Evaluation of Bioelectrical Impedance Analysis Methods for Use on Channel Catfish

by

Julie Sharp

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Approved by

Elise Irwin, Chair, Associate Professor of Fisheries, Aquaculture and Aquatic Sciences, Auburn University
Allen Davis, Alumni Professor of Fisheries, Aquaculture and Aquatic Sciences, Auburn University
Amit Morey, Assistant Professor of Poultry Science, Auburn University
Keith Cox, Assistant Professor of Marine Fisheries Arts and Sciences, University of Alaska Southeast

Abstract

Assessment of body composition is an accurate measure of condition. This study evaluates bioelectrical impedance analysis (BIA) methods for Channel Catfish *Ictalurus punctatus*. BIA shows promise as a quick, inexpensive, and non-lethal technique to estimate body composition. Models were developed by correlating BIA measures to total body water (TBW). The seven anatomical locations where measures were taken had a significant influence on predictive ability of models. Dorsal-lateral measurements were highly correlated with volumetric resistance in series and had the highest predictive ability for TBW (R^2=0.9651 and 0.9816). TBW converted to percent dry mass (%DM) was used as a proxy for fat and protein. Significant relations were found between %DM and percent fat (R^2=0.8319) as well as percent protein (R^2=0.7033). Temperature had a significant negative effect on BIA measures. Measures of wet weight and total length also had significant relations to TBW, therefore, further analysis of BIA models is necessary.

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BIA	Bioelectrical impedance analysis
TBW	Total body water (g)
DM	Dry mass (g)
TBP	Total body protein (g)
TBF	Total body fat
DM	Dorsal midline
DTL	Dorsal total length
LL	Lateral line
VTL	Ventral total length
VML	Ventral midline
DTVpost	Dorsal to ventral post-dorsal fin
DTVpre	Dorsal to ventral pre-anal fin
R_s	Resistance in series
X_c	Reactance in series
R_p	Resistance in parallel
X_p	Reactance in parallel
C_{pf}	Capacitance
Z_s	Impedance in series
Z_p	Impedance in parallel
A_P	Phase angle
D_LA_P	Standardized phase angle

Introduction

The Tallapoosa River is in the Mobile River basin (Alabama, U.S.A.), the most diverse river system in North America. The river historically supported recreational fisheries of black basses, channel catfish, and flathead catfish (Lydeard and Mayden 1995; Irwin and Freeman 2002). The construction of dams throughout the river system, as well as the southeastern U.S., has negatively impacted the productivity and diversity of several river-dependent fish species (Neves and Angermeier 1990; Travnichek and Maceina 1994; Freeman et al. 2001). In 1982, R.L. Harris Dam was constructed for hydropower generation which regulates the river flow regime through releases of water from the dam (Irwin and Freeman 2002; García et al. 2011). Irwin and Freeman (2002) reported that operation of R.L. Harris Dam poses a threat to native fauna and recreation due to depleted low flows, flow instability, and thermal-regime alteration. Bunn and Arthington (2002) states that because many fish prefer specific habitat types and fauna richness increases with increasing habitat complexity. Because of this, when hydrologic regimes are regulated, leading to reduced habitat complexity, fish communities become more generalist in nature.

Channel Catfish *Ictalurus punctatus* are a native species to and an important recreational fishery in the Tallapoosa River. In the United States, catfishes rank in the top three most important sportfish (USDOI 2014). However, populations in the Tallapoosa River below the R.L. Harris Dam are believed to be in decline as indicated by low catch rates during electrofishing surveys and capture of underweight and unhealthy individuals (E. R. Irwin, USGS, unpublished data). Juvenile Channel Catfish have been stocked in the river to improve populations but, similarly, sampling has not recaptured any of these stocked fish. Additionally, Channel Catfish typically spawn in June and July based on optimal temperatures (Hubert 1999) and young juveniles have routinely been captured in late summer, indicating a protracted spawning period (Sakaris 2006). It is likely that late spawned fish will have high over-winter mortality due to lack of development and energy stores. These data provide inference that the habitat for spawning and recruitment is not favorable and river regulation is potentially the controlling variable.

Hydrologic regimes in lotic systems are responsible for transfer of nutrients and energy downstream, availability and persistence of flow-associated habitats, and are an important determinant of community structure and function (Petts 1984). While adult Channel Catfish prefer middle pool habitat (Lobb and Orth 1991), the preferred habitat of juveniles is riffle/shoal habitat of variable depth (Irwin et al. 1999). Sakaris (2006) indicated that in regulated reaches of the Tallapoosa River age-0 Channel Catfish growth rates were significantly slower than in unregulated reaches. This was attributed to frequent pulses of water from the dam which created a variable and energetically demanding environment.

There are many environmental variables that may affect fish health such as contaminants, fluctuating thermal or hydrologic regimes, sediment loading, low oxygen concentrations, and changes in food or habitat availability (Wedemeyer et al. 1984; Adams et al. 1993; Adams 1999). These potential stressors contribute to various physiological effects on fish as they divert energy resources to maintain homeostasis and survive fluctuating environmental conditions (Adams et al. 1993). Fish experiencing stress will utilize energy from stored lipids, potentially having a lasting impact on growth and reproduction of the individual, which can ultimately affect recruitment and subsequent population size (Wedemeyer et al. 1984; Adams 1999). Reduced energy reserves can negatively impact populations through reduced survival, fecundity, and egg quality or delayed maturation (Wedemeyer et al. 1984).

These energy reserves are commonly associated with levels of fat in an individual (Pope and Kruse 2007; McPherson et al. 2011) and can be used as a determinant of a fish's condition, which is generally defined as the well-being of an individual (Le Cren 1951; Blackwell et al. 2000) and is often equated to fat, or energy reserves. Condition can be measured using morphometric, biochemical, or physiological analysis (Adams 1999; Shulman and Love 1999; McPherson et al. 2011; Lloret et al. 2013). It can be an indicator of the overall health of the ecosystem by providing insight into habitat quality, food availability, competition, overwintering mortality, seasonal lipid storage, and maturation (Pope and Kruse 2007). Lipid content is a good indicator of fish condition as individuals in good condition are assumed to have larger energy reserves than those in poor condition (Lloret et al. 2013).

In poikilothermic animals, such as Channel Catfish, the main depots of lipid storage are mesenteric fat, liver, and muscle (Sheridan 1994). Lipids stored secondarily in liver and muscle are utilized for life history events, such as reduced winter foraging, while mesenteric fat tends to serve as primary storage for a longer-term reserve (Sheridan 1994; Jolley 2003). Research has shown that whole body fatty acid patterns are more reflective of the proportion of fatty acids consumed by Channel Catfish than are liver lipid patterns (Stickney and Andrews 1972). Therefore, whole body lipid content may provide a better representation of energy stores and condition of Channel Catfish than organosomatic indices.

The most accurate methods of determining magnitude of energy stores are biochemical and physiological analyses (Shulman and Love 1999). However, this approach includes lengthy processing time for analyzing samples in the laboratory and, often, increased financial investment (Lloret et al. 2013). In addition, these methods are lethal to the animal, thereby eliminating any long-term monitoring of individuals or the use on rare and endangered species.

In contrast, morphometric indices, such as Fulton's K, Le Cren's relative condition factor, and relative weight, are most often used as measures of condition. These indices are non-lethal, inexpensive, and based on simple length and weight measurements (McPherson et al. 2011; Lloret et al. 2013) and thus lend themselves to field studies. However, morphometric indices are based on the fresh weight of the whole body which consists of 60-80% water and can vary significantly through time (Shulman and Love 1999). Additionally, their use and validity is limited due to frequently violated assumptions which lead to incorrect conclusions about fish condition (Cone 1989). Any study using these indicators should define what is being measured and validate the index against a benchmark, such as a biochemical index (Davidson and Marshall 2010; McPherson et al. 2011).

The evaluation of whole body fat content is an accurate way to monitor condition of fish (Adams 1999; Shulman and Love 1999; Lloret et al. 2013). Yet, quantification of total fat is typically costly, time consuming, and particularly cumbersome when analyzing larger animals (Hartman et al. 2011). However, bioelectrical impedance analysis (BIA) has been shown to be an accurate, low-cost, and non-lethal method of measuring percent dry mass (%DM) of fishes (Cox and Hartman 2005; Hafs and Hartman 2011, 2014; Hartman et al. 2015), which can be back calculated to whole body fat and protein content using simple equations developed from proximate body composition analyses (Hartman and Margraf 2008).

Bioelectrical impedance analysis measures reactance and resistance (impedance) of a small current (50kHz, AC) passed through an organism which can then be regressed to predict actual body composition measurements of the organism (Cox and Hartman 2005). Resistance (R_s) is a measure of a substances ability to conduct electricity while reactance (X_c) measures its ability to hold a charge (Lukaski 1987). Because fat is a poor conductor of electricity there is a strong relation between resistance and the amount of fat in an individual (Hafs and Hartman 2011). Also, the lipid bilayer acts as a capacitor and therefore measures of reactance relate to cell volume and condition of the individual (Hafs and Hartman 2011).

BIA is based on the principle expressed in the following equations in which resistance (R; Ohms) of a cylinder is proportional to its length (L) and inversely proportional to cross-sectional area (A) multiplied by a resistivity constant (p):

$$R = \frac{pL}{A}$$

By multiplying the numerator and denominator by L we are left with:

$$R = \frac{pL^2}{V}$$

Where area multiplied by length is equal to volume (V). By this equation, a measure of volume should be able to be predicted based on resistance:

$$V = \frac{pL^2}{R}$$

Water in the body contains electrolytes which conduct electricity and allow the current to pass through the body of an organism (Kyle et al. 2004). BIA uses this current and measures the resistance and reactance in between two sets of electrodes. Resistance is a measure of how quickly the current is able to flow through the interstitial space and is impeded by the non-conductive lipid bilayer of cell membranes. By this reasoning, as lipid content increases in a fish, resistance should similarly increase. The cell membranes in an organism act as capacitors and reactance is a measure of the ability to hold a charge (Lukaski 1987). Because of this, reactance should increase in healthy fish.

The body essentially functions as a circuit as the current passes through intra- and extra-cellular material yet it is unclear if it is a in series or parallel. Electrical parameters which reflect body composition are often calculated both ways to account for the complexity of the cellular makeup of an organism (Kyle et al. 2004). Additionally, although a fish is not a true cylinder, having an approximate cylindrical shape and lack of appendages lends itself to establishment of empirical relations (Kyle et al. 2004). The resistivity constant, *p*, is based on the assumption of homogeneity, which is violated by the internal composition of a fish. Because of this, specific coefficients must be empirically derived to establish relations between total body water mass and L^2/R.

BIA has been used to successfully predict proximate body composition values in Brook Trout *Salvelinus fontinalis* (Cox and Hartman 2005; Rasmussen et al. 2012), Rainbow Trout *Oncorhynchus mykiss* (Bourdages 2011), Dolly Varden Char *Salvelinus malma* (Stolarski et al. 2014), and Cobia *Rachycentron canadum* (Duncan et al. 2007). Using BIA to predict %DM is useful because it can be calculated at a fraction of the cost of proximate body composition or bomb calorimetry (Hafs and Hartman 2011). Dry weight has been successfully predicted for Bluefish *Pomatomus saltatrix* (Hartman et al. 2011) and Brook Trout (Hafs and Hartman 2011) from impedance measurements. The values of %DM can then be correlated to proximate composition estimates using previously established relations (Hartman and Brandt 1995; Hartman and Margraf 2008).

Electrode location is an important consideration when conducting BIA because the impedance values are measurements of the body tissue in between the two electrodes. Several studies have solely used the dorsal total length (DTL) location for electrodes (Figure 2; Cox and Hartman 2005; Pothoven et al. 2008; Bourdages 2011; Rasmussen et al. 2012), or the DTL location plus one dorsal-to-ventral or lateral-ventral measurement (Duncan et al. 2007; Hartman et al. 2011; Stolarski et al. 2014; Hafs and Hartman 2014, 2015), to collect impedance measurements. However, Hafs and Hartman (2011) determined that by analyzing several locations the reliability of the BIA model could be improved, leading to a more accurate prediction of %DM.

Temperature can have significant effects on BIA measurements and reactance and resistance both have been shown to decrease as temperature increases (Hafs and Hartman 2015). These effects were documented at 15°C and 27°C on Bluefish (Hartman et al. 2011). Hartman et al. (2011) also suggest that more data are needed to determine the linear or nonlinear relation of impedance measurements to temperature so corrections can be easily included in BIA models. By testing individual fish at each temperature, differences in impedance measurements related to different body conditions can be eliminated (Hartman et al. 2011).

In this study, predictive models were developed with which body composition of Channel Catfish can be evaluated using BIA. To achieve this, there were three main objectives: 1) determine the optimal electrode location on the fish that has highest predictive ability of total body water with validation 2) develop independent models which established the relation between %DM and body composition values of total body water (TBW), dry mass (DM), total body protein (TBP), and total body fat (TBF), and 3) establish the relation between temperature and BIA measurements to develop temperature correction equations. These models and temperature corrections will presumably allow for an accurate estimation of fish body composition and condition when data are collected in the field.

Methods

Animals

A total of 134 Channel Catfish were obtained from the E.W. Shell Fisheries Center in Auburn, Alabama which had a size range of 179-358 mm. These fish were divided into two size groups, small (179 – 272 mm) and large (260 – 358 mm), and about eight fish were placed in each of sixteen 15-gallon re-circulating tanks and acclimated for a minimum of two weeks. All fish were considered to be in a fat condition owing to their daily ad libitum feeding schedule during the acclimation period. The fish were fed a catfish feed diet consisting of 32% protein and 6% fat. Upon the start of the study, the fish were fasted and periodically sampled in attempt to achieve a wide range of body conditions with a fat range of at least 29% (Hartman et al. 2015). The fish were sampled at seven approximately evenly spaced intervals over the course of the fasting period with the leanest fish sampled last. The smaller fish were fasted for 12 weeks and the larger fish for 16 weeks. The work was conducted under the IACUC protocol number 2017-3003.

BIA Measurement

Bioelectrical impedance was measured using two electrodes, each of which consisted of two needles, one signal and one detecting. The needle electrodes were built by setting two stainless steel needles (28 gauge, 13mm) with epoxy in plastic blocks 10 mm apart with a 6 mm depth for puncturing the skin of the fish (Figure 1). The depth of the needles was doubled from the methods of Hafs and Hartman (2011) due to thickness of the catfish skin and inability of shorter needles to puncture skin and provide stable measures.

Seven anatomical electrode placement locations were tested and analyzed to determine which would achieve the best estimates of total water content (Hafs and Hartman 2011). These include dorsal midline (DM), dorsal total length (DTL), lateral line (LL), ventral total length (VTL), ventral midline (VML), dorsal to ventral post-dorsal fin (DTVpost), and dorsal to ventral pre-anal fin (DTVpre; Figure 2). The electrodes were oriented parallel to the fish (Hafs and Hartman 2014). The signal needle on each electrode was positioned interior to the detector electrode for consistency, although Hafs and Hartman (2011) noted that the orientation of the signal and detector needles had no effect on the readings during their study.

The order of electrode location was randomized for each fish to avoid any bias due to temperature changes, handling, or repeated BIA measures. The detector length – distance between the inner needles of the two electrodes – was recorded for every measurement. Latex gloves were worn while taking BIA measurements to prevent interference with measurements and arms were stabilized to ensure relatively constant pressure for measurements across fish.

On every sample date before measurements were taken, the Quantum IV bioelectrical impedance analyzer (RJL Systems, Clinton Township, Michigan) was calibrated with a 500 Ohm test resistor to ensure accurate readings. When fish were selected for BIA measurement they were anesthetized in a clove oil solution of 15 g/L (AVMA 2013). Once anesthetized, each fish was blot dried, weighed to the nearest 0.1 g (WW), and placed on a nonconductive wooden board on its right side with the head facing to the left. Total length (TL) was measured to the nearest mm and recorded. While on the nonconductive board, BIA measures were taken using the Quantum IV. The Quantum IV passes an electrical current at 50 kHz through the fish and measures reactance and resistance in Ohms. On each fish, every position was measured five times by adjusting needle placement, without reinserting to avoid additional harm to the fish. These measurements were used to analyze reliability of measurements. The average of these measurements was used in model development.

Proximate Composition Analysis

After all measurements were taken and recorded, fish were euthanized in a 45 g/L overdose solution of clove oil (AVMA 2013) and promptly processed individually under high pressure to soften bones for homogenization and further analysis. Individual fish were homogenized using a food processor for proximate composition analysis. Duplicate wet samples of 9-12 g were oven dried to a constant weight at 95°C and percent DM was calculated by dividing dry weight by wet weight (WW) of the sample and multiplying by 100. A subsample of 18 fish (%DM range = 19.09 – 31.52%, mean = 25.90%) were analyzed for fat and 48 fish (%DM range = 19.09 – 31.52%, mean = 27.02%) for protein. These samples were analyzed in duplicate for fat and crude protein using the Folch and Dumas methods respectively (AOAC 1990). The estimates were averaged for each individual fish.

Temperature

Temperature corrections were calculated by measuring the BIA of 60 fish at 12, 22.5, and 28°C. These temperatures are considered within the normal range experienced by Channel Catfish in the Tallapoosa River basin in both regulated and unregulated reaches (Hoxmeier and Devries 1997). A control group of 20 fish was measured at 22.5°C over the course of the three temperature treatments to evaluate any changes in impedance values within the measurement period. All 80 fish were acclimated for 24 hours before any measurements were taken to allow their core temperatures to reach equilibrium. The control fish were acclimated at 22.5°C and the temperature treatment fish were acclimated at 28°C. The BIA measurements were taken in two optimal locations determined prior to temperature correction trials as described previously. After, BIA measurements were collected the temperature treatment fish were placed in a tank to recuperate and acclimate to 22.5°C for another 24 hours. This was repeated at 12°C. The control fish were placed back in the 22.5°C tanks to recuperate for 24 hours. It was assumed that each fish's body composition would not change significantly over the period of temperature change (Hartman et al. 2011; Hafs and Hartman 2015), however, the control group was used to validate that assumption. Measurements on each fish were again taken 5 times to assess reliability with changing temperatures. Once the temperature treatments were complete, the fish were euthanized in an overdose (45 g/L) of clove oil.

Statistical Analysis

Detector length (DL) is directly related to fish size therefore all electrical parameters were standardized to electrical conductor volume by dividing detector length squared by each parameter (Cox and Hartman 2005; Cox et al. 2011). Electrical properties that were calculated are resistance in series (R_s), reactance in series (X_c), resistance in parallel (R_p), reactance in parallel (X_p), capacitance (C_{pf}), impedance in series (Z_s), impedance in parallel (Z_p), phase angle (A_P), and standardized phase angle (D_LA_P; Table 1; Cox and Hartman 2005). Except A_P and D_LA_P, all measurements were volumetric as they were divided into DL^2.

Single parameter regression models were developed to predict total water (TBW) from BIA measurements and electrical parameters at all locations. TBW was calculated from measured %DM by: TBW = WW - (%DM · WW). These models were compared with a model using only WW and TL as predictive parameters. The measured fish were divided into two groups: 100 in a training group and 34 in a test group. The predictive models were developed using the training group and those models were validated using the test group. Generalized linear mixed-effect models were used to determine relations between temperature and BIA measurements for the two selected locations. Each individual fish was treated as a random effect and water temperature as a fixed effect. Lastly, equations were developed to predict lipid and protein, expressed as a percentage of total dry weight, using regression analysis.

Results

The Channel Catfish used to develop predictive models using BIA measurements were 179 - 358 mm TL (Figure 3) and 34.55 - 370.8 g WW. Over the course of the experiment, wide ranges were achieved for %DM (19.09 - 31.52%), %TBF (4.99 - 36.29%) and %TBP (44.63 - 68.51%), on a dry weight basis. Hartman et al. (2015) established recommendations that a sample size of 60 and a minimum fat range of 29% was necessary to obtain an $R^2 \geq 0.80$, which is arbitrarily set, yet achievable and yields highly predictive models. The fish used in this experiment fall comfortably within these limits.

Single parameter models developed for seven unique morphological locations showed strong linear relations between calculated volumetric BIA parameters and TBW. The best model from each position indicated high correlation between calculated volumetric electrical parameters and TBW (Table 2): resistance in series for LL ($R^2 = 0.9822$), DTL ($R^2 = 0.9816$), DML ($R^2 = 0.9651$), VTL ($R^2 = 0.9326$), DTVpost ($R^2 = 0.8766$), resistance in parallel for VML ($R^2 = 0.9192$), and reactance in parallel for DTVpre ($R^2 = 0.9161$). Resistance in series had the highest correlation for five out of the seven positions. The last model correlating WW and TL to TBW was also highly significant ($R^2 = 0.9968$).

Of the seven anatomical positions, DTL and DML were selected for validation and temperature corrections. Although LL had the highest R^2 value, it was eliminated due to substantially increased bleeding when needles punctured the fish. Models for DML and DTL demonstrated the highest predictive ability and thus were validated using the test group of Channel Catfish. This validation showed high correlation between the measured and predicted TBW (Figure 4). The model for the DTL position was the best supported (R2 = 0.9901) although the DML position also had high predictive ability (R2 = 0.9568).

In the temperature correction trial, the acclimation period began when the water was within 1° of the desired temperature (Figure 5). In the control groups, there was no change in resistance measurements in the DML position (p-value = 0.6718); whereas in the DTL position there was a slight increase (p-value = 0.0041) over the course of the experiment (Figure 6). In the experimental group, which were measured at incrementally decreasing temperatures, the measurements in the DTL position were adjusted to reflect the slight increase in the corresponding control group measurements. Temperature had a significant influence on resistance and the generalized linear mixed effect models indicated that it decreased as temperature increased (Figure 6). In the DML position, resistance measurements decreased by -4.7628 ohms for every 1° decrease in temperature (p-value = <0.0001). In the DTL position, resistance decreased by - 9.8262 ohms for every 1° decrease in temperature (p-value = <0.0001).

Percent TBF and TBP contents were calculated on a dry weight basis and regressed with %DM. These predictive equations demonstrated significant correlations. There was a significant positive relation between %TBF and %DM (R^2 = 0.8319, p-value = <0.0001; Figure 7). Relative TBP also had a significant relation to %DM (R^2 = 0.7033, p-value = <0.0001) but decreased with increasing %DM (Figure 8).

Discussion

The value in BIA as a tool to estimate body composition values lies in its potential to produce accurate results quickly, inexpensively, and in a manner that is repeatable and non-invasive to the individual. The high correlations and strong linear relations in the regressions of TBW and BIA measurements indicate that there may be promise in using BIA as a reliable means of predicting body composition of Channel Catfish. Strong correlations were observed between TBW and R_s in the DTL (R^2 = 0.9816) and DML (R^2 = 0.9651) positions. However, these correlations were not better than those of WW and TL to TBW (R^2 = 0.9968). Successful model development for other species indicated that the best predictor of TBW was R_p for Brook Trout (R^2 = 0.9746), and Cobia (adj. R^2 = 0.9894; Cox and Hartman 2005; Duncan et al. 2007). Alternately, in juvenile steelhead *Oncorhynchus mykiss*, X_p was found to be most predictive of TBW (R^2 = 0.95; Hanson et al. 2010). However, the results of Hanson et al. (2010) used a very small sample size (n = 15) to develop the models.

Reaching the recommended fat range (29%) and sample size (n = 60) established by Hartman et al. (2015) may be a challenge in some studies if the fish are small or difficult to acquire in large numbers. Acquiring Channel Catfish for this study was not an issue and, with 134 individuals, a fat range of 31.3% was achieved; despite the necessary educated predictions due to limited literature on proximate composition of fish under long-term fasting conditions. Models developed for Atlantic Salmon *Salmo salar* postsmolts using BIA measures were unsuccessful in predicting body composition (g or %) more accurately than size related variables (Caldarone et al. 2012). This study by Caldarone et al. (2012) only included mean ranges of percent fat (4.9 - 7.1%) which likely are well below the range necessary to use BIA effectively. Similarly, Pothoven et al. (2008) failed to improve models for three species predicting body composition using BIA measures over those using only body mass. However, this may be due to a combination of small sample sizes (n = 30 - 38) and likely a limited range of percent fat. The Channel Catfish sampled in this study exceeded the minimum requirements, yet the BIA models did not outperform the length-weight model.

Temperature was found to have a significant influence on resistance, however, the impact varied between positions. Whereas the impact of temperature on resistance was negative, the slope for the DTL position was doubled from the DML position. Temperature corrections developed for Brook Trout and Dolly Varden similarly indicate that these corrections were dependent on positioning as well as being species specific (Stolarski et al. 2014; Hafs and Hartman 2015). If measurements were not corrected for temperature, relative dry mass was shown to be underestimated when using models calibrated at lower temperatures (Klefoth et al. 2013). Additionally, Pothoven et al. (2008) did not account for temperature effects, which in this instance probably contributed to the inability to develop predictive models. Correcting for temperature clearly influences the predictive ability of BIA models and must be accounted for in model development, especially for new species.

Few studies have evaluated positioning of electrodes, and most use only the DTL position, however, many fish store lipids in different parts of their body so it is necessary to include this evaluation to best predict body condition (Jacobs et al. 2008). Model development for Brook Trout, which included an evaluation of seven positions, indicated that using two positions, instead of just one, improved predictive power for both adult (R_2 = 0.82) and age-0 (R_2 = 0.86) Brook Trout (Hafs and Hartman 2011, 2014). Interestingly, the two best positions included a dorsal-lateral and dorsal-to-ventral measurement. The best dorsal-lateral position was either DML or DTL, which corresponds with the results for Channel Catfish. Stolarski et al.

(2014) used lateral dorsal and ventral positions and found that models including both positions were an improvement from those using only one. Additionally, an evaluation of position and detector length on Pink Salmon *Oncorhynchus gorbuscha* indicated that in a dorsal-lateral position, there was a significant difference in resistance and reactance measures when the posterior electrodes were moved forward and the detector length was halved (Cox et al. 2011). This finding illustrated that positioning of electrodes can lead to variable measures and placement of the posterior electrodes should also be considered.

Studies using only dorsal-lateral measures, however, have successfully produced models with high predictive ability, indicating that alternate or additional positions may not be necessary for all species of fish (Cox and Hartman 2005; Duncan et al. 2007; Hanson et al. 2010; Rasmussen et al. 2012; Andrade et al. 2014). Wuenschel et al. (2013) suggested dorsal placement may even avoid confounding results due to stomach fullness or gas bladder inflation. However, this multi-position evaluation has not been approached, aside from Brook Trout and, now, Channel Catfish. Hartman et al. (2015) stated that because extensive analysis has been done to evaluate optimal electrode positioning in Brook Trout, additional research may not be necessary for other salmonids due to their similarity. This shows promise for use across other taxa and it is suggested that additional positions be evaluated in all further model development.

BIA techniques are still being developed and improved and for many species they are not currently reliable enough for routine use. Model development for Channel Catfish was not considered to be successful so further exploration of this method is necessary prior to field validation and use. In preparation, it was decided to lengthen the needle electrodes due to the thick and tough nature of catfish skin. The length of the needles (6 mm) allowed us to get reliable measurements whereas the shorter 3 mm needles did not; although, this longer length was at times problematic for smaller fish. Some fish were very small and had thin tails, especially towards the end of the fasting period, so that they were not thick enough to fully insert the needle electrodes. On these smaller individuals, it is possible that the length of the needles could impact internal organs when inserted into the body of the fish. Shorter needles would also allow for full needle insertion. It would be beneficial to determine a needle length for Channel Catfish which minimizes harm to the individual, yet optimizes ease of use in making a good connection.

There was relative consistency in the measurements at the DML and DTL positions, however, there was still some variation in resistance and reactance within each position. It is suggested in future collection that two measurements be taken to ensure consistent and accurate readings. If the difference between these two measurements is greater than an acceptable pre-determined range, a third measurement should be taken. Klefoth et al. (2013) took three measurements and used the mean value for analysis. Although they did not report on this procedure, adding this step could eliminate any unrealized measurement error.

A potential source of error in this experiment was long handling times for each fish when evaluating electrode position. Because repeated measures were taken for each fish, the handling times for the fish in which seven positions were evaluated were up to 20 minutes per fish. In this amount of time it is entirely possible that the body temperature of the fish fluctuated. When measuring fish in the temperature treatments, handling times were roughly 3-4 minutes because only two positions were evaluated. It may be beneficial to collect additional data on fish in which only two to three measurements are taken at one position and handling times are more reasonable.

In temperature correction trials, fish were anesthetized, probed with needle electrodes, and then placed back in the tank for recovery twice before being euthanized on the last round. Throughout the course of handling these fish there was no mortality or outwardly visible harm to the fish. This is a positive result which supports the use of repeated BIA measurement on individual fish over the course of their lifetime. Additional long-term monitoring may be valuable to assess infection or mortality rates of fish subject to repeated measurement.

Predicting TBW using a single parameter is simple and theoretically effective. Cox and Hartman (2005) were able to successfully develop similar single parameter models for Brook Trout which predicted TBW, as well as other proximate composition values. Studies in which all electrical parameters are evaluated for inclusion in a single model, particularly when a second position is included, have resulted in potentially overparameterized models of up to 16 parameters (Stolarski et al. 2014). Because all predictive variables are calculated from measured values of resistance and reactance, some of these variables are potentially collinear and that should be accounted for. Dibble et al. (2017) suggested the use of principle component analysis (PCA) to identify uncorrelated variables. Additionally, more attention should be paid to inclusion of variables with physiological relevance to the compositional component being estimated. This may lead to improved accuracy, reduced redundancy and simplification of models.

As BIA research progresses and techniques are improved, there is potential to be able to evaluate and compare energy storage levels of fishes at specific times and locations. Temporally, this may include age and growth, overwinter energy storage and survival, or reproductive and recruitment success. Spatially, variance in energy storage in distinct habitat types, river reaches, river systems, and regions could be evaluated. Margraf et al. (2005) proposed that BIA models for Chinook Salmon, with minor refinements, could be used to predict energy content of migrating fish. In combination with tagging, radio-tracking, and genetic studies, estimates of energy-levels could lead managers to an improved understanding of energy use during migration to spawning grounds.

There is also potential for use on small, native, or endangered species, however, very little research has been done to develop these techniques. Determining the validity of a model across species with similar morphology can elucidate this capability. Species of concern may be difficult to acquire and use for the necessary testing in model development. However, it may be feasible to determine a correction factor for these species from BIA models of a similar species. This could provide insight into the variability and corrections needed to make models suitable for fish smaller than those for which they were developed. Some factors to consider if using one species as a proxy are size, morphology, energy storage, and life history characteristics. Some error is likely to be inherent in these types of models which would need to be defined to determine an acceptable level for continued use or further model development.

Dibble et al. (2017) evaluated the use of BIA on three small threatened and endangered species in Arizona and determined that adding BIA measurements only improved morphometric models, based on length and weight, by 2-3%. The sample size for each species exceeded the requirements mentioned previously, however, the limited capabilities of these BIA models is likely due to a small range of %TBF. This may be an inherent limitation of working with threatened or endangered species for which the solution may be using similar species as proxies. Additionally, one of the three species exhibited high mortality following BIA measurements. Further research is needed to evaluate species that may have increased vulnerability to stress due to BIA and handling before it can be supported as a reliable method for evaluation of body composition.

The development of BIA techniques has implications for use in river systems in which Channel Catfish populations are of interest. Particularly, in the Tallapoosa River, development of a baseline for health and condition would complement information about movement and population dynamics. However, further evaluation of BIA techniques and development of predictive equations are necessary before it will ready for field use. Until that time, indices based on length and weight measurements may be of the greatest benefit for evaluating the status of this population of fish.

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Table 1. A list of electrical parameters measured and calculated that were used for model development. Detector length (D_L) is included to convert parameters to electrical volume.

Parameter	Symbol	Units	Calculation
Resistance	r	Ohms	Measured by Quantum IV
Reactance	x	Ohms	Measured by Quantum IV
Volumetric resistance in series	R_s	Ohms	D_L^2/r
Volumetric reactance in series	X_c	Ohms	D_L^2/x
Volumetric resistance in parallel	R_p	Ohms	$D_L^2/ [r + (x^2/r)]$
Volumetric reactance in parallel	X_{cp}	Ohms	$D_L^2/ [r + (r^2/x)]$
Volumetric impedance in series	Z_s	Ohms	$D_L^2/ (r^2 + x^2)^{0.5}$
Volumetric impedance in parallel	Z_p	Ohms	$D_L^2/ [r \cdot x (r^2 + x^2)^{0.5}]$
Phase angle	A_P	Degrees	$\text{atan}(x/r) \cdot 180/\pi$
Standardized phase angle	D_LA_P	Degrees	$D_L [\text{atan}(x/r) \cdot 180/\pi]$

Table 2. Relations between actual total body water and impedance equations for Channel Catfish, *Ictalurus punctatus*, at each measured position. (n = 134) Lateral line (LL), dorsal total length (DTL), dorsal midline (DML), ventral total length (VTL), ventral midline (VML), and dorsal to ventral pre-anal fin (DTVpre), dorsal to ventral post-dorsal fin (DTVpost), including the model using only body measurements of wet weight (WW) and total length (TL).

Model	Position	Intercept (SE)	Slope (SE)	Predictor	*p* value	R^2
1	n/a	-14.2975 (5.7490)	0.6878 (0.0155)	WW	<0.0001	0.9968
			0.0756 (0.0300)	TL		
2	LL	-7.0552 (1.7707)	4.5731 (0.0622)	R_s	<0.0001	0.9822
3	DTL	-3.7048 (1.7572)	4.3316 (0.0598)	R_s	<0.0001	0.9816
4	DML	-7.6877 (2.5046)	5.6434 (0.1084)	R_s	<0.0001	0.9651
5	VTL	0.5547 (3.3336)	4.8054 (0.1305)	R_s	<0.0001	0.9326
6	VML	13.9097 (3.3352)	7.0480 (0.2112)	R_p	<0.0001	0.9192
7	DTVpre	-12.9940 (4.1010)	44.5680 (1.3630)	X_p	<0.0001	0.9161
8	DTVpost	-18.5509 (5.2452)	17.2444 (0.6536)	R_s	<0.0001	0.8766

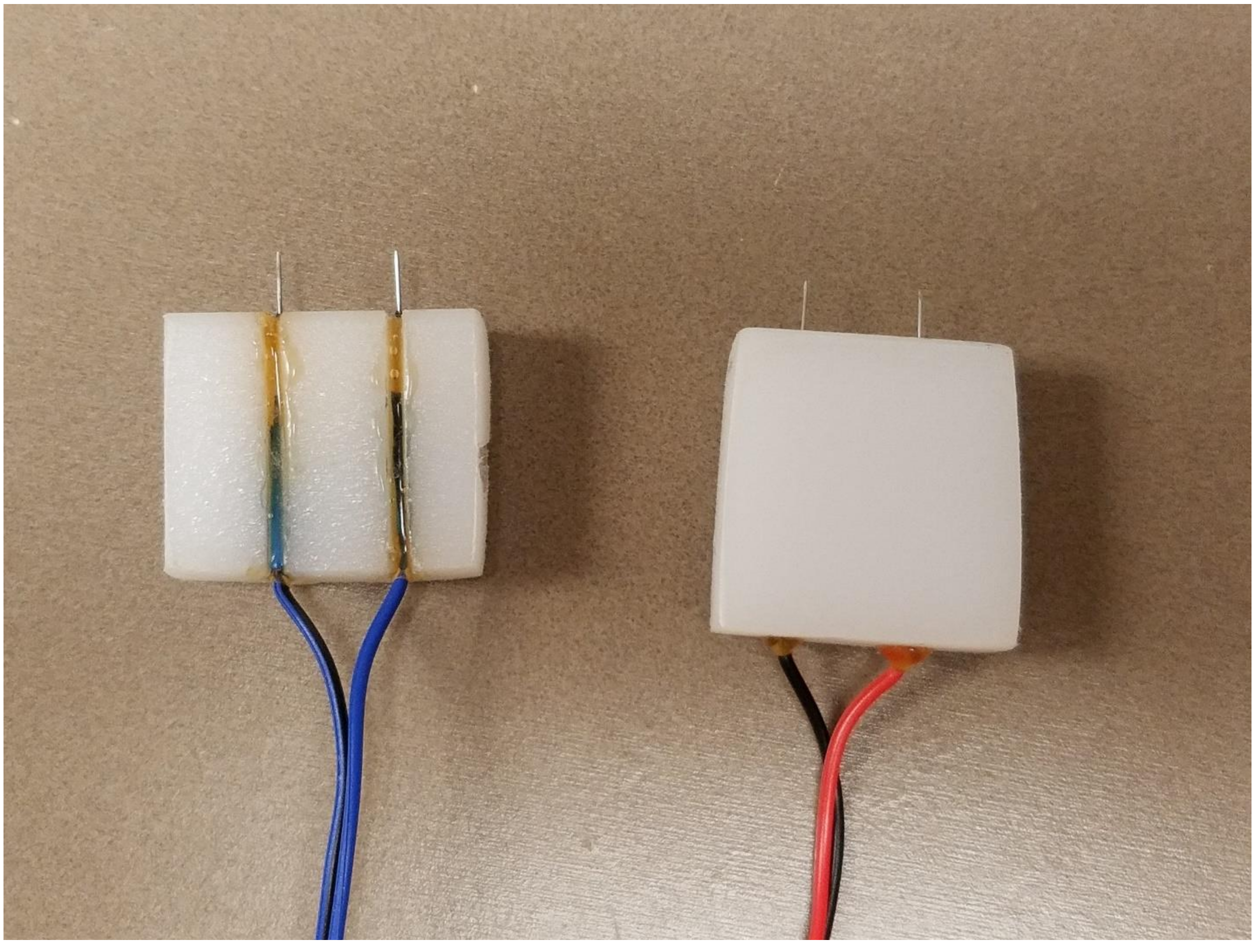

Figure 1. Electrodes used to take BIA measures on individual fish are set. Two sets of electrodes are necessary to take measures. Electrodes are 6mm in length and set 10mm apart in a plastic block.

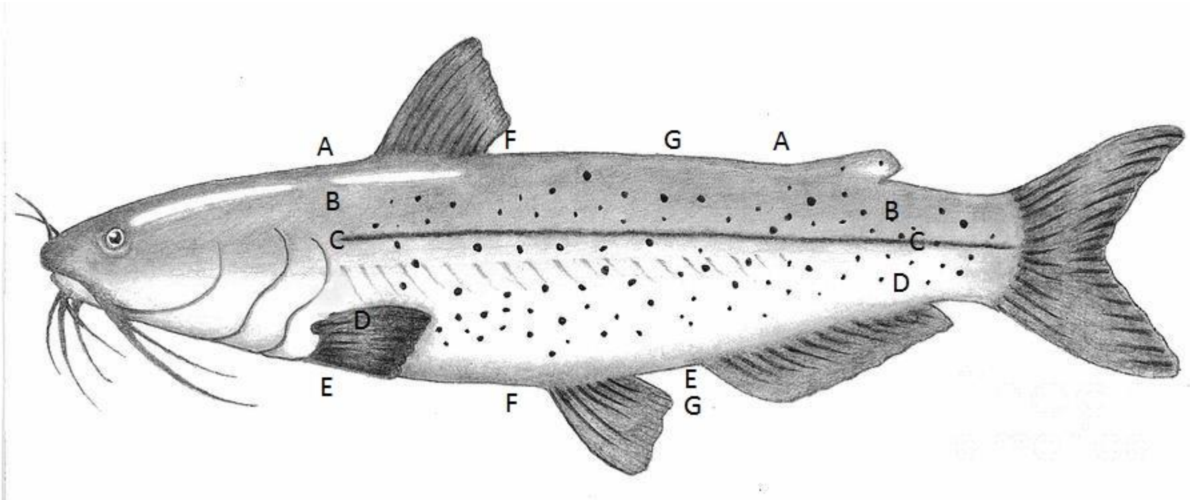

Figure 2. Electrode locations: (A) dorsal midline (DML), (B) dorsal total length (DTL), (C) lateral line (LL), (D) ventral total length (VTL), (E) ventral midline (VML), (F) dorsal to ventral post-dorsal fin (DTVpost), and (G) dorsal to ventral pre-anal fin (DTVpre).

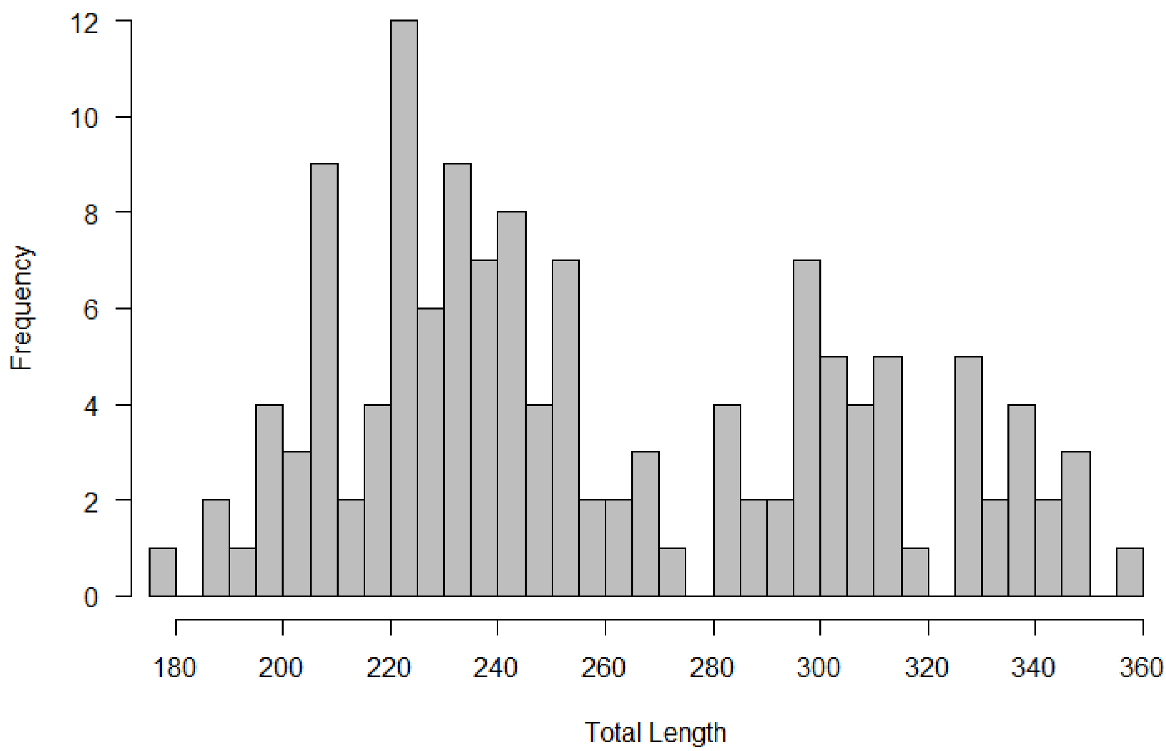

Figure 3: Frequency histogram of the total length distribution of the Channel Catfish, *Ictalurus punctatus* (n = 134).

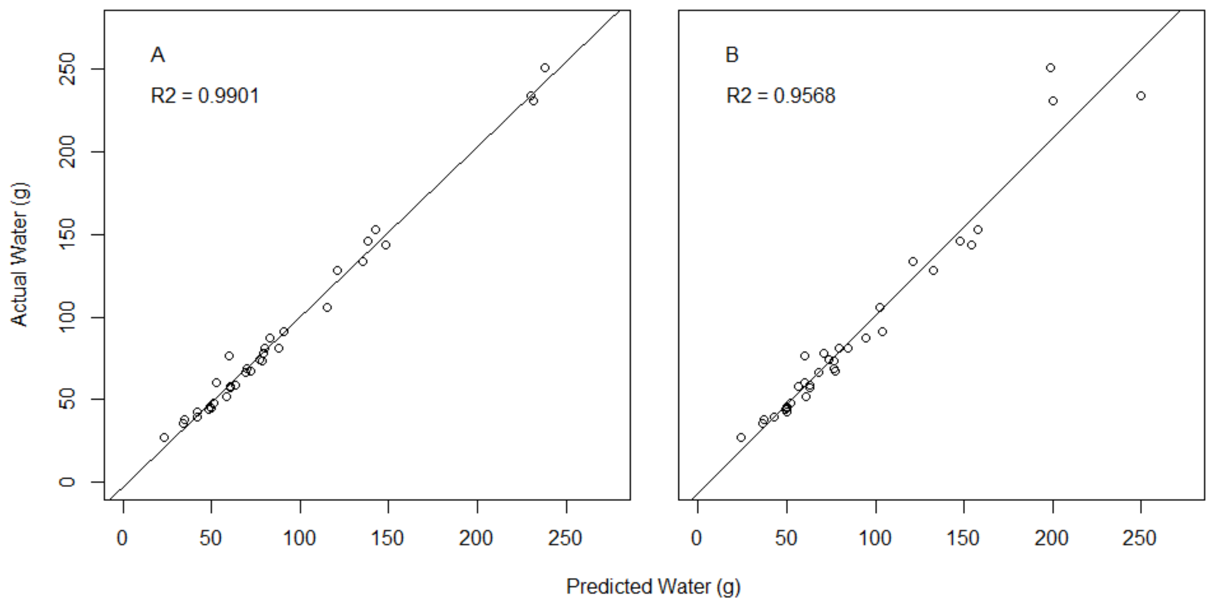

Figure 4: Correlations of predicted versus actual water content in Channel Catfish, *Ictalurus punctatus*, including (A) dorsal total length (DTL) and (B) dorsal midline (DML) positions, which were the best supported models.

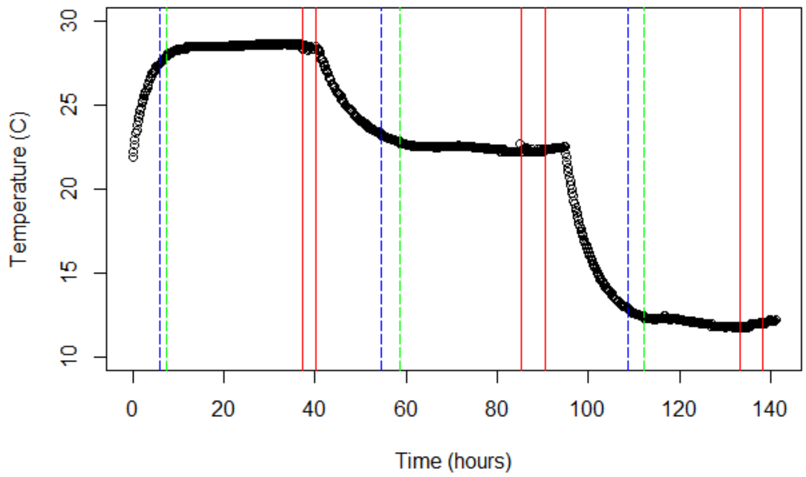

Figure 5. Actual changes in temperature of the holding tanks for the temperature experiment. The acclimation period began when water reached a temperature within 1° of the measurement temperature, indicated by the dotted blue line. The dotted green line indicates the time at which the water was within 0.5° and the red lines indicate the time period of measurement.

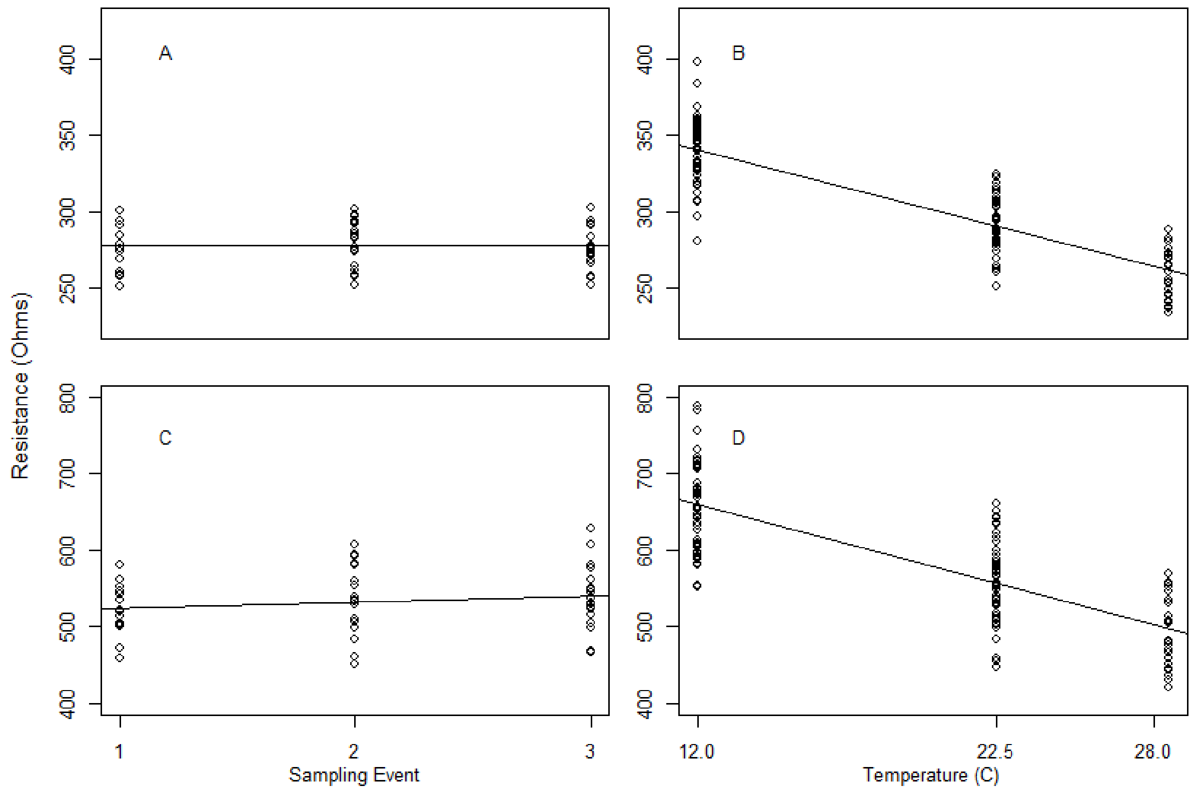

Figure 6. Linear models of temperature effects on resistance (ohms), including (A,C) control fish and (B,D) experimental fish. The DML position is represented in plots A and B, while the DTL position is in C and D. The sampling events (A,C) are the days on which control fish were measured, corresponding to the high, mid, and low temperature BIA measurements (B,D).

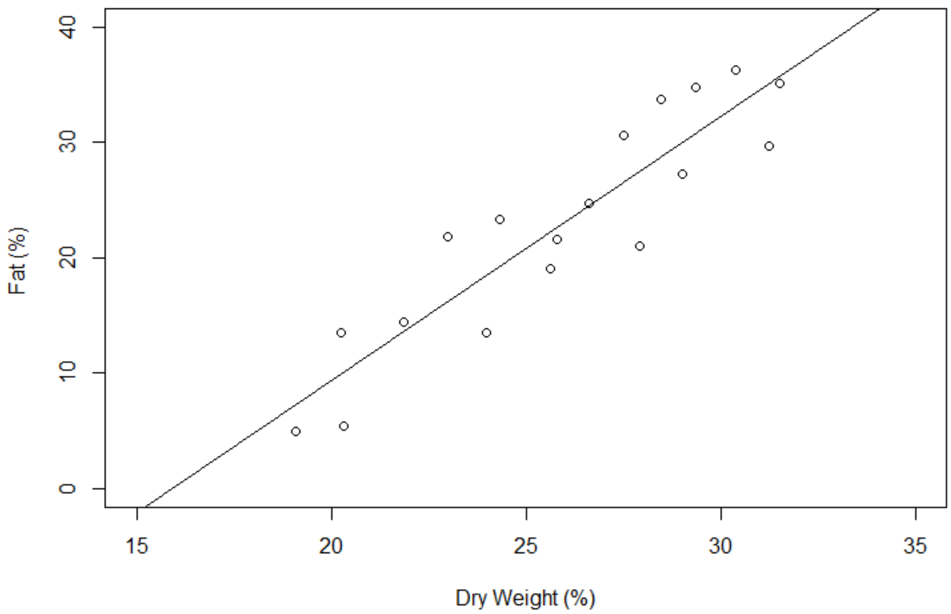

Figure 7. Correlation demonstrating strong relation between %DM and %TBF (on a dry weight basis). This relation is fitted by: $y = 2.29012\, x - 36.4793$

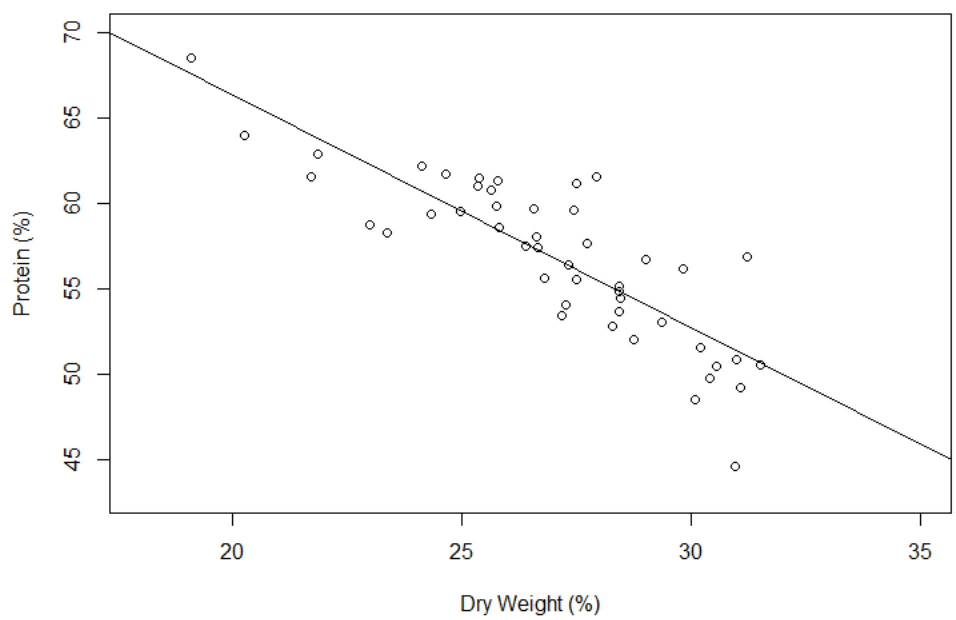

Figure 8. Correlation between %DM and %TBP (on a dry weight basis). This relation is fitted by: $y = -1.3611\,x + 93.5642$