The Use of Bioelectrical Impedance Analysis for Estimating the Body Composition of Various Fish Species

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ABSTRACT

The reliable measurement of growth and condition is vital for effective fisheries assessments. Biologists have long attempted to estimate condition for their assessments, but a reliable method to nonlethally estimate body composition is lacking. Proximate analysis is the most dependable and accurate method for estimating internal composition, but it is lethal, time consuming, and expensive. Recent research has shown bioelectrical impedance analysis (BIA) to be an effective method for estimating proximate composition in some fishes. The technique is quick, inexpensive, and, most importantly, nonlethal, which is vital when examining endangered species or cultured fish. My research focused on developing BIA indices for several new species of fish, using those indices to evaluate the body composition of fish in the field, and determining whether water temperature influenced resistance and reactance measurements. I found that BIA accurately estimated the body composition of bluegill *Lepomis macrochirus*, redear sunfish *Lepomis microlophus*, brook trout *Salvelinus fontinalis*, and northern logperch *Percina caprodes* ($r^2 \geq 0.71$, $p < 0.0001$). I also determined that bluegill and redear regressions were not significantly different ($P \geq 0.10$) suggesting they can be used interchangeably during future studies. Laboratory studies revealed that water temperature did not significantly influence resistance and reactance measurements of bluegill, redear, and largemouth bass *Micropterus salmoides* ($P \geq 0.18$). These results, along with previous literature, indicate that BIA may be an accurate and reliable assessment tool for fisheries biologists.

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CHAPTER 1

Introduction to Assessing Condition and Proximate Composition

Energy and condition

Animals must obtain food for the production of new tissue, repair and maintenance of existing tissue, and reproduction as well as ongoing processes such as metabolism and locomotion (Randall et al.1997). Excluding water, the body is primarily composed of proteins and lipids (Higgs et al. 1995), which are essential in the growth process of animals (Hill et al. 2004). Lipids are also crucial energy stores on which organisms often must rely during times of starvation. Lipids produce nearly twice as much energy per unit weight as do proteins or carbohydrates, thus enabling animals to limit their body mass without constraining energy stores (Randall et al. 1997). This makes lipids the preferred source for stored energy, as evidenced by the high lipid levels in many fishes (Tocher 2003). Seasonal changes in the environment and the reproductive cycle lead to the deposition or utilization of these energy sources (MacKinnon 1972; Reznick and Braun 1987). If current energy expenditure is not met by food intake, existing energy stores are used to make up the difference. High body condition is thought to reflect the ability of an organism to meet these energy requirements (Baker 1989).

Brown and Murphy (1991) described physiological condition as the ability of an individual to compete successfully, maintain its bodily functions, and cope with the surrounding environment. Schulte-Hostedde et al. (2001) more simply referred to condition as the energetic state of an animal. It has also been defined as the relative fatness (Jakob et al. 1996) or plumpness (Simpkins et al. 2003) of a fish. In theory, an increase in condition should translate to higher fitness, which has been defined as the relative probability of genetic transfer from an individual to the population (Brown et al. 1993), or simply growth, survivability, and reproductive success of an organism (Pope and Kruse 2007). Animals that more efficiently

acquire and assimilate necessary energy supplies have the ability to sustain a higher level of fitness than others (Arrington et al. 2002).

The health of an organism is critical to the survival of not only itself, but also its population and potentially the species as a whole. Understanding the body condition of animals living within a given ecosystem may be informative about not only the fitness of the organism and the status of the population, but the condition of the surrounding area as well (Speakman 2001) . Biologists have developed and researched a variety of methods to assess condition or body composition of fish, but each has its limitations.

Review of condition indices

As described above, condition refers to the ability of an individual to compete successfully, maintain vital functions, and survive in its environment (Brown and Murphy (1991). Researchers have attempted to estimate or quantify condition through a variety of statistical methods using length and weight measurements to create condition indices. Biologists have used condition indices to assess the effects of habitat (Colle and Shireman 1980), trophic interactions (Lammens et al. 1985), parasitism (Morton and Routledge 2006), anthropogenic disturbances (Rice et al. 2001), water chemistry (Wiener and Hanneman 1982), and harvest limits (Eder 1984) on fishes. Their quick and simple results, coupled with their noninvasive nature, make condition indices ideal for field studies. There are three primary morphological indices used to evaluate the condition of fish: Fulton condition factor (K for metric units or C for English units; Fulton 1904), relative condition factor (K_n; Le Cren 1951), and relative weight (W_r; Wege and Anderson 1978).

The Fulton condition method was developed as one of the first condition indices for use in fisheries science, and is calculated as

$$K = (W/L^3) \text{ x } 10,000 \tag{1.1}$$

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where W represents weight and L represents total length when using metric units (g and mm). A flaw of the index is that it assumes isometric growth (Richter et al. 2000), which is not the case for most fishes (Le Cren 1951). Isometric growth refers to growth that occurs at the same rate for all parts of an organism throughout its life. Another drawback of K is that direct comparisons cannot be made between species, or between fish with considerable length differences (Murphy et al. 1991; Blackwell et al. 2000). Biologists have developed more complex indices to help solve many of the problems associated with K.

The relative condition factor was developed as an alternative index that does not assume allometric growth, and is calculated as

$$K_n = (W/W') \tag{1.2}$$

where W represents weight of a subject fish and W' is a length-specific mean weight for the population of interest. This approach prohibited comparisons between populations until Swingle and Shell (1971) created length-weight regression standards for 25 species of fish using statewide data sets from Alabama. This development allowed biologists to make local and even regional comparisons using the K_n index (Murphy and Willis 1992). Although these more-comprehensive data sets expanded the capabilities of the K_n index, Murphy et al. (1990) emphasized the importance of including data from the entire range of the species when developing standards. The index may accurately estimate the condition of fish populations within the region where researchers developed the species-specific standards, but biologists should be conscious of potential mischaracterizations when K_n regressions are used outside of the area in which they were developed. Additionally, the index continues to assume the slopes of all sample regressions are equivalent to those of regressions used during initial model development. Many times that assumption is not accurate, which may prevent accurate results (Cone 1989). Sutton et al. (2000) found that the K_n index misinterpreted changes in total body fat of Atlantic salmon *Salmo salar* due to the confounding effects of body-water dynamics.

Trudel et al. (2005) also found poor correlations between the K_n index and energy density of coho salmon *Oncorhynchus kisutch* and Chinook salmon *O. tshawytscha*. Due to the drawbacks associated with the K_n index, biologists created an even more comprehensive index -- relative weight.

Wege and Anderson (1978) created the relative weight index, currently the most commonly used condition index in fisheries science (Blackwell et al. 2000), with the intention of providing fisheries scientists with a viable option for inter-populational comparisons between regions. Relative weight is calculated as

$$W_r = (W/W_s) \text{ x } 100 \tag{1.3}$$

where W is the weight of the individual and W_s represents a length-specific standard weight predicted by a geographically broad species-specific weight-length regression. These species-specific standards, rather than the population or state-specific standards used for the K_n index, allow biologists to use the W_r index across large geographic areas. Rather than using what is considered to be the average-sized fish for W_s development as was the case with the development of the K_n index, the W_r index uses the species 75th percentile of weight at a given length as the input for regression development. Biologists may prefer the W_r index to other indices since agencies typically manage for above-average fisheries. The careful development of the index has eliminated many of the inherent problems associated with the previously described indices and may reflect the general nutritional status of fishes, but it falls short as a reliable and precise indicator of body composition in wild fish (Copeland et al. 2008).

Although many researchers have experienced success using condition indices to estimate body composition (e.g., Salam and Davies 1994; Copeland and Carline 2004; Pangle and Sutton 2005), many biologists have also failed to find significant relationships between condition indices and body composition (e.g., Simpkins et al. 2003; Trudel et al. 2005). Simpkins et al. (2003) found that the W_r index did not accurately reflect the lipid content of rainbow trout *O.*

mykiss. Jonas et al. (1996) also experienced similar difficulties estimating the energy content of muskellunge *Esox masquinongy* using the W_r index. Mommsen (1998) claimed that changes in body water can reduce the correlation between the W_r index and lipid content for reasons similar to those reported by Sutton et al. (2000) with regards to the influence of body water on the K_n index. Additionally, the W_r index may have difficulty predicting the condition of plastic species such as bluegill *Lepomis macrochirus* (Copeland et al. 2008).

Condition indices may save time and money, but biologists must be aware of the potential inaccuracies of these methods. Murphy et al. (1990) asserted that an ideal index must use similar methodology regardless of test subject, be subject to statistical analysis, be reliable even with small sample sizes, and be useable with various sampling methods. Although some indices may meet these criteria some of the time, that is not always true. The inaccuracy of condition indices in certain situations emphasizes the importance of using a more-reliable technique when attempting to evaluate body composition.

Techniques for determining body composition

Proximate composition analysis is widely accepted in the scientific community as the most reliable method for assessing body composition. Biologists can use a variety of chemical analyses to assess protein, lipid, ash, and water content of specific organs or the entire animal. Although it is the only direct method available to researchers for determining body composition, the technique is lethal, costly, time consuming, technically demanding, and potentially dangerous for researchers. Proximate analysis requires toxic chemicals, which creates safety and disposal concerns. The detailed and lengthy laboratory methods make large sample sizes difficult to analyze quickly and cost efficiently. The lethality of proximate composition analysis to test subjects negates applicability in research with endangered species, and the inability to repeatedly test live subjects makes the technique undesirable for some experiment designs. Due to these

inadequacies, researchers have attempted to develop nonlethal methods for evaluating the body composition of animals.

Total body electrical conductivity (TOBEC) is one of a few nonlethal methods developed to estimate multiple proximate body composition parameters. This method was originally developed for the agricultural industry but has found its way into numerous studies with fish (Bai et al. 1994; Lantry et al. 1999; Novinger and Del Rio 1999) and other wildlife (Castro et al. 1990; Scott et al. 1991; Golet and Irons 1999). The TOBEC equipment consists of a solenoid coil that produces an electromagnetic field. The study subject, which must be small, is placed within the coil where the instrument measures the conductivity of the organism through disturbances in electromagnetic fields. These measurements are then correlated with proximate analysis results to estimate body composition. A major drawback to TOBEC is that it uses sensitive and bulky instruments, thereby inhibiting field use. Although some researchers have had some success using TOBEC (e.g., Bai et al. 1994), most have discovered inherent problems with the equipment and methodology. Lantry et al. (1999) found that TOBEC accurately measured the body composition of yellow perch *Perca flavescens* and alewife *Alosa pseudoharengus* under ideal conditions, but errors increased as the test subject's weight increased or compositional changes took place. Novinger and Del Rio (1999) found that TOBEC did not provide accurate estimates of brook trout *Salvelinus fontinalis* lipid mass even under ideal conditions. The unreliability and inefficiency of this methodology prohibits widespread use of TOBEC in scientific research.

Another recently developed method for estimating the body composition of live fish, microwave transmission, uses a handheld instrument that emits low-powered microwaves used to measure body water. Like TOBEC, microwave transmission is nonlethal and minimally invasive, but the technique produced inconsistent results during testing. Colt and Shearer (2001) could only qualitatively characterize Chinook salmon as having "high" or "low" lipid levels.

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Although Crossin and Hinch (2005) demonstrated greater success estimating energy stores of Pacific salmon *Oncorhynchus* spp., they did not report the instrument's ability to estimate the protein or ash content of fish, and cautioned that accuracy may decline with low lipid levels (< 2.5 %). Therefore, microwave transmission has produced mixed results and remains relatively untested.

The complexity of these methodologies, costs associated with analysis, field limitations, and insensitivity place serious limitations on the methods described above. These restrictions emphasize the need for a broadly applicable technique that biologists can use to accurately estimate body composition. Researchers need a nonlethal technique that can be implemented reliably in the field, in a variety of systems, throughout the year, and on a variety of species.

Bioelectrical impedance analysis

Widespread research on human condition using impedance measurements did not occur until the 1980's (Lukaski et al. 1985; Kushner and Schoeller 1986; Lukaski et al. 1986), even though the relationship between body water and impedance was reported earlier (Thomasett 1962). As of 2003 (Kyle et al. 2004), there were over 1600 papers available in medical journals on bioelectrical impedance analysis (BIA). In recent years the method has been used to assess patients suffering from liver disease (Pirlich 2000), obesity (Ko et al. 2001), HIV (Schwenk et al. 2000), and cancer (Toso 2000).

To utilize bioelectrical impedance analysis, researchers measure the impedance (i.e., resistance and reactance) of the test subject's tissue using a tetrapolar bioelectric impedance analyzer. Ohm's law states that resistance is proportional to the voltage of an applied current as it passes through a substance. Reactance is the opposition to a current by a capacitor. The electrical conductance of an organism is determined by its fluids and tissues. Lipids and bone act as insulators and are much less conductive than body water and tissue. An increase in fat or bone will increase the resistance of an organism, which is directly measured by the instrument.

Cell membranes are surrounded by a layer of lipid that is enveloped by two conductive protein layers. The cells maintain an ionic gradient, allowing certain materials to pass through the outer membrane via protein channels. The channels permit the passage of the electrical signal from the BIA instrument through the membrane, thereby charging the inside of the cell. This leads the cell to act as a capacitor, which the analyzer detects and measures as reactance of tissue. The instrument uses low voltages and high frequencies (800 µA, AC and 50 kHz), which allow the current to pass through extracellular fluids but not through the cell membranes. These properties allow BIA to measure the conductance of several body components; including total body water, total body protein, dry mass, total body ash, fat-free mass, and total body fat. Resistance and reactance are used to calculate common electrical property equations, which include resistance in series and parallel, reactance in series and parallel, and capacitance (Cox and Hartman 2005). Researchers can develop indices for body components by relating the electrical property equations with actual composition values determined in the laboratory. These capabilities make BIA a valuable asset for a variety of studies on not only humans, but also livestock, wildlife, and fishes.

The BIA technique takes only seconds to complete, whereas proximate analysis requires significantly more time (Scott et al. 2001). The simple methodology results in very precise readings (Segal et al. 1985) and does not require extensive training as do other techniques. Unlike other methods, BIA uses a small handheld instrument that can easily be transported into the field. The equipment is also significantly less expensive than other methods used to measure proximate composition. Most importantly the procedure is nonlethal which is vital when examining valuable subjects such as endangered species, trophy game species, and cultured fish populations. The method allows for repeated measurements of an individual subject whereas proximate analysis is lethal. Another favorable attribute of BIA is that it estimates the composition variables of the individual (e.g., total body fat), which are more recognizable to the

general public than results produced by condition indices, thus allowing biologists to more effectively communicate with stakeholders. Better communication between biologists and the public can lead to more-effective management of fisheries.

Researchers first applied BIA to livestock species in the late 1980's (Cosgrove et al. 1988; Jenkins et al. 1988). After become established in the medical literature, BIA received a great deal of attention from the agriculture industry and found its way into numerous studies (e.g., Swantek et al. 1992; Marchello and Slanger 1994; Thomson et al. 1997). Concurrently, wildlife biologists were using BIA to assess the condition of wombats *Lasiorhinus latifrons* (Woolnough et al. 1997); seals *Halichoerus grypus* (Gales et al. 1994; Arnould 1995); bears *Ursus americanus, U. arctos, and U. maritimes* (Farley and Robbins 1994; Hilderbrand et al. 1998); moose *Alces alces* (Hundertmark and Schwartz 2002); and skunks *Mephitis mephitis* (Hwang et al. 2005). Some researchers reported discouraging results due to their inability to properly restrain test subjects (Bowen et al. 1998), inadequate sample sizes (Hilderbrand 1998), inconsistent methodology (Hundertmark and Schwartz 2002) or imprecision due to injured test subjects (Farley and Robbins 1994). The technique first appeared in the fisheries literature when Bosworth and Wolters (2001) examined fat content of channel catfish *Ictalurus punctatus* fillets using BIA. Cox and Hartman (2005) made the first attempt to assess live fish using BIA, and Margraf et al. (2006) and Duncan et al. (2007) are the only other studies to date using BIA on fish. All three studies experienced notable success, possibly due in part to the less-complicated body forms of fish relative to other vertebrates. The fusiform body shape of most fish allows simple readings to effectively characterize whole-body impedance. Cox and Hartman (2005) also reported only slight bruising of the subject fish with no effect on swimming, coloration, or feeding. Even with the newfound success of BIA in fisheries science, there are still important questions that need to be answered such as the influence of temperature on BIA readings.

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Understanding the effects of temperature on BIA will allow biologists to account for changes in body temperatures encountered in wild fish. Since most fish are ectotherms, their body temperature fluctuates with changing water temperatures. Cox and Hartman (2005) found that temperature may affect BIA. Conversely, Hill et al. (1967) observed only minor conductivity changes in various unfrozen animal tissues in response to temperature changes, suggesting that temperature may only have negligible effects on BIA readings. The dynamic health of fish requires multiple assessments throughout the year to fully understand their true condition. A better understanding of the effects of temperature on BIA readings would permit biologists to make more-precise temporal and spatial comparisons.

Although condition indices may be suitable for evaluating the condition of some species during certain times of the year, BIA may provide biologists with an additional option for estimating the body composition of fish when traditional methods (e.g., proximate analysis) are inadequate. Additionally, BIA could provide the aquaculture industry with a valuable tool to continually assess the fat content of their product, which is often monitored meticulously throughout the life of the fish. Quickly and nonlethally estimating the condition of fish will help increase profit margins for the industry, while helping to eliminate wasteful feeding regimes and resultant water quality degradation. The new method might provide biologists with a means of determining the body composition of endangered species after developing BIA indices using a similarly shaped species as a surrogate. While the technique is still in its infancy, BIA appears to be a promising approach for assessing the body composition of fish.

I applied BIA to a variety of species and field situations to help better understand the potential use of the method. Only a handful of species and field studies have been evaluated using the new method (Cox and Hartman 2005; Margraf et al. 2006; Duncan et al. 2007). For this study, I applied BIA to various fish in lentic and lotic environments as well as coldwater and

warmwater systems over a variety of temporal and geographic scales. The specific objectives for this study were as follows:

1) Develop BIA indices for bluegill, redear sunfish *L. microlophus*, brook trout, and logperch *Percina caprodes*.

2) Evaluate cross-species similarity and potential cross-species applicability of BIA indices.

3) Test BIA methodologies in the field.

4) Determine whether temperature has a significant effect on impedance readings of largemouth bass *Micropterus salmoides*, bluegill, and redear sunfish.

5) Determine whether needle-insertion methodology for BIA influences BIA index models.

CHAPTER 2

The Use of Bioelectrical Impedance Analysis to Evaluate the Body Composition of Bluegill, Redear Sunfish, Brook trout, and Northern Logperch

Abstract

Until recently, fisheries biologists lacked a nondestructive method capable of reliably estimating the body composition of fish. Recent research has shown bioelectrical impedance analysis (BIA) to be an effective method for nonlethally estimating the body composition of some fish. Using linear regression, I developed BIA indices for bluegill, redear sunfish, brook trout, and northern logperch that can be used to accurately estimate the body composition of these species in the field. I regressed BIA readings against specific proximate composition parameters (e.g., total body fat) to describe their functional relationships. I also determined that bluegill and redear regressions were not significantly different indicating that these regressions could be applied across similarly shaped species such as salmonids or other sunfish. Using BIA to nonlethally estimate the body composition of fish may help fisheries biologists better assess their respective fisheries and potentially decrease the environmental impact of aquaculture facilities. The technique could also allow biologists to more directly assess the body composition of endangered species by using similarly shaped and related species as surrogates for index development. Although it is a relatively new technique, it appears that BIA could provide biologists with a viable technique for nonlethally estimating the body composition of fish.

Introduction

Animals must obtain food for the production of new tissue, repair and maintenance of existing tissue, and reproduction (Randall et al. 1997). If current energy expenditure is not met by food intake, energy stores in lipids and proteins are used in an attempt to make up the difference. The high energy content of lipids makes them the primary source of metabolic energy for many fishes (Tocher 2003). Lipid availability is important for overwinter survival (Oliver et al. 1979; Thompson et al. 1991) and reproduction (Wootton 1985; Reznick and Braun 1987). Consequently, biologists have long attempted to evaluate these lipid stores to characterize the health or energetic status of an individual (i.e., condition). However, the lack of a reliable, nonlethal method for estimating body composition may limit fisheries biologists' understanding of the productivity and status of aquatic ecosystems – especially for researchers evaluating threatened and endangered species.

Biologists currently rely on proximate analysis to evaluate body composition and morphometric condition indices to assess condition. Proximate analysis serves as the standard for estimating the body composition of animals (AOAC 2002) and is the only direct method for determining body composition. Unfortunately, the method is lethal, time consuming, and technically demanding. These drawbacks make it impractical for studies with large sample sizes or research on endangered species. Its lethality also precludes the analysis of repeated measures on the same individual. As an alternative to proximate analysis, researchers often rely on condition indices for their assessments but, depending on the time of year, particular indices may produce inconsistent results thereby making the selection of an appropriate index crucial (Bolger and Connolly 1989). For example, gonad development during the spawning season may confound the results of certain indices, leading to inaccurate characterizations of test subjects.

Biologists can misinterpret the results of condition indices, leading to inappropriate management strategies (Murphy et al. 1991).

In recent decades, nonlethal electronic methods have been developed for estimating body composition. A once-promising method for estimating the internal composition of fishes, total body electrical conductivity (TOBEC), uses an electromagnetic field to measure the conductivity of the animal, thereby estimating its internal composition. The method has been examined by several researchers but they have demonstrated limited success. A major disadvantage of TOBEC is that the large instrument used during analysis is not well-suited for field studies, and environmental conditions such as wind and rain can interfere with the analysis (Scott et al. 2001). Novinger and Del Rio (1999) found that, even under ideal conditions, TOBEC did not reliably estimate lipid mass. Lantry et al. (1999) reported that errors in TOBEC readings increased with weight and changes in total body water. A method new to fisheries biologists, bioelectrical impedance analysis (BIA), may provide researchers with a viable alternative to proximate analysis and condition indices (Cox and Hartman 2005; Margraf et al. 2006; Duncan et. al. 2007).

Bioelectrical impedance analysis is a quick, inexpensive and, most importantly, nonlethal technique for estimating body composition. The handheld instrument, a tetrapolar bioelectric impedance analyzer (RJL Systems, Detroit, MI), measures the resistance and reactance of the subject fish. The device can easily be transported and used in the field, even during harsh weather conditions. Biologists can take readings quickly and analysis causes only slight bruising from the needles used during testing (Cox and Hartman 2005). Toxic chemicals are not required after index development, thereby reducing BIA's environmental footprint.

The BIA analyzer uses low voltages and high frequencies that allow the current to pass through extra cellular fluids, but not through the cell membranes. Due to increased water and electrolyte content, lean tissues experience higher electrical conductivity than tissues high in

lipids. These electrical properties permit researchers to distinguish differences or changes of tissues within the test subject. Researchers can determine the common electrical properties of the test subject using the resistance and reactance measured by the analyzer. Using regression analysis, researchers can then correlate the estimated body composition (BIA) with proximate analysis results to predict total body fat (TBF), total body protein (TBP), total body water (TBW), dry mass (DM), fat-free mass (FFM) and total body ash (TBA). These regressions provide future researchers with 'BIA indices', which are nonlethal estimators of body composition for each species examined.

The primary objectives for this study were as follows: 1) develop BIA indices for bluegill *Lepomis macrochirus*, redear sunfish *L. microlophus*, brook trout *Salvelinus fontinalis*, and northern logperch *Percina caprodes*; 2) determine similarities of species-specific BIA indices; and 3) test BIA methodologies in the field. The species that I selected for this study represent a wide range of body shapes and sizes, and include several popular game fishes as well as a surrogate species (northern logperch) for the endangered Roanoke lopgerch *Percina rex*.

Methods

I measured the resistance and reactance of anaesthetized fish in the field using a tetrapolar bioelectric impedance analyzer (RJL Systems, Detroit, MI). After analysis, fish were euthanized and subjected to proximate analysis. The measured body-composition components (proximate analysis) were regressed on the field-estimated components (BIA) for each species to develop an individual regression for each body composition parameter. I tested the cross-species utility of bluegill and redear sunfish indices by estimating the body composition of independent test groups for each species. I used the coefficients of determination for the regressions developed using the index-estimated body compositions and the proximate analysis values to evaluate the predictive strength of the BIA regressions. The BIA indices for bluegill and brook

trout were applied to additional fish analyzed in the field to further evaluate their field

applicability. Details of the each aspect of the study are provided below.

BIA Index Development and Validation

I collected bluegill and redear sunfish from Briery Creek Reservoir (BCL) in Prince

Edward County, Virginia, brook trout from Laurel Branch in Giles County, Virginia, and

northern logperch from the New River also in Giles County, Virginia to develop new BIA

indices for each species. I anesthetized fish using clove oil (Sigma-Aldrich, St. Louis, MO,

USA) and placed them on a nonconductive measuring board for analysis. In addition to

individual weights and total lengths, I measured resistance and reactance with a bioelectric

impedance analyzer using two sets of 12-mm, 28-gauge stainless steel subdermal needles (Grass

Telefactor, West Warwick, Rhode Island); each set has one signal electrode and one detecting

electrode spaced 1.0 cm apart with the signal electrodes positioned on the outside. I placed one

set of electrodes at the posterior apex of the operculum and the second set along the posterior

region of the caudal peduncle (at the posterior edge of the adipose fin for brook trout and

approximated for all other species; see Cox and Hartman 2005 for diagram). I inserted the

electrodes approximately 3 mm through the skin. I measured the distance between the signal

electrodes and recorded resistance and reactance. These two impedance values then were used to

calculate common electrical properties: resistance in series and parallel, reactance in series and

parallel, and capacitance (Cole 1932; Cox and Hartman 2005). These values were later used as

independent variables in the regression models.

After I completed BIA, fish were euthanized using an overdose of clove oil, immediately

placed on ice, and stored at -20º C. I later homogenized fish using an industrial blender and

collected samples for proximate analysis at the Virginia Tech Aquaculture Center. Using the

method described in Official Methods of Analysis of AOAC International (2002), I estimated

DM, TBA, and TBP, using the block digestion method. I measured TBF using the

chloroform/methanol extraction method originally described by Bligh and Dyer (1959). For all

species except brook trout, I estimated TBW by subtracting DM from total body weight and

determined FFM by subtracting TBF from body mass. I determined the TBW of brook trout by

freeze drying whole fish and then subtracting dry mass from wet mass. All proximate

measurements are expressed in grams and were performed in duplicate and averaged for the final

value. I used these values as the dependent variables for regression analysis. I selected the best-

fit model (i.e., the regression resulting in the highest r^2 value) for each body composition

component after completing linear regression analyses using each one of the common electrical

properties described above. Independent BIA indices were developed for each proximate

component for all four species.

To test the predictive strength of the bluegill and redear indices, I used the best-fit BIA

indices described above to estimate the body composition of two independent test groups

(validation group) - one for each species. After incorporating the BIA measurements into the

appropriate indices, I used linear regression analyses to determine the coefficients of

determination of the estimated (BIA) and actual (proximate analysis) body composition

components.

Cross-validation of Species-specific Indices

After determining the best-fit model for each body composition parameter, I compared

the similarity of the slopes and intercepts of the redear sunfish and bluegill indices. Using

parallel and intercept tests (see Paulson 2007), I compared the slopes and intercepts for each

independent calibration model to determine if there was a significant difference ($P \leq 0.05$)

between the two species. Using ANOVA, I simultaneously compared the slopes and intercepts

of all four species-specific indices to determine whether the regressions were significantly

different ($P \leq 0.05$). To further validate the similarity and accuracy of the bluegill and redear

sunfish indices, I used both sets of sunfish models to estimate the body composition of the

independent redear group. Using ANOVA, I compared the body compositions derived from bluegill and redear indices with the proximate analysis results to determine if there was a significant difference ($P \leq 0.05$) among the three estimates.

Field Applications

Using the bluegill TBF index, I monitored the body composition of several bluegill and redear sunfish populations biweekly from May through July of 2006 in two Virginia reservoirs: BCL and Sandy River Reservoir (SRR; also located in Prince Edward County, Virginia). I measured total length, weight, and impedance for the first 30 bluegill and redear collected during electrofishing surveys using the same BIA methodologies described above with one exception: all fish were released following BIA. Immediately following analysis, I placed fish in freshwater, allowing them to recover before release. Using the bluegill BIA index described above, I estimated the TBF (g) of bluegill and redear for each sample throughout the summer and converted those estimates to percentages using the body mass of each fish. The mean TBF was calculated for bluegill and redear in each lake, on each sampling date.

I also evaluated the TBF (g) of native brook trout in 11 Virginia streams. Initial survey reaches were 50 m, but I extended my sampling effort if I collected less than 10 adult brook trout within the original survey reach. For BIA, I used the same procedures described above for the reservoir study. I estimated the TBF (%) for all trout using the same methods described in the reservoir study. I also estimated the TBF for brook trout collected in streams containing only natives (n = 5 streams) as well as brook trout in streams co-occurring with nonnative trout (n = 6 streams).

Results

BIA Index Development

All fish that I did not euthanize for index development or cross validation recovered from the anesthetic quickly and were released under their own power. Proximate analysis revealed a wide range of body composition values for the fish that I used for development of BIA indices, leading to broad impedance ranges (Table 1; Table 2). I found highly significant correlations between BIA and proximate composition for each BIA body composition index for all species examined: bluegill ($r^2 \geq 0.90$; Figure 1; Table 3), redear sunfish ($r^2 \geq 0.91$; Figure 2), brook trout ($r^2 \geq 0.86$; Figure 3), and logperch ($r^2 \geq 0.71$; Figure 4). Each BIA index was significant at $p < 0.0001$. The logperch TBA ($r^2 = 0.71$) and TBF ($r^2 = 0.75$) indices exhibited the two lowest correlations for this study. The greatest correlations for each species were: bluegill (FFM and TBW, $r^2 = 0.99$), redear (TBP and DM, $r^2 = 0.94$), brook trout (DM, $r^2 = 0.98$), logperch (TBW, $r^2 = 0.94$). Proximate analysis revealed similar ranges of body composition for fish in the cross validation groups to those used for development of BIA indices (Table 2). Linear regression analyses revealed high coefficients of determination between the estimated (BIA) and actual (proximate analysis) body composition masses for bluegill ($r^2 > 0.77$, $p < 0.0001$) and redear sunfish ($r^2 > 0.71$, $p < 0.0001$) validation groups (Table 4).

Cross-validation of Species-specific Indices

I found that the slopes of all six body composition indices for bluegill and redear sunfish were not significantly different (df = 26, $P \geq 0.10$) nor were the intercepts significantly different (df = 26, $P \geq 0.10$). I also found that there was not a significant difference among the actual (proximate analysis) and estimated body composition (derived from the bluegill or redear indices) for the independent bluegill validation group (df = 2, $P \geq 0.78$; Table 5). However, when comparing the indices for all four species included in the study, I found that the intercepts for the TBF, TBP, and TBA indices were significantly different (df = 59, $P \leq 0.01$). The slopes

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and intercepts of the FFM, TBW, and TBA indices were not significantly different among the four species (df = 70; $P \geq 0.09$).

Field Applications

Sampling efforts yielded hundreds of bluegill and redear sunfish from both reservoirs and included fish ranging in length from 79 to 264 mm (Table 6). The bluegill TBF index estimated the fat content of bluegill and redear sunfish in Briery Creek Lake throughout the summer to be 1.9 – 3.2 % and 2.8 – 3.8%, respectively (Figure 5). The same index estimated the fat content of bluegill and redear in Sandy River Reservoir during the same time period to be 2.0 – 3.6 % and 2.4 – 2.7 %, respectively (Figure 6). All four populations began the spring with low BIA-estimated fat and ended the summer with slightly higher estimated fat reserves. The relative weight of each population was below the optimum of 100 during most sampling dates and remained stable throughout the summer (Figure 7; Figure 8).

Stream electrofishing produced over 130 brook trout in a total of 11 streams and included multiple year classes for BIA analysis (Table 6). Using BIA, I estimated the average brook trout TBF to be slightly greater than 6.2 % (SD = 1.0), which was lower than the observed fat content of the trout I used for development of BIA indices. The estimated TBF for brook trout in streams containing only brook trout was 6.6 % ($n = 5$, SD = 1.1,) while the TBF for brook trout in streams also inhabited by rainbow, brown, or both nonnative trout species was 5.9 % ($n = 6$, SD = 1.0). Although I observed a lower brook TBF in streams containing nonnative trout, the difference was not significant ($P \geq 0.05$)

Discussion

The BIA indices I developed were strongly correlated with and accurately estimated the body composition for each species. Measures of precision for my indices were similar to those reported by other researchers using BIA (Cox and Hartman 2005; Margraf et al. 2006; Duncan et

al. 2007). My field and lab TBF estimates for bluegill and redear sunfish were relatively low but within or close to the low range of TBF reported in other studies (Table 7). The overall low relative weight of fish analyzed in the field supports my low TBF estimates. Although I did observe differences in body composition among species due to a variety of reasons including seasonal and species-specific differences (Table 2), internal composition remained relatively similar among fish of the same species throughout the year. Future studies where researchers manipulate body composition by fasting or feeding test subjects may help to determine the ability of BIA to monitor gradual compositional changes of a given species. The fish analyzed in this study represent most of the size range for each species encountered in Virginia, but they may not reflect the full range of sizes reached in other systems. Additional research is needed to expand the upper length limits of the regressions presented in this study.

Ohm's law and Kirchhoff's rules state the path of least resistance (i.e., TBW and TBP) will carry more of the applied current; therefore, Cox and Hartman (2005) hypothesized that ventral impedance readings may increase the strength of correlations between BIA and TBF. Nevertheless, the results from my study confirm that dorsal readings accurately estimate TBF and eliminate the probability of causing serious injury. Unlike ventral readings, researchers can insert the subdermal needles through muscle when using dorsal readings, which removes the likelihood of puncturing vital organs during analysis. Ventral readings may be useful if future researchers wanted to analyze fecundity (i.e., egg production) or specific lipid stores, but dorsal readings appear to suffice for whole body analysis.

The technique also provides biologists with a means of estimating the body composition of small fish rather than relying on simple length or weight measurements when condition indices are not available. The standard weight equations for relative weight have species-specific minimum length requirements (e.g., bluegill 80 mm (Hillman 1982); redear sunfish 70 mm (Pope et al. 1995); and brook trout 130 mm (Whelan and Taylor 1984). Several species

such as lake trout *Salvelinus namaycush*, paddlefish *Polyodon spathula*, and muskellunge *Esox masquinongy* have length requirements that exceed 250 mm (Brown and Murphy 1993; Piccolo et al. 1993; Neumann and Willis 1994) making relative weight unavailable throughout the early life-history of these species as well as other fish that do not meet the length requirements. The electrode assembly I used permitted the analyses of fish ≥70 mm, but future researchers could reduce the spacing between the signal and detecting electrodes, which would allow them to analyze even smaller fish using BIA. The ability to more closely analyze younger fish could provide biologists with additional information about early growth and factors limiting recruitment.

A review of the fisheries literature revealed very few studies evaluating the body composition of the species used in this study, which indicates the underutilization of, or inability to, estimate the body composition of some fishes. The new technique provides biologists with a reliable nonlethal method for approximating body composition, which could supply data for more-accurate estimates of test-subject condition and population health. After the initial development of indices, BIA may provide biologists with an additional tool to assess the status of fisheries without substantially increasing overhead costs (Table 8). The cost efficiency and simple methodology makes BIA an ideal candidate as a new assessment tool for fisheries biologists.

Table 1. Ranges of length and mass of fish used for bioelectrical impedance analysis index development (ID) and cross validation (CV).

Species	Test group	n	Length (mm)	Mass (g)
Bluegill	ID	15	136 - 235	38 - 260
	CV	15	92 - 224	13 - 245
Redear	ID	15	100 - 205	18 - 150
	CV	15	105 - 218	22 - 160
Brook trout	ID	30	140 - 235	31 - 137
Logperch	ID	15	130 - 164	17 - 34

Table 2. Ranges of total body fat, protein, water, and ash (%) as well as resistance and reactance measurements (Ω) of fish used for bioelectrical impedance analysis index development (ID) and cross validation (CV).

Species	Test group	Fat	Protein	Water	Ash	Resistance	Reactance
Bluegill	ID	1.7 - 4.1	15.0 - 17.6	73.4 - 79.4	3.8 - 7.2	324 - 523	101 - 185
	CV	1.4 - 4.7	15.4 - 18.7	72.5 - 78.5	4.2 - 6.2	334 - 649	105 - 233
Redear	ID	1.2 - 3.2	15.0 – 17.0	74.5 - 78.1	4.3 - 6.2	372 - 516	145 - 214
	CV	1.1 - 4.1	15.1 – 18.2	71.4 - 78.2	3.8 - 6.6	372 - 530	103 - 207
Brook trout	ID	8.3 - 12.6	14.8 – 20.7	72.7 - 78.5	3.1 - 5.3	409 - 662	153 - 228
Logperch	ID	7.8 - 10.9	14.5 – 20.3	69.5 - 72.2	4.5 - 6.4	961 - 1297	146 - 224

Table 3. Best-fit models developed using linear regression analysis of bioelectrical impedance analysis (BIA) and proximate body composition components for BIA indices: total body fat (TBF), fat-free mass (FFM), total body protein (TBP), total body water (TBW), dry mass (DM), and total body ash (TBA). Impedance variables and proximate composition were significantly correlated for every model ($p < 0.0001$). Calibration models are calculated as: proximate composition $= a + b\mathrm{E}$

Species, component	n	a	b	E^a	r^2
Bluegill TBF	15	-0.263	0.161	$L^2/\mathrm{res(p)}$	0.90
FFM	15	-8.056	4.950	$L^2/\mathrm{res(p)}$	0.99
TBP	15	-0.736	2.133	$L^2/\mathrm{reac(p)}$	0.97
TBW	15	-5.991	3.826	$L^2/\mathrm{res(p)}$	0.99
DM	15	-2.417	1.289	$L^2/\mathrm{res(p)}$	0.98
TBA	15	-0.817	0.307	$L^2/\mathrm{res(p)}$	0.96
Redear TBF	15	-0.957	0.190	$L^2/\mathrm{res(p)}$	0.91
FFM	15	-18.076	6.575	$L^2/\mathrm{res(p)}$	0.93
TBP	15	-2.791	2.799	$L^2/\mathrm{reac(p)}$	0.94
TBW	15	-13.842	5.097	$L^2/\mathrm{res(p)}$	0.93
DM	15	-5.191	1.668	$L^2/\mathrm{res(p)}$	0.94
TBA	15	-1.286	0.377	$L^2/\mathrm{res(p)}$	0.91
Brook trout TBF	31	0.289	0.938	$L^2/\mathrm{reac(p)}$	0.94
FFM	31	-5.030	3.253	$L^2/\mathrm{res(p)}$	0.97
TBP	31	-1.521	0.644	$L^2/\mathrm{res(p)}$	0.96
TBW	31	-2.023	2.613	$L^2/\mathrm{res(p)}$	0.97
DM	31	-3.008	0.980	$L^2/\mathrm{res(p)}$	0.98
TBA	31	-0.038	2.528	$L^2/\mathrm{reac(p)}$	0.85
Logperch TBF	15	-1.447	0.433	$L^2/\mathrm{res(p)}$	0.75
FFM	15	-9.928	3.817	$L^2/\mathrm{res(p)}$	0.92
TBP	15	-1.483	0.673	$L^2/\mathrm{res(p)}$	0.76
TBW	15	-8.299	3.039	$L^2/\mathrm{res(p)}$	0.94
DM	15	-3.107	1.215	$L^2/\mathrm{res(p)}$	0.86
TBA	15	-0.559	0.223	$L^2/\mathrm{res(p)}$	0.71

[a] - The squared length between signal electrodes is represented by L^2. The specific impedance equation for each component is represented by E while res(p) and reac(p) represent resistance and reactance in parallel.

Table 4. Linear regression coefficients of determination (r^2) for estimated (bioelectrical impedance analysis) and measured (proximate analysis) body composition (g) cross validation groups ($n = 15$): total body fat, fat-free mass, total body protein, total body water, dry mass, and total body ash.

	Fat	Fat-free	Protein	Water	Dry	Ash
Bluegill	0.77	0.97	0.96	0.91	0.96	0.96
Redear	0.71	0.95	0.95	0.94	0.95	0.89

Table 5. Actual (proximate analysis) and estimated (bioelectrical impedance analysis; BIA) body composition masses (g) of redear sunfish ($n = 15$) using either bluegill or redear indices with associated SD in parentheses. *P*-values representing the difference among the three body composition estimates were not significant (Wilcox rank-sum test).

Body composition parameter	Proximate analysis	Bluegill index estimates	Redear index estimates	*P*
Total body fat	2.8(2.5)	2.9(2.0)	2.8(2.5)	0.97
Fat-free mass	100.4(72.2)	90.0(62.9)	112.2(83.5)	0.86
Total body protein	16.7(11.9)	13.8(9.1)	16.2(12.0)	0.86
Total body water	74.2(55.3)	69.8(48.6)	87.1(64.7)	0.88
Dry mass	25.6(19.0)	23.1(16.4)	27.9(21.2)	0.95
Total body ash	5.8(4.5)	5.3(3.9)	6.2(4.8)	0.78

Table 6. Ranges of length and mass of fish evaluated using bioelectrical impedance analysis during field studies.

Species	n	Length (mm)	Mass (g)
Bluegill	371	79 - 227	8 - 265
Redear	348	79 - 264	8 - 330
Brook trout	136	119 - 304	14 - 250

Table 7. Total body fat ranges derived from bioelectrical impedance analysis, proximate analysis, and peer-reviewed literature for each species evaluated in this study.

Species	Range of Fat			References
	BIA field estimates	Proximate analysis	Literature	
Bluegill	0.7 – 5.9%	1.4 – 4.7%	3.3 – 9.4%	McCormish et al. 1974, Lemly and Esch 1984, Fischer et al. 1996
Redear sunfish	0.9 – 4.2%	1.1 – 4.1%	1.5 – 9.0%	Cook and Scurlock 1998
Brook trout	3.5 – 13.3%	8.3 – 12.6%	0.6 – 12.3%	Hutchings et al. 1999, Novinger and Del Rio 1999, Rasmussen and Ostenfeld 2000, Cox and Hartman 2005
Northern Logperch	NA	7.8 – 10.9%	NA	NA

Table 8. Cost comparison of bioelectrical impedance analysis (BIA) and proximate analysis for both in-house and contracted determination of body composition (n=100). Comparative analysis does not include BIA equipment, model development, common costs associated with field collection, glassware and equipment found in most working laboratories, or disposal costs of chemical waste. Chemical costs from www.fishersci.com. Technician costs include time spent analyzing fish using BIA or time spent completing proximate analysis. In-house technician costs were estimated using $8/hr pay-rate while technician costs for contracted analysis were estimated using average quoted costs from laboratories found on the internet.

	BIA	In-house proximate analysis	Contracted lab analysis
Chemical costs [a]	$162	$1,919.04	NA
Safety concerns	None	Use and disposal of toxic chemicals	Use and disposal of toxic chemicals
Mortalities	0 %	100 %	100 %
Technician	$58.11	$2,400	$9,033.33
Total costs	$220.11	$4,319.04	$9,033.33

[a] - includes anesthetic, methanol and chloroform for lipid analysis as well as copper

Kjeldahl tablets, sulfuric, hydrochloric and boric acids for protein analysis
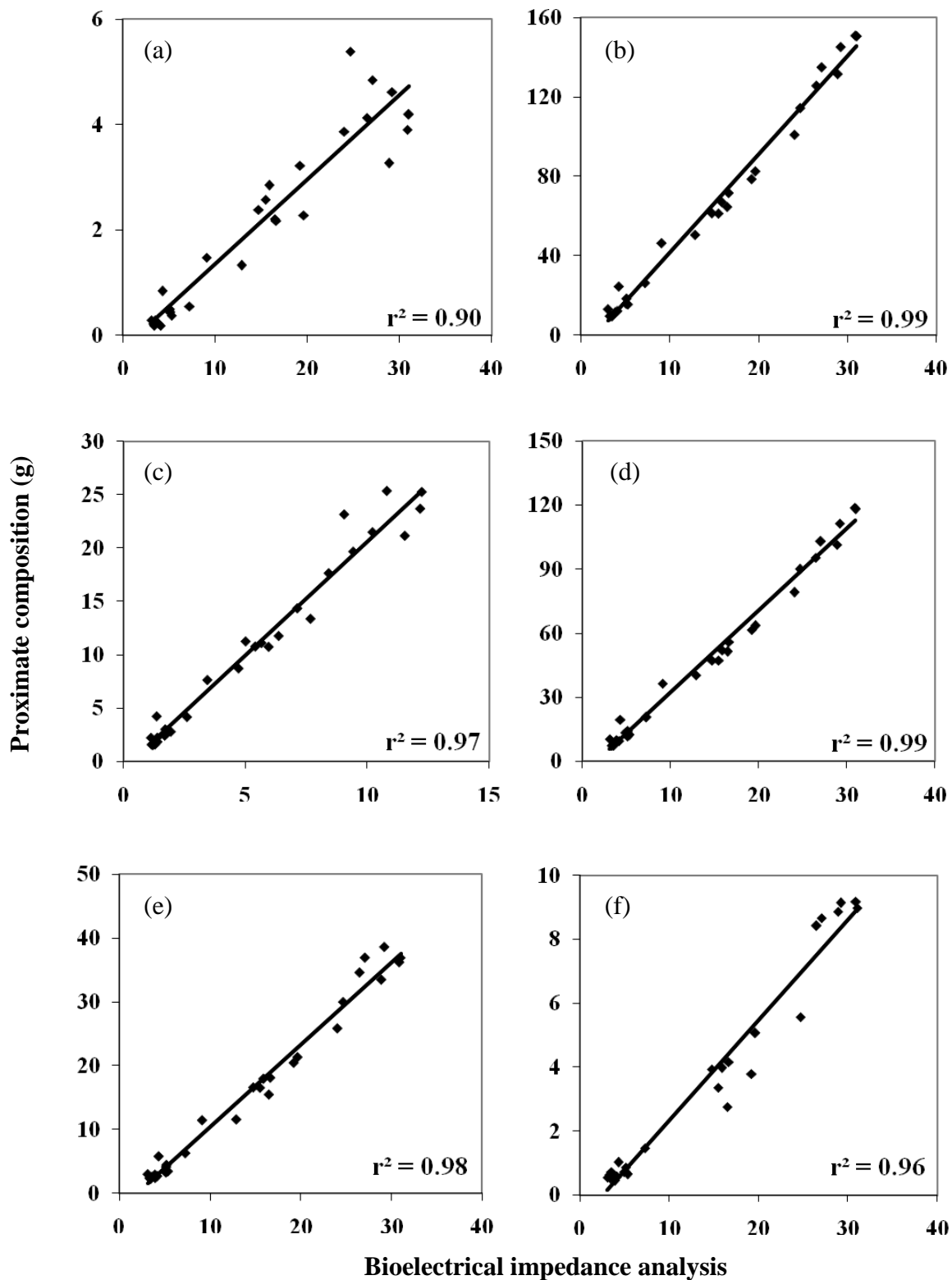
Figure 1. Regressions illustrating relationships between proximate body composition components (total body fat (a), fat-free mass (b), protein (c), body water (d), dry mass (e), and ash (f)) and bioelectrical impedance analysis for bluegill.

31
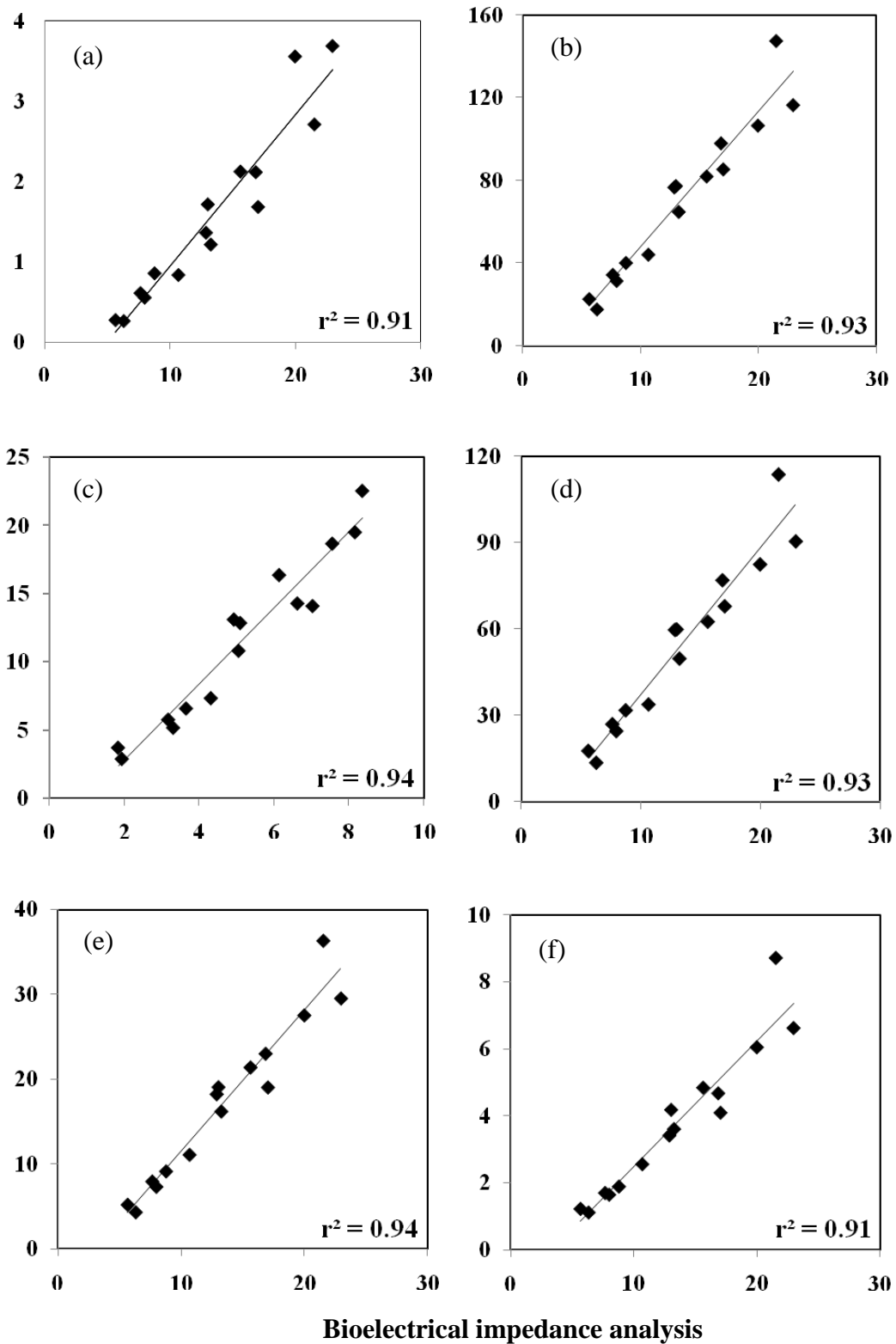
Figure 2. Regressions illustrating relationships between proximate body composition components (total body fat (a), fat-free mass (b), protein (c), body water (d), dry mass (e), and ash (f)) and bioelectrical impedance analysis for redear sunfish.

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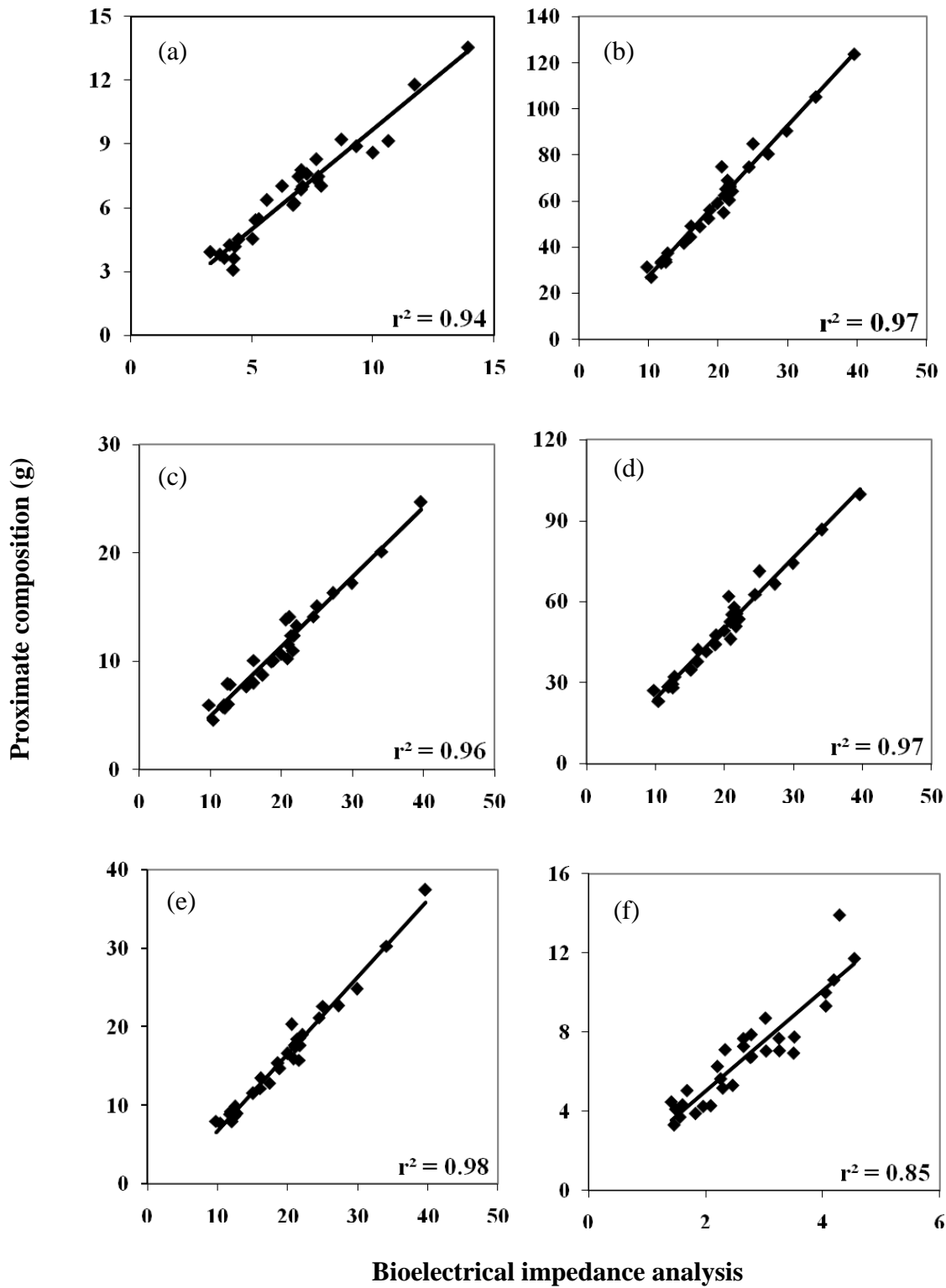
Figure 3. Regressions illustrating relationships between proximate body composition components (total body fat (a), fat-free mass (b), protein (c), body water (d), dry mass (e), and ash (f)) and bioelectrical impedance analysis for brook trout.

33
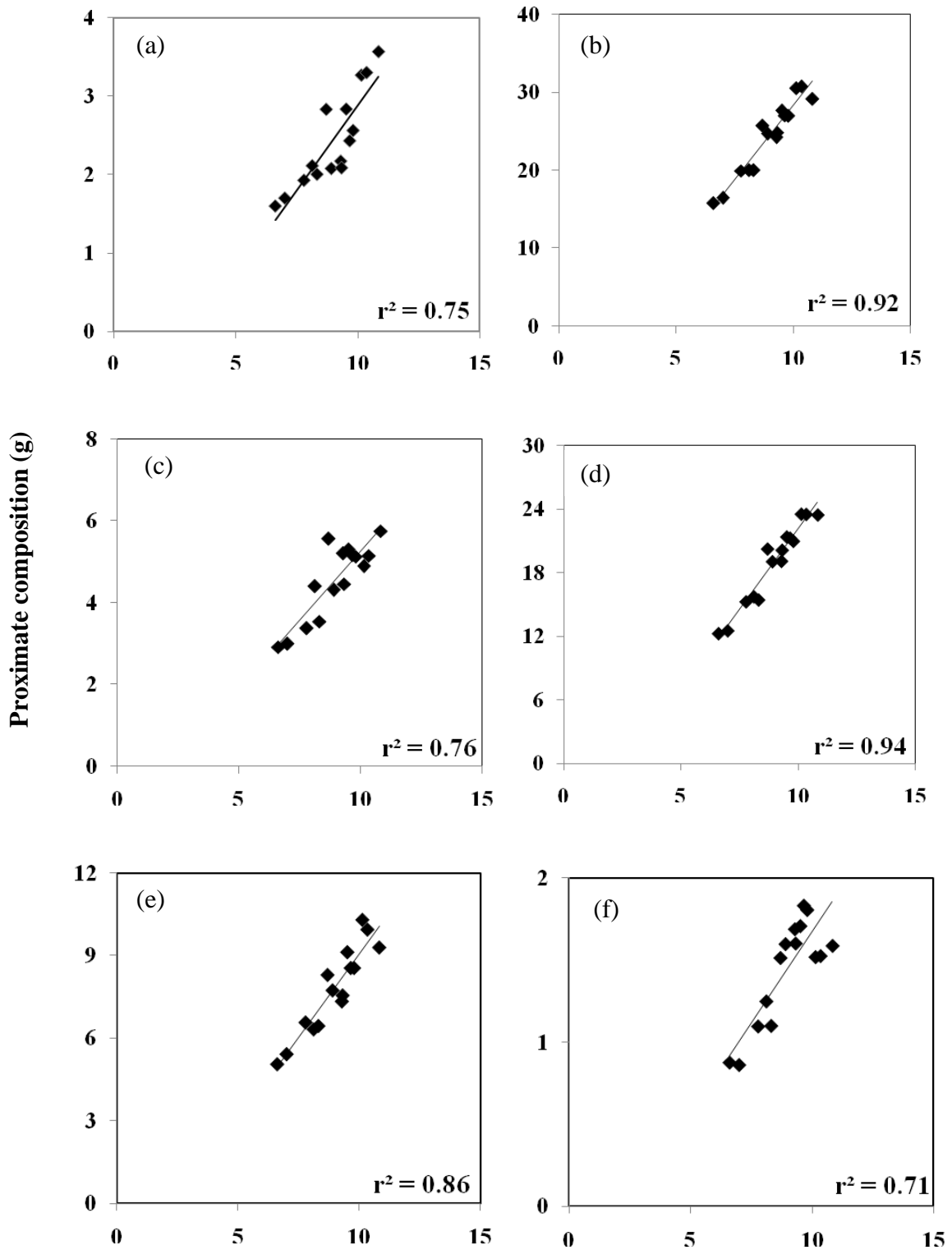
Figure 4. Regressions illustrating relationships between proximate body composition components (total body fat (a), fat-free mass (b), protein (c), body water (d), dry mass (e), and ash (f)) and bioelectrical impedance analysis for northern logperch.

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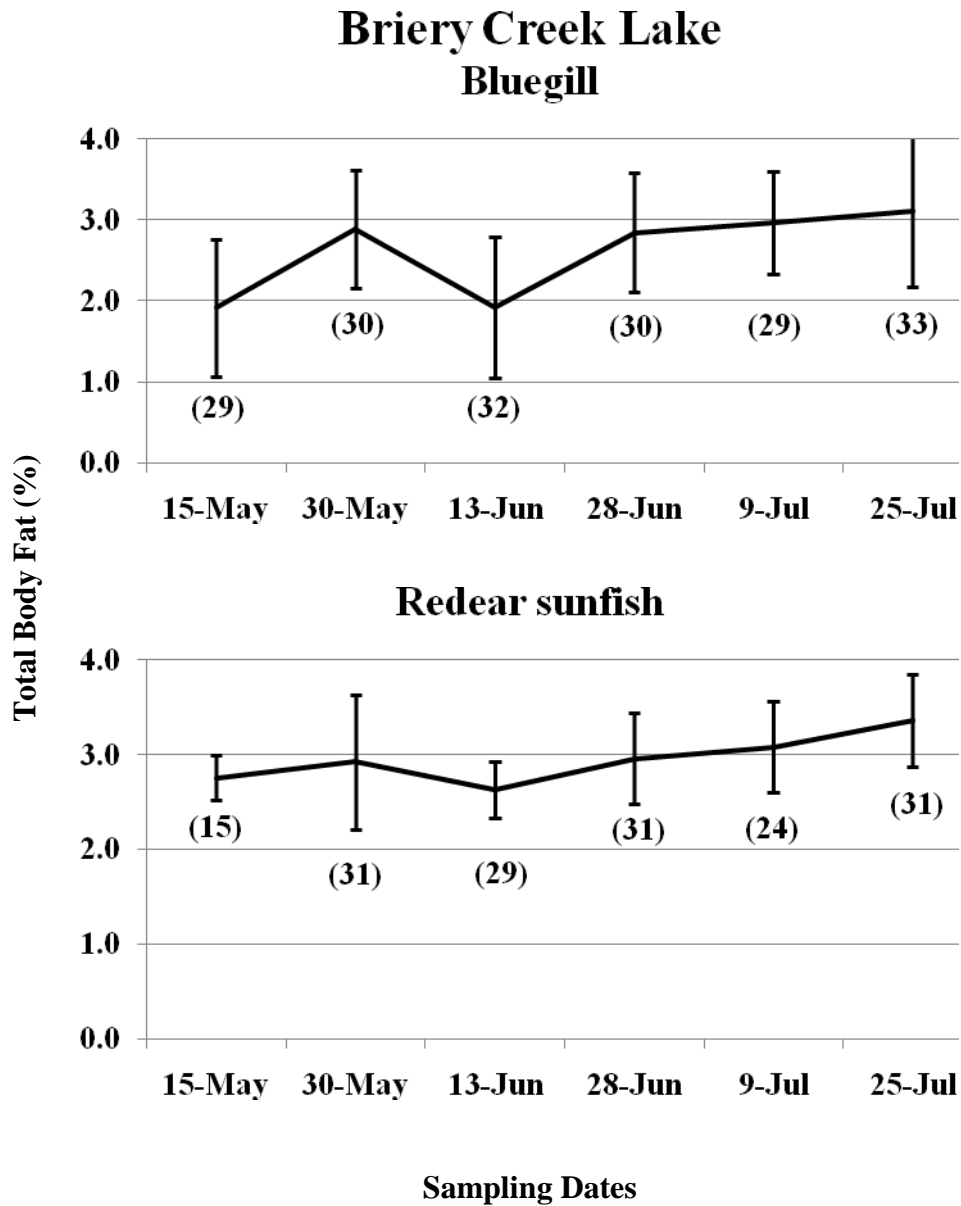
Figure 5. Average estimated total body fat ± SE using bioelectrical impedance analysis of bluegill and redear sunfish in Briery Creek Lake with sample sizes in parentheses.
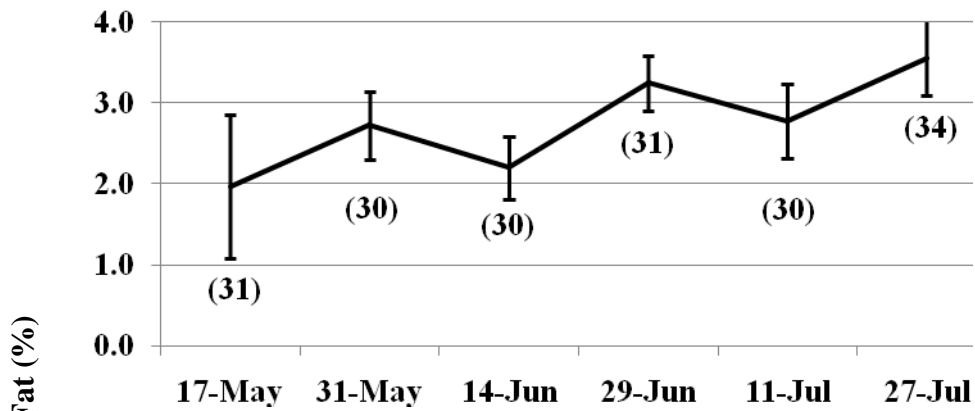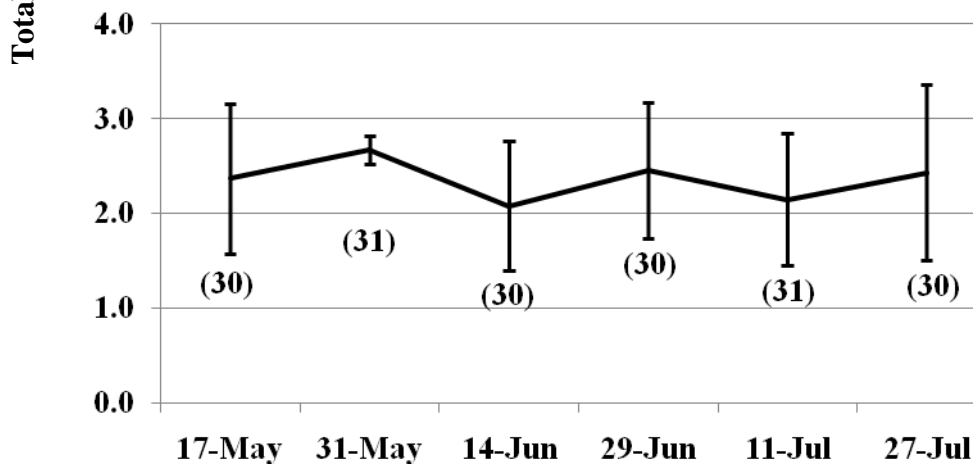
Figure 6. Average estimated total body fat ± SE using bioelectrical impedance analysis of bluegill and redear sunfish in Sandy River Reservoir with sample sizes in parentheses.
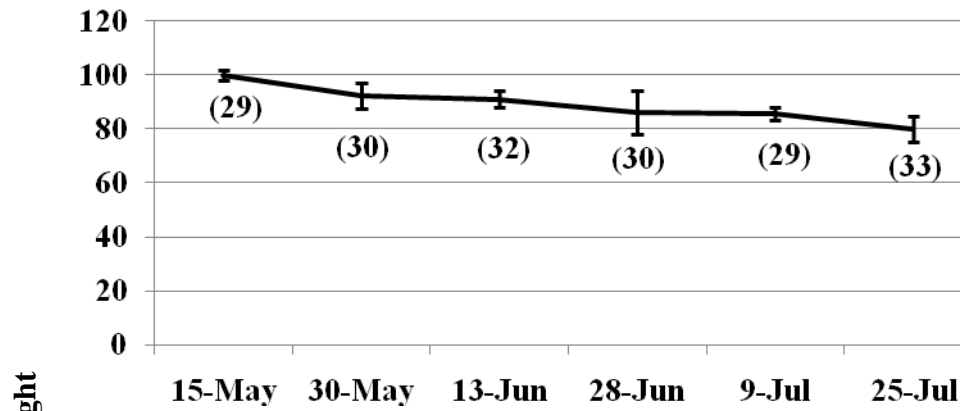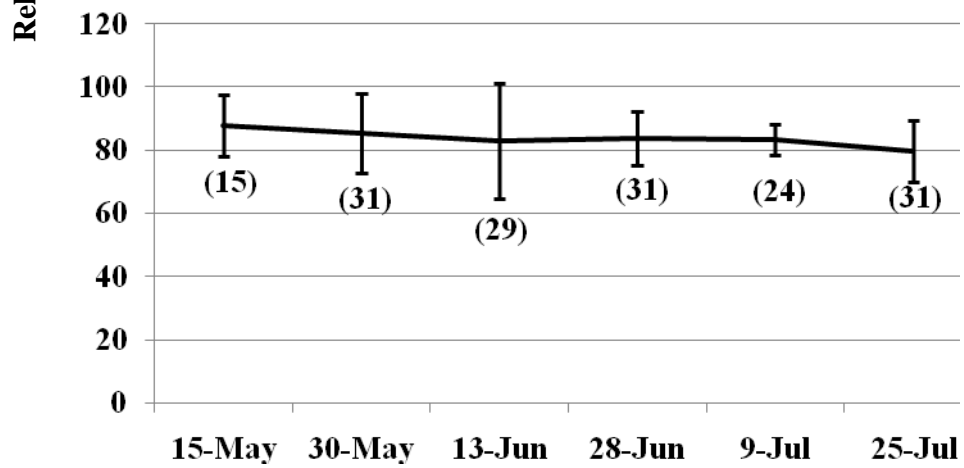
Figure 7. Average relative weight ± SE of bluegill and redear sunfish in Briery Creek Lake with sample sizes in parentheses.
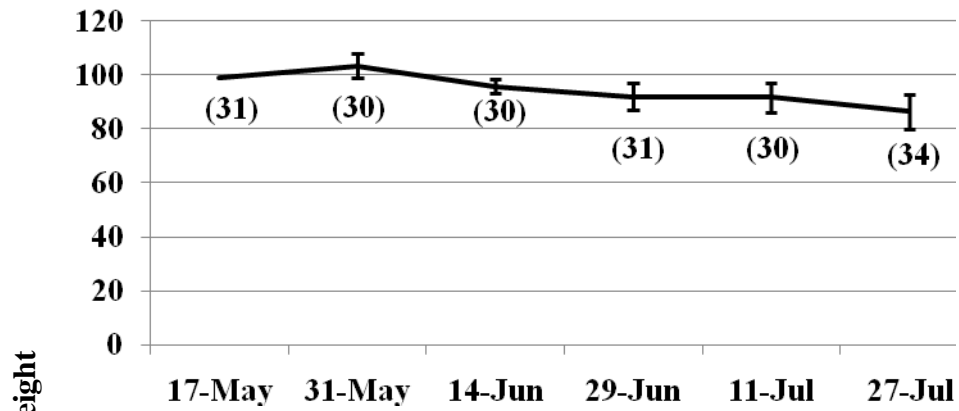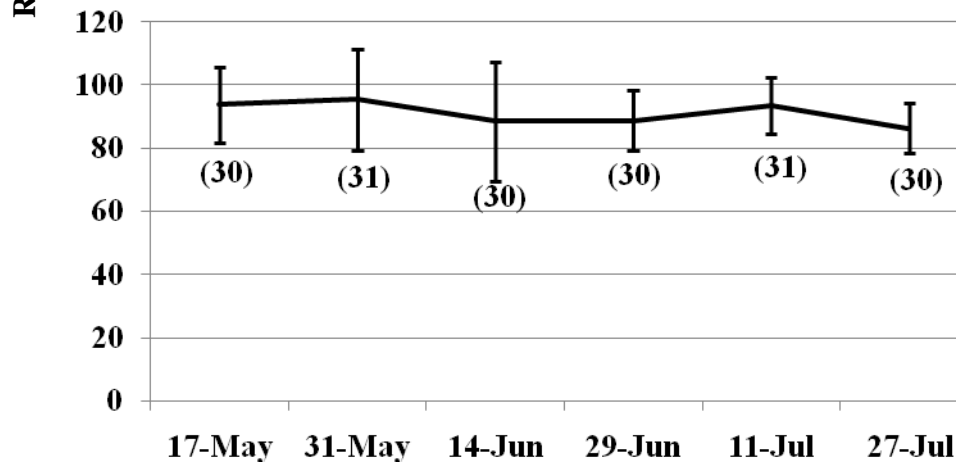
Figure 8. Average relative weight ± SE of bluegill and redear sunfish in Sandy River Reservoir with sample sizes in parentheses.

CHAPTER 3

The Influences of Water Temperature and Electrode Application on Bioelectrical Impedance Analysis

Abstract

Recent research has shown bioelectrical impedance analysis to be an accurate technique for nonlethally estimating the body composition of fish. Bioelectrical impedance analysis (BIA) indices have been developed for a variety of species from a multitude of environments. There is potential to apply this technique to fish across a variety of temporal and geographic scales, but little research has been conducted to evaluate the influence of temperature and electrode application on BIA. I determined that a nearly 10 °C change in temperature did not significantly influence resistance and reactance readings for largemouth bass, bluegill, or redear sunfish. Approximated or precise needle insertion did not significantly affect bluegill and most northern logperch linear regressions. These results indicate that biologists could use the method in the field without accounting for changes in water temperature throughout most of the year in many environments. They can also use BIA to analyze multiple species with the same equipment by approximating depths for needle insertion through the skin of test subjects. This study helps to further increase the efficiency and applicability of BIA, and strengthen the applicability of the method for estimating the body composition of fish.

Introduction

Bioelectrical impedance analysis (BIA) is a quick, inexpensive and, most importantly, nonlethal method for estimating the body composition of fish. The technique uses a small, handheld instrument to measure the resistance and reactance of a fish's tissue, which then are correlated with the results of proximate analysis performed on the same sample. Resultant linear regression equations can then be used in the future to estimate body composition parameters including total body fat, protein, water, and ash. Hartman and Cox (2005) found BIA to be an accurate predictor of brook trout *Salvelinus fontinalis* body composition, while resulting in only slight bruising. Other researchers have had success using the method on chum salmon *Oncorhynchus keta* (Margraf et al. 2006) and cobia *Rachycentron candadum* (Duncan et al. 2007). This new technique may aid in management and conservation programs as an additional assessment tool, but several questions about the method remain that prevent biologists from relying on BIA for field evaluations.

Biologists evaluate fish populations throughout the year for a variety of reasons, which include assessing overall condition (Jonas et al. 1996), growth (Olson and Young 2003; Bonvechio et al. 2005), overwinter survival (Hurst et al. 2000; Fullerton et al. 2000), and movement (Hilderbrand and Kershner 2004; Ebersole et al. 2006). Water temperatures not only vary seasonally but also fluctuate daily, which leads to varying body temperatures of fish among sampling dates or times. Margraf et al. (2006) developed temperature-correction equations for resistance and reactance readings of chum salmon to remove variance due to changes in temperature, but this may be an unnecessary step when developing BIA regressions. Hill et al. (1967) found that differences in temperature produced only minor changes in the conductivity of unfrozen animal tissues. If the same is true for live fish, researchers could further simplify the

development process for BIA regressions and reduce the complicating effects of unnecessary correction equations for regression analysis.

Oftentimes, fisheries biologists collect and analyze several species of various sizes during the same sampling period (e.g., Sammons and Bettoli 2000). Biologists could benefit from a technique that could be quickly and reliably used to estimate the body composition of any fish collected in the field. Methodologies should be as efficient as possible to reduce the time required to employ them in the field. However, researchers in previous BIA studies precisely controlled needle penetration depth, which may prohibit the analyses of different-sized fish without changing the equipment used for analysis. Hartman and Cox (2005) used 12-mm subdermal needles for analyses but limited the length of the needle that was exposed for penetration to 2 mm. This approach may be suitable for fish within a certain size range, but biologists may need to increase the length of needle exposed for analysis to penetrate large scales and thicker skin if they are evaluating very large test subjects. Conversely, smaller fish may require biologists to shorten the length of exposed needle to prevent over-penetration. Sorting fish into similar size groups and switching electrodes will increase the time required for field analyses, thus making BIA less appealing to biologists. Biologists will more likely implement BIA into existing sampling regimes if analyses are quick and simple.

The primary objectives for this study were as follows: 1) determine whether temperature has a significant effect on the impedance readings of largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, and redear sunfish *Lepomis microlophus*; 2) determine whether the depth of needle penetration influences the results obtained from BIA analysis.

Methods

Temperature Effects

     I collected largemouth bass, bluegill, and redear sunfish from Sandy River Reservoir in Prince Edward County, Virginia and immediately transported all fish to the Aquaculture Center at Virginia Tech. All fish were initially kept in a recirculating system comprised of four 1600 L tanks and a 1600 L KMT-based (Kaldnes Miløjteknologi, Tønsberg, Norway) biofilter for nitrification. I used a bead filter (Aquaculture Technologies Inc., Metaire, Louisiana) for solids removal. The system also included a protein skimmer (R&B Aquatic Distribution, Waring, Texas) and a 40 W UV sterilizer (Emperor Aquatics, Pottstown, Pennsylvannia). To prevent predation by the largemouth bass, I isolated the larger largemouth bass into two of the tanks, the smaller largemouth bass into the third tank, and all of the sunfish into the fourth tank. To limit impedance readings to changes in water temperature rather than digesting prey tissue in the stomach or intestines of test fish, I withheld food one week prior to initial analysis. I anesthetized fish using clove oil (Sigma-Aldrich, St. Louis, MO, USA) and placed them on a nonconductive measuring board for analysis. In addition to individual weights and total lengths, I measured resistance and reactance with a tetrapolar bioelectric impedance analyzer (RJL Systems, Detroit, MI) using two sets of 12-mm, 28-gauge stainless steel subdermal needles (Grass Telefactor, West Warwick, Rhode Island); each set has a signal electrode and a detecting electrode spaced 1.0 cm apart with the signal electrode positioned towards the outside of the fish. I placed one set of electrodes at the posterior apex of the operculum and the second set along the posterior region of the caudal peduncle (see Cox and Hartman 2005 for diagram). I inserted the electrodes approximately 3 mm through the skin. I measured the distance between the signal electrodes and recorded resistance and reactance. Initial BIA measurements occurred at 24.5° C before I separated the fish into five groups: one control (24.5° C) and four treatment groups (16.4, 20.9, 29.2, 33.2° C; $n = 5$ for each group for each species). I transferred the 24.5, 29.2,

and 33.2º C groups to separate, but identical, systems as described above. I relocated the other two groups to separate 284 L Living Stream systems (Frigid Units, Inc., Toledo, Ohio). Both systems included a chiller unit (Frigid Units, Inc., Toledo, Ohio), which recirculated water and maintained water temperature. The living stream systems did not contain filters or any other sterilization equipment, so I replenished the tanks with fresh water throughout the first 36 hours. Fish were gradually acclimated to their respective water temperatures throughout the first 24 hours. After three days, I measured resistance and reactance again using the same methods described above. Fish were placed in anesthetic of the same water temperature as the tank I removed them from. I completed BIA immediately after anesthetizing each fish, which prevented changes in internal body temperature. I tested the changes in resistance and reactance using oneway ANOVA.

Needle Insertion Methodology

I collected bluegill from Briery Creek Lake and northern logperch *Percina caprodes* from the New River in Giles County, Virginia to analyze the accuracy of BIA indices developed using approximated needle insertion (Table 10). I collected and analyzed both species using the same BIA procedures as described in the temperature study. I precisely controlled electrode penetration by inserting the needles through a nonconductive piece of rubber, which left exactly 3 mm of the subdermal needle exposed. I collected a second group of bluegill from Sandy River Reservoir and another group of northern logperch from the New River in Giles County, Virginia. I used the same BIA methodologies described above except that the rubber block was not used - this permitted slight under or over-penetration of the needles. I used the impedance readings to determine the following common electrical properties: resistance in both series and parallel, reactance in series and parallel, and capacitance for the two test groups of each species. Immediately following BIA, I euthanized the fish using an overdose of clove oil and placed them on ice until being frozen at -20º C. Later in the lab at Virginia Tech, I homogenized the fish

using an industrial blender for proximate analysis. Using the method described in AOAC

(2002), I determined dry mass, total body ash, and total body protein (using the block digestion

method). I determined total body fat using the chloroform/methanol extraction method

originally described by Bligh and Dyer (1959). Total body water was determined by back-

calculating dried samples while fat-free mass was calculated by subtracting total body fat from

body mass. All proximate measurements were performed in duplicate and averaged for use in

regression analysis.

For linear regression analyses, I used the chemically derived body composition masses as

the dependent variables and the common electrical properties as the independent variables. I

then selected the best-fit model (i.e., regression with highest r^2 value) for each body composition

parameter. I compared the slopes and intercepts of the corresponding regressions from the

approximated and precise needle-insertion techniques for each body composition model using a

Students t-test to determine whether there was a significant difference ($P \leq 0.05$) between the

different needle applications.

Results

Temperature Effects

Table 9 represents the fish used in the temperature study. I determined that there were no

significant differences in resistance and reactance readings among water temperatures for

bluegill, redear sunfish or largemouth bass (df = 4, $P \geq 0.18$; Table 10) nor did observed changes

in impedance readings follow predictable trends (i.e., gradually increasing or decreasing with

changes in temperature). I did observe a slight decrease between initial and final resistance and

reactance readings for the control groups for each species. After Day 2 of the experiment, I

observed two mortalities: 17- and 25-g redear sunfish. One mortality occurred in the control

group and one was in the 20.9º C experimental group. No other mortalities were observed during analyses.

Needle Insertion Methodology

Table 10 presents results for the fish included in each needle insertion test group. Table 11 shows the body composition and impedance measurements for the approximated and precise needle-insertion groups that I used for analysis. I determined that the precision of needle insertion did not significantly affect the slopes of any of the six individual regressions (TBF, FFM, TBP, TBW, DM, and TBA) for bluegill (df = 45, $P \geq 0.10$). I also determined that the intercepts for these same regressions were not significantly different (df = 45, $P \geq 0.10$). For logperch TBF and FFM, I found that the intercepts of the regressions developed using the precise and approximated needle insertion method were significantly different (df = 44, $P \leq 0.05$); however, the slopes for each of these indices were not different (df = 44, $P \geq 0.10$). The slopes and intercepts of the remaining indices (TBP, TBW, DM, and TBA) were not significantly different (df = 44, $P \geq 0.10$).

Discussion

Although there was a slight decrease in resistance and reactance following initial BIA for all test groups including the control, these changes, in addition to those resulting from water temperature, were not significant. Although resistance and reactance of tissues may change with temperature, the differences may not be large enough to significantly change impedance readings (J. Margraf, Alaska Cooperative Fish and Wildlife Research Unit, personal communication), which is consistent with the results of this study. The initial decline in resistance and reactance was most likely due to an inadequate recovery time (3 days). Lengthening the time between analyses or applying needles to the opposite side of the fish may reduce or eliminate these changes. The temperature range I used for this experiment includes the temperatures observed in

the reservoir field study reported in Chapter 2, but more extreme water temperatures may be observed in the field at other times. Many systems experience nearly freezing water temperatures (e.g., Foy and Paul 1999), while desert streams can exceed 40° C (e.g., Deacon and Minckley 1974). The mortalities that I observed in the temperature study were most likely a result of the high ammonia levels (> 1.0 mg/L) in two of the redear tanks. All other fish analyzed recovered quickly from the anesthetic and showed no lasting effects of analysis, thus supporting the claim that BIA is nonlethal and minimally invasive. Although I did not observe significant temperature-induced changes in impedance readings, further research evaluating additional species and larger temperature ranges are needed to confidently assert that my findings are applicable to all fish in all environments.

Comparisons of the slopes and intercepts between the two sets of bluegill and logperch regressions show that generally there is not a significant difference between the two needle insertion methodologies except in the case of several of the logperch regressions. I developed the electrode arrangement used for this study with more flat-bodied fish (e.g., bluegill) in mind. The small, cylindrical body shape of the logperch made consistent application of the needles difficult. The shifting of needles after insertion may have produced enough imprecision to create differences in some of the regressions. An electrode assembly designed especially for the unique body shape of logperch may help eliminate these discrepancies. This could be done by adjusting the angle of insertion or the angle at which the electrodes are secured to the lead cables. Biologists should strive for consistency in their methodologies, but approximating needle penetration will allow them to more easily analyze numerous size classes of multiple species with the same equipment.

The capability of BIA to reliably estimate the body composition of numerous fishes, the cross-species utility of the technique, and the insensitivity of BIA to changes in temperature and needle application reveal the practicality of the technique as an assessment tool for most fisheries

biologists. The equipment is relatively inexpensive making it an economically viable option for most agencies or businesses. The use of BIA in fisheries science is relatively new, but the method appears to be a promising addition to the well-established techniques that biologists commonly use for population evaluations.

Table 9. Ranges of length (mm) and mass (g) for fish in temperature study. Each group consisted of five individuals except for the 16.4 and 29.2º C redear test groups, which included only four fish.

Temperature °C	Bluegill		Redear sunfish		Largemouth bass	
	Length	Mass	Length	Mass	Length	Mass
16.4	114 - 169	20 - 91	109 – 247	17 – 221	302 – 500	330 - 1760
20.9	116 - 187	22 - 118	130 – 245	25 – 236	255 - 425	206 - 1157
24.5	110 – 189	19 - 110	115 – 225	18 – 182	273 - 429	240 – 989
29.2	124 - 207	28 - 199	110 – 255	15 – 235	235 - 505	152 - 2340
33.3	118 – 178	20 - 88	112 – 229	18 – 185	255 - 468	208 - 1401

Table 10. Average changes (%) in resistance (res) and reactance (reac) readings (Ω) for test groups in the temperature study. Initial bioelectrical impedance analysis (BIA) of bluegill, redear sunfish, and largemouth bass occurred at 24.5° C. For each species, one group remained at the 24.5° C (control group), and the remaining fish were separated into four test groups (16.4, 20.9, 29.2, and 33.3° C) to evaluate the effects of temperature on BIA.

Temperature	Bluegill		Redear sunfish		Largemouth bass	
	Res	Reac	Res	Reac	Res	Reac
16.4	-10.3	-10.3	-9.3	-11.5	-15.4	-13.0
20.9	-8.0	-14.5	-5.0	-11.4	-12.2	-9.5
24.5	-5.0	-0.5	-9.5	-10.1	-5.5	-7.8
29.2	-6.3	-9.5	-1.2	-4.5	-8.2	-15.9
33.3	-8.1	-9.3	-0.5	3.5	-9.6	-14.3

Table 11. Ranges of length and mass for fish used to determine the effects of using approximated or precise needle insertion for the development of bioelectrical impedance analysis indices.

Species	Needle insertion	n	Length (mm)	Mass (g)
Bluegill	Approximate	31	88 - 201	10 - 155
	Precise	15	92 - 224	13 - 245
Logperch	Approximate	30	117 - 152	12 - 27
	Precise	15	130 - 164	17 - 34

Table 12. Ranges of total body fat, protein, water, and ash (%); and resistance and reactance

measurements (Ω) for fish used to determine the effects of approximated (AP) and precise (PR)

needle insertion methodologies for the development of bioelectrical impedance analysis indices.

Species	Test group	Fat	Protein	Water	Ash	Resistance	Reactance
Bluegill	AP	1.8 - 4.1	15.2 - 17.3	72.9 - 78.4	3.9 - 5.2	252 - 603	92 - 211
	PR	1.7 - 4.1	15.0 - 17.6	73.4 - 79.4	3.8 - 7.2	324 - 523	101 - 185
Logperch	AP	5.0 - 12.8	16.6 - 18.4	68.9 - 74.6	3.0 - 4.9	568 - 914	129 - 219
	PR	7.8 - 10.9	14.5 – 20.3	69.5 - 72.2	4.5 - 6.4	961 - 1297	146 - 224

SUMMARY AND CONCLUSIONS

1. I did not observe any BIA-related mortalities during the study.

2. BIA accurately estimated the body composition (total body fat, fat-free mass, total body protein, total body water, dry mass, and total body ash) for each species examined: bluegill ($r^2 > 0.90$), redear sunfish ($r^2 > 0.91$), brook trout ($r^2 > 0.86$), and logperch ($r^2 > 0.71$). The regression relationships that form the basis of each BIA index were significant at $p < 0.0001$.

3. Using independent validation groups, I determined the precision of the bluegill and redear BIA indices for estimating body composition ($r^2 \geq 0.71$, $p < 0.0001$).

4. The slopes and intercepts of bluegill and redear sunfish regressions were not significantly different, thus confirming cross-species applicability of the indices for similarly shaped species.

5. Laboratory studies indicated that a 17° C change in water temperature did not significantly influence resistance and reactance for the species tested.

6. Results from this study reveal that there is not a significant difference between most BIA regressions produced using approximated or precise electrode insertion methodologies.

Until recently, fisheries biologists lacked a reliable and nonlethal method for estimating the body composition of fishes. The portable equipment, quick sampling protocols, and lack of mortalities during model development and field application make BIA an ideal candidate for evaluating the body composition of fish. The small size of the analyzer is easily transportable into the field -- even to remote areas. The short time required for analysis permits biologists to use the method during existing sampling efforts. Most importantly, BIA is nonlethal allowing biologists use of the method with most species.

By using indices developed for surrogate species, biologists could use the technique to estimate the internal composition of threatened and endangered species. This feature further increases the functionality of BIA especially for biologists working in species-rich systems. For example, biologists may be able to use my brook trout models to estimate the body composition of numerous salmonid species. Although biologists may be able to apply indices across species, they should be cautious of applications when greater differences exist in body shape or size than were analyzed here, even within the same genus.

The reservoir and stream studies demonstrate the usefulness of the bluegill and brook trout indices during field analyses. My use of bluegill indices to estimate the fat content of redear sunfish further illustrates the cross-species utility of BIA indices. The combined use of relative weight and BIA may provide biologists with more accurate information regarding the population of interest and facilitate more efficient and successful management. Although the small sample sizes prohibited definitive conclusions on the ecological significance of my results and the potential differences between BIA and relative weight, these studies help to further present means by which these indices can be used by fisheries biologists.

Biologists are responsible for evaluating a diverse assortment of fishes, in different environments, on a variety of temporal and spatial scales. These differences reduce the applicability of certain assessment techniques and have historically limited the ability of fisheries biologists to quickly and accurately evaluate some fisheries. My laboratory experiments revealed that temperature and electrode application do not significantly influence impedance readings for the species and temperatures included in the study. These results demonstrate the potential to increase the efficiency and applicability of BIA while simplifying the field protocols for the new technique. Biologists can better monitor growth, overwinter survival, and the effects of migration by using BIA throughout the year.

The results from this study illustrate the accuracy of BIA in estimating the body composition of several previously untested species and support the findings reported in Cox and Hartman (2005) for brook trout. It also accurately estimated the body composition of several differently shaped and sized fishes, which is important for people working in species-rich systems. Researchers can develop BIA indices for fishes that do not have species-specific condition indices (e.g., nongame and marine species) allowing for the long-term monitoring of body composition rather than relying on simple morphometric measurements or population estimates for assessments. The technique could help reduce the environmental impact of aquaculture facilities in additional to increasing profit margins. Future applications for BIA could include monitoring physiological changes in fish (e.g., spawning females) or predicting the migration potential of fish using estimated energy stores. Biologists should be cautious of solely relying on BIA for assessments until the regression can be used to estimate body composition components in percentages. This would remove the influence of length, which currently plays a role in the ability of BIA to estimate body composition. Although it is a relatively new technique, BIA shows promise as a reliable method for nonlethally estimating the body composition of fish.

LITERATURE CITED

AOAC (Association of Official Analytical Chemists). 2002. Official methods of analysis of AOAC, 17th edition. W. Horwitz, editor. AOAC, Gaithersburg, Maryland.

Arnould, J.P. 1995. Indices of body condition and body composition in female Antarctic fur seals (*Arctocephalus gazella*). Marine Mammal Science 11:301-313.

Arrington, D.A., K.O. Winemiller, W.F. Loftus, and S. Akin. 2002. How often do fishes "run on empty"? Ecology 83:2145-2151.

Bai, S.C., G.R. Nematipour, R.P. Perera, F. Jaramillo, Jr., B.R. Murphy, and D.M. Gatlin III. 1994. Total body electrical conductivity for nondestructive measurement of body composition in red drum. The Progressive Fish-Culturist 56:232-236.

Baker, R.L. 1989. Condition and size of damselflies: a field study of food limitation. Oecologia 81:111-119.

Blackwell, B.G., M.L. Brown, and D.W. Willis. 2000. Relative weight (Wr) status and current use in fisheries assessment and management. Reviews in Fisheries Science 8:1-44.

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.

Bolger, T., and P.L. Connolly. 1989. The selection of suitable indices for the measurement and analysis of fish condition. Journal of Fish Biology 34:171-182.

Bonvechio, T.F., M.S. Allen, and R.L. Cailteux. 2005. Relative abundance, growth, and mortality of Suwannee bass in four Florida Rivers. North American Journal of Fisheries Management 25:275-283.

Bosworth, B.G., and W.R. Wolters. 2001. Evaluation of bioelectric impedance to predict carcass yield, carcass composition, and fillet composition in farm-raised catfish. Journal of the World Aquaculture Society 32:72-78.

Bowen, W.D., D.J. Boness, and S.J. Iverson. 1998. Estimation of total body water in harbor

    seals: how useful is bioelectrical impedance analysis? Marine Mammal Science 14:765-

    777.

Brown, J.H., P.A. Marquet, and M.L. Taper. 1993. Evolution of body size: consequences of an

    energetic definition of fitness. The American Naturalist 142:573-584.

Brown, M.L., and B.R. Murphy. 1991. Relationship of relative weight (Wr) to proximate

    composition of juvenile striped bass and hybrid striped bass. Transactions of the

    American Fisheries Society 120:509-518.

Brown, M.L., and B.R. Murphy. 1993. Management evaluation of body condition and population

    size structure for paddlefish: a unique case. Prairie Naturalist 25:93-108.

Castro, G., B.A. Wunder, and F.L. Knopf. 1990. Total body electrical conductivity (TOBEC) to

    estimate total body fat of free-living birds. The Condor 92:496-499.

Cole, K.S., 1932. Electric phase angle of cell membranes. The Journal of General Physiology

    15:641-649.

Colle, D.E., and J.V. Shireman. 1980. Coefficients of condition for largemouth bass, bluegill,

    and redear sunfish in hydrilla-infected lakes. Transactions of the American Fisheries

    Society 109:521-531.

Colt, J., and K.D. Shearer. 2001. Evaluation of the use of the Torry Fish Fatmeter to nonlethally

    estimate lipid in adult salmon. U.S. Army Corps of Engineers, Seattle, Washington.

Cone, R.S. 1989. The need to reconsider the use of condition indices in fishery science.

    Transactions of the American Fisheries Society 118:510-514.

Cook, S.B., and G.D. Scurlock, Jr. 1998. Potential feed conversion of redear sunfish fed

    nutritionally complete formulated diets. The Progressive Fish-Culturist 60:55-58.

Copeland, T., B.R. Murphy, and J.J. Ney. 2008. Interpretation of relative weight in three
populations of wild bluegills: a cautionary tale. North American Journal of Fisheries
Management 28:368-377.

Copeland, T., and R.F. Carline. 2004. Relationship of lipid content to size condition in walleye
fingerlings from natural and aquacultural environments. North American Journal of
Aquaculture 66:237-242.

Cosgrove, J.R., J.W.B. King, and D.A. Brodie. 1988. A note on the use of impedance
measurements for the prediction of carcass composition in lambs. Animal Production
47:311.

Cox, M.K., and K.J. Hartman. 2005. Nonlethal estimation of proximate composition in fish.
Canadian Journal of Fisheries and Aquatic Sciences 62: 269-275.

Crossin, G.T., and S.G. Hinch. 2005. A nonlethal, rapid method for assessing the somatic energy
content of migrating adult Pacific salmon. Transactions of the American Fisheries
Society 134:184-191.

Deacon, J.E., and W.L. Minckley. 1974. Desert fishes. Pages 385-488 *in* G.W. Brown, Jr., editor.
Desert biology; special topics on the physical and biological aspects of arid regions,
volume 2. Academic Press, New York.

Duncan, M., S.R. Craig, A.N. Lunger, D.D. Kuhn, G. Salze, and E. McLean. 2007.
Bioimpedance assessment of body composition in cobia *Rachycentron candadum* (L.
1766). Aquaculture 271: 432-438.

Ebersole, J.L., P.J. Wigington, Jr., J.P. Baker, M.A. Cairns, M. Robbins Church, B.P. Hansen,
B.A. Miller, H.R. LaVigne, J.E. Compton, and S.G. Leibowitz. 2006. Juvenile coho
salmon growth and survival across stream network seasonal habitats. Transactions of the
American Fisheries Society 135:1681-1697.

Eder, S. 1984. Effectiveness of an imposed slot length limit of 12.0-14.9 inches on largemouth bass. North American Journal of Fisheries Management 4:469-478.

Farley, S.D., and C.T. Robbins. 1994. Development of two methods to estimate body composition of bears. Canadian Journal of Zoology 72:220-226.

Fischer, R.U., J.D. Congdon, and M. Brock. 1996. Total body electrical conductivity (TOBEC): a tool to estimate lean mass and nonpolar lipids of an aquatic organism? Copia 1996:459-462.

Foy, R.J., and A.J. Paul. 1999. Winter feeding and changes in somatic energy content of age-0 Pacific herring in Prince William Sound, Alaska. Transactions of the American Fisheries Society 128:1193-1200.

Fullerton, A.H., J.E. Garvey, R.A. Wright, and R.A. Stein. 2000. Overwinter growth and survival of largemouth bass: interactions among size, food, origin, and winter severity. Transactions of the American Fisheries Society 129:1-12.

Fulton, T.W. 1904. The rate of growth of fishes. Fisheries Board of Scotland Annual Report 22 (part 3): 141-241, Edinburgh.

Gales, R., D. Renouf, and G.A.J. Worthy. 1994. Use of bioelectrical impedance analysis to assess body composition of seals. Marine Mammal Science 10:1-12.

Golet, G.H., and D.B. Irons. 1999. Raising young reduces body condition and fat stores in black-legged kittiwakes. Oecologia 120:530-538.

Higgs, D.A., J.S. Macdonald, C.D. Levings, and B.S. Dosanjh. 1995. Nutrition and feeding habits of Pacific salmon (*Onchorhynchus* species) in relation to life history stage. Pages 159-315 *in* C. Groot, L. Margolis, and W.C. Clarke, editors. Physiological ecology of Pacific salmon. University of British Columbia Press, Vancouver.

Hilderbrand, G.V., S.D. Farley, and C.T. Robbins. 1998. Predicting body composition of bear via two field methods. Journal of Wildlife Management 62:406-409.

Hill, J.E., J.D. Leitman, and J.E. Sunderland. 1967. Thermal conductivity of various meats. Food Technology 21:1143-1148.

Hill, R.W., G.A. Wyse, and M. Anderson. 2004. Animal physiology. Sinauer Associates, Inc., Sunderland, Massachusetts.

Hillman, W.P. 1982. Structure and dynamics of unique bluegill populations. Master's thesis. Univeristy of Missouri, Columbia.

Hundertmark, K.J., and C.C. Schwartz. 2002. Evaluation of bioelectrical impedance analysis as an estimator of moose body composition. Wildlife Society Bulletin 30:915-921.

Hurst, T.P., E.T. Schultz, and D.O. Conover. 2000. Seasonal energy dynamics of young-of-the-year Hudson River striped bass. Transactions of the American Fisheries Society 129:145-157.

Hutchings, J.A., A. Pickle, C.R. McGregor-Shaw, and L. Poirier. 1999. Influence of sex, body size, and reproduction on overwinter lipid depletion in brook trout. Journal of Fish Biology 55:1020-1028.

Hwang, Y.T., S. Larivière, and M. Messier. 2005. Evaluating body condition of striped skunks using non-invasive morphometric indices and bioelectrical impedance analysis. Wildlife Society Bulletin 33:195-203.

Jakob, E.M., S.D. Marshall, and G.W. Uetz. 1996. Estimating fitness: a comparison of body condition indices. OIKOS 77:61-67.

Jenkins, T.G., K.A. Leymaster, and L.M. Turlington. 1988. Estimation of fat-free soft tissue in lamb carcasses by use of carcass and resistive impedance measurements. Journal of Animal Sciences 66:2174-2179.

Jonas, J.L., C.E. Kraft, and T.L. Margenau. 1996. Assessment of seasonal changes in energy density and condition in age-0 and age-1 muskellunge. Transactions of the American Fisheries Society 125:203-210.

Ko, G.T.C., J. Tang, J.C.N. Chan, R. Sung, M.M.F. Wu, H.P.S. Wai, and R. Chen. 2001. Lower BMI cut-off value to define obesity in Hong Kong Chinese: an analysis based on body fat assessment by bioelectrical impedance. British Journal of Nutrition 85:239-242.

Kushner, R.F., and D.A. Schoeller. 1986. Estimation of total body water by bioelectrical impedance analysis. American Journal of Clinical Nutrition 44:417-424.

Kyle, U.G., I. Bosaeus, A.D. De Lorenzo, P. Deurenberg, M. Elia, J.M. Gómez, B.L. Heitmann, L. Kent-Smith, J. Melchior, M. Pirlich, H. Scharfetter, A.M.W.J. Schols, and C. Pichard. 2004. Bioelectrical impedance analysis – part II: utilization in clinical practice. Clinical Nutrition 23:1430-1453.

Lammens, E.H.R.R., H.W. de Nie, J. Vijverberg, and W.L.T. van Densen. 1985. Resource partitioning and niche shifts of bream (*Abramis brama*) and eel (*Anguilla Anguilla*) mediated by predation of smelt (*Osmerus eperlanus*) on *Daphnia hyaline*. Canadian Journal of Fisheries and Aquatic Sciences 42:1342-1351.

Lantry, B.F., D.J. Steward, P.S. Rand, and E.L. Mills. 1999. Evaluation of total-body electrical conductivity to estimate whole-body water content of yellow perch, *Perca flavescens,* and alewife, *Alosa pseudoharengus*. Fisheries Bulletin 97:71-79.

Le Cren, E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (Perca fluviatilis). Journal of Animal Ecology 20:201-219.

Lemly, A.D., and G.W. Esch. 1984. Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: ecological implications. The Journal of Parasitology 70:475-492.

Lukaski, H.C., P.E. Johnson, W.W. Bolonchuk, and G.I. Lykken. 1985. Assessment of fat-free
mass using bioelectrical impedance measurements of the human body. American Journal
of Clinical Nutrition 41:810-817.

Lukaski, H.C., W.W. Bolonchuk, C.B. Hall, and W.A. Siders. 1986. Validation of tetrapolar
bioelectrical impedance method to assess human body composition. Journal of Applied
Physiology 60:1327-1332.

MacKinnon, J.C. 1972. Summer storage of energy and its use for winter metabolism and gonad
maturation in American plaice (*Hippoglossoides platessoides*). Journal of Fisheries
Research Board of Canada 29:1749-1759.

Marchello, M.J., and W.D. Slanger. 1994. Bioelectrical impedance can predict skeletal muscle
and fat-free skeletal muscle of beef cows and their carcasses. Journal of Animal Science
72:3118-3123.

Margraf, F.J., K.J. Hartman, and M.K. Cox. 2006. Non-lethal estimation of energy content of
Yukon River chum salmon. U.S. Fish and Wildlife Service. Anchorage.

McComish, T.S., R.O. Anderson, and F.G. Goff. 1974. Estimation of bluegill (*Lepomis
macrochirus*) proximate composition with regression models. Journal of the Fisheries
Research Board of Canada 31:1250-1254.

Mommsen, T.P. 1998. Growth and metabolism. Pages 65-97 *in* D.H. Evans, editor. The
physiology of fish, 2nd edition. CRC Press, New York.

Morton, A., and R.D. Routledge. 2006. Fulton's condition factor; is it a valid measure of sea lice
impact on juvenile salmon? North American Journal of Fisheries Management 26:56-62.

Murphy, B.R., M.L. Brown, and T.A. Springer. 1990. Evaluation of the relative weight index
with new application to walleye. North American Journal of Fisheries Management
10:85-97.

Murphy, B.R., D.W. Willis, and T.A. Spring. 1991. The relative weight index in fisheries

> management: status and needs. Fisheries 16(2):30-38.

Murphy, B.R., and D.W. Willis. 1992. Proper distinction between relative weight and relative

> condition factor. North American Journal of Fisheries Management 12:665-666.

Neumann, R.M., and D.W. Willis. 1994. Relative weight as a condition index for muskellunge.

> Journal of Freshwater Ecology 9:13-18.

Novinger, D.C., and C.M. Del Rio. 1999. Failure of total body electrical conductivity to predict

> lipid content of brook trout. North American Journal of Fisheries Management 19: 942-
> 947.

Olson, M.H., and B.P. Young. 2003. Patterns of diet and growth in co-occurring populations of

> largemouth bass and smallmouth bass. Transactions of the American Fisheries Society
> 132:1207-1213.

Pangle, K.L., and T.M. Sutton. 2005. Temporal changes in the relationship between condition

> indices and proximate composition of juvenile *Coregonus artedi*. Journal of Fish Biology
> 66:1060-1072.

Paulson, D.S. 2007. Regression and modeling: applications for the clinical and pharmaceutical

> industries. Chapman & Hall/CRC. New York.

Piccolo, J.J., W.A. Hubert, and R.A. Whaley. 1993. Standard weight equation for lake trout.

> North American Journal of Fisheries Management 13:401-404.

Pirlich, M., T. Schütz, T. Spachos, S. Ertl, M.L. Weiss, H. Lochs, and M. Plauth. 2000.

> Bioelectrical impedance analysis is a useful bedside technique to assess malnutrition in
> cirrhotic patients with and without ascites. Hepatology 32:1208-1215.

Pope, K.L., M.L. Brown, and D.W. Willis. 1995. Proposed revision of the standard weight (*Ws*)

> equation for redear sunfish. Journal of Freshwater Ecology 10:129-134.

Pope, K.L., and C.G. Kruse. 2007. Condition. Pages *in* 423-472. C.S. Guy and M.L. Brown, editors. Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda, Maryland.

Oliver, J.D., G.F. Holeton, and K.E. Chua. 1979. Overwinter mortality of fingerling smallmouth bass in relation to size, relative energy stores, and environmental temperature. Transactions of the American Fisheries Society 108:130-136.

Randall, D., W. Burggren, and K. French. 1997. Animal physiology: mechanisms and adaptations. W.H. Freeman and Company, New York.

Rasmussen, R.S., and T.H. Ostenfeld. 2000. Effects of growth rate on quality traits and feed utilisation of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Aquaculture 184:327-337.

Reznick, D.N., and B. Braun. 1987. Fat cycling in the mosquitofish (*Gambusia affinis*): fat storage as a reproductive adaptation. Oecologia 73:401-413.

Rice, S.D., R.E. Thomas, R.A. Heintz, A.C. Wertheimer, M.L. Murphy, M.G. Carls, J.W. Short, and A. Moles. 2001. Synthesis of long-term impacts to pink salmon following the *Exxon Valdez* oil spill: persistence, toxicity, sensitivity, and controversy. Final Report to the *Exxon Valdez* Trustee Councili, Project 99329.

Richter, H., C. Luckstadt, U. Focken, and K. Becker. 2000. An improved procedure to assess fish condition on the basis of length-weight relationships. Archive of Fishery Marine Research 48:255-264.

Salam, A., and P.M.C. Davies. 1994. Body composition of northern pike (*Esox lucius* L.) in relation to body size and condition factor. Fisheries Research 19:193-204.

Sammons, S.M., and P.W. Bettoli. 2000. Population dynamics of a reservoir sport fish

 community in response to hydrology. North American Journal of Fisheries Management

 20:791-800.

Schulte-Hostedde, A.I., J.S. Millar, and G.J. Hickling. 2001. Evaluating body condition in small

 mammals. Canadian Journal of Zoology 79:1021-1029.

Schwenk, A., A. Beisenherz, K. Römer, G. Kremer, B. Salzberger, and M. Elia. 2000. Phase

 angle from bioelectrical impedance analysis remains an independent predictive marker in

 HIV-infected patients in the era of highly active antiretroviral treatment. American

 Journal of Clinical Nutrition 72:496-501.

Scott, I., M. Grant, and P.R. Evans. 1991. Estimation of fat-free mass of live birds: use of total

 body electrical conductivity (TOBEC) measurements in studies of single species in the

 field. Functional Ecology 5:314-320.

Scott, I., C. Selman, P.I. Mitchell, and P.R. Evans. 2001. The use of total body electrical

 conductivity (TOBEC) to determine body composition in vertebrates. *In* Body

 composition analysis of animals: a handbook of nondestructive methods. *Edited by* J. R.

 Speakman. Cambridge University Press, Cambridge. pp. 127-160.

Segal, K.R., B. Gutin, E. Presta, J. Wang, and T. Van Itallie. 1985. Estimation of human body

 composition by electrical impedance methods: a comparative study. Journal of Applied

 Physiology 58:1565-1571.

Simpkins, D.G., W.A. Hubert, C.M. Del Rio, and D.C. Rule. 2003. Physiological responses of

 juvenile rainbow trout to fasting and swimming activity: effects on body composition and

 condition indices. Transactions of the American Fisheries Society 132:576-589.

Speakman, J.R. 2001. Introduction. Pages 1-7 *in* J.R. Speakman, editor. Body composition of

 analysis of animals: a handbook of non-destructive methods. Cambridge University

 Press, Cambridge.

Sutton, S.G., T.P. Bult, and R.L. Haedrich. 2000. Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. Transactions of the American Fisheries Society 129:527-538.

Swantek, P.M., J.D. Crenshaw, M.J. Marchello, and H.C. Lukaski. 1992. Bioelectrical impedance: a nondestructive method to determine fat-free mass of live market swine and pork carcasses. Journal of Animal Science 70:169-177.

Swingle, W.E., and E.W. Shell. 1971. Tables for computing relative conditions of some common freshwater fishes. Alabama Agricultural Experiment Station, Auburn University, Circular 183.

Thomasett, A. 1962. Bioelectrical properties of tissue impedance. Lyon Medicine 207:107-118.

Thompson, J.T., E.P. Bergersen, C.A. Carlson, and L.R. Kaeding. 1991. Role of size, condition, and lipid content in the overwinter survival of age-0 Colorado squawfish. Transactions of the American Fisheries Society 120:346-353.

Thomson, B.C., B.J. Thomas, L.C. Ward, and M.N. Sillence. 1997. Evaluation of multifrequency bioelectrical impedance data for predicting lean tissue mass in beef cattle. Australian Journal of Experimental Agriculture 37:743-749.

Tocher, D.R. 2003. Metabolism and functions of lipids in fatty acids in teleost fish. Reviews in Fisheries Science 12:107-184.

Toso, S. 2000. Altered tissue electric properties in lung cancer patients as detected by bioelectric impedance vector analysis. Nutrition 16:120-124.

Trudel, M., S. Tucker, J.F.T. Morris, D.A. Higgs, and D.W. Welch. 2005. Indictors of energetic status in juvenile coho salmon and Chinook salmon. North American Journal of Fisheries Management 25:374-390.

Wege, G.J., and R.O. Anderson. 1978. Relative weight (Wr): a new index of condition for largemouth bass. Pages 79-91 in G.D. Novinger and J.G. Dillard, editors. New

approaches to the management of small impoundments. American Fisheries Society, Bethesda, Maryland.

Whelan, G.E., and W.W. Taylor. 1984. Fisheries report. ELF Communications System Ecological Monitoring Program. Annual report for ecosystems – tasks 5.8, 5.9, 5.10 for ITT Research Institute, Chicago, Illinois. U.S. Navy Electronics Systems Command Technical Report E06548-8, Washington, DC.

Wiener, J.G., and W.R. Hanneman. 1982. Growth and condition of bluegills in Wisconsin lakes: effects of population density and lake pH. Transactions of the American Fisheries Society 111:761-767.

Woolnough, A.P., W.J. Foley, C.N. Johnson, and M. Evans. 1997. Evaluations of techniques for indirect measurement of body composition in free-ranging large herbivore, the southern hairy-nosed wombat. Wildlife Research 24:649-660.

Wootton, R.J. 1985. Energetics of reproduction. Pages 231-254 *in* P. Tytler and P. Carlow, editors. Fish energetic: new perspectives. Johns Hopkins University Press, Baltimore, Maryland.