred}}$  than the model using all lengths of fish. The 15°C model for large bluefish had a poorer fit than the model for all lengths and had an  $R^2_{\text{pred}}$  of only 26%. For 27°C data the model for large bluefish provided a slightly better fit and higher  $R^2_{\text{pred}}$  than the model for all lengths, but the model for small bluefish at 27°C explained only 77% of variation in the data and had a relatively low  $R^2_{\text{pred}}$ . Based upon these results, we determined that within the confines of our data, a single model incorporating all lengths of bluefish was a better approach to using BIA measures to predict percent dry weight than models for different length-groups of bluefish. The resulting model to predict PDW from BIA measures in

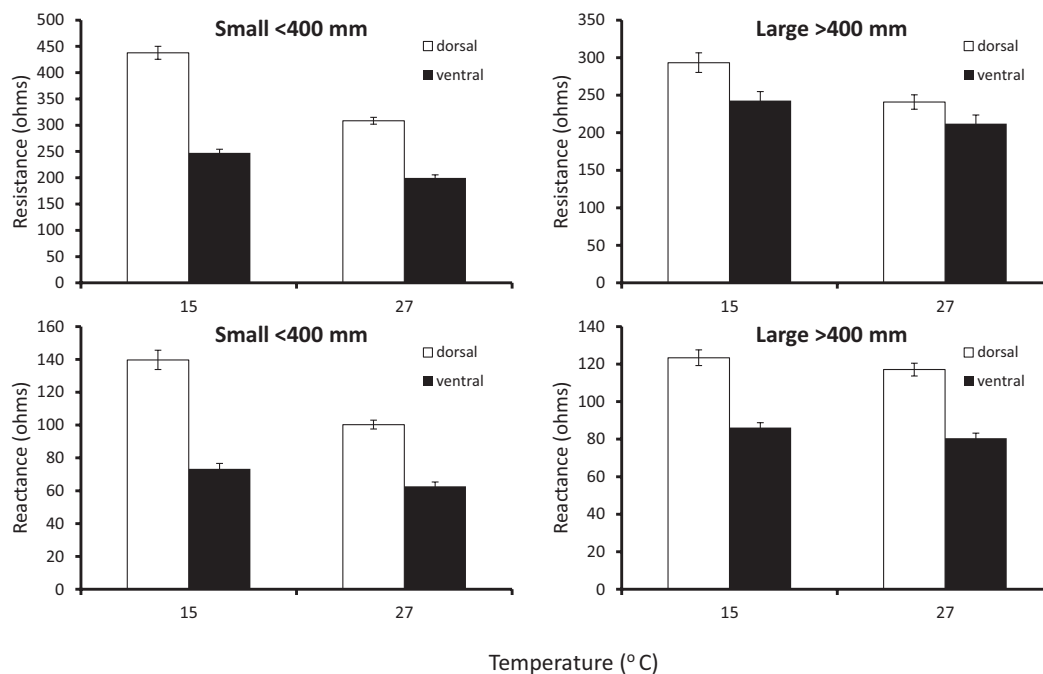


FIGURE 3. Dorsal and ventral resistance and reactance for small and large bluefish at 15°C and 27°C, showing that the effects of temperature on impedance were negative and significant. Error bars represent 95% confidence intervals about the means.

TABLE 2. Regression models using all bluefish observations to evaluate whether size-specific (small, <400 mm total length; large, ≥400 mm) or all-size-inclusive models are needed to accurately predict percent dry weight from electrical properties calculated from bioelectrical impedance analysis measures of bluefish at 15°C and 27°C. The variables (defined in Table 1) are differentiated here as dorsal (D) or ventral (V) (e.g., DE8 refers to the dorsal E8 variable). Fits were compared between models using all sizes of bluefish and individual models based on fish length-groups.

Data set	Variables	$R^2$	$N$	df	$F$	$P$	$R^2_{\text{pred}}$
15°C							
All lengths	DE8, DE9, VE7	0.86	60	3, 56	112.6	<0.0001	0.834
Small	DE2, DE4, DE7, VE5	0.83	38	5, 32	30.9	<0.0001	0.757
Large	VE3, VE9, VE3, VE8	0.72	22	4, 17	10.9	0.001	0.260
27°C							
All lengths	DE2, DE5, DE7, DE8, DE9 VE1, VE3, VE5, VE7, VE9	0.86	95	10, 84	52.6	<0.0001	0.818
Small	DE3, DE7, VE1, VE3, VE4, VE5, VE9	0.77	59	7, 51	24.6	<0.0001	0.716
Large	DE8, DE9, VE5, VE8	0.91	36	4, 31	77.6	<0.0001	0.875

bluefish of all lengths at 15°C was

$$\text{PDW} = 52.19 - 9.3832 (\text{DE8}) + 21.2225 (\text{DE9}) - 45.2875 (\text{VE7}), \quad (1)$$

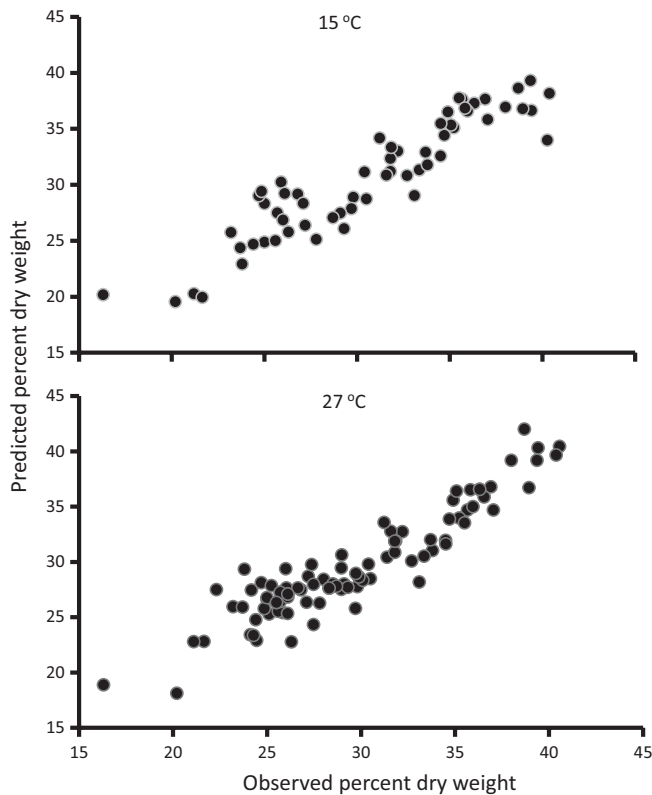


FIGURE 4. Relationships between the percent dry weight (PDW) predicted by the full bioelectrical impedance analysis models given in Table 2 and observed PDW in bluefish at two temperatures; the relationships were significant (all lengths and both dorsal and ventral measures included;  $P < 0.0001$ ). The models incorporating both size-groups of fish explained 86% or more of the variability in the data at 15°C and 27°C.

where DE8 is dorsally measured standardized resistance, DE9 is dorsally measured standardized reactance, and VE7 is ventrally measured phase angle (Table 1).

At 27°C the model for all lengths of bluefish was

$$\text{PDW} = 69.89 + 0.0385 (\text{DE2}) - 2.1466 (\text{DE5}) - 51.5251 (\text{DE7}) - 18.0264 (\text{DE8}) + 42.0259 (\text{DE9}) + 0.1781 (\text{VE1}) - 0.1084 (\text{VE3}) + 25.0913 (\text{VE5}) - 72.3870 (\text{VE7}) + 5.6953 (\text{VE9}), \quad (2)$$

where the electrical variable abbreviations (e.g., DE2, DE5, etc.) are those reported in Table 1.

### Influence of Position on BIA Measures

Models with the highest coefficients of determination were achieved when both dorsal and ventral measures were included (Table 3). Using the model data set at 27°C, predictive models using only the dorsal BIA measures explained 71.5% of variation and ventral-only BIA measures explained 65.5% of variation. Models including both dorsal and ventral BIA measures explained 78.3% of variation. The  $R^2_{\text{pred}}$  was 72.5%, suggesting strong future predictive power of the model.

Similarly, predictive models based on BIA measures at 15°C explained between 73.0% (ventral only) and 82.6% (dorsal only) of the variation in percent dry weight (Table 3). When both dorsal and ventral BIA measures were included in the candidate variables, 85.5% of the variation was explained by the model. Future predictive power of the full (dorsal and ventral measures) model was 81.4% (Table 3).

### BIA Model Validation

Models using all lengths of bluefish with BIA measures taken at both dorsal and ventral positions at 15°C and 27°C (Table 3) were validated using independent test data sets for each temperature and found to provide reasonable estimates of percent dry

TABLE 3. Equations using the model data sets to predict bluefish percent dry weight (PDW) at 15°C versus 27°C from electrical properties calculated from dorsal-only, ventral-only, and dorsal-and-ventral bioelectrical impedance analysis measures.

Model	$R^2$	$F$	$P$	$N$	$R^2_{\text{pred}}$
<b>Holding temperature of 15°C</b>					
Dorsal only: PDW = 36.14 – 8.1296(LE8) + 19.4718(LE9)	0.826	92.6	<0.0001	42	0.795
Ventral only: PDW = 64.33 – 78.684(VE7) – 9.729(VE8) + 25.635(VE9)	0.730	34.3	<0.0001	42	0.669
Dorsal and ventral: PDW = 50.23 – 9.718(LE8) + 22.554(LE9) – 39.353(VE7)	0.855	74.4	<0.0001	42	0.814
<b>Holding temperature of 27°C</b>					
Dorsal only: PDW = 70.86 + 0.0197 (LE2) – 0.0609 (LE3) – 123.282 (LE7) – 19.16 (LE8) + 54.3025 (LE9)	0.715	31.6	<0.0001	69	0.652
Ventral only: PDW = 17.12 – 0.028 (VE3) + 30.577 (VE5) + 30.881 (VE7)	0.655	41.2	<0.0001	69	0.616
Dorsal and ventral: PDW = 21.32 + 2.126 (LE5) – 10.983 (LE8) + 22.935 (LE9) + 9.336 (VE5) + 3.055 (VE8)	0.783	45.4	<0.0001	69	0.725

weight. Correlations between predicted and observed percent dry weight were highly significant ( $R^2$  values of 0.87 for both 27°C and 15°C), neither relationship between observed and predicted values differing significantly from a 1:1 line (Figure 5).

### BIA Model Sensitivity

The bluefish models using all lengths at 15°C and 27°C (Table 3) were not sensitive to errors of  $\pm 10\%$  in the measurement of resistance, reactance, or detector length (Figure 6). The most sensitive parameter at either temperature was resistance measured dorsally (DRES), where a 10% error in DRES resulted in a change in predicted PDW of  $\pm 10.5\%$  at 15°C or  $+7.0\%$  at 27°C. Overall, however, PDW was insensitive to all other errors of  $\pm 10\%$  in measured variables at both 15°C and 27°C (Figure 6).

### DISCUSSION

The BIA approach used in this paper offers several improvements over previously published work with fish. First, most previous studies used BIA to estimate masses of body constituents such as water mass, lipid mass (Bosworth and Wolters 2001; Cox and Hartman 2005; Duncan et al. 2007; Duncan 2008). Estimating masses from BIA using the electrical properties presented in Table 1, as was previously done, yields high coefficients of determination, largely because of the high correlation between fish length and weight and the use of detector length (highly correlated with fish length) in the numerator of most of the electrical equations. Although we might expect a relationship

between fish length and percent composition (e.g., longer fish may also have a higher lipid and lower water percentage) this relationship is much weaker (explaining 55% of variability in PDW) than the ones between detector length and mass or total length and mass, which each explain more than 99.6% of variation in bluefish mass. In fact, in the models presented in Table 3, the variables retained in the models tended to be those for which impedance measures were standardized by detector length. Thus, predictive capabilities of BIA models developed here for bluefish appear relatively unaided by underlying length relationships, similar to previous studies.

In addition to limiting length bias, our study also documented significant temperature affects on BIA observations. Bioelectrical impedance analysis has been widely used in humans to estimate body composition, particularly water masses, but applications to fish add challenges. Because electrical conductivity of materials is affected by temperature, the poikilothermic status of most fish means that resistance and reactance will differ for a given fish under different water temperatures. With all other variables constant, resistance will increase as temperature declines in fish. The model presented by Cox and Hartman (2005) included data gathered at a narrow range of temperatures (12–14°C) and did not consider temperature effects. Attempts to use BIA with field-caught fish by Pothoven et al. (2008) did not account for temperature differences because fish samples were pooled for May–September and June–October collections. Duncan (2008) suggested that temperature had no significant effect on BIA measures over a 10°C range and advocated that field researchers need not consider temperature effects on BIA measures. However, Duncan's experiments used only five



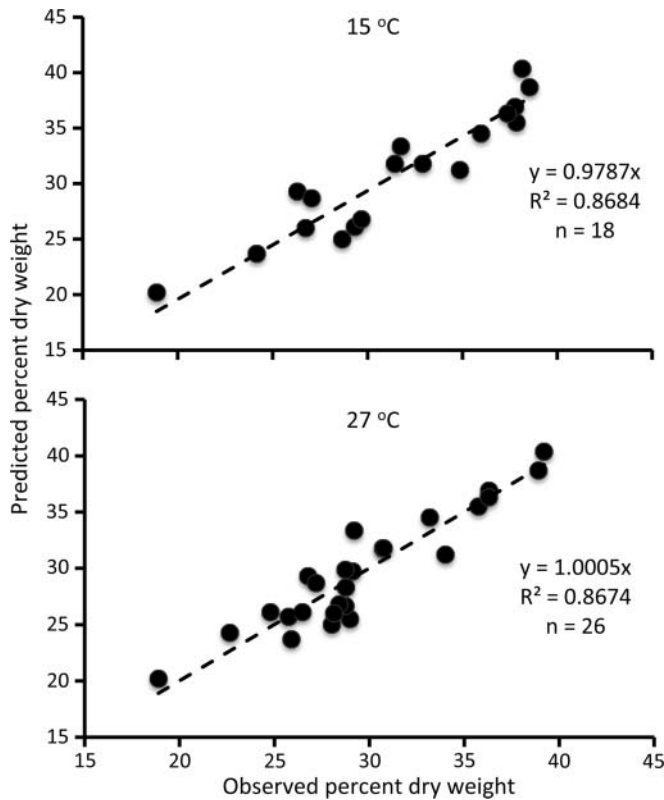


FIGURE 5. Comparison of the full bioelectrical impedance analysis models given in Table 3 (all lengths and both dorsal and ventral measures included) with an independent test data set at 15°C and 27°C. The models accurately predicted percent dry weight (PDW) in bluefish (note that the predicted and observed PDW yielded  $R^2 = 0.867$  for both 15°C and 27°C, the resulting relationships not differing from 1:1 [dashed line] at either temperature).

individuals at each test temperature without measuring each fish at each temperature. As a result, differences in impedance among fish related to different body composition and low sample size limited the ability to detect temperature influence on BIA. In our study, 59 bluefish were each measured at 15°C and 27°C, and temperature was found to significantly affect resistance and reactance. As a result, we believe temperature must be accounted for in using BIA to assess fish composition or condition.

In this paper we presented BIA models to estimate PDW at two temperatures. While these temperatures nearly cover the range of water temperatures typically occupied by bluefish (12–29°C; Olla and Studholme 1971), more data on the influence of temperature on resistance and reactance measures are needed to determine the shape (linear or nonlinear) of the temperature relationship so temperature corrections can be incorporated into BIA models. For now, we recommend using models formulated by equations (1) and (2) because they provide relatively higher  $R^2$  and  $R^2_{\text{pred}}$  for bluefish measured at 15°C or 27°C. Of note, we differentiate measurement temperature from collection temperature because fish body temperature can significantly change in a short time on deck or on ice, which

can affect the accuracy of BIA. If temperature effects on resistance and reactance in bluefish are determined to be linear in future studies, then our measures suggest that resistance and reactance measures decline by less than 2.5% per 1°C increase in temperature. Such relationships with temperature should be easily incorporated into corrections that permit use of these established BIA models for bluefish at 15°C and 27°C.

It is interesting that across the BIA models presented in Tables 2 and 3 relatively few consistent candidate variables were retained across temperatures and length-groups. When all observations were included at 15°C and 27°C (no test data set) the standardized dorsal resistance (DE8), standardized dorsal reactance (DE9), and ventral phase angle (VE7) were retained in models for each temperature, but the 27°C model also retained seven other variables. In contrast, the 27°C model from the model data set (Table 3) retained a maximum of five variables. This difference in numbers of parameters retained suggests some

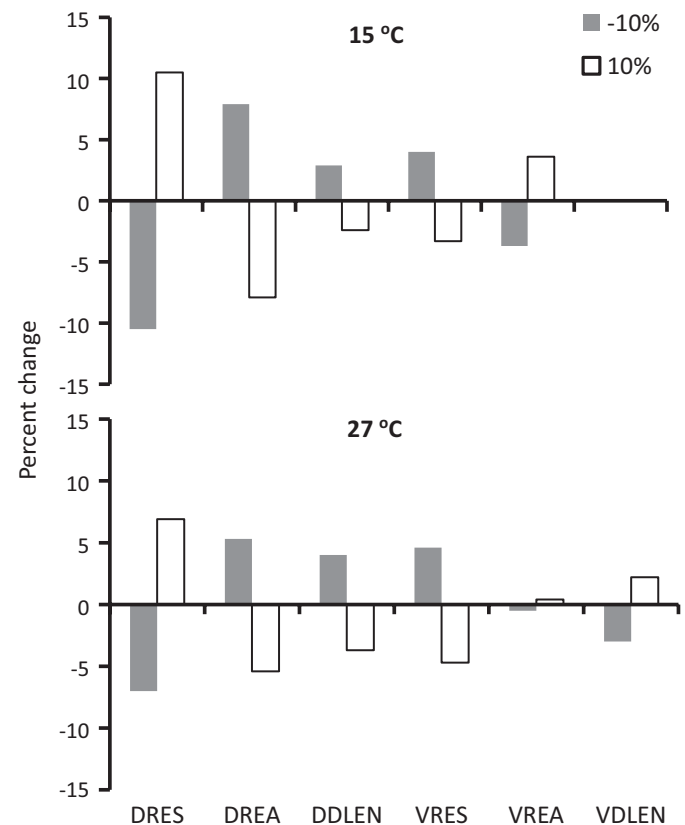


FIGURE 6. Parameter sensitivity analysis of the full bioelectrical impedance analysis models given in Table 3, showing the effects of varying the measured parameters by +10% (unshaded bars) or –10% (shaded bars). Abbreviations are as follows: DRES = dorsally measured resistance, DREA = dorsally measured reactance, DDLEN = dorsally measured detector length, VRES = ventrally measured resistance, VREA = ventrally measured reactance, and VDLEN = ventrally measured detector length. Only dorsally measured resistance at 15°C was considered marginally sensitive (i.e., a 10% change in the parameter resulted in a 10.5% change in the estimate of PDW); up to 10% errors in measurement of other parameters had little effect on the estimates of PDW.

models could be over-parameterized. However, Mallow's  $C_p$  statistic for the 27°C model was 8.9, indicating good fit. While the exact reason for a lack of common variables retained across all data sets is unknown, several factors could have contributed to the differences. First, the stepwise regression approach we used considered 9–18 different candidate variables for single-location or two-location models, and with such a large number of variables each derived from three to six measured properties ( $R$ ,  $X_c$ ,  $DL$  in Table 1), it is unlikely the same variables will be retained from each data set. Differences in retained variables across models of different fish length-groups can also be partially explained by differences in where and how fish of different sizes store lipids (Shearer et al. 1994). While it would be assuring to always retain the same suite of candidate variables in these BIA models, our goal was to develop models that accurately predict PDW in bluefish. The  $R^2_{\text{pred}}$  values for models of all lengths of bluefish exceeded 0.82 at each temperature, suggesting we can accurately predict PDW of bluefish with the models.

The ability to use BIA to estimate fish composition from PDW has several advantages. Duncan (2008) determined that the cost to estimate body composition using BIA was 2.4–5.1% of the cost using traditional proximate composition analytical methods. This relative cost suggests 20–40 times more observations can be gathered using BIA than could be processed using analytical methods. This low relative cost makes it possible to greatly enhance the spatial and temporal coverage of measures that can be afforded in fisheries studies, which has special relevance for coastal migratory species such as bluefish. Other advantages of BIA are that once a model is developed and validated it can be used nonlethally on other fish of the same species (Cox and Hartman 2005), and when using BIA models to estimate percent dry weight, the other body composition percentages can be estimated using body composition models. Hartman and Margraf (2008) found percent dry weight can be used with high precision and accuracy to estimate lipid, protein, and ash percentages in several species of fish, including bluefish. Combining BIA with models such as those in Hartman and Margraf (2008) or Sutton et al. (2000) may greatly reduce or eliminate the need for chemical analysis of fish for proximate analysis, thereby further reducing costs.

For highly migratory species such as bluefish, assessing population-level changes is often complicated by the difficulty of obtaining population estimates and other vital statistics. Such difficulties may prevent the detection of population responses to climate change, habitat degradation, and competition. The bluefish BIA models presented in this paper provide the tool necessary to begin monitoring bluefish populations via compositional measurements of individuals collected over broad spatial and temporal scales, which may be boosted by piggybacking on existing fisheries assessment and monitoring programs. Equipment needed for BIA is relatively inexpensive (under US\$2,500 based on 2010 prices) and very minimal training is required to operate the instrument. Thus, BIA can be added to ongoing fish-

eries sampling programs that commonly handle bluefish at both a very low cost and with the potential to greatly improve our understanding of spatial and temporal population demographics.

### Suggestions for Future BIA Model Development

Several factors that may affect BIA model precision and accuracy should be considered when using existing models or developing models for new species. These recommendations are based on our experience developing BIA models for brook trout, Pacific salmon, striped bass *Morone saxatilis*, and bluegills *Lepomis macrochirus* (Cox and Hartman 2005; Hartman unpublished data) and are meant to help guide future BIA applications on fish. First, fish temperature must be accounted for in impedance measures during model development and model use. Fish temperatures can easily be measured internally by inserting a temperature probe into the esophagus (for live fish) or rectally (for dead fish). The BIA measurements must also be taken in consistent locations across individual fish and in the same location used in model development. Measurements at different locations will assess different fish body substrates (tissues, fats, and inert materials) with different impedance measures and circuit lengths than those for which a model was developed, which will therefore yield inaccurate predicted values. Researchers should explore impedance measurement locations for untested species to determine the best location or combination of locations to produce the most accurate and precise results. Electrode needles should also match those for which the model was developed in terms of penetration length and distance between signal and detecting electrodes on a probe. In developing models, it is also important for the fish sampled to adequately span the range of lengths and body conditions for the species. Often, this is not possible with fish caught in the wild, so model development in the controlled conditions of the laboratory may be necessary.

### ACKNOWLEDGMENTS

We are grateful to J. Howell, G. Staines, and J. Nye for assistance in field collections and measures and to J. Rosendale for collection and husbandry of bluefish used at Sandy Hook. A. Hafs provided comments that improved this manuscript. Funding for this project was provided by the 2004 Bluefish-Striped Bass Dynamics Research Program to KJH. All procedures involving fish were conducted under guidelines approved by the West Virginia University Animal Care and Use Committee under protocol 05-0201.

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