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# Developing bioelectrical impedance analysis methods for age-0 brook trout

A. W. HAFS & K. J. HARTMAN

*Wildlife and Fisheries Resources Program, West Virginia University, Morgantown, WV, USA*

**Abstract** Year class strength of many fishes often is determined by survival through the first winter. Increased fat reserves improve survival and overall cohort success. Bioelectrical impedance analysis (BIA) methods are established for estimating proximate composition of adult brook trout, *Salvelinus fontinalis* (Mitchill), but none have been developed for early life stages. Small-fish BIA would provide information about age-0 percent dry weight, a proxy for fat reserves, allowing for better prediction of cohort success. The objective of this study was to develop BIA methods that provide reliable estimates of percent dry weight for age-0 brook trout. BIA measurements were taken at seven anatomical locations from 48 to 115 mm fish. A model developed using BIA measures taken by subdermal needle electrodes precisely predicted percent dry weight (best model, RMSE = 1.03, $R^2$ = 0.86). Although lacking support, as determined by information theoretical analysis, BIA measured with non-invasive external rod electrodes also precisely predicted percent dry weight (RMSE = 1.09, $R^2$ = 0.85). Models developed using two electrode locations performed better than models developed with only one location. For small brook trout, a dorsal to ventral pre-dorsal fin electrode location should be used in conjunction with a dorsal total length location when measuring BIA to predict percent dry weight.

KEYWORDS: age-0, BIA, brook trout, condition, percent dry weight, *Salvelinus fontinalis*.

## Introduction

For many fishes, cohort strength is often determined by age-0 survival through winter (Hubbs & Trautman 1935; Garvey *et al.* 2004). Fisheries researchers often are interested in estimating specific proximate composition of fish, particularly fat content, because increased fat reserves improve the probability of winter survival for small fish (Thompson *et al.* 1991; Miranda & Hubbard 1994; Sogard & Olla 2000; Biro *et al.* 2004; Finstad *et al.* 2004). The ability to estimate fat content will allow researchers to provide reliable estimates of cohort survival.

Bioelectrical impedance analysis (BIA) has been found to be a low-cost, non-lethal method of estimating proximate composition of humans (Lukaski *et al.* 1985; Kushner & Schoeller 1986), other animals (Berg *et al.* 1996; Hwang *et al.* 2005; Pitt *et al.* 2006) and, recently, fish (Hanson *et al.* 2010; Krimmer *et al.* 2011; Garner *et al.* 2012; Rasmussen *et al.* 2012). The basic methods for conducting BIA on fish are relatively simple. A microampere electrical current (425 μA, 50 kHz) is passed through fish tissue and the resistance and reactance values are measured, typically using a Quantum II bioelectrical body composition analyser (RJL Systems, Clinton Township, MI, USA). The concept is that resistance and reactance values or other electrical parameters calculated from resistance and reactance, will be related to measures of proximate composition. This occurs because resistance is a measure of how well electricity can pass through a substance; as fat is an insulator (Lukaski 1987), resistance should be increased in fish with more fat. Reactance measures the ability of a substance to hold a charge; because the lipid bilayer of cells acts as a capacitor (Lukaski 1987), reactance should increase in healthy fish. To establish relationships between BIA measurements and proximate composition, fish are sacrificed to measure proximate composition and regression procedures are used to develop models that predict proximate composition values from the suite of electrical parameters and measured variables. The BIA models can then be used to estimate proximate composition without sacrificing additional fish.

Previous BIA with fishes has focused on specimens larger than 110 mm leaving the utility of BIA for assessing the proximate composition of small fish unresolved

Correspondence: A. W. Hafs, Aquatic Biology Program, Department of Biology, Bemidji State University, Bemidji, MN 56601, USA. (e-mail: ahafs@bemidjistate.edu)

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(Cox & Hartman 2005; Pothoven *et al.* 2008; Caldarone *et al.* 2012). Predictive models have been developed that describe relationships between fat content and percent dry weight ($R^2 = 0.81$; Hartman & Margraf 2008). Therefore, if BIA can be used to provide reliable estimates of percent dry weight, fat content can be estimated without expensive laboratory analysis of proximate composition.

Cox and Hartman (2005) developed BIA models that predicted proximate composition for adult brook trout, *Salvelinus fontinalis* (Mitchill). Cox and Hartman (2005) used two electrodes, each consisting of two needles spaced 10 mm apart that penetrated to a depth of 3 mm. Hafs and Hartman (2011) found that percent dry weight of adult brook trout could be predicted with greater precision by obtaining BIA measurements from two anatomically perpendicular axes rather than one as used by Cox and Hartman (2005). Previous researchers have provided a good foundation for the use of BIA on small specimens, but the penetration depth of the electrodes needs to be reduced to avoid injuring the spine or internal organs, specifically when electrodes are placed in the caudal or ventral areas of small fish. Bioelectrical impedance analysis methods need to be tested with electrode dimensions suitable for use with fish that are thinner and shorter than the adult brook trout used in previous studies. Furthermore, electrode locations need to be tested to determine if the findings of Hafs and Hartman (2011) are similar for smaller fish. Finally, altering the dimensions of the electrode is likely to have substantial influence on the BIA measurements suggesting new models will have to be developed that can predict percent dry weight of small fish. Therefore, the objective of this study was to develop a BIA method for age-0 brook trout that provides reliable predictions of percent dry weight.

## Methods

Age-0 brook trout [~50 mm total length (TL)] were obtained from Bowden State Fish Hatchery, Bowden, West Virginia, USA and transported to the West Virginia University Ecophysiology Laboratory where fish were maintained in 284 L recirculated tanks ($0.5 \times 0.5 \times 1.5$ m, flow rate ~11 L $min^{-1}$) at $14 \pm 1$ °C. Bioelectrical impedance analysis methods previously had been developed for age-1 and older brook trout (Cox & Hartman 2005; Hafs & Hartman 2011), which are usually >100 mm (Hakala 2000; Sweka 2003). Therefore for this study, fish were sampled from three sizes classes (50, 75, 100 mm TL) to cover the normal range of sizes for age-0 brook trout. At the time fish were received from the hatchery, 45 fish were randomly selected to represent the 50-mm-size class (range = 48–58 mm) and were isolated from the rest of the fish in a separate tank identical to the others. The remaining fish were fed floating pellets multiple times daily to satiation until their selected size classes [75 mm TL (range = 70–78 mm) and 100 mm TL (range = 93–115 mm)] were reached. All fish were acclimated to the recirculation system at West Virginia University Ecophysiology Laboratory for at least 2 weeks before any BIA was carried out.

Bioelectrical impedance analysis models that predict percent dry weight should be developed from fish that provide the widest possible range of percent dry weight. Fish fed multiple times daily to satiation until they reached their appropriate size were assumed to have the highest possible percent dry weight. To achieve a wide range of percent dry weights while controlling for interactive effects of size and percent dry weight, fish from each size class were fasted for varying lengths of time before being selected for BIA. For the 50-mm-size class, fasting began on 19 March 2009; 10 fish were randomly sampled on 20 March 2009, 10 more fish were sampled on 27 March 2009 and the remaining 25 fish were sampled on 3–5 April, 2009. A similar protocol was followed for the 75- and 100-mm-size classes; but, because it takes larger fish a longer time to use up fat reserves and reduce percent dry weights (Hafs & Hartman 2011), fasting periods were extended. The fish with the lowest percent dry weights in the 75-mm-size class were fasted 4 weeks, and fish from the 100-mm-size class were sampled over an 18-week period.

### *Bioelectrical impedance analysis*

Resistance and reactance were measured using a Quantum II bioelectrical body composition analyser (RJL Systems). The Quantum II passes a microampere current (425 μA, 50 kHz) between two electrodes through the fish and measures resistance and reactance in ohms. To assess whether subdermal needles or external electrodes worked better, two sets of electrodes were built and used on each fish. Subdermal needle electrodes were 27 gauge (0.4-mm diameter) needles set into epoxy 5 mm apart and such that they could penetrate into the fish a maximum of 1.5 mm (Fig. 1). This was the shortest penetration depth possible that would still allow the needles to pass entirely through the skin. External rod electrodes were built by setting two stainless steel rods (1.6-mm diameter) 5 mm apart in epoxy. It is important to note that each electrode has two needles or rods with one serving as the signal electrode and the other as the detector electrode (Cox & Hartman 2005). The electric current is passed from the signal needle or rod on one

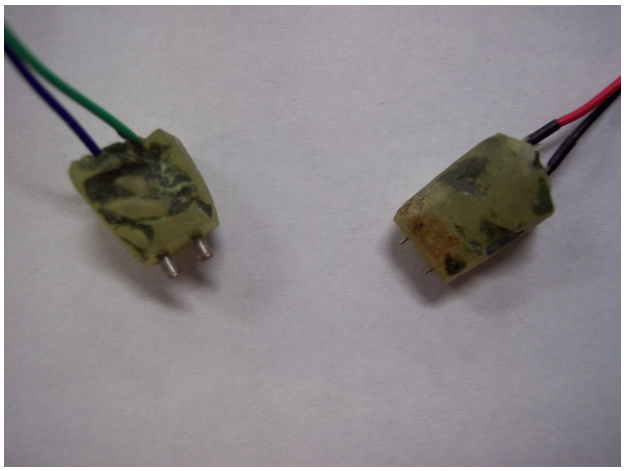

**Figure 1.** External rod (left) and subdermal needle (right) electrodes used in this study.

electrode to the detector on the other electrode. Signal needles or rods were always kept in an anterior position relative to the detector electrode. To assess which location on the fish electrodes should be placed to produce the best estimates of percent dry weight, seven different locations also used by Hafs and Hartman (2011) were tested: dorsal midline (DML), dorsal total length (DTL), lateral line (LL), ventral total length (VTL), ventral midline (VML), dorsal to ventral pre-dorsal fin (DTVpre) and dorsal to ventral post-dorsal fin (DTVpost; Fig. 2).

Fish were acclimated to water temperature equal to ambient room temperature (range 18.0–21.0 °C) for at least 12 h prior to all BIA measurements to minimise the influence of air temperature on measurements (Gudivaka *et al.* 1996). After the 12-h acclimation period, the fish were euthanised in an overdose of MS-222, blotted dry and wet weight (WW; g), fork length (FL; mm) and TL (mm) were measured. The fish were then placed on a non-conductive board with the head facing to the left. Resistance and reactance were measured at all seven

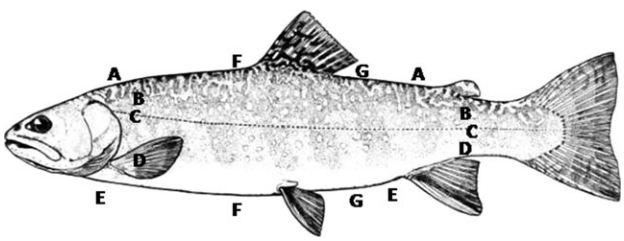


**Figure 2.** Electrode locations: (A) dorsal midline, (B) dorsal total length, (C) lateral line, (D) ventral total length, (E) ventral midline, (F) dorsal to ventral pre-dorsal fin and (G) dorsal to ventral post-dorsal fin. When taking measurements at any individual location, one electrode would be positioned at each of the two letters on the figure with the electrodes oriented parallel to the fish.

locations with both electrode types. To avoid bias due to temperature changes from handling or repeated BIA measures, the order of both the electrode type and measurement location was chosen randomly for every fish during the study. The distance between the needles or rods of the two electrodes (detector length) was recorded for every measurement. So that detector length was equal to the distance between the signal needles or rods, 5 mm was added to all lateral measurements. The person holding the electrodes wore rubber gloves so that BIA measurements were not corrupted. While taking the BIA measurements the person holding the electrodes stabilised their arms on a secure surface to ensure pressure was relatively constant across fish and would therefore not result in bias. After all BIA measurements were completely taken, fish were oven-dried to a constant weight at 80 °C. Percent dry weight was calculated by dividing dry weight by wet weight and multiplying by 100. The Quantum II was checked with a 500-Ohm resistor on every sample date, and accurate readings were confirmed.

### *Data analysis*

A suite of electrical parameters was included in BIA models to predict percent dry weight (Table 1). Because detector length is directly related to fish size, all electrical parameters were standardised to electrical conductor volume by dividing detector length squared ($DL^2$) by

**Table 1.** Electrical parameters [converted to electrical volume when detector length squared ($DL^2$) is included in the equation] used to develop biological impedance analysis models [from Cox and Hartman (2005)]

| Parameter | Symbol | Units | Calculation |
|---|---|---|---|
| Resistance | $r$ | Ohms | Measured by Quantum II |
| Reactance | $x$ | Ohms | Measured by Quantum II |
| Resistance in series | $R_s$ | Ohms | $DL^2/r$ |
| Reactance in series | $X_c$ | Ohms | $DL^2/x$ |
| Resistance in parallel | $R_p$ | Ohms | $DL^2/(r + (x^2/r))$ |
| Reactance in parallel | $X_{cp}$ | Ohms | $DL^2/(x + (r^2/x))$ |
| Capacitance | $C_{pf}$ | PicoFarads | $DL^2/((1/(2\cdot\pi\cdot 50000\cdot r))\cdot(1\cdot 10^{12}))$ |
| Impedance in series | $Z_s$ | Ohms | $DL^2/(r^2 + x^2)^{0.5}$ |
| Impedance in parallel | $Z_p$ | Ohms | $DL^2/(r\cdot x/(r^2 + x^2)^{0.5})$ |
| Phase angle | PA | Degrees | $\text{atan}(x/r)*180/\pi$ |
| Standardised phase angle | DLPA | Degrees | $DL\cdot(\text{atan}(x/r)*180/\pi)$ |

each parameter (Cox & Hartman 2005; Cox *et al.* 2011; Caldarone *et al.* 2012). Standardised phase angle (PA) was calculated by multiplying PA and DL. Total length, FL, WW and a parameter termed 'Residual' were also included for model building process. The Residual parameter was the residual for each fish from the weight-length equation developed for the fish from this study ($WW = 0.0000072 \cdot TL^{3.0056}$; $n = 135$, $r^2 = 0.97$).

A BIA model predicting percent dry weight was developed for each electrode location for both external and subdermal electrodes. Bioelectrical impedance analysis often is carried out by taking measurements at two different locations on each fish; therefore, BIA models were also developed for all two-electrode location combinations for both external and subdermal electrodes. A total of 56 models were developed, which included 7 single-location models and 21 two-location models for both needle and rod electrodes. Models were developed by ordinary least squares regression using the function ols (Harrell 2009), part of the package rms in program R (R Core Team 2012). Forty-five fish from each size class were included in regression models. Mallows' $C_p$ (Mallows 1973) was calculated for every possible model using the function leaps (Lumley 2009), which is part of the package LEAPS in program R (R Core Team 2012). Mallows' $C_p$ is based on the total square errors of the model and is therefore unaffected by collinearity (Yu 2000).

From every electrode type–location combination, the model with the lowest Mallows' $C_p$ value from each possible model size was selected for validation. Bioelectrical impedance analysis models were validated using the function validate (Harrell 2009), part of the package RMS in program R (R Core Team 2012). The validate function uses bootstrapping methods developed by Efron (1983) to randomly separate the original data into training data sets and test data sets. The training data sets are used to develop the models, and the test data sets are used to validate the model. $R^2$ and root-mean-square error (RMSE) are then calculated based on how well the test data sets fit the models. The validate function used 10 000 permutations to develop each model and estimate the $R^2$ and RMSE values. RMSE were included to estimate how close model predictions were to actual values on average. Akaike's information theoretical criterion (Akaike 1973) corrected for small sample size ($AIC_c$; McQuarrie & Tsai 1998) was used to determine model support.

After model development and validation were complete, a sensitivity analysis was carried out on the best-supported BIA model for each electrode type to determine which of the measured variables were most likely to cause large changes in prediction of percent dry weight. Thirty fish were randomly selected from each electrode type, and values for each measured variable present in the model were altered individually by ±10% similar to the methods of Hartman *et al.* (2011). Subsequent changes in predicted percent dry weight were then compared with original predictions from unaltered BIA models, and percent change resulting from the ±10% alteration was calculated. Model predictions were considered sensitive to a variable when a 10% change in the variable resulted in changes in predicted percent dry weight greater than 10% (Bartell *et al.* 1986).

## Results

Brook trout used to develop BIA models were 48–115 mm TL and 0.63–10.61 g WW and had percent dry weights ranging from 15.09–26.08% (Fig. 3). Models including BIA parameters were better at predicting percent dry weight than the best model developed using only TL, FL, WW and Residual ($AIC_c = 99.18$, RMSE = 1.41, $R^2 = 0.74$). Of the 56 models considered, the six models with the lowest $AIC_c$ estimates all contained two electrode locations, and the DTVpre-location was one of the two locations (Table 2). The best-supported model predicted percent dry weight from BIA measurements obtained from subdermal needles at the DTL and DTVpre-locations (Fig. 4). The best model developed using two electrode locations and the external rods was also developed using the DTL and DTVpre-electrode locations; however, the $\Delta AIC_c$ was ~6 and this model was not considered to have similar support to the

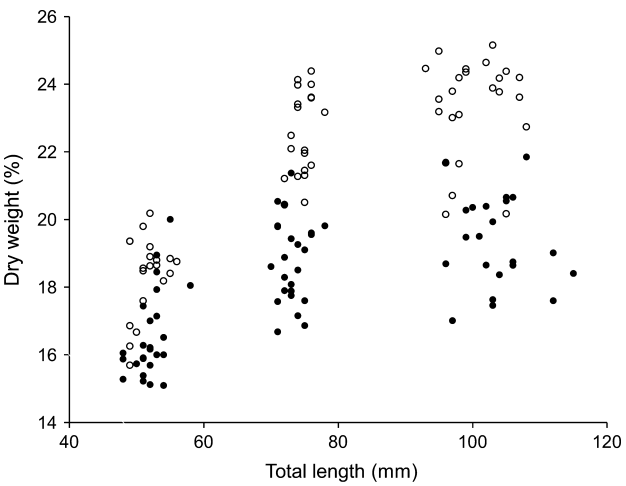


**Figure 3.** Ranges of percent dry weights and total lengths of the brook trout ($n = 135$) used to develop the bioelectrical impedance analysis (BIA) models. Fish from the 50-, 75- and 100-mm-size classes were sampled for BIA over 17-day, 4- and 18-week periods, respectively. Within each individual size class, fish represented by filled circles were sampled later in the fasting period.

**Table 2.** Results of regression analysis to predict percent dry weight from biological impedance analysis for the six best-supported models developed using two electrode locations and the four best-supported models using only one electrode location. Also included are regression analysis results from the morphometric model including fork length, wet weight and residuals from the weight-length equation ($WW = 0.0000072 \cdot TL^{3.0056}$). Electrode locations are defined in Fig. 2

| Location(s) | Electrode type | $R^2$ | RMSE | $AIC_c$ | $\Delta AIC_c$ |
|---|---|---|---|---|---|
| DTL DTVpre | Needles | 0.86 | 1.03 | 33.67 | 0.00 |
| DTL DTVpre | Rods | 0.85 | 1.09 | 39.63 | 5.96 |
| DML DTVpre | Needles | 0.85 | 1.06 | 40.20 | 6.53 |
| DML DTVpre | Rods | 0.85 | 1.07 | 44.40 | 10.73 |
| LL DTVpre | Needles | 0.84 | 1.12 | 47.43 | 13.76 |
| VML DTVpre | Rods | 0.84 | 1.11 | 48.40 | 14.73 |
| DTVpre | Needles | 0.82 | 1.17 | 57.42 | 23.75 |
| DTVpost | Rods | 0.82 | 1.19 | 61.73 | 28.06 |
| DML | Rods | 0.82 | 1.17 | 61.75 | 28.08 |
| DTVpre | Rods | 0.81 | 1.20 | 63.15 | 29.48 |
| Morphometric | | 0.74 | 1.41 | 99.18 | 65.51 |

RMSE; root-mean-square error; $AIC_c$; Akaike information criterion adjusted for small sample size; $\Delta AIC_c$; the difference between the $AIC_c$ for each individual model in comparison with the model with the lowest $AIC_c$.

**Table 3.** Regression coefficients for predicting percent dry weight of 48–115 mm total length brook trout from bioelectrical impedance analysis measured with subdermal needle and external rod electrodes. The models presented require bioelectrical impedance analysis measurements to be taken from two locations (DTL and DTVpre). The parameter column indicates which location's resistance and reactance measurements should be used when calculating the electrical parameter in parentheses. Formulas for calculating the electrical parameters are given in Table 1; the measurement location notation is defined in Fig. 2

| | Model | |
|---|---|---|
| Parameter | Subdermal needles | External rods |
| Intercept | 10.3260 | 26.1464 |
| DTL($r$) | | −0.0059 |
| DTL($x$) | −0.0219 | |
| DTL($R_s$) | −45.0939 | |
| DTL($R_p$) | 46.9168 | 72.3372 |
| DTL($C_{pf}$) | | 15.6299 |
| DTL($Z_s$) | | −70.9148 |
| DTL(PA) | 0.8428 | |
| DTL(DLPA) | 0.0089 | |
| DTVpre($r$) | 0.0074 | |
| DTVpre($x$) | −0.0505 | |
| DTVpre($R_s$) | −246.9491 | −9.8058 |
| DTVpre($R_p$) | 230.2351 | |
| DTVpre($C_{pf}$) | | 300.4741 |
| DTVpre(DLPA) | 0.0825 | |
| Residual | 0.8160 | 1.1298 |

model with needle electrodes. Coefficients for the parameters of the best-supported model are provided in Table 3.

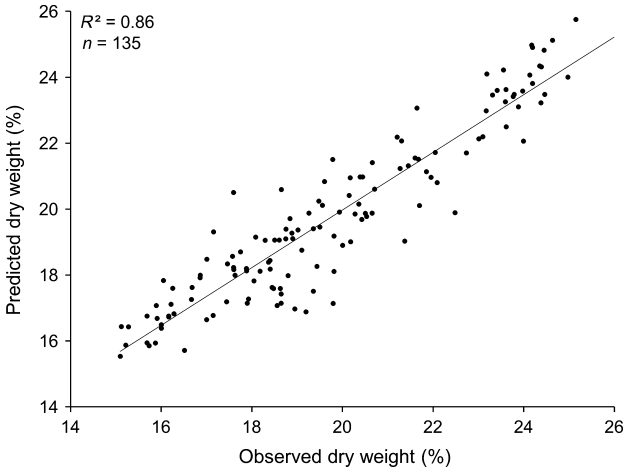


**Figure 4.** Predicted percent dry weights (line) resulting from the best-supported model [Percent dry weight = 10.33 – 0.02·DTL($x$) – 45.09·DTL($R_s$) + 46.92·DTL($R_p$) + 0.84·DTL(PA) + 0.01·DTL(DLPA) + 0.01·DTVpre($r$) – 0.05·DTVpre($x$) – 246.95·DTVpre($R_s$) + 230.24·DTVpre($R_p$) + 0.08·DTVpre(DLPA) + 0.82·Residual] developed using subdermal needle electrodes to measure resistance and reactance for age-0 brook trout at ambient room temperature (range 18.0–21.0 °C). Measurement locations in the model (DTL and DTVpre) are in Fig. 2. Formulas for calculating the electrical parameters included in the model (DLPA, PA, $r$, $R_s$, $R_p$, and $x$) are in Table 1.

Sensitivity analysis indicated that none of the measured BIA variables, when altered by ±10%, changed predicted percent dry weight by more than ±10% (Fig. 5). Total length was the most sensitive variable, and resistance measures at the DTL location had the second greatest influence in the subdermal needle and the external rod models.

## Discussion

The models developed in this study demonstrate that it is possible to predict percent dry weight of the early life stages of fish with precision and accuracy using BIA. Previous attempts to predict percent-based estimates have had varying success. For example, Pothoven *et al.* (2008) used BIA models to predict percent lipids of lake whitefish, *Coregonus clupeaformis* (Mitchill); their best model had a $R^2 = 0.53$. More precise models for predicting percent dry weight from BIA measures were found for tank-reared brook trout in a laboratory setting [$R^2 = 0.82$; Hafs and Hartman (2011)] and bluefish, *Pomatomus saltatrix* (Linnaeus) [$R^2 = 0.86$; Hartman *et al.* (2011)]. Temperature significantly affects BIA measurements (Gudivaka *et al.* 1996; Cox *et al.* 2011); therefore, it is likely that the high $R^2$ values in this study and that of Hafs and Hartman (2011) are probably attributed, at least in part, to the temperature being held constant.

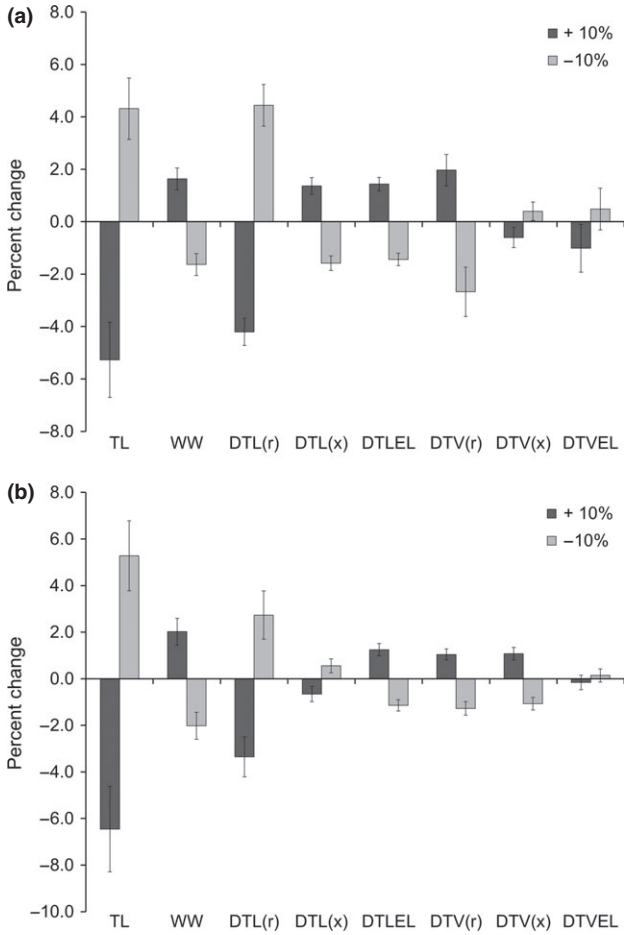


**Figure 5.** Results from the sensitivity analysis of the DTL DTVpre-model for predicting percent dry weight for both subdermal needle (a) and external rod electrodes (b). Percent change refers to change in predicted percent dry weight that occurred by altering measured biological impedance analysis variables by ±10%. Whiskers represent ±2 standard errors. Total length and wet weight are represented by TL and WW, respectively. DTLEL is the electrode length from the DTL location and DTVEL is the measured distance between the electrodes from the DTVpre-location. Resistance and reactance are represented by (*r*) and (*x*), respectively; measurement locations are in Fig. 2.

Two other factors that likely contributed to the precision of the BIA models developed in this study were the inclusion of fish with a wide range of percent dry weight and that multiple locations on fish were tested to determine which provided the best estimates. Hafs and Hartman (2011) also used a wide range of percent dry weights and multiple measurement locations and determined that models including BIA measurements at both DML and DTVpre explained 13% more of the variation in percent dry weight than models developed from measurements taken at locations used by previous researchers [DTL and VTL; Cox and Hartman (2005)]. Further, results from this study provide evidence to suggest that the DTVpre explained should be used in conjunction with the DTL location when measuring BIA on small brook trout. Based on the results of this study and those of Hafs and Hartman (2011), BIA models can be substantially improved by determining which locations should be used for model development.

Similar to the results of Hafs and Hartman (2011), the results from this study demonstrate that although the resulting model had less support, little precision was lost when using external rod electrodes while measuring BIA. Even though the subdermal needles used in this study only penetrated 1.5 mm, external rod electrodes are less invasive. Taking measurements at two locations results in eight small puncture wounds on the fish when using subdermal needle electrodes. Furthermore, more pressure was applied initially to make the subdermal needles penetrate the skin than when external rods were just placed on the surface of the fish. Age-0 fish would have to allocate a portion of their energy intake to heal the eight small puncture wounds and any injuries that would have occurred due to the added pressure applied during initial penetration of subdermal needle electrodes. While energy reserves can be depleted in any size fish, energy reserves are more likely to be lower for age-0 fish; therefore, energy needed for tissue repair may constitute a larger portion of those energy reserves. Because age-0 fish with higher energy reserves are more likely to survive winter (Miranda & Hubbard 1994) age-0 fish that are forced to use energy to heal BIA wounds could be less likely to survive stressful periods. Given the minor loss in precision, external rod electrodes should be used to obtain BIA measurements to predict percent dry weight until more research is carried out to assess the effects of BIA measurement on small fish health.

Species-related factors that are more likely to affect BIA models are scale thickness, fat storage location, body morphology and bone structure. As scale thickness increases external rod electrodes will less effectively transmit electric current through the scales into the fish tissue. Because other age-0 salmonids have small cycloid scales similar in thickness to those of brook trout, scale thickness should have little influence on BIA measurements. Additionally, all salmonids store fat reserves in the muscle (Ackman 1989), and differences in bone structure and body shape are minimal. Therefore, it is likely the models provided in Table 3 would produce similar results when used on other age-0 salmonids. Nevertheless, evaluation of similar models for other species is warranted.

Sensitivity analysis indicated measurement errors of ±10% in BIA variables such as detector length, resistance, and reactance had limited influence on model

predictions of percent dry weight for age-0 brook trout. These results are similar to those of the sensitivity analysis carried out by Hartman *et al.* (2011) on their BIA models that predict the percent dry weight of bluefish. In the current study, model predictions were most sensitive to changes in TL. The model developed that included only length- and weight-based measurements (traditional condition analysis) accounted for 74% of the variability in percent dry weight. The addition of BIA variables into models helped explain an additional 12% of the variation in percent dry weight. Thus, the low model sensitivity found for BIA variables results from length and weight measurements explaining the majority of the variation in percent dry weight, while BIA variables are essentially fine tuning the models and allowing greater precision than was previously achieved.

By demonstrating that it is possible to predict percent dry weight of small fish using BIA, future researchers can use the methods outlined in this study as a framework for the development of BIA models for small fish. The models provided in Table 3 should allow future researchers to improve assessment of changes in percent dry weight of small fish. Although this study provides a good starting point for BIA on small fish, the models provided should be field validated, and the effects of temperature need to be considered. The models provided by this study were developed in a laboratory setting where temperature was controlled (18–21 °C). Because temperature has a large influence on BIA (Gudivaka *et al.* 1996; Cox *et al.* 2011; Hartman *et al.* 2011), these models should only be applied when BIA is measured for age-0 brook trout at 18–21 °C. Use of the models presented in this study outside of the 18–21 °C temperature range would require future researchers to account for temperature following the methods of Hartman *et al.* (2011), Hafs (2011) or Stolarski *et al.* (2014).

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