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NOTE

Influence of Electrode Type and Location upon Bioelectrical Impedance Analysis Measurements of Brook Trout

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Abstract

In recent years, bioelectrical impedance analysis (BIA) has started to develop into a low-cost tool that can provide accurate estimates of fish condition. Past researchers have had success predicting mass-based proximate condition components, but attempts to predict percent-based components have not been as successful, suggesting that methodological improvements are needed. The percent dry weight (%DW) of a fish is a desirable value because energy density and body composition estimates can be obtained from it using previously developed or easily developable equations. The primary objective of this study was to determine the locations at which electrodes should be placed to provide the best estimates of %DW for brook trout *Salvelinus fontinalis* ranging from 140 to 330 mm (total length). A second objective was to determine the effect that electrode type has on the ability to predict %DW. Models developed using two electrode locations performed better than those with only one location. One set of measurements should be made by placing the electrodes along the dorsal midline (DML) of the fish. A second set should be made by placing one electrode on the dorsal midline directly in front of the dorsal fin and another on the ventral midline directly below the first electrode (DTVpre). On average, models developed using these locations explained 13.2% more of the variation in%DW than models developed using the same locations as previous researchers. Validation of the BIA models demonstrated that both subdermal needle (root mean square error [RMSE] = 1.34, $R^2 = 0.82$) and less-invasive external rod electrodes (RMSE = 1.37, $R^2 = 0.79$) provided accurate estimates of %DW using the DML and DTVpre locations. More research is needed to determine whether these patterns hold true for smaller fish and species with distinctly different morphologies, bone structures, or scale types.

Bioelectrical impedance analysis (BIA) can be used as a low-cost, nonlethal method for estimating the proximate composition of fish (Cox and Hartman 2005). Bioelectrical impedance analysis is done by passing an electrical current through the subject of interest and the resistance and reactance is measured. Resistance measures the ability of a substance to conduct electricity (Cox and Hartman 2005). Because fat is a poor conductor of electricity, there should be a relationship between the amount of fat in the subject and the resistance measured by BIA. Reactance measures a substance's ability to hold a charge. Because the lipid bilayer of a cell serves as a capacitor (Lukaski 1987), reactance is subsequently a measure of total cell volume and should be related to the size and condition of the subject. Simple regression models have been developed that can predict mass-based proximate composition estimates from BIA measurements (Bosworth and Wolters 2001; Cox and Hartman 2005; Duncan et al. 2007; Pothoven et al. 2008). Although previous models predict mass-based proximate composition estimates, it would be useful if models predicting percent-based estimates of proximate composition were developed. By obtaining reliable predictions of percent dry weight (%DW), we could use equations developed by previous research to estimate both energy density for use in bioenergetics (Hartman and Brandt 1995) and body composition values (Hartman and Margraf 2008). Previous models attempting to predict percent-based estimates using BIA have been unreliable (Pothoven et al. 2008), suggesting improvements in the method are needed.

Past BIA models for fish have been developed by measuring the resistance and reactance of a small electrical current (425 μA, 50 kHz) that is passed between two electrodes placed on the side of the fish. The resistance and reactance measures are then regressed against measures of proximate composition. Recent researchers have used subdermal needle electrodes. Cox and Hartman (2005) used 28-gauge needles that penetrated 2 mm into brook trout *Salvelinus fontinalis* ranging from 110 to 285 mm in total length (TL). Pothoven et al. (2008) used 23-gauge needles that penetrated 3 mm into yellow perch *Perca flavescens* (138–358 mm), walleye *Sander vitreus* (328–639 mm), and lake whitefish *Coregonus clupeaformis* (246–564 mm). Willis and Hobday (2008) used 20 and 28

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gauge needles that penetrated approximately 10 mm into juvenile southern bluefin tuna *Thunnus maccoyii* ranging from 410 to 1,090-mm fork length (FL). Although past researchers have used different electrodes, little research has been done to see how the type of electrode influences BIA measurements.

In addition to the type of electrodes used, the locations at which they are placed may affect BIA measures. Past researchers have placed the electrodes along the side of the fish, typically with one electrode just posterior to the head and the other electrode anterior to the tail. Often one set of measurements is taken above the lateral line and a second set is taken below the lateral line (Cox and Hartman 2005). Although these locations have provided reliable mass-based estimates of proximate composition, alternative locations need to be tested to see if improvements in the method are available that will provide reliable percent-based estimates.

The objective of this study was to determine the electrode location on fish that provides the best estimate of %DW for brook trout, a streamlined fish with small cycloid scales. A second objective was to determine how well BIA models developed using three different electrode types could predict %DW of brook trout and to test whether results from one electrode can be applied to a model developed with another electrode type.

METHODS

Brook trout (~150 mm TL) were donated by Bowden State Fish Hatchery, Bowden, West Virginia, and transported to the West Virginia University Ecophysiology Laboratory, where fish were maintained in recirculating tanks (0.58 × 0.58 × 2.13 m) at 12.5 ± 0.5°C. Cox and Hartman (2005) had previously developed models for fish ranging from 110 to 285 mm, so for this study we sampled fish from three size-classes similar to that range (150, 225, and 300 mm). At the time the fish were received from the hatchery, 45 fish were randomly selected to represent the 150-mm size-class and were isolated from the rest of the fish in a separate recirculating tank. The remaining fish were fed ad libitum daily until their selected size-class (225- and 300-mm TL) was reached. All fish were acclimated to the recirculating system at West Virginia University Ecophysiology Laboratory for at least 2 weeks before any BIA was done.

In developing BIA models, it is desirable to include fish with the full range of possible body conditions. Because the fish were fed ad libitum daily until they reached their appropriate size-class, it was assumed that those fish were in the best possible condition at that time. In order to have fish at a wide range of body conditions while controlling for interactive effects of size and condition, fish from each size-class were fasted (no food was provided) for varying lengths of time before being selected for BIA. To accomplish this, fish were sampled at approximately seven evenly spaced intervals over each of the individual fasting periods. Within the 150-mm size-class, the leanest fish were fasted for approximately 4 months. The leanest fish in the 225-mm size-class were fasted approximately 5 months, and lastly, the 300-mm fish were randomly sampled over the course of a 6-month fasting period.

Bioelectrical impedance analysis.—Resistance and reactance were measured with a Quantum II bioelectrical body composition analyzer (RJL Systems, Clinton Township, Michigan). The Quantum II passes a small current (425 μA, 50 kHz) through the fish and measures resistance and reactance in ohms. For this study, two sets of electrodes (subdermal needle and external electrodes) were created by the experimenters and one set (subdermal needle electrodes) was manufactured by a medical supply company (Model FE24; The Electrode Store, Enumclaw, Washington) following the experimenters' designs (Figure 1). Subdermal needles used were 29-gauge mounted 10 mm apart, set to penetrate to a depth of 3 mm. External electrodes consisted of stainless steel rods 3.2 mm in diameter, the center of the rods mounted 10 mm apart (Figure 1). For the remainder of the manuscript, the subdermal needles created by the experimenters will be called Epoxy because the needles were set in epoxy; the Electrode Store subdermal needles will be called FE24, and the external rod electrodes will be called Rods.

We also assessed the location on the fish at which electrodes should be placed to produce the best estimates of %DW. To do this, resistance and reactance was measured at seven different locations: dorsal midline (DML), dorsal total length (DTL), lateral line (LL), ventral total length (VTL), ventral midline (VML), dorsal to ventral predorsal fin (DTVpre), and dorsal to ventral postdorsal fin (DTVpost). These seven electrode locations are shown in detail in Figure 2. It is important to note that each electrode has two needles or rods, one serving as the signal and the other serving as the detector electrode (Cox and Hartman 2005). Although it appears from our own unpublished observations that the orientation of the signal and detector needles or rods has no influence on the readings, for this study signal electrodes were always kept towards the head of the fish.

Because ambient air temperature can influence BIA measurements (Gudivaka et al. 1996), fish were acclimated in water with temperature equal to the room temperature (range = 18.0–21.0°C) for at least 12 h prior to all BIA measurements. This was done to minimize the influence of air temperature on BIA measurements. After the 12-h acclimation period, the fish was anesthetized using MS-222 (tricaine methanesulfonate) and blotted dry, and the wet weight (WW; g), FL (mm), and TL (mm) were measured. The fish was then placed on a nonconductive board with the head facing left. Resistance and reactance was measured at all seven locations with all three electrode types on each fish. The distance between the inner needles or rods of the two electrodes was recorded for every measurement. So that detector length was equal to the distance between the signal needles or rods, 10 mm was added to all lateral measurements. The person holding the electrodes was wearing rubber gloves. To avoid bias due to temperature changes from handling or repeated BIA measures, the order of both the electrode type and location that the measurements were taken was changed for every fish during the study. After all BIA measurements were

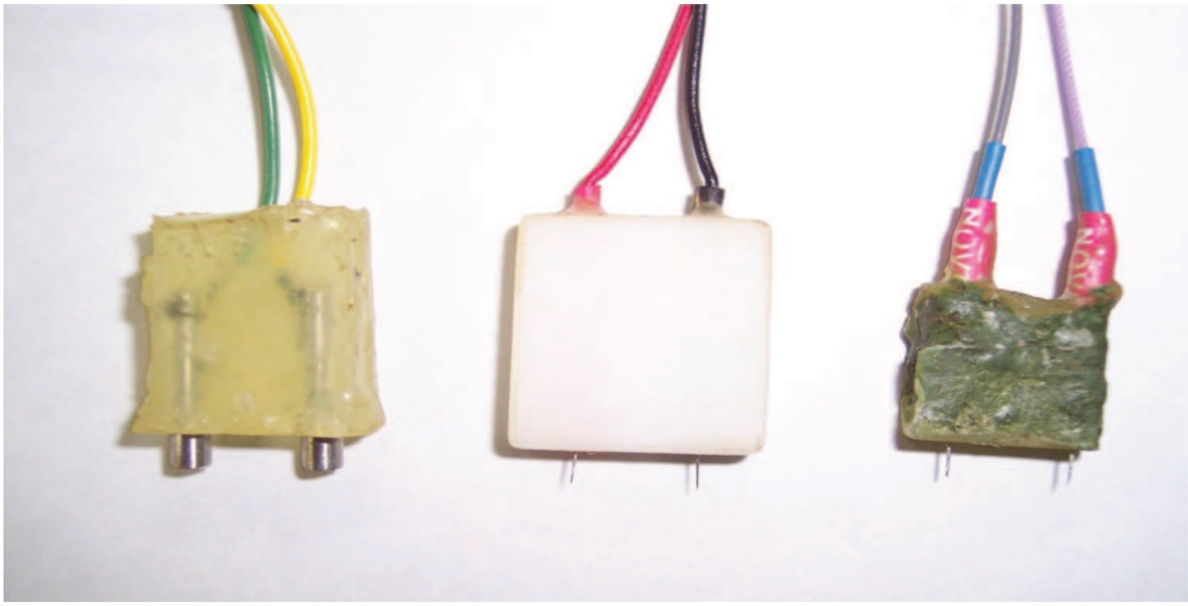

FIGURE 1. Electrode types used in this study. The subdermal needle (Epoxy; right) and external (Rod; left) electrodes were created by the experimenters; the Model FE24 subdermal needle electrode (center) was manufactured by The Electrode Store. [Figure available in color online.]

completed for a fish, it was euthanatized in an overdose of MS-222 and the whole fish was oven-dried to a constant weight at 80°C. Percent dry weight was calculated by dividing dry weight by wet weight and multiplying by 100.

Data analysis.—From the measured resistance and reactance for each location and electrode type, a suite of electrical parameters were calculated following the methods outlined by Cox and Hartman (2005) and Cox et al. (2011). Table 1 outlines the calculations for parameters used in regression analysis: resistance (r), reactance (x), resistance in series (R_s), reactance in series (X_c), resistance in parallel (R_p), reactance in parallel (X_{cp}), capacitance (C_{pf}), impedance in series (Z_s), impedance in parallel (Z_p), phase angle (PA), and standardized phase angle (DLPA). Because detector length is correlated to fish size, all electrical parameters were standardized to electrical volume by dividing DL^2 with each parameter (e.g., DL^2/R_s) following the methods of Cox and Hartman (2005). Standardized phase angle was calculated by multiplying PA and DL.

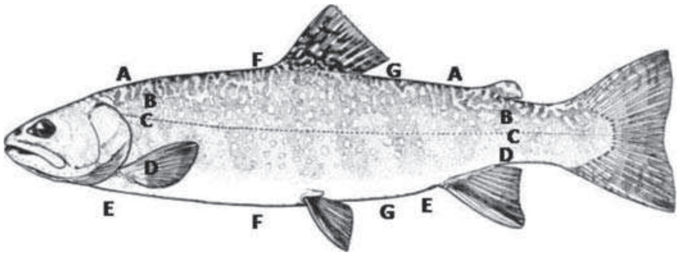

FIGURE 2. Electrode locations used in the study: **(A)** DML, **(B)** DTL, **(C)** LL, **(D)** VTL, **(E)** VML, **(F)** DTVpre, and **(G)** DTVpost; see text for additional details.

Bioelectrical impedance analysis models predicting %DW were developed by running ordinary least squares regression using the function ols (Harrell 2009), part of the package rms in program R (R Development Core Team 2009). Fish from all three size-classes ($n = 45$–47 per each size-class) were included for model development, and models were also developed for each size-class individually. A BIA model was developed for each electrode location and type combination individually. To determine if using two electrode locations improved predictive ability, BIA models were also developed for all two electrode location combinations for each electrode type individually. The function leaps (Lumley 2009), part of the package leaps in program R (R Development Core Team 2009), was used to calculate Mallows' C_p (Mallows 1973) for every possible model for each electrode location and type combination (Figure 3). From every electrode type–location combination, the model with the lowest Mallows' C_p-value from each possible model size was selected for validation.

Bioelectrical impedance analysis models were validated using the function validate (Harrell 2009), which is part of the package rms in program R (R Development Core Team 2009). The validate function uses bootstrapping methods developed by Efron (1983) to randomly select training data sets of size n. The original whole data set is used as the test data set. The training data sets are used to develop the models, and the test data are used to validate the model; R^2 and root mean square error (RMSE) values are then calculated based on how well the test data fit the models. The validate function was set so 10,000 permutations were run to develop each model and estimate the R^2 and RMSE values. Akaike's information theoretical criterion (Akaike 1973) corrected for sample size (AIC$_c$; McQuarrie and

TABLE 1. Electrical parameters used in bioelectrical impedance analysis model development. Parameters are converted to electrical volume when the square of detector length (DL^2) is included in the equation.

Parameter	Symbol	Units	Calculation
Resistance	r	Ohms	Measured by Quantum II
Reactance	x	Ohms	Measured by Quantum II
Resistance in series	R_s	Ohms	DL^2/r
Reactance in series	X_c	Ohms	DL^2/x
Resistance in parallel	R_p	Ohms	$DL^2/[r + (x^2/r)]$
Reactance in parallel	X_{cp}	Ohms	$DL^2/[x + (r^2/x)]$
Capacitance	C_{pf}	Picofarads	$DL^2/\{[1/(2\cdot\pi\cdot 50{,}000\cdot r)]\cdot[1\cdot 10^{12}]\}$
Impedance in series	Z_s	Ohms	$DL^2/(r^2 + x^2)^{0.5}$
Impedance in parallel	Z_p	Ohms	$DL^2/[r\cdot x/(r^2 + x^2)^{0.5}]$
Phase angle	PA	Degrees	$\text{Arctan}(x/r)\cdot 180/\pi$
Standardized phase angle	DLPA	Degrees	$DL\cdot[\arctan(x/r)\cdot 180/\pi]$

Tsai 1998) was used to determine the best model from those previously selected by Mallows' C_p-values.

After the validation was complete and the best models had been determined, we randomly selected 80% of the fish to represent a training data set and the other 20% to represent a test data set. To compare needle electrode types, we then entered the resistance and reactance values from the test data set for the Epoxy subdermal needles into the regression model that was developed using the FE24 training data set. Root mean square estimates were calculated to determine if a model would be applicable for sets of electrodes not used during model development. We also tested all other model–electrode combinations in a similar manner.

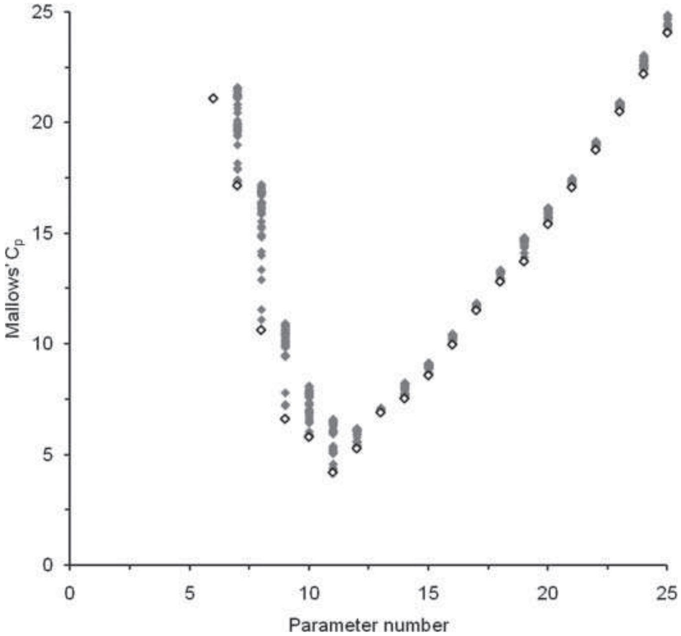

FIGURE 3. Mallows' C_p-values for Electrode Store subdermal needle electrodes using DML and DTVpre electrode locations. Mallows' C_p-values (shaded diamonds) for all possible models are plotted for each model size (parameter number); the model with the lowest value for each size was selected for validation (open diamonds).

Finally, because the distance between the electrodes could be related to the %DW (especially for the DTVpre and DTVpost locations, where the detector length is basically the body depth), we wanted to make sure that the BIA measurements and not the detector lengths were the driving force behind our models. To test this possible pitfall, we selected the best model after all validation results were complete. For each fish that had previously been used to develop the model, we changed the measured resistance and reactance values to 1 while leaving the measured detector lengths unchanged. The electrical parameters were calculated as normal using the new resistance and reactance values and the unchanged detector lengths. The calculated electrical parameter estimates were then entered into the model to predict %DW for each fish. The resulting RMSE and R^2 estimates were compared with the results obtained using actual resistance and reactance values. In addition to changing all resistance and reactance values to 1, we also developed a model that attempted to predict %DW using only TL, FL, WW, and Rod DL from all seven locations.

RESULTS

The percent dry weight of brook trout sampled from 150-, 225-, and 300-mm size-classes ranged from 17.64 to 27.14, 17.80 to 28.38, and 17.93 to 32.55, respectively (Figure 4). Model validation demonstrated that all three electrode types were able to accurately predict %DW. When the three fish size-classes were analyzed individually, on average the best models were developed using the VML DTVpre locations or the DML DTVpre locations. On average, across models for all size-classes and electrode types, the VML DTVpre location combination produced models having an AIC_c of 23.00, RMSE of 1.11, and R^2 of 0.84. The DML DTVpre location combination provided

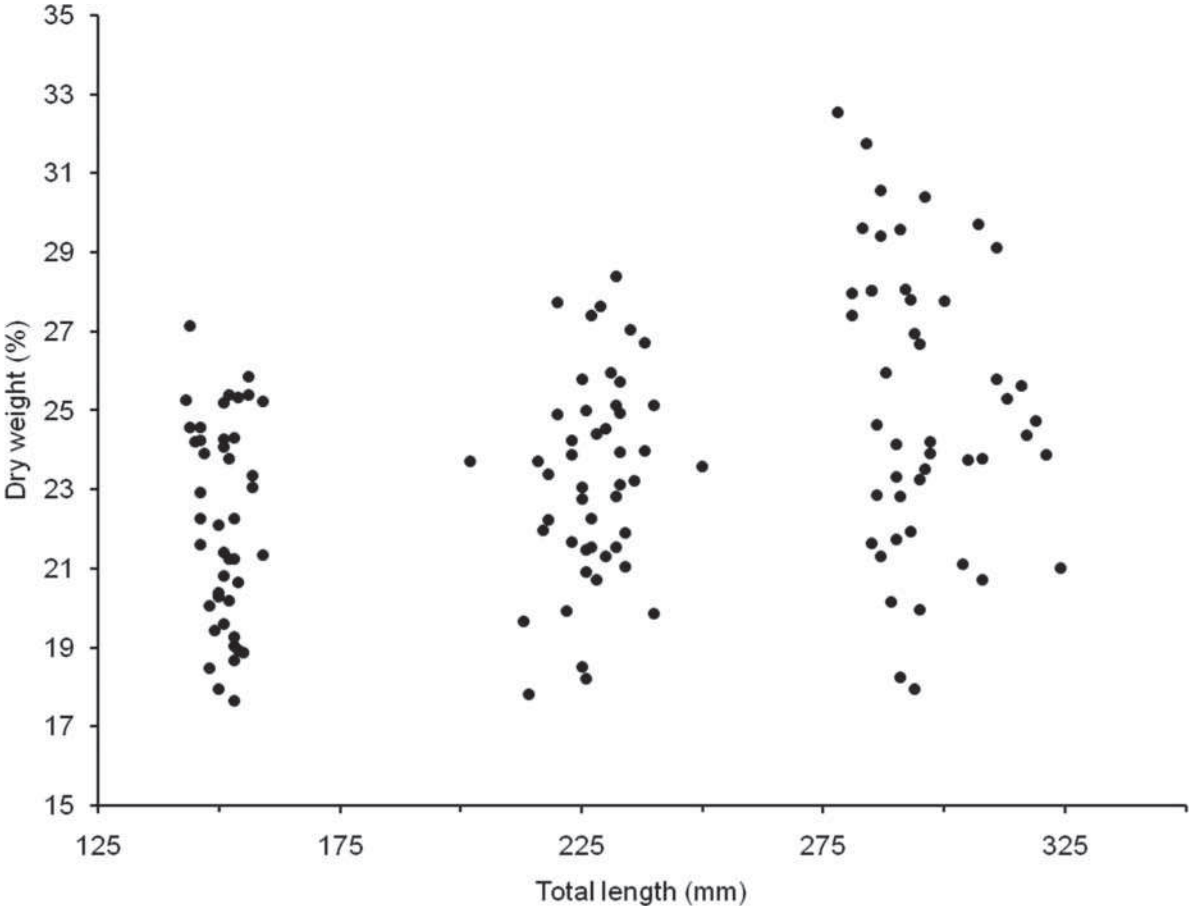

FIGURE 4. Ranges of the %DWs and total lengths of the brook trout ($n = 139$) used to develop the BIA models in this study.

similar results on average ($\mathrm{AIC}_c = 23.61$, RMSE = 1.08, $R^2 = 0.85$).

Models developed for individual size-classes performed only slightly better than models including all size-classes. The best model developed while including all size fish resulted in RMSE (1.34) and R^2 (0.82) estimates that were only slightly worse than the models developed for individual size-classes (RMSE = 1.11, $R^2 = 0.84$). Since models developed using all size-classes of fish performed similarly to models for individual size-classes, the rest of the Results and the Discussion section focus on models that were developed using all size-classes of fish.

Models developed using two locations performed better than those using only one location. The best model developed using only one measurement location was the DTVpre location using the Epoxy electrodes ($\mathrm{AIC}_c = 115.19$, RMSE = 1.43, $R^2 = 0.79$), and it performed similarly to models developed with two locations. However, Rod ($\mathrm{AIC}_c = 144.01$, RMSE = 1.59, $R^2 = 0.72$) and FE24 ($\mathrm{AIC}_c = 125.32$, RMSE = 1.51, $R^2 = 0.77$) models developed using only the DTVpre location did not perform quite as well (Figure 5). There were 21 different models developed using two locations that outperformed the best single-location model. The regression coefficients for the FE24 and Rod DTVpre models are located in Table 2.

The locations at which the electrodes were placed did have a large influence on the ability to accurately predict %DW. The best 27 models all were developed using DTVpre or VML as at least one of the two locations. On average across electrode types the models developed using the DML and DTVpre locations performed the best (Epoxy: $\mathrm{AIC}_c = 95.90$, RMSE = 1.32, $R^2 = 0.82$; FE24: $\mathrm{AIC}_c = 100.28$, RMSE = 1.34, $R^2 = 0.82$; Rods: $\mathrm{AIC}_c = 111.19$, RMSE = 1.37, $R^2 = 0.79$; Figure 5). Models developed using locations from previous research (DTL and VTL) on average explained 13.2% less variability in comparison with the DML and DTVpre locations. The regression coefficients for the FE24 and Rod models developed using the DML and DTVpre locations can be found in Table 2.

Because the models developed using the DML and DTVpre locations provided the most reliable results across all three electrode types, that was the location combination used to determine whether models developed for one electrode type could be used for data collected with other electrodes. Entering the Epoxy test data set into the training Epoxy model resulted in an RMSE estimate of 0.99. The test data from FE24 subdermal needles and the Epoxy training model produced an RMSE estimate of 1.36, and the Rod test data set RMSE estimate was 1.31. When the Rod training model was developed, the resulting RMSE

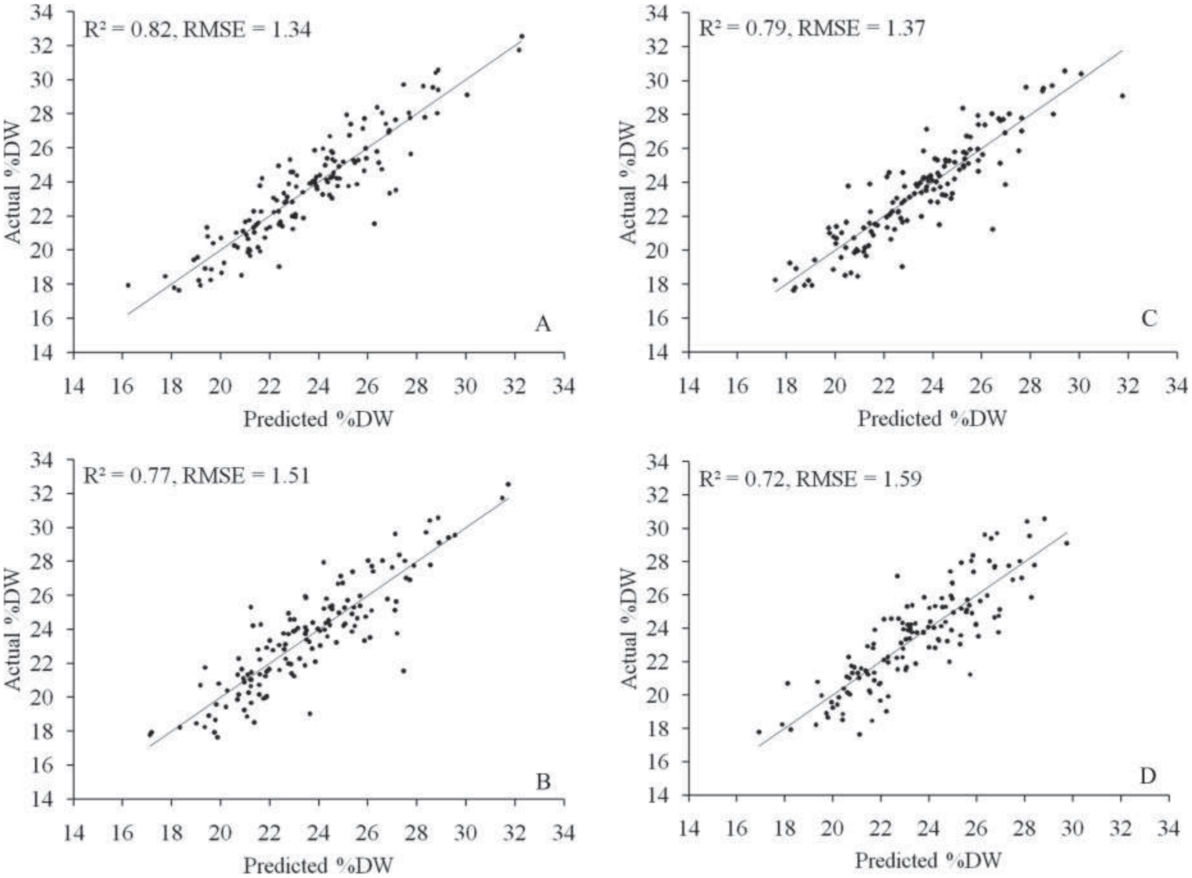

FIGURE 5. Predicted versus actual %DW for Model FE24 subdermal needle electrodes and external electrodes created by the experimenters using models based on **(A)** and **(C)** the DML and DTVpre locations and **(B)** and **(D)** the DTVpre location only.

estimates were 4.00, 0.96, and 2.74 for the Epoxy, Rod, and FE24 test data sets, respectively. Finally, when the FE24 training model was created, the resulting RMSE estimates were 1.17, 1.17, and 0.96, for Epoxy, Rod, and FE24 training data sets, respectively. In summary, the models developed for subdermal needle electrodes (FE24 and Epoxy) performed well when data from either subdermal needles or external rod electrodes were entered. However, the model developed for the Rod electrodes did not perform as well when data from the subdermal needle electrodes was entered.

Bioelectrical impedance analysis models developed using only detector length did a much poorer job at predicting %DW than models that included measured resistance and reactance values. For example, the Epoxy model developed using the DML and DTVpre locations was able to predict %DW with an RMSE of 1.32 and an R^2 of 0.82. Conversely, when only detector length from the DML and DTVpre were used and all resistance and reactance values were changed to 1, the best model that could be developed was only able to predict %DW with an RMSE of 2.53 and an R^2 of 0.36. The model that attempted to predict %DW using only TL, FL, WW, and Rod DL from all seven locations resulted in an RMSE of 2.77 and an R^2 of 0.13.

DISCUSSION

Previous researchers attempting to predict percent-based composition estimates have had limited success (Pothoven et al. 2008), suggesting that improvements in the methods were needed. By determining at which location the electrodes should be placed on the brook trout, we were able to substantially improve the reliability of our BIA models, thereby allowing accurate prediction of %DW. Future researchers can now use the methods and models provided in this paper to accurately predict %DW. Hartman and Brandt (1995) have previously established relationships between %DW and energy density. In addition to the relationship established by Hartman and Brandt (1995), relationships have also been established among proximate composition estimates and %DW (Hartman and Margraf 2008). Therefore, once %DW has been predicted researchers can relate %DW to energy density and body composition values at a fraction of the cost needed for laboratory analysis of proximate composition or bomb calorimetry.

Past researchers have commonly used what we call in this paper the DTL and VTL locations to take their BIA measurements (Cox and Hartman 2005; Pothoven et al. 2008). In this study when we used the locations from previous research (DTL

TABLE 2. Regression coefficients for the prediction of %DW for brook trout ranging from approximately 140 to 330 mm TL. Four models are presented, two for FE24 subdermal needle electrodes and two for external rod electrodes. The models presented allow bioelectrical impedance analysis measurements to be taken from two locations (DML and DTVpre) or only one (DTVpre). The parameter column tells which location's resistance and reactance mesurements should be used when calculating the electrical parameter in parentheses. Formulas for calculating the electrical parameters are given in Table 1; the measurement location notation is explained in Figure 2.

	Model			
	FE24 location(s)		Rod location(s)	
Parameter	DML–DTVpre	DTVpre	DML–DTVpre	DTVpre
Intercept	14.2881	7.6944	42.1160	26.01171
FL			–0.0765	–0.04109
WW	0.0504	0.0211	0.0878	0.02553
DML(r)	–0.0159		–0.0233	
DML(R_s)			3.1429	
DML(X_c)			–0.4166	
DML(X_{cp})	–0.4690		–7.5129	
DML(C_{pf})	30.0180		21.4788	
DML(PA)	0.9160			
DML(DLPA)	–0.0123			
DTVpre(r)	0.0390	0.0518		
DTVpre(x)			0.0720	0.06974
DTVpre(X_c)			–0.0430	
DTVpre(X_{cp})		–0.9262		–1.83278
DTVpre(Z_p)	–0.0262			
DTVpre(PA)			–0.3170	–0.63769
DTVpre(DLPA)		0.0060		0.01875

and VTL) and the Epoxy subdermal needle electrodes, resulting models could only predict %DW with an R^2 of 0.61 and an RMSE of 1.96. By testing seven different locations, we were able to determine that the DML and DTVpre locations produced models that performed much better ($R^2 = 0.82$, RMSE $= 1.32$). The Epoxy model developed using the DML and DTVpre locations was able to explain an extra 21 % of the variability in comparison to the methods provided by previous researchers. The other two electrode types used in this study also provided similar results. The DTVpre location resulted in models that did a much better job at predicting %DW than models developed using other electrode locations. Because the detector length for the DTVpre location is essentially the body depth in front of the dorsal fin, it can be measured very accurately, minimizing a source of error present in the models developed not using this location. Additionally, it is likely that by taking measurements from the DTVpre location and one lateral location (DML) the electrical current is forced to pass through a greater proportion of the fish than when two similar lateral locations are used, ultimately resulting in better prediction from the models. For future BIA research on brook trout or other fish species with similar body morphology, we suggest that taking BIA measurements at the DML and DTVpre locations will improve results and should allow for accurate prediction of percent-based estimates. If time or money permits that only one measurement is taken per fish, the DTVpre location should be used, but researchers should expect some loss in the accuracy of their predictions compared with when two measurement locations are used.

This is the first study that we are aware of in which external electrodes were used to take BIA measurements on fish. The external rod electrodes used in this study produced estimates of %DW that were comparable to those estimates provided from subdermal needle electrode models. This is important because external rod electrodes are far less invasive than subdermal needles. The less-invasive external rod technique may be required when working with small, fragile fish or endangered species. Even though the external rod electrodes worked well on brook trout, a salmonid with very small cycloid scales, researchers should use caution. It is likely that external rod electrodes will have limited success on other fish species with larger or thicker scales. More research is needed to determine if these patterns hold true for brook trout smaller than 140 mm or fish species with different morphologies or bone or scale structure.

A total of 21 measurements (seven locations with three electrodes types) were taken on each fish. Although air temperature and water temperatures were controlled, we assume that over the course of taking 21 measurements (although gloved), the contact with the experimenter's hands would cause a slight rise in the fish's body temperature. Both the order of the locations and electrode type was changed for each fish so the results should not be biased in any way, but the changing body temperatures would affect the BIA measurements (Gudivaka et al. 1996; Cox et al. 2011), incorporating an amount of variation into our models that could not be explained. This suggests that our results are conservative and that if only two measurements (DML and DTVpre for example) were taken on each fish and a model was then developed, the RMSE would likely be lower than 1.34.

Another important result from this research was that models developed for subdermal needle electrodes provided accurate predictions of %DW even when resistance and reactance values measured from a different electrode type were entered. This means that as long as future researchers follow our methods and electrode specifications, they should be able to build their own electrodes or purchase some from The Electrode Store and the models given in this paper should provide reliable predictions ($R^2 > 0.80$) of %DW. That being stated, future research is needed to determine if other researchers can replicate our accuracy levels using the models and methods provided in this paper. Furthermore, we strongly encourage researchers that plan on using our methods and models to independently validate them on a subset of the fish sampled. Lastly, both types of subdermal needle electrodes used in this study were the same gauge (29) and penetrated the same distance (3 mm), and it is unclear if our models would provide reliable results when using electrodes with different specifications. Research is warranted that attempts to determine the effect that gauge and penetration depth has on BIA measurements.

The models presented in this paper were developed under strict laboratory conditions in which both air and water temperatures were held constant. Because temperature can have a large influence on BIA measurements (Gudivaka et al. 1996; Cox et al. 2011), future researchers should use care when attempting to use our models outside of the range of temperatures that were present during our laboratory experiments (18.0–21.0°C). There is a need to develop temperature corrections for BIA measurements so the models provided in this paper can be used in the field where large fluctuations in both air and water temperature are common. It is our opinion that if BIA is used in the field where variable water temperatures are present, too much unexplained variation will be incorporated into the models to allow for any reliable predictions. Until temperature corrections are developed, BIA models will be limited to the conditions that they were developed under in the laboratory.

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