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Empirical relationships between body tissue composition and bioelectrical impedance of brook trout *Salvelinus fontinalis* from a Rocky Mountain Stream

J. B. Rasmussen*†, A. N. Krimmer*, A. J. Paul‡ and A. Hontela*

**Department of Biological Sciences, 4400 University Dr. W., University of Lethbridge, Lethbridge, Alberta, T1K 3M4 Canada and ‡Fish and Wildlife Division, Alberta Sustainable Resource Development, Box 1420, 2nd Floor, Provincial Bldg. 213-1st Street W. Cochrane, Alberta, T4C 1B4 Canada*

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Bioelectrical impedance analysis (BIA) analysis was carried out in the field on anaesthetized *Salvelinus fontinalis* electrofished from a mountain stream in Alberta, Canada; the fish were then sacrificed for subsequent analysis of tissue composition. Water content was assessed by comparing wet and dry mass, and total body lipid content was measured by Soxhlet extraction with petroleum ether. A multivariate analysis of body composition and size metric against impedance measurements was carried out, and the main findings were (1) body size and related metrics were strongly related to volumetric impedance measures, as shown in several previous studies, (2) lipid content (%) and water content (%) were both well predicted by regression models whose main predictor was reactance and (3) reactance and resistance measures that were series-based produced excellent predictions of tissue composition, whereas the corresponding parallel-based models were crude. The BIA measurements are quick and easy to conduct and appear to provide excellent predictions of a number of proximate body components, without the need to kill the fish; however, more studies are required to provide improved understanding of possible effects of region, season, life stage and species on measurements.

Key words: lipid content; phase angle; reactance; resistance; water content.

INTRODUCTION

Bioelectrical impedance analysis (BIA) is a relatively new tool with potentially important applications in fish ecology and fisheries research; it provides a non-invasive index of the composition of body tissues that can be used to evaluate body condition. While there is extensive literature citing the use of BIA on humans (Kyle *et al.,* 2004), applications to fishes are limited and very recent (Bosworth & Wolters, 2001; Cox & Hartman, 2005; Duncan *et al*., 2007; Willis & Hobday, 2008). Because BIA has the capacity to detect small change in the electrical properties of a body (Foster & Lukaski, 1996) and because of its non-lethal and non-invasive nature,

†Author to whom correspondence should be addressed: Tel.: +1 403 892 7982; email: joseph.rasmussen@uleth.ca

it can be used as a tool to monitor changes in proximate body composition and therefore changes in physical condition and energy expenditure.

When electrodes are inserted into biological tissues and a voltage is applied, a field is established and electric current, conducted by aqueous electrolytes, will flow. While there is no defined circuitry and indeed the medium is anisotropic, the principles defined by Ohm's and Kirchoff's laws are still pertinent; however, variables such as voltage, current and impedance must be viewed as bulk properties, *i.e.* cross-sectional averages across the field of flow. As such, impedance (Z; ohms) across a pair of electrodes separated by a distance (D; m) would be

$$Z = \rho D A^{-1} = \rho D^2 V^{-1}, \text{or } V = \rho D^2 Z^{-1},$$

where ρ (ohm m) is a point property relating to the insulative properties of the tissue and would be termed impeditivity, resistivity or reactivity depending on whether impedance, resistance or reactance was being measured, $A(\text{m}^2)$ is cross-sectional area of the field and $V(\text{m}^3)$ is a volumetric representation ($= DA$). Thus, Z, whether it pertains to impedance in general, or to resistance or reactance in particular, is a cross-sectional measure and thus related to average tissue properties (*e.g.* % water or % lipid) and $D^2\text{Z}^{-1}$ is a three-dimensional measure that reflects whole organism properties such as total body water, total dry mass or lipid-free body mass (Liedtke, 1997; Cox & Hartman, 2005). Most BIA studies on fishes have focussed on volumetric measures and their relationship to body mass metrics (Duncan *et al.*, 2007; Willis & Hobday, 2008; Hanson *et al.*, 2010), although cross-sectional measures have also been used to reflect body composition (Bosworth & Wolters 2001; Pothoven *et al.,* 2008). Biological tissues are far from uniform in composition and, therefore, electrode placement can have significant effects on impedance measurements, as can the degree of gut fullness, and ambient temperature (5–10 Ω decrease per °C) (Cox *et al.,* 2011).

There are two components of impedance: resistance and reactance, both of which resist the flow of current (Cox *et al.,* 2011). Resistance [R (ohms, Ω) $= VI^{-1}$, where V is applied voltage (V) and I is current (A)] applies equally to direct and alternating current and is provided by the non-conductive lipid bi-layer sandwiched between two conductive layers with high protein content. When the current is weak, R reflects the composition of extracellular material adjacent to membranes. Reactance [$\chi_\text{c}(\Omega) = (2\pi f C)^{-1}$, where f is frequency in Hertz and C is capacitance in F] reflects the tendency of biological membranes to absorb charge from an alternating current and reflects the total amount of membrane material through which the current is flowing. Body tissues containing large amounts of water and electrolytes are highly conductive and therefore provide low resistance pathways, whereas materials such as bone and fat are poor conductors and correspond to high resistance (Liedtke, 1997).

An alternative type of impedance measure is derived from the fact that capacitors shift the phase of alternating current as they accumulate charge. Thus, the phase angle ϕ ($\tan \phi = \chi_\text{c} R^{-1}$), which equals the ratio of reactance to resistance, is thought to be sensitive indicator of the functional integrity of membrane systems (Liedtke, 1997), although its relationship to particular aspects of membrane function (*e.g.* ion flux, osmotic regulation, membrane selectivity or cell signalling) have never been studied. In human studies, ϕ has been shown to decrease as the extracellular water: intracellular water ratio increases (Segal *et al.*, 1987), which reflects malnutrition (Kushner, 1992). Recently, ϕ has been shown to decline rapidly as fishes were fasted under both laboratory and field conditions, and as such may be a promising indicator of short-term changes in condition (Cox & Heintz, 2009).

There are two very different ways of modelling the relationship between resistance and reactance to total impedance, and this has contributed to some confusion in the literature. R and χ_c, the direct resistance and reactance measurements output by the analyser, are based on the premise that the elements of impedance are arranged in series (*i.e.* total impedance, $Z^2 = R^2 + \chi_c^2$, which assumes that the current passing through each element is the same). Alternatively, R_p and χ_{cp} are the corresponding measures based on Kirchoff's law ($Z^{-2} = R_p^{-2} + \chi_{cp}^{-2}$), which assumes that the impedance elements are arranged in parallel, *i.e.* the voltage drop across each element is the same. Pothoven *et al.* (2008) used only the parallel-transformed versions of resistance and reactance, whereas several other studies have used the untransformed, series-based versions or both, and still others have not clearly indicated which version was used. No detailed comparisons of the relationships of these different formulations to fish tissue composition have been investigated.

Although most authors have considered BIA as a promising tool for non-lethal assessment of tissue composition, there have been concerns expressed over its strong reliance on empirical relationships (Lukaski, 1999). The concern is that a host of inter-correlated body size and compositional metrics can be expected to yield strong correlations to a variety of equally inter-correlated impedance measures. As such, many of the patterns that can emerge from a piece-meal analysis of such data may be statistically spurious, or at least have no mechanistic basis and thus probably lack generality, while others that may be mechanistically important may escape notice. Multivariate methods permit a holistic and systematic approach to data of this kind but have not been used in BIA studies. Such methods as MANOVA or canonical regression allow efficient isolation of patterns, even those that may be less dramatic and, as a result, reduce the likelihood of such problems (Tabachnick & Fidell, 2007).

The purpose of this study was to calibrate impedance measures of brook trout *Salvelinus fontinalis* (Mitchill 1814) against proximate measures of tissue composition, to provide a non-lethal and non-invasive index of body condition that could be applied repeatedly to the same fish over time. This was done as part of a study on flow manipulation of overwintering *S. fontinalis*, wherein it was necessary to evaluate and monitor changes in condition over winter under different imposed flow regimes (Krimmer *et al.,* 2011). Total body mass (M_T), dry mass (M_D), total body water (W_T), total lipid content (O_{LT}), lipid-free dry mass (M_{LFD}), total length (L_T) and fork length (L_F), as well as average tissue composition metrics such as % lipid (O_L%) and % water (W%) and Fulton's condition index (K) were regressed against a series of impedance measures, including volumetric metrics, such as D^2R^{-1} and $D^2\chi_c^{-1}$, as well as cross-sectional measures, such as R and χ_c, and the phase angle (tan ϕ), using canonical regression (Tabachnik & Fidell, 2007). Resistance and reactance were tested both in their series-based form (R and χ_c) and in their parallel formulation (R_p and χ_{cp}) as employed by Pothoven *et al.* (2008). This comparison was intended to shed light on how important this distinction is for different types of impedance measurements.

MATERIALS AND METHODS

Thirty-two *S. fontinalis* were collected from two small tributaries of the Kananaskis River, Alberta, Canada (50° 59′ 13″ N; 115° 05′ 14″ W) on 18 October 2007; water temperature was 3·5° C at both sites. The study area is described in more detail by Krimmer *et al*. (2011). The creeks were fished using a backpack electrofisher and each sampled fish was anaesthetized using a 40 mg l^{-1} solution of clove oil before M_T and L_F were measured.

BIA measures were then taken using a tetrapolar, Quantum X Bioelectrical Body Composition Analyzer (RJL Systems; www.rjlsystems.com), which delivers a current of 800 μA at 50 kHz. The analyser was adapted for use on fish by using stainless steel needle electrodes (Grass Telefactor; www.grasstechnologies.com). The anaesthetized fish were placed on a dry paper towel for electrode insertion. The analyser has two sets of needle electrodes (28 gauge, 12 mm), each set consisting of a signal and a detector electrode placed 1 cm apart. Each set of electrodes was inserted ipsilaterally into the fish midway between the lateral line and the dorsal midpoint, with the anterior set placed near the operculum, and the posterior set in the caudal peduncle just below the adipose fin, in the manner described by Cox & Hartman (2005) (position A of Cox *et al.,* 2011). The electrodes penetrated the skin and entered the subdermal tissue to a depth of 2 mm.

Resistance and reactance readings were recorded, and the distance between the two inner detecting electrodes (D) was measured. Electrode placement was consistent in all trials. Impedance data were expressed in two ways: R and χ_c, the direct resistance and reactance measurements output by the analyser, and R_p and χ_{cp}, based on the assumption that impedance elements are arranged in parallel. Thus, $R_p = R + \chi_c^2 R^{-1}$ and $\chi_{cp} = \chi_c + R^2 \chi_c^{-1}$ (Liedtke, 1997).

The sampled *S. fontinalis* were euthanized by an overdose of clove oil, individually bagged and returned to the laboratory for processing to measure body composition variables. The whole bodies were dried at 50° C in individual foil containers until a constant dry mass was reached (*c*. 48 h) to obtain dry mass (M_D). Total body water (W_T) was determined as $M_T - M_D$, and $W\%$ was calculated relative to M_T. Each whole dried fish was homogenized in a small grinder. Aliquot samples, containing a minimum of 1·5 g of homogenized tissue were run using the hot Soxhlet extraction method, with petroleum ether as a solvent (Cabrini *et al*., 1992; Manirakiza *et al*., 2001). The samples were run in duplicate for a minimum of 3 h until the sample maintained a constant mass. Total body lipid (O_{LT}) was determined as M_D before minus M_D after lipid extraction, and $O_L\%$ was calculated relative to M_T.

The relationships between the proximate compositional variables and the BIA measurements were first explored in a holistic manner using the general linear model (GLM in JMP IN; www.jmp.com), $\boldsymbol{Y} = \boldsymbol{a} + \boldsymbol{bX} + \boldsymbol{\varepsilon}$, where $\boldsymbol{Y}$ is a set of dependent variables, $\boldsymbol{X}$ is a set of independent variables, a and b represent sets of estimated coefficients and ε denotes a matrix of residuals, the $\boldsymbol{Y}$ matrix being a set of vectors composed of M_T, W_T, L_T and L_F and the X matrix, D, R, χ_c and tan ϕ. Univariate regression is of limited use for analysis of data sets with strong correlations among the dependent variables. Because GLM involves dimensional reduction and identifies a set of orthogonal (canonical) components of the covariance matrix between $\boldsymbol{X}$ and $\boldsymbol{Y}$, it is a flexible tool that allows efficient exploration of data sets where strong correlations exist within, as well as across, both $\boldsymbol{X}$ and $\boldsymbol{Y}$. The core $\boldsymbol{X}$ and $\boldsymbol{Y}$ relationships are expressed by canonical correlations between the orthogonal components of the dependent and independent variables and are evaluated using a set of F-tests based on standard multivariate methods (*e.g.* Wilks' λ and Pillai's trace). All of the variables tested in this study were continuous variables, and as such, the GLM procedure is often referred to as canonical regression, whereas, when applied to data sets with categorical independent variables, it is referred to as MANOVA or MANCOVA (Tabachnik & Fidell, 2007). While the multivariate analysis is purely empirical, each significant canonical relationship was then transcribed as a univariate regression relating its primary compositional variable (or ratio) to a primary impedance variable, in a manner that best reflects the principles behind the method.

RESULTS

The *S. fontinalis* sampled ($n = 32$) spanned a considerable range in body size (M_T 13–76 g, mean ± s.d. 35 ± 17 g; L_F 110–183 mm, mean ± s.d. 150 ± 23 mm) and body composition (O_L 2–16%, mean ± s.d. 8·0 ± 3·9% and W 73–80%, mean ± s.d. 7·7 ± 2%). Impedance measures also spanned a wide range (R 445–776 Ω, mean ± s.d. 607 ± 87 Ω and χ_c 120–197 Ω, mean ± s.d. 153 ± 20 Ω). Total impedance measures ($Z = \sqrt{R^2 + \chi c^2}$) were very similar to R, since R values were always $> \chi_c$. The value of K ranged from 0·86 to 1·16 (mean ± s.d. 0·97 ± 0·08).

The multivariate GLM revealed highly significant relationships between body composition and impedance measurements; univariate F-values ranged from 3·43 ($P < 0·05$) to 86·9 ($P < 0·001$) and a series of multivariate test metrics including Wilks' λ (*c.* $F = 24·6$, $P < 0·001$) were all highly significant (Table I). The analysis yielded canonical components ($\boldsymbol{C}_1, \boldsymbol{C}_{2,\ldots}$) for both the dependent variable set, *i.e.* body metrics ($\boldsymbol{C}_{1Y}, \boldsymbol{C}_{2Y} \ldots$) and the independent variable set, *i.e.* impedance measurements ($\boldsymbol{C}_{1X}, \boldsymbol{C}_{2X} \ldots$). $\boldsymbol{C}_{1Y}$ was mainly a reflection of body size, and all metrics of total body composition except total lipids loaded strongly to it [Fig. 1(a)]. The $\boldsymbol{C}_{1X}$ reflected mainly a positive loading from D and a negative one from R [Fig. 1(b)]. The canonical correlation between $\boldsymbol{C}_{1Y}$ and $\boldsymbol{C}_{1X}$ (r_{C1}) was 0·99, reflecting the very strong relationship between body size and volumetric impedance. A second component, with a strong contribution from relative compositional metrics ($\boldsymbol{C}_{2Y}$), reflected mainly the influence of lipids (O_{LT}, O_L% and W%), and on the independent side ($\boldsymbol{C}_{2X}$) reflected mainly the influence of the cross-sectional impedance measures, χ_c and ϕ [Fig. 1(b)]; the canonical correlation ($r_C = 0·84$) across this component was not nearly as strong as $\boldsymbol{C}_1$. The $\boldsymbol{C}_3$ reflected the relationship between mass and length (body shape) on the dependent side, and the effect of ϕ on the independent side; however, the relationship was weak ($r_C = 0·48$), and the loadings are not shown. The $\boldsymbol{C}_4$ was too weak to consider ($r_C = 0·01$).

Table I. Parameter estimates and F-values from four multivariate tests on the general linear model $\boldsymbol{Y} = \boldsymbol{a} + \boldsymbol{bX} + \boldsymbol{\varepsilon}$ using data on body composition ($\boldsymbol{Y}$) and bioelectrical impedance* ($\boldsymbol{Y}$) of *Salvelinus fontinalis*. $\boldsymbol{Y}$ variables were total mass (M_T), fork Length (L_F), total body water (W_T) and total lipid (O_{LT}); $\boldsymbol{Y}$ variables were bioelectrical impedance analysis (BIA) length (L_D), resistance (R), reactance (χ_c) and phase angle (tan ϕ)

Parameter estimates						Whole model tests				
	M_T	L_F	W_T	O_{LT}	$F_{4,24}$; $P > F$	Test	Value	F	d.f.	$P > F$
a	−205.00	−7·29	−173.00	23·80	3·90, <0·05	Wilks' λ	0·00	24·6	16	<0·001
D	1·08	1·61	0·85	0·05	86·90, <0·0001	Pillai's trace	1·93	6·32	16	<0·001
R	0·28	0·13	0·25	−0·05	3·43, <0·05	Hotelling–Lawley	62·8	90·6	16	<0·001
χ_c	−1·39	−0·90	−1·22	0·23	5·21, <0·005	Roy's max root	60·0	405.0	4	<0·001
tan ϕ	876	503	752	-119	4·30, <0·01	$n = 32$, d.f. $\varepsilon = 27$				

*Total impedance measures ($Z = \sqrt{R^2 + \chi c^2}$) were not used in modelling since they were very similar to R (R values $\gg \chi_c$).

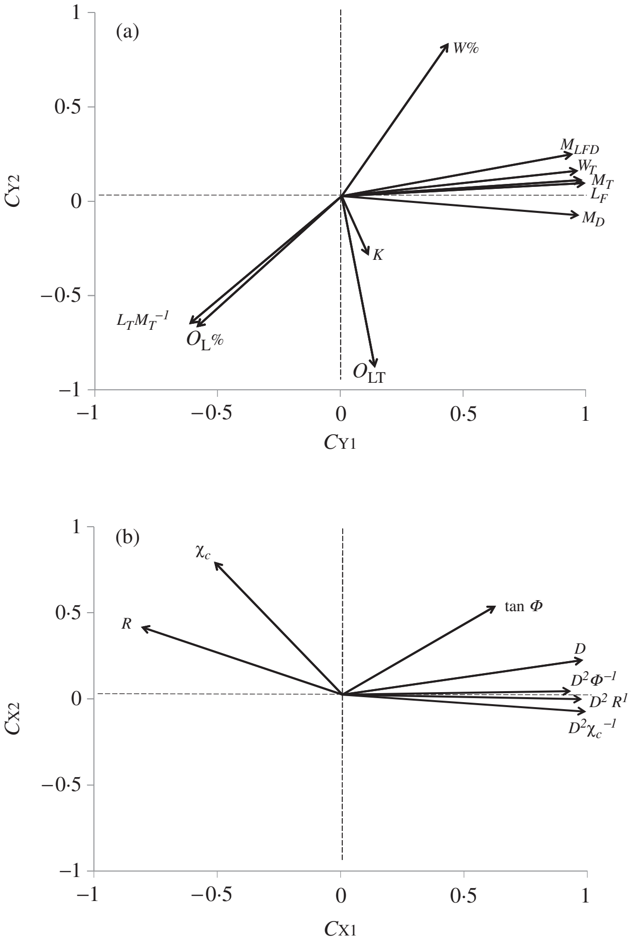

FIG. 1. Loadings obtained from canonical regression analysis of data on body composition (***Y***) and bioelectrical impedance (***X***) of *Salvelinus fontinalis*: (a) loadings from ***Y*** variables to components $\boldsymbol{C}_{1Y}$ and $\boldsymbol{C}_{X2}$, the two dominant principal components of the dependant variable data set, and (b) loadings from independent variables to components C_{X2} and C_{X2}, the two dominant components of the independent variable data set. ***Y*** variables are: M_{T}, total mass g; L_{T}, total length mm; L_{F}, fork length mm; K, Fulton's condition factor; W_{T}, total body water g; O_{LT}, total lipid g; M_{D}, dry mass g; $W\%$, water content %; $O_{\mathrm{L}}\%$, lipid content %; M_{LFD}, lipid-free dry mass. ***X*** variables are R, resistance (series) Ω; χ_{c}, reactance (series) Ω; tan ϕ, phase angle; D, electrode distance mm.

Univariate regressions based on the canonical relationships were almost as strong as the canonical correlations but were simpler to formulate and easier to relate to the principles of impedance analysis. The strongest and most interesting relationships are presented here and the remainder can be found in Supporting Information Appendix SI (Tables SIII–SV). The strongest of the relationships among the dependent variables reflect body composition. While total lipid (O_{LT}) was not significantly correlated with any body size metric, lipid content ($O_{\mathrm{L}}\%$) and water content ($W\%$) were strongly negatively related: $O_{\mathrm{L}}\% = 147{\cdot}5 - 1{\cdot}81$ (S.E. $\pm$ 0·16) $W\%$ ($r^2 = 0{\cdot}82$, root mean square (RMS) $= 1{\cdot}7$, $P < 0{\cdot}001$). $\boldsymbol{C}_1$ reflected the influence of M_{T},

M_D, W_T and M_{LFD} on D^2R^{-1}, and the strongest linear regressions reflecting this component were: M_T (g) $= 2{\cdot}37 + 6{\cdot}24\ (\pm\ 0{\cdot}15)\ D^2R^{-1}$ ($r^2 = 0{\cdot}98$, RMS $= 2{\cdot}18$, $P < 0{\cdot}001$) and M_{LFD} (g) $= -0{\cdot}82 + 1{\cdot}18\ (\pm\ 0{\cdot}05)\ D^2R^{-1}$ ($r^2 = 0{\cdot}94$, RMS $=$ $0{\cdot}73$, $P < 0{\cdot}001$). Linear relationships between total body metrics based on resistance were slightly stronger than those based on reactance, and relationships based on series and parallel formulations were very similar. Strong univariate regressions that reflected $\boldsymbol{C}_2$ were also obtained; this component reflected the contribution of lipids to body tissues, and strong regressions linking O_L% and W% to χ_c (series) were obtained: $O_L\% = -16{\cdot}9 + 0{\cdot}16\ (\pm\ 0{\cdot}02)\ \chi_c$ ($r^2 = 0{\cdot}72$, RMS $= 2{\cdot}10$, $P < 0{\cdot}001$) and $W\% = 89{\cdot}9 - 0{\cdot}08\ (\pm\ 0{\cdot}01)\ \chi_c$ ($r^2 = 0{\cdot}72$, RMS $= 2{\cdot}10$, $P < 0{\cdot}001$). While O_L% was well predicted by χ_c, tan ϕ also loaded strongly to $\boldsymbol{C}_2$ and enhanced the relationship between O_L% and χ_c (series) to yield: $O_L\% = -7{\cdot}65 + 0{\cdot}17\ (\pm\ 0{\cdot}02)$ $\chi_c - 0{\cdot}71\ (\pm\ 0{\cdot}3)\ \phi$ ($r^2 = 0{\cdot}70$; RMS $= 1{\cdot}99$; $F_{2,29} = 44{\cdot}5$, $P < 0{\cdot}001$).

The corresponding relationships to parallel-transformed reactance (χ_{cp}) were much weaker than their series counterparts and are not given here (Supporting Information, Appendix SI). While resistance (R) was also a good predictor of these tissue composition metrics, relationships were not nearly as strong as those based on χ_c and are not shown here. None of the proportional body composition metrics were significantly related to tan ϕ. K was not strongly related to any single impedance measure; however, it was weakly related to both series and parallel-based estimates of tan ϕ, which is consistent with $\boldsymbol{C}_3$.

The best multiple regression to predict O_L% from morphological variables was: $O_L\% = -3{\cdot}65 - 0{\cdot}16\ (\pm\ 0{\cdot}03)\ M_T + 17{\cdot}8\ (\pm\ 6{\cdot}4)\ K$ ($r^2 = 0{\cdot}53$, RMS $= 2{\cdot}80$, $F_{2,29} = 16{\cdot}3$, $P < 0{\cdot}001$.

The best multiple regression to predict water content from morphological variables was: $W\% = 85{\cdot}3 + 0{\cdot}06\ (\pm\ 0{\cdot}02)\ M_T - 10{\cdot}3\ (\pm\ 3{\cdot}7)\ K$ ($r^2 = 0{\cdot}39$; RMS $= 1{\cdot}58$, $F_{2,29} = 9{\cdot}12$, $P < 0{\cdot}001$).

DISCUSSION

For several decades, it has been known that changes in hydration status of tissues and body composition are reflected in changes in resistance and capacitive reactance of tissues (Liedtke, 1997), and the development of safe, portable, rapid and standardized tissue impedance measurement techniques led to therapeutic applications during the 1980s (Kushner, 1992). With the advent of commercially available units, BIA was applied in animal studies on a wide range of taxa including mammals (Gales *et al*., 1994; Hilderbrand *et al*., 1998; Hundertmark & Schwartz, 2002; Barthelmess *et al*., 2006), aquatic reptiles (Ultsch, 1989) and fishes (Bosworth & Wolters, 2001; Cox & Hartman, 2005).

Three types of impedance measurements were commonly used, and all three have been applied to a fish, but only to a limited range of species: (1) volumetric measures, which provide a three-dimensional view of the current field (*e.g.* D^2R^{-1} or $D^2\ \chi_c^{-1}$), (2) cross-sectional measures which reflect impedance to current flux across the plane of the field (*e.g.* R or χ_c) and (3) phase angle measurements (ϕ), which measure the ratio of reactance to impedance (Liedtke, 1997). These are essentially the three types of measurements that dominate the independent variable loadings to the three main canonical components ($\boldsymbol{C}_X, \boldsymbol{C}_{2X}$ and $\boldsymbol{C}_{3X}$) of the GLM; therefore, it seems unlikely that these analyses have overlooked other combinations of variables with significant explanatory potential.

Previous studies have found very strong linear relationships between volumetric impedance quotients (especially D^2R^{-1}) and whole body measures including M_T, M_D, W_T, M_{LFD} and others (Cox & Hartman, 2005; Duncan *et al.,* 2007; Willis & Hobday, 2008; Hanson *et al.,* 2010). This is reasonable since such three-dimensional measures should strongly reflect the overall mass of tissue in the current field. This is the relationship reflected by the first canonical correlation in the present data set, and the linear regressions predicting M_T, M_D and M_{LFD} were all very similar to those of previous studies. No relationship between O_{LT}) and volumetric impedance, however, was found; while some previous studies have reported strong volumetric relationships to O_{LT} (Cox & Hartman, 2005), others have found little or no relationship (Duncan *et al.,* 2007). Volumetric impedance is most strongly related to M_T, but in the present study, O_{LT} and M_T were uncorrelated and $O_L\%$ was negatively correlated to M_T, which could explain the poor relationship observed. In this study, volumetric reactance always provided slightly weaker models than volumetric resistance, and series and parallel representations for both impedance measures were equally strong. Similar observations were reported by Duncan *et al.* (2007), and the volumetric reactance-based models of Pothoven *et al.* (2008) and Hanson *et al.* (2010) were of similar strength to those obtained in this study.

The strong relationship between volumetric impedance and body size was derived from fish ranging in L_F from 110 to 183 mm (13–76 g). The *S. fontinalis* studied by Cox & Hartman (2005) ranged somewhat more widely in size (110–285 mm), and they also found that the relationships of volumetric impedance to body size extended over a similar size range of other fishes (centrarchids, percids, clupeids and a sciaenid). The relationship for steelhead *Oncorhynchus mykiss* (Walbaum 1792) reported by Hanson *et al.* (2010) was based on fish ranging in L_F between 131 and 193 mm, and that of Bosworth & Wolters (2001) was derived from channel catfish *Ictalurus punctatus* (Rafinesque 1818) of a considerably larger size (440–891 g). Willis & Hobday (2008) found similarly strong relationships for large southern bluefin tuna *Thunnus maccoyii* (Castelnau 1872) ranging in L_F from 400 to 1100 mm. Thus, while volumetric BIA measures have been shown to correlate well to body size metrics of a wide range of species and sizes (*c.* 100–1000 mm L_F), no studies have yet examined very small fishes, such as fry, and this may be a potentially important area of application for BIA techniques since a great deal of laboratory experimentation is carried out on small fishes.

It would be expected that cross-sectional impedance measures should provide a reasonable reflection of average, as opposed to total, tissue conductive properties, and this is the type of relationship that the second canonical correlation in the GLM represents. Fewer studies have, however, taken this approach to BIA analysis and those that have (Bosworth & Wolters, 2001; Pothoven *et al.,* 2008) reported fairly weak relationships for both $O_L\%$ and $W\%$. In the present study, however, the univariate regressions derived from $\boldsymbol{C}_2$, relating $O_L\%$ (+) and $W\%$ (−) to reactance were fairly strong. The strong inverse relationship between $O_L\%$ and $W\%$ seen here has also been reported in many previous studies (Cunjak & Power, 1986; Peters *et al.*, 2007). Although O_{LT} was not related to body size, both O_{LT} and $O_L\%$ were weakly positively related to K; however, these relationships were too weak to support any consideration of K as an indicator of fish health or condition.

Most BIA studies on human subjects have advocated the use of parallel-transformed impedance measures (R_p and χ_{cp}), as opposed to their series-based analogues, to predict tissue composition (Liedtke, 1997). The present study, however, did not support this and found that both $O_L\%$ and $W\%$ were much more strongly related to χ_c than χ_{cp} (although as noted previously, parallel transformation had no effect on the volumetric impedance relationships). Pothoven *et al*. (2008) found that $O_L\%$ and $W\%$ were only weakly related to impedance metrics in yellow perch *Perca flavescens* (Mitchill 1814), lake whitefish *Coregonus clupeaformis* (Mitchill 1818) or walleye *Sander vitreus* (Mitchill, 1818) and provided predictions that were little better than relationships obtained using body size metrics alone. As such, they questioned the general utility of BIA measurements for predicting this type of tissue composition metric. It should be noted, however, that the only reactance measures tested by Pothoven *et al*. (2008) were the parallel-transformed formulations (R_p and X_{cp}) and that they never reported regressions using series-based values, which in the present study provided the only strong relationships to $O_L\%$ and $W\%$. The weak relationships between $O_L\%$ (and $W\%$) and χ_{cp} in the present study were consistent with the relationships obtained using reactance by Pothoven *et al*. (2008) and Bosworth &Wolters (2001).

Phase angle (tan ϕ) made only a minor contribution to the regressions in this study. It slightly strengthened the prediction of $O_L\%$ from χ_c and was also weakly but significantly correlated with K, as reflected in the third canonical correlation. The measurements made in this study were, however, not short-term acute stress studies of, for example, food deprivation, where the ratio of reactance to resistance would be expected to change greatly. In human studies, ϕ has been shown to decrease with the ratio of extracellular:intracellular water (Segal *et al*., 1987), which reflects malnutrition (Kushner, 1992). The BIA studies on humans have also shown that malnutrition is accompanied by changes in cellular membrane integrity and alterations in fluid balance; therefore, BIA readings can provide insight into nutritional status by indirectly measuring these changes (Barbosa-Silva *et al*., 2003). Recently, ϕ has been used as an indicator of fishes' condition, and declined as fishes were fasted under both laboratory and field conditions, indicating that ϕ may have promise as an indicator of acute nutritional stress (Cox & Heintz, 2009).

BIA is a convenient tool because it is a safe, inexpensive, rapid and portable means to determine proximate body composition (Kushner, 1992). Reactance provides a non-lethal index to assess the nutritional state of fishes that can be repeated over time to provide an accurate indication of proximate tissue composition variables and energetic condition. In an analysis of the effect of flow manipulation on overwintering *S. fontinalis*, reactance measured prior to, and at the end of, an *in situ* flow manipulation experiment showed that fish lost on average 21% of their body lipids over the course of the winter experiment; however, there was no difference in the condition of fish exposed to flow reduction pulses, or of reference fish which experienced normal flow (Krimmer *et al.,* 2011). More BIA studies, especially experimental studies are, however, needed on fishes to test the generality of existing relationships between impedance measures and body composition, across seasons, among life stages, among regions and among species and to evaluate their sensitivity to environmental stresses.

SUPPORTING INFORMATION

Supporting Information may be found in the online version of this paper:

Appendix SI. Additional statistical information and other relationships between body measures and impedance.

Table SI. Matrix of correlations showing the strongly intercorrelated nature of the data set. $*P < 0{\cdot}05$; $**P < 0.01$, $***P < 0.001$.

Table SII. Canonical loadings and correlations between body composition and bioelectrical impedance measurements obtained from multivariate analysis of the general linear model $\boldsymbol{Y} = \boldsymbol{a} + \boldsymbol{bX} + \boldsymbol{\varepsilon}$. $\boldsymbol{Y}$ variables were total mass (M_T), fork length (L_F), total body water (W_T) and total lipid (L_{IT}); $\boldsymbol{X}$ variables were BIA length (L_D), resistance (R), reactance (χ_c) and phase angle (tan ϕ).

Table SIII. Best linear relationships among body composition metrics, $n = 32$.

Table SIV. Best linear relationships between volumetric impedance and total body measures, $n = 32$.

Table SV. Best linear relationships between cross-sectional impedance and proportional body composition, $n = 32$. A comparison of the predictive power of series-based and parallel-based measures of reactance.

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