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Development of temperature correction equations for bioelectrical impedance analysis models for brook trout *Salvelinus fontinalis*

A. W. Hafs*† and K. J. Hartman‡

**Aquatic Biology Program, Department of Biology, Bemidji State University, Bemidji, MN 56601, U.S.A. and ‡Wildlife and Fisheries Resources Program, West Virginia University, Morgantown, WV 26506-6125, U.S.A.*

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The objective of this study was to establish the relationships between bioelectrical impedance analysis (BIA) measures (resistance and reactance) and temperature and to determine if corrections improve BIA models for brook trout *Salvelinus fontinalis* when used over a wide range of temperatures. Both resistance and reactance significantly decreased as temperature increased. Application of temperature corrections to BIA models attempting to predict per cent dry mass reduced root-mean-squared error by an average of 32%. Researchers taking BIA measures on fishes in the field where temperature varies will need to correct resistance and reactance to the temperature at which the BIA model was developed for successful predictions of per cent dry mass to be possible. This study presents a clear description of methods that can be used to developed temperature correction equations so that future researchers can use BIA in any field setting and increase the accuracy of BIA-based estimates of per cent dry mass.

Key words: BIA; condition; error; percent dry mass; proximate composition; salmonid.

INTRODUCTION

The recent success of multiple independent researchers using bioelectrical impedance analysis (BIA) to assess body condition and proximate composition of fishes has highlighted the potential of the method for use in fisheries research and management (Bosworth & Wolters, 2001; Cox & Hartman, 2005; Duncan *et al.*, 2007; Willis & Hobday, 2008; Fitzhugh *et al.*, 2010; Cox *et al.*, 2011; Hafs & Hartman, 2011; Hartman *et al.*, 2011; Krimmer *et al.*, 2011; Rasmussen *et al.*, 2012; Stolarski *et al.*, 2014). Although many of the past studies have demonstrated positive results when using BIA, some studies have reported mixed results indicating that more work is needed to improve the methods (Pothoven *et al.*, 2008; Hanson *et al.*, 2010; Mehner *et al.*, 2011; Caldarone *et al.*, 2012; Garner *et al.*, 2012). Once well-developed methods have been established, BIA will be an attractive tool for researchers and managers. The major benefits of BIA are that it is cost efficient and after models are developed the procedure is non-lethal (Cox & Hartman, 2005). Other major benefits are that the

†Author to whom correspondence should be addressed. Tel.: +1 218 755 2789; email: ahafs@bemidjistate.edu

equipment required is compact, light-weight and easy to use making it ideal for remote field locations.

The basic principle of BIA is that when a small electrical current (425 µA, 50 kHz) is passed through fish tissue the resistance and reactance values measured will be correlated to measures of proximate composition. Resistance is a measure of how well electricity can pass through a substance and since fat is an insulator resistance should be increased in fishes with more fat (Lukaski, 1987). Reactance measures the ability of a substance to hold a charge and because the lipid bilayer of cells serves as a capacitor reactance should be increased in healthy fatter fishes (Lukaski, 1987).

Temperature has been shown to have large influences on BIA measurements (Gudivaka *et al.*, 1996; Marchello *et al.*, 1999; Buono *et al.*, 2004; Cox *et al.*, 2011; Hartman *et al.*, 2011; Stolarski *et al.*, 2014), but few of the models relating BIA measures to fish proximate composition have accounted for temperature. Previous models for fishes that have provided sound predictions of proximate composition have either held the animals at a constant temperature (26° C, Bosworth & Wolters, 2001; 27° C, Duncan *et al.*, 2007; 20° C, Hafs & Hartman, 2011), applied a temperature correction (Stolarski *et al.*, 2014) or sampled within a relatively narrow range of temperatures (Cox & Hartman, 2005). Slanger & Marchello (1994) measured resistance and reactance on several cuts of meat from beef cows *Bos taurus* over a wide range of temperatures (*c.* 0–16° C). They concluded that impedance measures were negatively related to temperature and that it was an important parameter in many of their models. Cox *et al.* (2011) concluded that temperature was a significant source of error in BIA models. Hartman *et al.* (2011) took BIA measurements on coastal bluefish *Pomatomus saltatrix* (L. 1766) at 15 and 27° C and determined that temperature had a significant effect on both resistance and reactance. Furthermore, they concluded that as temperature increased from 15 to 27° C, average resistance declined by 35·8 and 20·4% at what they called the dorsal and ventral locations. In their study, reactance measures also declined as temperature increased at rates of 12·7 and 12·9% for the dorsal and ventral locations. Temperature correction equations have been developed for dolly varden *Salvelinus malma* (Walbaum 1792) by Stolarski *et al.* (2014). Additional correction equations are still needed for the electrode locations that have been shown to produce optimal results for other salmonids (Hafs & Hartman, 2011). Furthermore, comparison between independent studies are needed to determine if temperature correction equations are comparable across species, electrode locations on the fishes in which the measurements are taken and for different sizes of electrodes typically used on salmonids.

Past research suggests that if BIA is used to estimate the per cent dry mass, condition or energy density of fishes under circumstances where temperature cannot be controlled, temperature will have to be accounted for in the BIA models. Because of the potential influence of temperature on BIA, a clear method to develop temperature corrections is needed. The objective of this study was to establish the relationships between BIA measures (resistance and reactance) and temperature taken on age 0 year and adult brook trout *Salvelinus fontinalis* (Mitchill 1814) and to determine if correction equations improve BIA models when used over a wide range of temperatures. Developing temperature corrections for BIA models should provide more accurate estimates of per cent dry mass when used in the field over the range of temperatures normally present in Appalachian streams containing *S. fontinalis*.

MATERIALS AND METHODS

A total of 270 *S. fontinalis* were collected from Bowden State Fish Hatchery, Bowden, WV, U.S.A. and were transported back to the West Virginia University Ecophysiology Laboratory, Morgantown, WV. Fish were maintained in recirculating tanks (0·5 m × 1·5 m) at 14° C, range ± 1° C and grown to one of the six sizes (50, 75, 100, 150, 225 and 300 mm total length, L_T; 45 fish per size). Once fish reached the selected size, they were randomly selected for BIA during a fasting period of either 2 (50 mm) or 7 (75 and 100 mm) weeks for age 0 year fish, and 4 (150 mm), 5 (225 mm) or 6 months (300 mm) for adults to ensure that fish were sampled from a wide range of both L_T and condition. All fish were acclimated to the recirculating system for at least 2 weeks before any BIA measurement was done.

BIOELECTRICAL IMPEDANCE ANALYSIS

Each fish had resistance and reactance measured at three different temperatures (5·0, 12·5 and 20·0° C) covering the range of water temperatures that would normally be present for field measurements of BIA with *S. fontinalis*. When a fish was selected for BIA it was acclimated to 20° C for at least 12 h prior to testing. Fish were anaesthetized using MS-222, blotted dry with paper towelling, then placed on a non-conductive surface with the head facing left and both resistance and reactance (Quantum II bioelectrical body composition analyser, RJL Systems; www.rjlsystems.com) as well as the distance between the inner two needles or rods were measured (mm). Previous research has used both external rod electrodes and subdermal needle electrodes (Hafs & Hartman, 2011); therefore, measurements were taken using both subdermal needle and external rod electrodes on each fish. Each electrode was composed of one signal and one detector needle or rod and for this study signal needles or rods were always keep towards the head of the fish. Because the distance between the inner needles or rods was measured, 5 mm was added to measured detector length for age 0 year fish and 10 mm for adults for only measurements taken laterally on the fish. This was done so that the detector length (D_L) was equal to the distance between detector needles or rods and not the inner needles or rods. Wet mass (M_W; g), L_T (mm) and fork lengths (L_F, mm) were measured after BIA was done at 20° C. Adult fish had their core temperatures measured before and after BIA measurements were taken by inserting an electronic meat thermometer down the oesophagus into the stomach.

For both age 0 year and adult fish, BIA measurements were taken at two locations. For age 0 year fish, BIA measurements were taken at the dorsal total length (L_{TD}) location as well as the dorsal to ventral pre-dorsal fin location (L_{DV}) following the recommendations of Hafs (2011). To take BIA measurements at the L_{TD} location, one electrode was positioned with the needles or rods oriented parallel to the lateral line midway between the lateral line and the dorsal midline directly above where the lateral line intersects the operculum. The other electrode was positioned with the needles or rods oriented parallel to the lateral line midway between the lateral line and the dorsal midline directly below the adipose-fin. For the L_{DV} location, one electrode was positioned along the dorsal midline directly anterior to the dorsal-fin and the other electrode was positioned along the ventral midline directly under the other electrode. For adult fish, BIA measurements were taken at the dorsal midline (L_{DM}) location and at the L_{DV} location as recommended by Hafs & Hartman (2011). For the L_{DM} location, both electrodes were positioned along the dorsal midline of the fish with one electrode directly posterior to the head and the other directly anterior to the adipose-fin. For age 0 year fish, both subdermal needle and external rod electrodes were built following the specifications of Hafs (2011). For adult fish, Model FE24 subdermal needle electrodes were used (The Electrode Store; www.electrodestore.com) and rod electrodes were built following the specifications of Hafs & Hartman (2011).

Once the measurements were taken at both locations with both electrode types, the fish were placed into 12° C water to recuperate. The adult fish were acclimated for 12 h and age 0 year fish for 6 h to allow the core temperature to reach equilibrium with the water and then the BIA measurements were repeated. This same process was then done for 5° C water. Once resistance and reactance had been measured at all three temperatures, the fish were euthanized in an overdose of MS-222, oven-dried at 80° C to a constant mass and per cent dry mass was calculated: $\%M_D = 100 M_D M_{W-1}$, where M_D is dry weight.

DATA ANALYSIS

Generalized linear mixed-effect models within the R software package LME (R Development Core Team; www.r-project.org) were used to establish relationships between BIA measurements (resistance and reactance) and temperature for all electrode types and locations. Within each model, individual fish were treated as random effects and water temperature was a fixed effect. The slopes from the model equations were then used to correct all BIA measurements to 12·5° C, the temperature treatment closest to the average water temperature present in Appalachian mountain streams containing *S. fontinalis* (11·5° C; A. W. Hafs, unpubl. data). Using the regression procedures from Hafs & Hartman (2011), BIA models were developed for age 0 year and adult fish separately, using only 12·5° C data. Per cent M_D was predicted using both temperature corrected and uncorrected BIA measurements at all three temperatures. Predicted $\%M_D$ was then compared with actual $\%M_D$ values and root-mean-square error (RMSE) and r^2 was used to assess the ability of temperature corrections to improve BIA models.

In addition to developing temperature correction equations for BIA measurements, taking measurements of adult fish body temperatures allowed the inclusion of temperature as a parameter in the regression models as an alternative to temperature correction equations. Therefore, 45 adult fish were randomly selected from each temperature treatment and used to develop regression models following the methods of Hafs & Hartman (2011). The remaining 90 fish were then used to calculate r^2 and RMSE values as a way to validate the temperature inclusive BIA model.

RESULTS

Age 0 year fish ranging from 50 to 110 mm L_T and 15·24 to 21·88% M_D ($n = 135$), and adults ranging from 145 to 320 mm L_T and 15·03 to 30·95% M_D ($n = 135$) were sampled to develop the temperature corrections. Because each fish had BIA measurements taken at all three temperatures, 405 BIA measurements were used to develop each temperature correction. Generalized linear mixed-effect model results indicated that BIA measures (resistance and reactance) decreased as temperature increased (Figs 1 and 2). This pattern held true independent of electrode type, location of measurements, or whether adult (Fig. 1) or age 0 year (Fig. 2) *S. fontinalis* were sampled (Table I). The 95% C.I. of the slopes, however, did not overlap in several instances suggesting that different correction equations would be needed for age 0 year and adults or rods and needles (Table I).

Application of temperature corrections clearly improved the ability of all BIA models to predict $\%M_D$ (Table II) and centred residuals on zero (Fig. 3). Prior to the application of temperature corrections, residuals from all four models (age 0 year needles and rods, adults needles and rods) were clearly influenced by temperature and were not centred on zero for predictions based on data collected at 5 and 20° C (Fig. 3). On average across all four models, temperature corrections reduced RMSE by 37 and 27% for predictions made using data collected at 5 and 20° C (Fig. 4). The BIA models used to make predictions of $\%M_D$ were developed using the data collected at 12·5° C (Table III); therefore, no changes occurred to estimates of $\%M_D$ at that temperature.

Changes in r^2 estimates due to application of temperature corrections were not as clear as RMSE results. On average across all four models, RMSE estimates for temperature corrected models were lower for age 0 year (0·78) than adult (0·92) fish; however, r^2 values for temperature corrected models were lower for age 0 year (0·48) than for adult models (0·91). Applying temperature corrections did result in improved r^2 values in most circumstances (Table II). For age 0 year models at 5° C, however, r^2 values decreased by an average of 0·125.

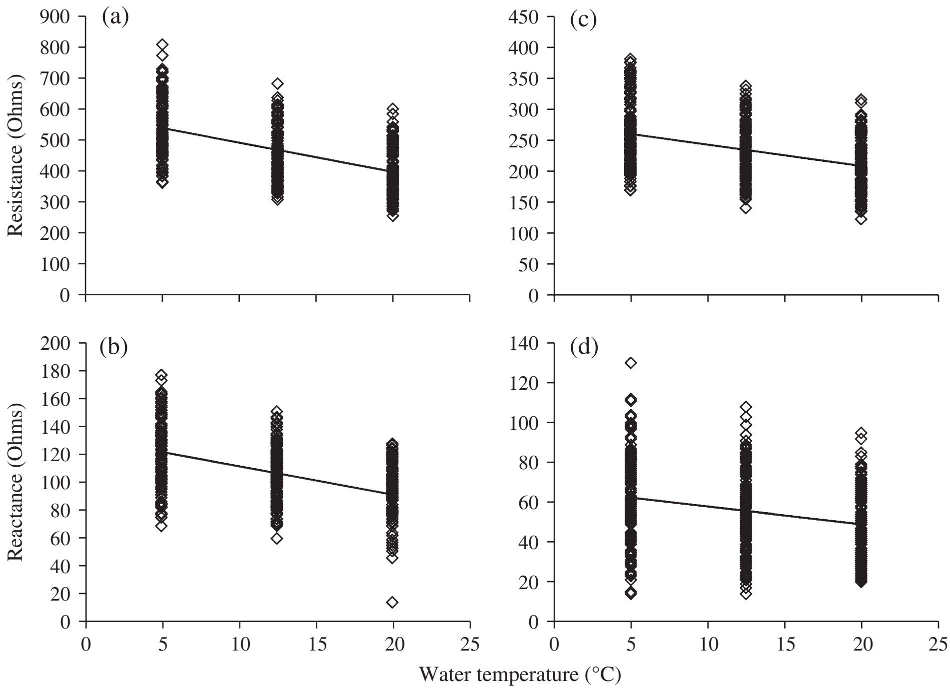

FIG. 1. Trends in measured (a, c) resistance and (b, d) reactance across a range of temperatures for adult *Salvelinus fontinalis* using subdermal needle electrodes at the (a, b) dorsal midline (L_{DM}) location and (c, d) dorsal to ventral pre-dorsal fin (L_{DV}) location. Similar results occurred when using external rod electrodes. The curves were fitted by: (a) $y = -9{\cdot}4040x + 589{\cdot}02$, (b) $y = -2{\cdot}036x + 132{\cdot}04$, (c) $y = -3{\cdot}446x + 277{\cdot}44$ and (d) $y = -0{\cdot}885x + 66{\cdot}751$. Regression coefficients for all temperature corrections are provided in Table I.

Models including BIA measurements were better supported and provided better predictions of $\%M_D$ than models that included only morphometric measurements (Table IV). Lastly, the 12·5° C BIA models developed from this research, when used in combination with temperature correction equations, were better supported than BIA models that included temperature as a parameter. The 12·5° C models had fewer parameters resulting in lower second-order Akaike's information criterion (AIC_c) scores and less complicated models than those where temperature was included as a regression parameter (Table IV).

DISCUSSION

The correction equations provided in this paper improved the ability of all four BIA models to predict $\%M_D$ of *S. fontinalis*. These results and those of previous literature (Gudivaka *et al.*, 1996; Marchello *et al.*, 1999; Buono *et al.*, 2004; Cox *et al.*, 2011; Hartman *et al.*, 2011; Stolarski *et al.*, 2014) clearly demonstrate that temperature has an influence on BIA measures and that temperature corrections are needed. The temperature corrections provided by this research were able to reduce the effect of temperature decreasing RMSE by as much as 56·9%. On average, RMSE was reduced by 36·7 and

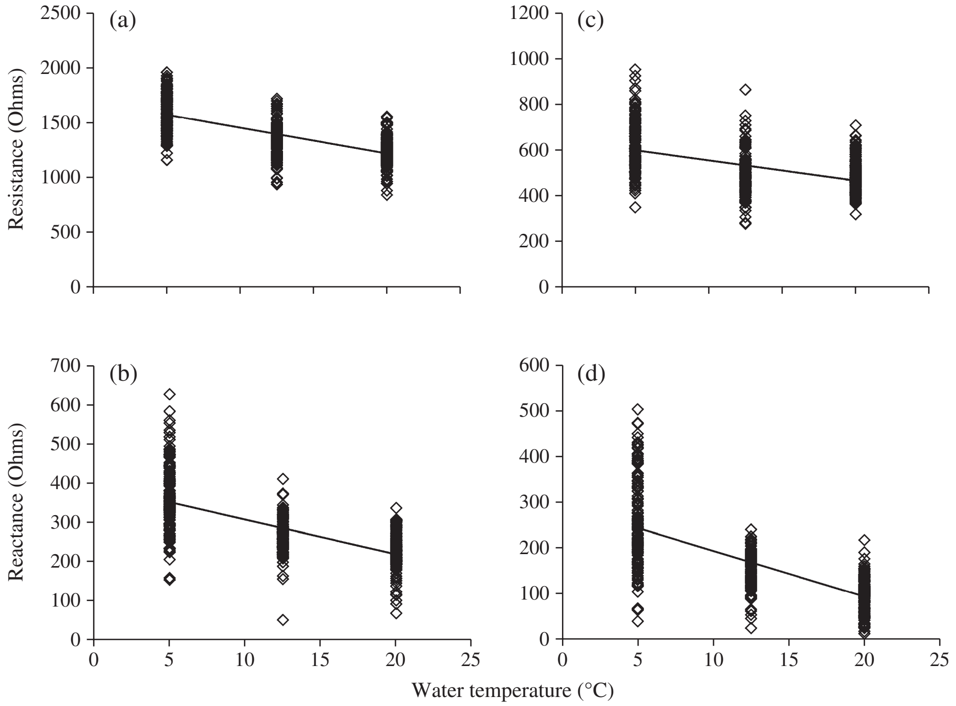

FIG. 2. Trends in measured (a, c) resistance and (b, d) reactance across a range of temperatures for age 0 year *Salvelinus fontinalis* using subdermal needle electrodes at the (a, b) dorsal total length (L_{TD}) location and (c, d) dorsal to ventral pre-dorsal fin (L_{DV}) location. Similar results occurred when using external rod electrodes. The curves were fitted by: (a) $y = -23{\cdot}588x + 1693{\cdot}2$, (b) $y = -8{\cdot}8444x + 394{\cdot}28$, (c) $y = -8{\cdot}8232x + 643{\cdot}44$ and (d) $y = -10{\cdot}018x + 295{\cdot}51$. Regression coefficients for all temperature corrections are provided in Table I.

27·3% when 5 and 20° C data, respectively, were corrected to 12·5° C (the temperature for BIA model development). The r^2 estimates for adult fish 12·5° C models developed during this study were all >0·87, slightly better than the r^2 (0·82) estimate of the best models developed by Hafs & Hartman (2011). For age 0 year fish, the r^2 estimates for both uncorrected and corrected models were lower than those reported in the study of Hafs (2011). Hafs (2011) reported r^2 estimates of 0·86 and 0·85 for subdermal needle electrodes and external rod electrodes, respectively. In this study, the best r^2 achieved for age 0 year fish was 0·66 using external rod electrodes. One reason for r^2 estimates of age 0 year fish to be lower than those reported by Hafs (2011) is that the range of $\%M_D$ for fish in this study was much narrower (15·24–21·88, this study; 15·09–26·08, Hafs, 2011). Evidence for this conclusion is provided by the RMSE estimates from this study which are lower than those provided by Hafs (2011). In this study, when temperature corrections were applied, the average RMSE across all temperatures and electrode types for age 0 year fish was 0·78. This is lower than the RMSE (1·03) for the best model from Hafs (2011). The substantial decreases in RMSE that occurred when corrected for temperature is encouraging and when future researchers use BIA models at temperatures outside the range they were developed, temperature correction equations should be used.

TABLE I. Temperature correctiaon equations along with upper (UCL) and lower (LCL) 95% C.L. Equations are provided for both resistance (r) and reactance (x) at both dorsal total length (L_{TD}) and dorsal to ventral (L_{DV}) locations for age 0 year *Salvelinus fontinalis* and for the dorsal midline (L_{DM}) and L_{DV} locations for adult fish. Correction equations are also provided for both subdermal needle and external rod electrodes. In the temperature correction equation provided, T = the temperature at which bioelectrical impedance analysis (BIA) measures were taken at and T_c = the temperature being corrected to (12·5° C in this study). The slopes within the correction equations all had P-values <0·001 based on generalized linear mixed-effect models

		Needles		Rods	
Model	Measurement	Equation	UCL:LCL	Equation	UCL:LCL
Age 0 year	L_{TD}r	$-23·5881\ (T_c - T) + r$	−22·08:−25·09	$-24·8632\ (T_c - T) + r$	−23·44:−26·29
	L_{TD}x	$-8·8444\ (T_c - T) + x$	−8·14:−9·56	$-8·7328\ (T_c - T) + x$	−8·02:−9·45
	L_{DV}r	$-8·8232\ (T_c - T) + r$	−7·47:−10·17	$-12·3012\ (T_c - T) + r$	−11·15:−13·45
	L_{DV}x	$-10·0183\ (T_c - T) + x$	−9·10:−10·94	$-10·3659\ (T_c - T) + x$	−9·51:−11·22
Adult	L_{DM}r	$-9·4040\ (T_c - T) + r$	−9·09:−9·72	$-9·3802\ (T_c - T) + r$	−8·95:−9·82
	L_{DM}x	$-2·0361\ (T_c - T) + x$	−1·89:−2·18	$-2·2484\ (T_c - T) + x$	−2·11:−2·38
	L_{DV}r	$-3·4464\ (T_c - T) + r$	−3·18:−3·72	$-3·3057\ (T_c - T) + r$	−2·97:−3·64
	L_{DV}x	$-0·8849\ (T_c - T) + x$	−0·68:−1·09	$-2·0677\ (T_c - T) + x$	−1·96:−2·18

The new 12·5° C BIA models developed in this study contained fewer parameters than those models previously developed by Hafs (2011) and Hafs & Hartman (2011). This probably occurred because in this study only four BIA measurements were taken on each fish at the temperature used to develop the models. In the Hafs & Hartman (2011) study, 21 measurements were taken on each fish. As handling time and contact with the researcher increases during BIA measurements, errors resulting from temperature changes are likely to occur. Therefore, more parameters are needed in the models to explain a similar amount of variation. This suggests that as BIA methods are improved, by studies such as this one, the models will be able to make more accurate predictions with less complicated models. The simplicity of the new 12·5° C models presented in this paper will be an attractive feature for researchers looking to minimize the use of the technical electrical parameters (Table V) often used in BIA models. Although the same fish were used to develop the 12° C models and the temperature corrections in this study, future researchers looking to minimize the number of parameters in BIA models should take measurements at as few locations and with as few electrode types as possible to minimize processing time. This also means that researchers should consider using different fish to develop the BIA model than are used to develop the temperature corrections.

The sampling design used in this study did potentially introduce some sources of error into the models. For example, because each fish was sampled at three different temperatures there could be a possible stress-related response to the experimental protocol. Wuenschel *et al.* (2013) performed repeated BIA measures at monthly intervals on black sea bass *Centropristis striata* (L. 1758) and reported that only three of the 51 fish died but it was impossible to determine if those deaths were attributed to stress from BIA or other sources. Garner *et al.* (2012) reported that only slight bruising occurred occasionally and infections did not occur during repeated BIA measures conducted on Atlantic croaker *Micropogonias undulatus* (L. 1766). These previous studies were

TABLE II. Results from the application of temperature corrections to *Salvelinus fontinalis* bioelectrical impedance analysis (BIA) models. Root-mean-square error (RMSE), r^2 and mean estimated percent dry mass (%M_D) are provided for both corrected and uncorrected BIA models as well as for the two regression models for adult fish that include temperature as a parameter. Per cent improvement (Improv.) in RMSE resulting from temperature corrections is also provided. Actual mean %M_D was 22·86 and 18·74 for adult and age 0 year fish, respectively

			RMSE			r^2		%M_D	
Model	Temperature (° C)	Electrode	Uncorrected	Corrected	Improv.	Uncorrected	Corrected	Uncorrected	Corrected
Age 0 year	5·0	Needles	1·23	0·97	21·14	0·39	0·26	19·82	19·12
	12·5	Needles	0·63	0·63	0·00	0·61	0·61	18·74	18·74
	20·0	Needles	0·75	0·72	4·00	0·58	0·61	18·29	19·17
	5·0	Rods	1·18	0·98	16·95	0·33	0·21	19·79	18·93
	12·5	Rods	0·58	0·58	0·00	0·66	0·66	18·75	18·75
	20·0	Rods	0·82	0·77	6·10	0·46	0·53	18·18	19·14
Adult	5·0	Needles	1·93	0·93	51·81	0·88	0·90	24·59	22·83
	12·5	Needles	0·81	0·81	0·00	0·92	0·92	22·86	22·86
	20·0	Needles	1·93	0·96	50·26	0·83	0·91	21·02	23·20
	5·0	Rods	2·32	1·00	56·90	0·88	0·87	25·06	22·97
	12·5	Rods	0·76	0·76	0·00	0·93	0·93	22·86	22·86
	20·0	Rods	2·02	1·03	49·01	0·78	0·91	20·94	23·42

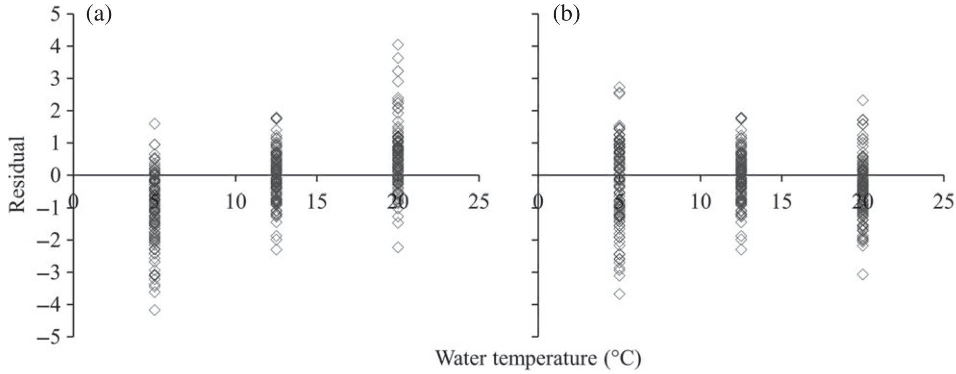

FIG. 3. The change in residuals resulting from the application of temperature corrections to the age 0 year *Salvelinus fontinalis* external rod electrode bioelectrical impedance analysis (BIA) model. (a) Uncorrected residuals that become less centred on zero as temperature deviates from 12·5° C and (b) residuals from the temperature corrected BIA model which are centred around zero at all temperatures. These results are representative of the influence that temperature corrections had on residuals, independent of age (age 0 year or adult) or electrode type (subdermal needle or external rod).

mainly interested in monitoring survival or outward appearance and since the effect of repeated BIA measures and stress-related physiological responses are factors that have not been researched previously, potential influence of these factors are unclear. The most likely influence is that stress over time from BIA sampling would cause plasma chloride levels to decrease (Davis & Parker, 1983). Reduced chloride in the extracellular fluid should cause resistance values to increase. Because higher temperatures were sampled first in this study and resistance decreases as temperature increases, it is possible that the temperature correction slopes are slightly farther from zero due to the influence of decreased plasma chloride levels. A comparison between the correction

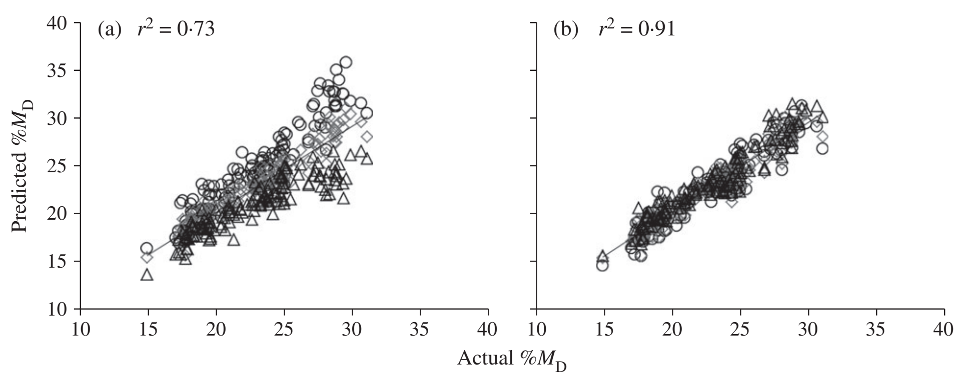

FIG. 4. Predicted per cent dry mass ($\%M_D$; 12·5° C adult model with needles) plotted against actual measured values. (a) Data not corrected for temperature. (b) The improvement that occurs when resistance and reactance values are corrected. All 135 *Salvelinus fontinalis* had measurements taken at 5·0 (○), 12·5 (◇) and 20° C (△). r^2 values reported here were calculated using the data from all three temperatures. These results are representative of the influence that temperature corrections had on residuals, independent of age (age 0 year or adult) or electrode type (subdermal needle or external rod).

TABLE III. Regression coefficients for the 12·5° C bioelectrical impedance analysis (BIA) models developed in this study that predict per cent dry mass of age 0 year or adult *Salvelinus fontinalis*. Models were developed for both subdermal needle and external rod electrodes. The parameter column tells which location's resistance and reactance measurements should be used when calculating the electrical parameter in parenthesis (see Tables I and V)

	Adults		Age 0 year	
Parameter (*)	Needles L_{DM} L_{DV}	Rods L_{DM} L_{DV}	Needles L_{TD} L_{DV}	Rods L_{TD} L_{DV}
Intercept	17·3855	26·6039	16·2400	14·6193
L_F	−0·0794	−0·1000		0·0888
M_W	0·0558	0·0663	0·6281	
L_{TD} (r)			−0·0033	−0·0019
L_{TD} (X_c)				36·0957
L_{TD} (X_{cp})			−22·9200	
L_{TD} (R_p)				7·7824
L_{TD} (Z_p)				−37·1532
L_{TD} ($D_L A_P$)			0·0153	
L_{DM} (R_s)		−6·4609		
L_{DM} (R_p)	7·7144			
L_{DM} (Z_s)	−7·7714	6·4777		
L_{DM} ($D_L A_P$)	0·0126	0·0108		
L_{DV} (r)	0·0268		0·0048	
L_{DV} (x)		0·0654		0·0165
L_{DV} (R_p)		−0·4466		
L_{DV} (A_P)		−0·3584	0·0602	
L_{DV} ($D_L A_P$)		0·0057		
Residual			0·7886	0·6473

L_{DM}, dorsal midline location; L_{DV}, dorso-ventral location; L_{TD}, total dorsal length location;L_F, fork length; M_W, wet mass.
*see Table V for symbols and units.

equations developed using the methods from this study and those developed using an alternative sampling technique that tests different fish, of similar $\%M_D$, at multiple temperatures may be warranted.

One other possible source of error is the effect of changing M_W over time. Adult and age 0 year fish had BIA measures taken over the course of 24 and 12 h, respectively. During these time periods, it is likely that some changes in the M_W of fish occurred by either uptake or release of water. This could have influenced RMSE estimates, especially for age 0 year fish with higher metabolism and surface area:volume ratios. For this study, an acclimation time was selected that would be long enough to allow body temperatures to stabilize and chloride levels to increase while being short enough to prevent changes in M_W. Age 0 year RMSE estimates were similar to or better than those reported in Hafs (2011) indicating that the effect of time was effectively minimized.

Hartman *et al.* (2011) took BIA measures at the same location (L_{TD}) on *P. saltatrix* as was done in this study for age 0 year fish and therefore some valuable insight can be gained about whether or not BIA temperature corrections are similar across species.

TABLE IV. r^2, root-mean-square error (RMSE), number of parameters and second-order Akaike's information criterion (AIC_c) estimates for the adult *Salvelinus fontinalis* bioelectrical impedance analysis (BIA) models developed in this study. The morphometric model was developed using only total length, fork length and wet mass. The temperature inclusive models contained the temperature of the fish as a parameter in the model instead of using the temperature corrections provided in Table I to adjust resistance and reactance

Model	Needles or rods	r^2	RMSE	Number of parameters	AIC_c
12·5° C	Needles	0·92	1·08	7	35·53
12·5° C	Rods	0·92	1·05	10	33·94
Morphometric	NA	0·54	6·46	3	258·04
Temperature inclusive	Needles	0·89	1·25	9	80·59
Temperature inclusive	Rods	0·91	1·13	12	59·24

NA, not applicable.

TABLE V. A list of parameters that are included in the bioelectrical impedance analysis (BIA) models used in this study. For the residual from total length (L_T) and wet mass (M_W) equations, the equations are given, and the residuals are calculated by subtracting the predicted from the observed M

Parameter	Symbol	Units	Calculation
Resistance	r	Ohms	Measured by Quantum II
Reactance	x	Ohms	Measured by Quantum II
Resistance in series	R_s	Ohms	$D_L{}^2$ r^{-1}
Reactance in series	X_c	Ohms	$D_L{}^2$ x^{-1}
Resistance in parallel	R_p	Ohms	$D_L{}^2$ [r + (x^2 r^{-1})]$^{-1}$
Reactance in parallel	X_{cp}	Ohms	$D_L{}^2$ [x + (r^2 x^{-1})]$^{-1}$
Impedance in series	Z_s	Ohms	$D_L{}^2$ (r^2 + x^2)$^{-0·5}$
Impedance in parallel	Z_p	Ohms	$D_L{}^2$ [r·x (r^2 + x^2)$^{-0·5}$]$^{-1}$
Phase angle	A_P	Degrees	atan(x r^{-1})·180π^{-1}
Standardized phase angle	$D_L A_P$	Degrees	D_L [atan (x r^{-1})·180 π^{-1}]
Residual from L_T and M_W equation	Resid.	N/A	$M_W = 0·0000072\ L_T{}^{3·0056}$

D_L = detector length

Hartman *et al.* (2011) determined that resistance measured on *P. saltatrix* decreased by 35·8% as temperature increased from 15 to 27° C. In this study, resistance decreased by an average of 22·1% for age 0 year *S. fontinalis* over a 15° C temperature increase, even though measurements were taken at the same location as in Hartman *et al.* (2011). For reactance, Hartman *et al.* (2011) reported a 12·7% decrease as temperature increased by 12° C, while in this study reactance decreased by 36·9% with a 15° C increase in temperature. Stolarski *et al.* (2014) developed temperature correction equations for BIA measurements; however, they used different locations and different size subdermal needle electrodes than this study. Although the slopes of the correction equations from Stolarski *et al.* (2014) were similar for both the L_{TD} and the ventral locations, in this study, the correction factors were different between the two locations used (L_{DM} and L_{DV} for adults and L_{TD} and L_{DV} in age 0 year fish). It appears that it may be possible to develop one correction equation for all readings that are oriented parallel to the fish, such as the L_{DM} location used in this study and the L_{TD} used in Stolarski *et al.* (2014); however, the correction equations for the L_{DV} location used in this study are vastly different. Comparisons between this study and Stolarski *et al.* (2014) provide evidence to suggest that BIA temperature relationships are at a minimum orientation specific and it is likely that equations developed for specific locations would be best. It is still too early to tell if correction equations are species or possibly family specific and more research in this area is warranted; however, the similarities in the correction equations between this study and those from Stolarski *et al.* (2014), at least for those locations that oriented along a similar plane in reference to the fish, provide reason for optimism.

Previous research has demonstrated that BIA measures are influenced by temperature (Gudivaka *et al.*, 1996; Marchello *et al.*, 1999; Buono *et al.*, 2004; Cox *et al.*, 2011; Hartman *et al.*, 2011; Stolarski *et al.*, 2014), but the development of correction equations, such as the one from this research and those from Stolarski *et al.* (2014), will help BIA produce more reliable estimates, and will allow use over a much wider range of field conditions. The detailed description of the methods provided by this study is a major first step in eliminating a source of error that has affected fisheries BIA researchers for the past decade. In conclusion, the BIA temperature correction equations from this study decreased RMSE estimates substantially and were very easy to administer. The next logical step in BIA *S. fontinalis* research is an in-depth field validation study that uses the temperature corrections and BIA models provided in this study and the BIA methods from previous literature to determine if laboratory results hold up in more variable field conditions.

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