

Nonlethal estimation of proximate composition in fish

M. Keith Cox and Kyle J. Hartman

Abstract: The need to precisely measure growth is a common denominator in many fisheries studies, but growth measures other than total masses or lengths are nearly nonexistent because more precise measurements such as body composition analysis are often too difficult and time consuming. Here, we present a means of estimating body composition in fish quickly, and after validation, without the need to sacrifice the animal. Models built with brook trout (*Salvelinus fontinalis*) were linear with strong validation group relationships ($R^2 > 0.96$) for composition parameters including water, protein, fat, fat-free, and dry masses. Subject responses to bioelectrical impedance analysis were minimal, with only slight bruising ($p < 0.001$) with no effect on swimming, color, bleeding, or feeding. The model was also tested on the water and dry masses of 10 warmwater fish species and found to have strong correlations ($R^2 > 0.86$), suggesting that more general relationships may exist. Nonlethal estimation of body composition using bioelectrical impedance analysis will permit increased precision in bioenergetics energy flow and compositional studies as well as permit study of community energetics and condition on spatial and temporal scales not previously possible.

Résumé : Une mesure précise de la croissance est nécessaire dans plusieurs études sur les poissons, mais il n'existe à peu près pas de variables mesurées autres que les masses ou les longueurs totales, parce que les variables plus précises, telles que la composition corporelle, sont souvent trop difficiles ou trop onéreuses à analyser. Nous proposons une méthode rapide de détermination de la composition corporelle des poissons qui, une fois validée, ne nécessite pas le sacrifice de l'animal. Les modèles élaborés pour l'omble de fontaine (*Salvelinus fontinalis*) sont linéaires et possèdent de fortes relations de validation de groupe ($R^2 > 0,96$) pour les variables de la composition, telles que l'eau, les protéines, les graisses, la masse sans les graisses et la masse sèche. La réaction individuelle à l'analyse par impédance bioélectrique est minimale et consiste en de légères ecchymoses ($p < 0,001$) sans effet sur la nage, la coloration, le saignement et l'alimentation. Le modèle a aussi été utilisé pour déterminer la masse hydrique et la masse sèche de 10 espèces de poissons d'eau chaude; les corrélations sont fortes ($R^2 > 0,86$), ce qui laisse croire qu'il peut exister des relations plus générales. L'estimation sans sacrifice du poisson de la composition corporelle par l'analyse par impédance bioélectrique permet une plus grande précision dans la détermination des flux dans les études bioénergétiques et dans les analyses de composition corporelle; elle permet aussi l'étude de la bioénergétique des communautés et de la condition physique à des échelles spatiales et temporelles impossibles à envisager jusqu'à maintenant.

[Traduit par la Rédaction]

Introduction

Growth is considered the ultimate expression of well being at the individual level and is also linked to the reproductive success of an organism (Shine and Schwarzkopf 1992; Brandt and Hartman 1993; Elliott and Hurley 1995). Actual growth reflects changes in the body composition or mass of growth components (e.g., fat and protein) relative to inert (ash) or compensating (water) components. However, the definition of growth in most studies has been limited by the ability to accurately measure energetic growth. Since most animals are between 60% and 90% water, changes in this compartment can greatly influence estimates of growth based on total mass. In most growth studies, the more precise measurements of body composition have often been

shelved because a quick, reliable, inexpensive method to estimate body composition is lacking.

A variety of in vivo and in vitro methods are available for assessing proximate composition in organisms, but most appear to be impractical, inconvenient, or cost-prohibitive for field or ecological type work (Lukaski 1987). Proximate analysis of compartments includes total body water (TBW), dry mass (DM), fat-free mass (FFM), total body protein (TBP), total body ash (TBA), and total body fat mass (TBF). Direct measurements of individual compartments using chemical analysis require sacrificing the organism followed by lengthy laboratory procedures (Association of Official Analytical Chemists 1990). This negates the ability for repeated measures on the same individual and suppresses compositional study of endangered species. Certain compo-

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M.K. Cox and K.J. Hartman.¹ Wildlife and Fisheries Resources Program, Division of Forestry, Davis College of Agriculture, Forestry, and Consumer Sciences, West Virginia University, Morgantown, WV 26506-6125, USA.

¹Corresponding author (e-mail: hartman@wvu.edu).

nents such as TBW are good predictors of other body composition estimations, but in vivo methods to estimate TBW involve using radioisotope tracers such as tritium, deuterium, and oxygen-18, which are difficult to analyze outside a specialized laboratory and are also cost-prohibitive (Lukaski 1987). FFM can be estimated nonsacrificially by counting intracellular potassium-40 with thallium-activated sodium iodide crystal detectors, but the high cost, instrumentation, and technicality diminish its practicality (Corcoran et al. 2000).

Electrical conductivity methods are nonsacrificial and include total body electrical conductivity (TOBEC) and bioelectrical impedance analysis (BIA), which both rely on impedance differences between fat and fat-free tissues. Lantry et al. (1999) found that the TOBEC method accurately estimates composition parameters for healthy individuals, but errors increase when subjects are undergoing mass or compositional changes or when subjects with dissimilar body sizes are compared (conductivity changes with body geometry and size). Furthermore, the TOBEC unit is large and nonportable, making field studies impractical. BIA is available in an inexpensive, small, portable unit that has provided accurate and reliable estimations of water mass in humans, but as with the TOBEC, complex body geometries tend to decrease the accuracy of the composition estimations. However, we hypothesize that if BIA technology is applied to organisms with a more simplified body geometry (majority of the mass located in the a single volume), accuracy of measurements would not be lost and proximate composition estimations could accurately and nonlethally be made in laboratory and field settings for fish. Furthermore, because of the generality of electrical resistivity laws across various mediums, models for fish would not seem to have the restrictions of individual species specificity.

To investigate the applicability of BIA technology on fishes, we measured brook trout (*Salvelinus fontinalis*) for bioelectrical impedance and sacrificed for analysis of total body proximate composition components. Independent predictive models were made for TBW, DW, FFM, TBP, TBA, and TBF using derivatives of resistance and reactance equations. These models were then tested on an independent validation group of brook trout. Values from each predicted composition parameter were compared with actual numbers obtained from laboratory proximate analysis. Generality of the brook trout model was tested on a second validation group that consisted of 10 species of warmwater fishes. Predicted values of DW, TBW, and total mass were compared with actual values. An additional group of brook trout was used to test for harmful responses to BIA methods.

Materials and methods

Principles of BIA

Proximate composition estimations are calculated by measuring the impedance (resistance and reactance) of a current (800 μ A AC and 50 kHz) passed through an organism and then regressing these measures with actual proximate body composition numbers for that organism. Resistance of a substance is proportional to the voltage of an applied current as it passes through a substance, or

$$(1) \quad R = V \cdot CT^{-1}$$

where R is resistance (ohms), V is applied voltage (volts), and CT is current (amps). Reactance X_c (ohms) is the opposition to alternating current by a capacitor (cell membranes) and can be mathematically expressed by the following equation:

$$(2) \quad X_c = 1 \cdot 2\pi f C^{-1}$$

where f is frequency (hertz) and C is capacitance (farads) (Keller et al. 1993). Impedance (R and (or) X_c) is related to the cross-sectional area, conductor length of the organism, and the signal frequency of the current (Lukaski 1987). Impedance can be expressed by the equation

$$(3) \quad Z = \rho L \cdot A^{-1}$$

where Z is impedance (ohms), ρ is a resistivity constant, L is measured length, and A is cross-sectional area (square centimetres). If the signal frequency and the configuration are held constant, the impedance measurements can be related to its volume. This is demonstrated by the following: if both sides of the equation are multiplied by L/L , then

$$(4) \quad Z = \rho L^2 L \cdot A^{-1}$$

Resistance represents conductive material more than X_c so Z can be represented by R

$$(5) \quad R = \rho L^2 L \cdot A^{-1}$$

where $L \cdot A$ represents a volume (cubic centimetres), and by substitution

$$(6) \quad V_\epsilon = \rho L^2 \cdot R^{-1}$$

where V_ϵ is volume epsilon (cubic millimetres) and ρ is determined statistically by regressions of V_ϵ on impedance. Cell membranes consist of a nonconductive lipid bilayer sandwiched between two conductive protein layers, and at the low voltages and high frequencies such as those used here, current mainly passes through extracellular fluids, while at higher frequencies, the cell walls become capacitive (Pethig 1979). This allows reactance and resistance numbers to be sensitive to changes in volume of extracellular and cellular material.

Model development and validation

A total of 50 brook trout, 22 from headwater streams and 28 from the Bowden State fish hatchery, Bowden, West Virginia, were placed into a data set from which the model and validation groups were selected (Table 1). The model group consisted of 30 fish that (i) satisfied conditions of having individual fish with the maximum and minimum values for length (110–285 mm), mass (10–241 g), resistance (389–786 Ω), and reactance (59–213 Ω) and (ii) were randomly selected until a total number of 30 had been reached. The remaining 20 fish not placed in the model group were placed in the validation group. It was important to include relatively fat and lean fish in the model to provide sufficient variation in fat masses to allow us to adequately evaluate the ability of BIA to predict this parameter. We assumed that the hatchery fish were in a fat condition owing to their regular, three times daily ad libitum feeding schedule. Therefore, BIA pro-

Table 1. Mean values for length, mass, fat mass, and impedance values for all species in this study.

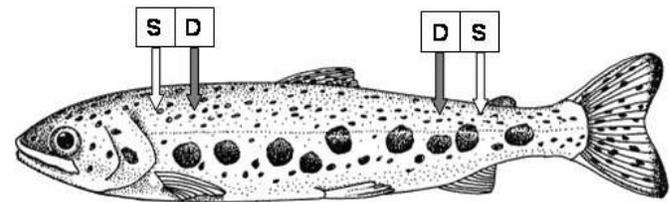
Species	<i>n</i>	Length (mm)	Mass (g)	Fat mass (g)	% fat (range)	Resistance (Ω)	Reactance (Ω)
Brook trout (<i>Salvelinus fontinalis</i>)	30	165.44 (53.25)	60.77 (67.54)	3.75 (5.88)	0.67–9.55	570.60 (115.75)	160.73 (34.18)
Validation	20	177.55 (51.16)	68.84 (64.51)	4.00 (6.04)	1.50–9.28	532.05 (118.16)	150.20 (26.40)
Freshwater drum (<i>Aplodinotus grunniens</i>)	11	236.45 (42.14)	140.82 (82.05)	na		431.91 (30.39)	132.09 (15.58)
Gizzard shad (<i>Dorosoma cepedianum</i>)	15	212.20 (30.24)	73.11 (37.16)	na		473.13 (73.30)	168.53 (43.42)
Longear sunfish (<i>Lepomis megalotis</i>)	10	124.90 (14.37)	45.47 (18.57)	na		318.10 (67.93)	91.30 (11.83)
Logperch (<i>Percina caprodes</i>)	6	115.67 (7.89)	11.39 (2.42)	na		509.00 (92.16)	163.33 (18.91)
<i>Moxostoma</i> spp.	11	186.55 (69.13)	97.55 (123.21)	na		392.27 (48.48)	118.45 (15.85)
Redbreast sunfish (<i>Lepomis auritus</i>)	4	126.00 (35.99)	44.71 (28.87)	na		329.75 (41.76)	69.75 (10.50)
Sauger (<i>Sander canadensis</i>)	11	273.55 (32.79)	156.50 (61.69)	na		386.36 (58.53)	107.00 (17.68)
Smallmouth bass (<i>Micropterus dolomieu</i>)	11	176.00 (36.62)	66.10 (38.67)	na		370.09 (34.63)	124.09 (32.11)

Note: Here, brook trout (*Salvelinus fontinalis*) refers to the fish used in the brook trout model and validation refers to fish used in the validation set for the brook trout model. Values in parentheses represent 1 SD. na, not applicable.

cedures (below) were performed on 10 hatchery fish on site (representing relatively fat fish) and the remaining 18 fish were transported back to the West Virginia University Ecophysiology Laboratory. Transported fish were placed and maintained in recirculating tanks (0.5 m \times 1.5 m) at 14 ± 1 °C and withheld from food for 30 days to achieve lower fat levels. The 22 stream fish were captured by electrofishing and analyzed with BIA (following the procedures below). Gender was not determined in fish.

Fish in both the model and validation groups were anesthetized and measured for BIA in an identical manner. Fish were anesthetized in a tricaine methanesulfonate solution of 1 g/9 L water, blot dried, and placed on a nonconductive board in a left lateral recumbent position. Electrical impedance (resistance and reactance) was measured with a tetrapolar bioelectrical impedance analyzer (RJL Systems, Detroit, Michigan). The analyzer has two sets of needle electrodes (stainless, 28 gauge, 12 mm) (Grass Telefactor, West Warwick, Rhode Island) with each set consisting of one signal and one detecting electrode placed 1.0 cm apart. One set was placed in the anterior dorsad region and the second set was placed in the caudal peduncle region of the fish (Fig. 1). Each set of electrodes was placed in a consistent position for each fish: midway between the lateral line and dorsal mid-point with the anterior set of electrodes positioned at the anterior apex of the operculum and the posterior set positioned even with the anterior edge of the adipose fin (Fig. 1). Electrode needles penetrated 2 mm into the fish. The distance between the two detecting electrodes was measured for each fish, and a current was introduced through the signal electrodes and the proximal detecting electrodes measured the voltage drop. These two electrical values, resistance and reactance, were then used to calculate values from common electrical property equations that included resistance in series and in parallel, reactance in series and in parallel, combined resistance and reactance in series and in parallel, and capacitance. These values were then used as independent variables in the regression model.

Fig. 1. Diagram of locations for signal (S) and detector (D) electrodes used in measuring bioelectrical impedance values of brook trout (*Salvelinus fontinalis*). Brook trout drawing modified from the Oregon Department of Fish and Wildlife, Salem, Oregon.



Dependent variables in the regression model were the body composition values measured in the laboratory immediately following BIA procedures. While still anesthetized, fish were measured for length and mass and euthanized with an overdose of tricaine methanesulfonate (solution of 3 g/9 L water) and frozen. The individual parameters and methods used to quantify each were analyzed by the West Virginia University Rumen Profiling Fermentation Laboratory and are as follows: TBP by the Kjalkahl method, TBA in an ashing oven at 500 °C, TBW by back-calculating dried samples, DM by oven drying at 100 °C, TBF by ether extraction, and FFM by mass balance equations (body mass minus TBF) (Association of Official Analytical Chemists 1990). Independent models for each parameter were built from the model group. Relationships between the actual and predicted values were explained with correlation analysis for strength of linear relationships and predictability, residual plots and *F* values (to test significance of slopes in linear regressions), and confidence limits on the regression coefficients (to test for 1:1 relationships).

Best-fit linear models were then used to predict parameter estimations in both the brook trout and warmwater species validation groups. Prediction values for each parameter in both validation groups were expressed by the following equation:

Table 2. Relationships between proximate body composition components and impedance equations for brook trout (*Salvelinus fontinalis*) in the model group.

Component	<i>n</i>	Intercept (SE)	Slope (SE)	E	<i>F</i> statistic (<i>p</i> value)	<i>R</i> ²
TBW	30	1.320 45 (1.948 26)	3.461 87 (0.105 60)	L^2/R_m	<0.0001	0.9746
DM	30	0.295 58 (0.778 43)	4.315 75 (0.136 79)	L^2/X_{cp}	<0.0001	0.9726
FFM	30	-0.518 81 (0.561 08)	0.999 68 (0.030 41)	L^2/R_m	<0.0001	0.9747
TBP	30	-0.673 52 (0.523 28)	0.896 30 (0.028 36)	L^2/R_m	<0.0001	0.9727
TBA	30	0.115 16 (0.060 89)	0.117 49 (0.003 70)	L^2/R_p	<0.0001	0.9730
TBF	30	-1.790 87 (0.320 15)	1.803 82 ⁻²³ (7.289 85 ⁻²⁵)	L^2/X_{cp}	<0.0001	0.9563

Note: Analysis results of the linear relationships between the measured bioelectrical impedance equations and actual numbers of proximate body composition components: total body water (TBW), dry mass (DM), fat-free mass (FFM), total body protein (TBP), total body ash (TBA), and total body fat mass (TBF). In the table, "E" represents the specific impedance equation providing the best fit, where *L* is length, *R* is resistance, *Z* is impedance, *X_c* is reactance, and subscripts "p" and "m" represent parallel and series circuitry, respectively.

Table 3. Correlation coefficient scores (*r*) of predicted and actual parameter values including dry mass (DM), total body water (TBW), and total mass (summation of DM and TBW versus actual wet mass of fish) for various warmwater species using the brook trout (*Salvelinus fontinalis*) bioelectrical impedance analysis (BIA) model.

Species	<i>n</i>	<i>r</i>		
		DM	TBW	Total mass
Freshwater drum (<i>Aplodinotus grunniens</i>)	11	0.992 06	0.999 76	0.999 74
Gizzard shad (<i>Dorosoma cepedianum</i>)	15	0.992 08	0.999 12	0.998 18
Longear sunfish (<i>Lepomis megalotis</i>)	10	0.861 70	0.998 63	0.996 08
Logperch (<i>Percina caprodes</i>)	6	0.878 16	0.995 29	0.990 83
<i>Moxostoma</i> spp.	11	0.997 91	0.999 45	0.999 81
Redbreast sunfish (<i>Lepomis auritus</i>)	4	0.858 39	0.999 41	0.996 69
Sauger (<i>Sander canadensis</i>)	11	0.942 27	0.999 69	0.996 02
Smallmouth bass (<i>Micropterus dolomieu</i>)	11	0.907 06	0.999 81	0.994 52

Note: Spotted bass (*Micropterus punctulatus*) and white crappie (*Pomoxis annularis*) were not included here because of low sample sizes (one fish each).

$$(7) \quad P\theta = (PP/PM) \times \text{mass}$$

where *Pθ* is the new predicted parameter value (grams), *PP* is the initial predicted parameter (grams), *PM* is the predicted total mass (grams), and *mass* is the measured actual wet mass of the fish. Statistical and analytical procedures were completed with SAS[®] 8.1 software. The best-fit linear models were then tested using the validation group.

Generality of the brook trout model

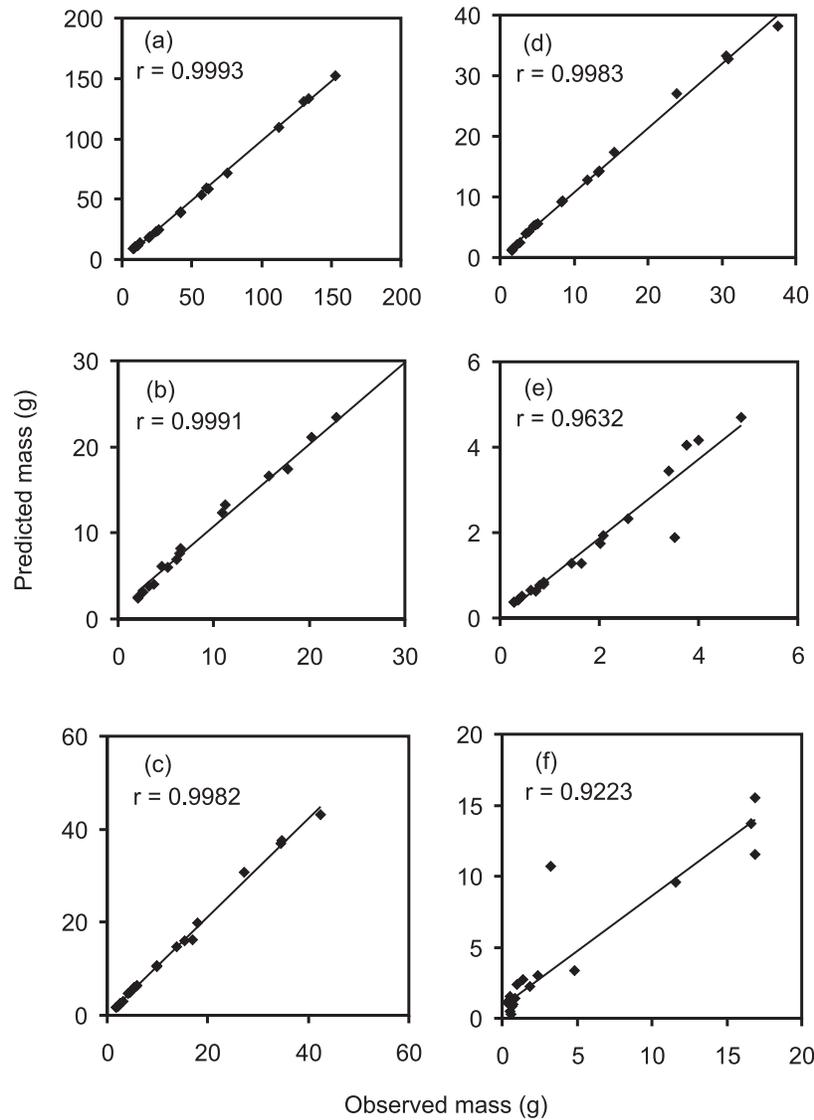
To test brook trout model generality, water and DM models developed for brook trout were used to predict TBW and DM of a group of warmwater fish. Eighty-one fish consisting of 10 species were collected in the summer of 2003 by nighttime electroshocking on the Kanawha River in Charleston, West Virginia. Species included white crappie (*Pomoxis annularis*), freshwater drum (*Aplodinotus grunniens*), gizzard shad (*Dorosoma cepedianum*), longear sunfish (*Lepomis megalotis*), logperch (*Percina caprodes*), *Moxostoma* spp., redbreast sunfish (*Lepomis auritus*), sauger (*Sander canadensis*), smallmouth bass (*Micropterus dolomieu*), and spotted bass (*Micropterus punctulatus*). Brook trout model equations were used to estimate TBW and DM in all species of fish. The sum of these terms (TBW and DM) was used to approximate a predicted wet mass of each fish and

were subsequently correlated with the actual wet mass of the fish. Actual water and dry masses were measured following Association of Official Analytical Chemists (1990) methods.

Physical response to BIA methods

To test subject responses to BIA procedures, 20 additional hatchery brook trout were placed individually into 37-L aquaria and fed a maintenance ration of fly larvae (Gruco Inc., Hamilton, Ohio). Half of the fish were randomly assigned to a treatment group and measured for impedance and the other assigned to a control group that were not measured for impedance. Regardless of group, once weekly for 3 weeks, each fish was anesthetized and measured for length and mass with the additional measurement of impedance taken from the treatment group fish. Following measures, fish were immediately returned to the aquaria. Observations of bleeding were noted immediately following BIA measures, and changes in color, feeding, swimming, and bruising were observed once daily. A flashlight was used to visually determine bruising and color, while feeding and swimming were scored as "not swimming, listless" or "not feeding". A dichotomous variable score (0 or 1; i.e., 0 = no bruising, 1 = bruising) was used to monitor basic observations. This repeated-measures experiment was analyzed with a logistic regression.

Fig. 2. Correlation analysis of predicted and actual values for the brook trout (*Salvelinus fontinalis*) validation group ($n = 20$) including (a) total body water, (b) dry mass, (c) fat-free mass, (d) total body protein, (e) total body ash, and (f) total body fat.



Results

Strong linear relationships were found between independent variables calculated from BIA values and laboratory-derived body composition values. Independent and dependent variables with associated correlation strengths (r values) are as follows: resistance (in series) with TBW ($r = 0.9872$), reactance (in parallel) with DM ($r = 0.9862$), combined resistance and reactance (in series) with FFM ($r = 0.9873$), resistance (in series) with TBP ($r = 0.9863$), resistance (in parallel) with TBA ($r = 0.9864$), and capacitance with TBF ($r = 0.9779$) (Table 2).

Tests of the linear regression models using the validation group of brook trout showed that the models were good predictors of all body composition values. Predicted and actual values for all body composition parameters were highly correlated ($p < 0.0001$) with r scores ranging from 0.9223 to 0.9993 in TBF and TBW, respectively (Fig. 2). The F value tests ($p < 0.0001$) and residuals reflected a linear relationship between impedance values and the predicted values

(Fig. 2). Correlations between predicted and observed values in all proximate composition categories indicated a strong linear relationship with values not differing from 1:1 (Fig. 2).

Linear regression models developed for brook trout were also good predictors of DM, TBW, and mass across all species of fish tested from the Kanawha River. Predicted values of DM, TBW, and mass were also strongly correlated with actual values (Fig. 3; Table 3). DM predicted and actual values were highly correlated, with r ranging from 0.8584 in redbreast sunfish to 0.9921 in gizzard shad. Predicted and actual values of TBW were highly correlated, with $r > 0.9500$. Predicted and actual total mass values were highly correlated, with $r > 0.9900$ for all species.

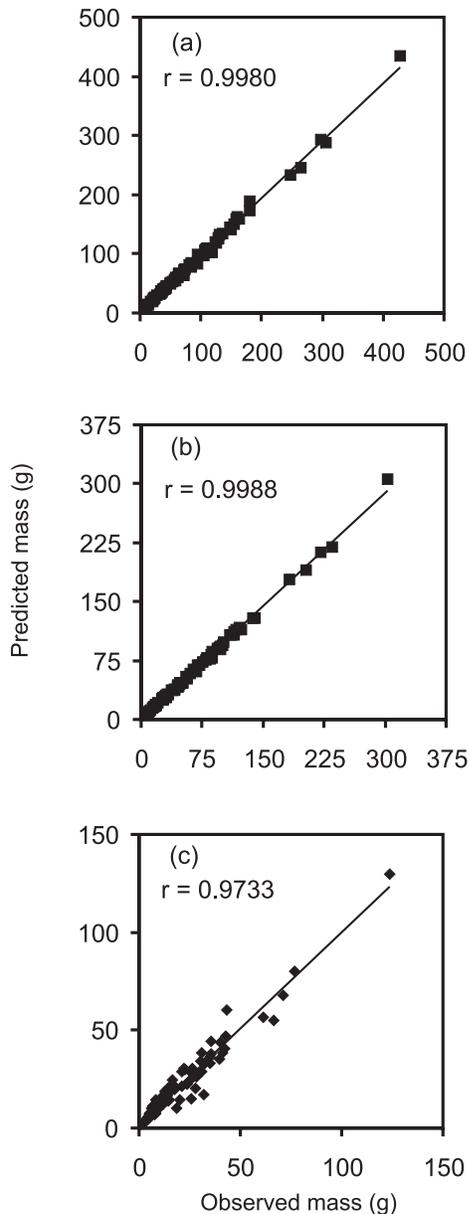
In repeated-measures laboratory experiments, brook trout showed little response to being measured with BIA. Measured parameters of swimming, feeding, bleeding, and color were not significantly different between the two groups (applied BIA and not). Bruising was a significant (logistic regression $p < 0.0001$) response to BIA but was slight and lasted only 2 days (Table 4).

Table 4. Number of negative responses (e.g., loss of color, not feeding or swimming, bruising, bleeding) of individual brook trout (*Salvelinus fontinalis*) that underwent bioelectrical impedance analysis (BIA) compared with those that received no BIA (control).

Treatment	<i>n</i>	Color	Feeding	Swimming	Bruising	Bleeding
Control	210	4	22	3	1	0
BIA	210	0	13	1	110 ^a	3

^aSignificant ($p < 0.0001$).

Fig. 3. Correlation analysis of predicted and actual values for the warmwater species validation group ($n = 81$). Predicted values of (a) wet mass, (b) dry mass, and (c) total body water were calculated using the existing brook trout (*Salvelinus fontinalis*) model.



Discussion

BIA models provided a means of estimating body composition in brook trout and a group of warmwater fish species with a high degree of predictability ($R^2 > 0.8507$). Addi-

tional experiments showed that the methods of BIA are nonlethal and appear to produce little measurable effect on fish health or behavior. The portability of the BIA unit makes this a useful method that can be utilized in the field. The BIA instrument is a small handheld unit that is powered by a 9-V battery. Once a model is built, BIA estimations are derived from three measurements: resistance (measure of extracellular resistance), reactance (measurement of “celled” mass), and distance between detecting electrodes. These three measurements are used to predict TBW, DM, FFM, TBP, TBA, and TBF and are obtained on live, anaesthetized organisms in about the time it takes to measure live mass.

The strong predictability and accuracy found in this fish data are a result of the body geometry of fish being more simplified than the higher vertebrates used in previous BIA studies (Berg and Marchello 1994; Marchello et al. 1999). More specifically, trout have a fusiform geometric shape that approximates a cylinder with a majority of the mass located in the thorax (Jobling 1995). The thorax accommodates all major body composition components and likewise is the main region for hypertrophic or hypotrophic growth. Since volume is proportional to the impedance and the length between detecting electrodes (see eqs. 1–4 in the Materials and methods section), a single impedance measurement represents the whole body and likewise compositional changes that occur within it. If the majority of mass is not located within a single volume (as with many higher vertebrates), it must be distributed into limbs or appendages. Since each limb or appendage has its own volume and tissue heterogeneity, a single measurement of impedance cannot represent the whole organism (e.g., two different-sized volumes with identical composition would have different impedance measurements) (Keller et al. 1993). Likewise, measuring impedances for each separate volume and combining them would result in complex methodology and conversely a complex model with error propagations occurring with each volume. For a single measurement of impedance to be representative of the whole body, simple body geometry with the majority of the mass located in one volume is desirable.

A validation of the brook trout BIA model with an independent BIA data set showed that the models accurately predicted actual values ($r > 0.9632$) except for lower predictability of fat mass ($r = 0.9223$). The reasoning for the weaker correlations for fat mass may be explained by electrical resistivity properties. The nature of current division described by Ohm’s law and Kirchhoff’s rules dictates that current will pass through an entire circuit, but the path with the least resistance will carry more current. Fat deposits (i) are concentrated in the ventral gut region of the fish and (ii) have a higher resistance than other visceral or somatic tissues. Since electrode placement was in the dorsal region, and resistance is higher in fat, it is possible that the current

does not represent the fat that is located in the lower ventral region. All other parameters such as TBW and TBP are more systemic, have a lower electrical resistance, and therefore are better represented by impedance values in this study. This leads to the possibility of localized or tissue-specific BIA modeling via electrode placement.

Furthermore, strong linear relationships in the model predictions for a data set of warmwater species suggest that the brook trout model may predict compositional parameters for other species. This may be due to the similar geometric shapes found among the species of fish used in this study. The warmwater species validation group was consistent with geometric shapes of a cylindrical shape with the majority of the mass (body composition components) located within one volume, and predictions with the brook trout BIA model were strongly correlated with actual parameter estimations. Some of the variation between predictions with the brook trout model and observed values for the warmwater species, especially in the DM parameter, may have been due to regional tissue deposits. Determining error constitutes is outside the scope of this study, but the authors feel that lower vertebrates such as fish, amphibians, reptiles, and some mammals that fit the criteria mentioned above would be eligible for BIA modeling. The brook trout models used here were to test for model generality and we conclude that general relationships observed for brook trout measured with BIA will hold for other species regardless of size if geometry is similar.

The ability to precisely and nonlethally estimate proximate composition through BIA will permit increased precision in energy flow and proximate composition studies on spatial and temporal scales that were previously impractical. At the individual level, BIA will permit repeated measures on the same individual during the course of investigation, yielding better tracking of energetics components and improved precision in bioenergetic models. At the population level, BIA will permit assessment of condition of cohorts over time and permit detailed comparisons across cohorts and temporal and spatial scales, such as evaluating body conditional properties of highly migratory species at different points during migration. At the community level, BIA will permit the evaluation of growth and energy flow dynamics across species that may elucidate community dynamics that were previously unknown or permit correlation of body composition with outbreaks of disease. This approach also has potential for the nonlethal study of threatened or endangered species by utilizing models developed for closely related species. Finally, this could be applied to other taxa

groups, particularly amphibians and reptiles, with similar results and uses as described here.

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