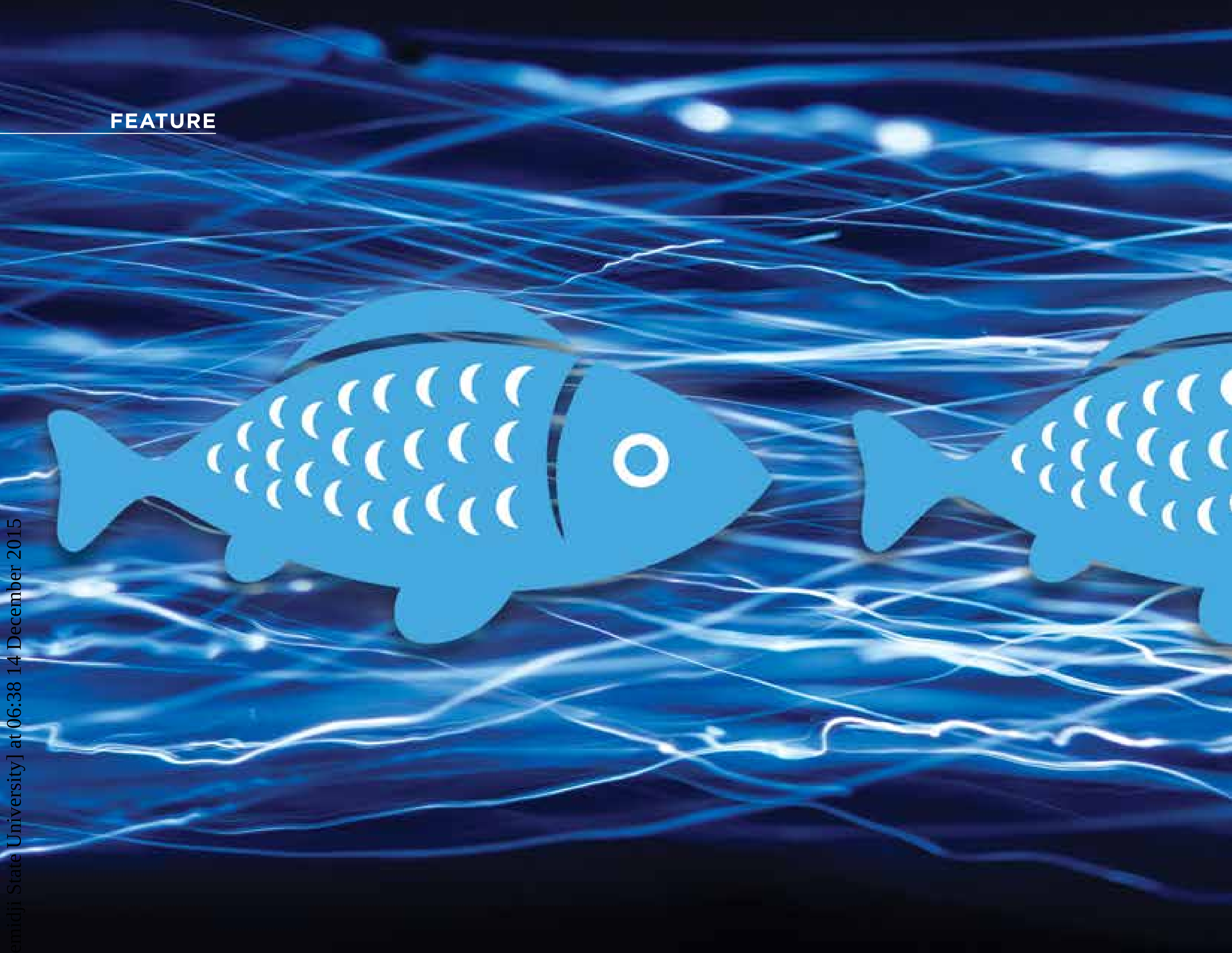
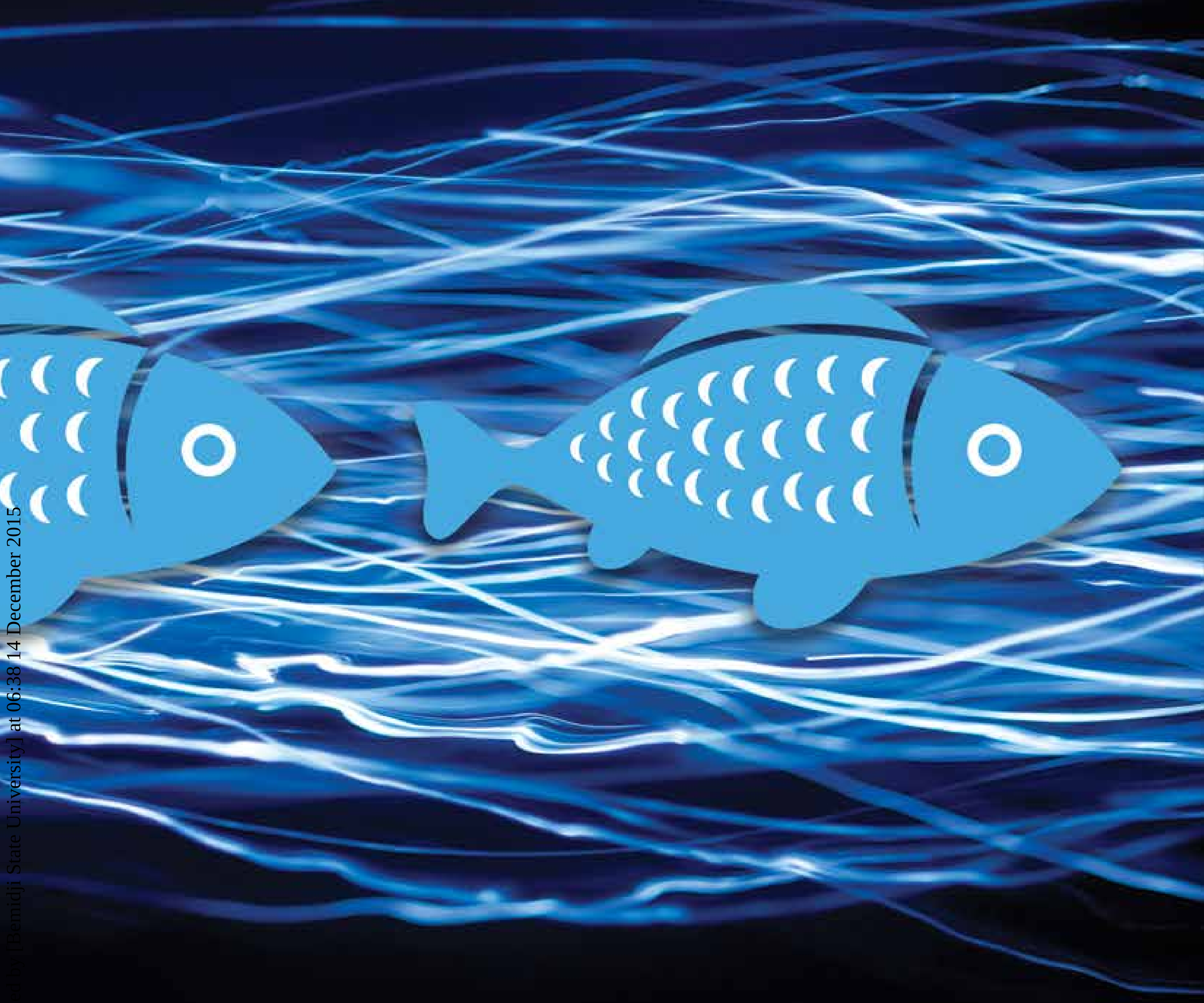


Bioelectrical Impedance Analysis:

A New Tool for Assessing Fish Condition



Kyle J. Hartman
Division of Forestry and Natural Resources, West Virginia University, 310A Percival Hall, Morgantown, WV 26506-6125. E-mail: Kyle.Hartman@mail.wvu.edu

F. Joseph Margraf
U.S. Geological Survey, Cooperative Research Units Program, Pagosa Springs, CO

Andrew W. Hafs
Aquatic Biology Program, Department of Biology, Bemidji State University, Bemidji, MN

M. Keith Cox
Seafood Analytics, Juneau, AK

Bioelectrical impedance analysis (BIA) is commonly used in human health and nutrition fields but has only recently been considered as a potential tool for assessing fish condition. Once BIA is calibrated, it estimates fat/moisture levels and energy content without the need to kill fish. Despite the promise held by BIA, published studies have been divided on whether BIA can provide accurate estimates of body composition in fish. In cases where BIA was not successful, the models lacked the range of fat levels or sample sizes we determined were needed for model success (range of dry fat levels of 29%, *n* = 60, yielding an R^2 of 0.8). Reduced range of fat levels requires an increased sample size to achieve that benchmark; therefore, standardization of methods is needed. Here we discuss standardized methods based on a decade of research, identify sources of error, discuss where BIA is headed, and suggest areas for future research.

Análisis de impedancia bioeléctrica: una nueva herramienta para evaluar la condición somática en peces

En análisis de impedancia bioeléctrica (AIB) se utiliza comúnmente en las áreas de salud y nutrición humana, pero solo hasta recientemente se ha considerado como una herramienta potencial para evaluar la condición somática en peces. Una vez que el AIB es calibrado, sirve para estimar niveles de grasa/humedad y contenido energético sin necesidad de sacrificar al animal. Pese a lo prometedor del AIB, algunos trabajos cuestionan la precisión de los estimados del AIB en cuanto a la composición corporal en peces. En los casos en los que la aplicación del AIB no resultó exitosa, los modelos carecían del rango de niveles de grasa o tamaños de muestra que en este trabajo se determinaron como necesarios para que el modelo fuera exitoso (rango de niveles de grasa seca de 29%, *n* = 60, produciendo una R^2 de 0.8). Si se desea tener un nivel aceptable de desempeño, una reducción en el rango de niveles de grasa requiere de un incremento en el tamaño de muestra; por lo tanto, la estandarización de métodos es indispensable. Aquí se revisan algunos métodos estandarizados que se han producido tras una década de investigación, se identifican fuentes de error, se discute hacia dónde se dirige el AIB y se sugieren áreas de investigación para el futuro.

Analyse d'impédance bioélectrique : Un nouvel outil d'évaluation de l'état du poisson

L'analyse d'impédance bioélectrique (BIA) est couramment utilisée dans les domaines de la santé et de la nutrition humaines, mais a récemment été considérée comme un outil potentiel pour évaluer l'état des poissons. Une fois que la BIA est calibrée, elle évalue les niveaux de graisse/d'humidité et la teneur en énergie sans qu'il ne soit nécessaire de tuer les poissons. Malgré la promesse tenue par la BIA, les études publiées se sont divisées sur le fait de savoir si la BIA peut fournir des estimations précises quant à la composition corporelle du poisson. Là où elle n'a pas été concluante, la gamme de niveaux de graisse ou la taille des échantillons que nous avions déterminée comme étant nécessaire à la réussite du modèle (gamme de niveaux de graisse sèche de 29%, *n* = 60, ce qui donne un R^2 de 0,8), étaient absente des modèles. Une gamme réduite des niveaux de graisse nécessite une augmentation de la taille de l'échantillon pour atteindre ce point de référence ; par conséquent, la standardisation des méthodes est nécessaire. Ici, nous discutons des méthodes normalisées basées sur une décennie de recherche, identifions les sources d'erreur, discutons de la direction que prend la BIA et proposons des pistes de recherche future.

BIOELECTRICAL IMPEDANCE ANALYSIS USE IN HUMANS

Bioelectrical impedance analysis (BIA) is a common tool in human health and physiology but only recently applied to fish or wildlife. Using BIA, body composition is estimated by measuring the impedance (resistance and reactance) of a current through an organism and then regressing (calibrating) these measures with actual body composition numbers for that organism (Cox and Hartman 2005). Bioelectrical impedance analysis has been widely used in human physiology to estimate hydration levels (Hoffer et al. 1969), water weights during pregnancy (Shaikh et al. 2011), fat levels (Lukaski et al. 1985; Lingwood et al. 2012), and altered tissue properties and reduced survival in AIDS and lung cancer patients (Schwenk et al. 2000; Toso et al. 2000). Such measures of condition and composition are also important to fisheries management and ecology.

MEASURES OF FISH CONDITION

Understanding and defining fish condition has been a goal of fisheries managers for decades. Many condition indices like Fulton's *K* and relative weight (W_r) rely on measures of length and weight and are nonlethal, making them attractive to users. However, Fulton's *K* has been shown to have serious size bias (Cone 1989), and some studies failed to find relationships between W_r and growth or lipid levels (Liao et al. 1995; Simpkins et al. 2003; Hartman and Margraf 2006).

Growth is considered to be the ultimate expression of well-being in fish. However, what is actually measured in most cases is change in total mass. Because most fish are 60–90% water, changes in this compositional component can greatly influence measures of growth or condition based on total mass. The propensity of fish to replace lipids with water when losing energy (Love 1970; Rottiers and Tucker 1982) complicates the accuracy of metrics like W_r.

Other measurements like energy content (bomb calorimetry) and body composition (proximate analysis) are more accurate but require euthanizing the fish, preventing repeated measures on individuals. Newer technologies like microwaves (Crossin and Hinch 2005) and BIA offer nonlethal alternatives to calorimetry or proximate analysis. In this article, we focus on BIA to measure fish composition and condition over microwave because the former is available in a very portable form that easily allows measures of impedance under a wide range of field and laboratory conditions and fish sizes.

Our purpose in this article is to inform and guide the fisheries management community in an understanding of what BIA is, its history, how it works, and how it should be used. The use of BIA in fisheries applications has increased rapidly in the decade since its first publication, but proper techniques for BIA measures and calibration model development have not been previously defined. We review successes and failures in the use of BIA on fish and show under what conditions BIA works best. We conclude by discussing where we think the use of BIA is headed as it gains acceptance in the fisheries management toolbox.

WHAT IS BIA, AND HOW DOES IT WORK?

The BIA device has a four-electrode array consisting of two signal electrodes and two detecting electrodes (Figure 1) with 800 μA, 50 kHz, AC current between 3.75 and 10.60 V (RJL Systems, Clinton Township, MI). The electrodes produce a parallel electrical field within the tissue in an approximately cylindrical shape. To compare impedance measures between fish, electrodes are placed in relation to anatomical landmarks to provide similar electrical pathways in each individual fish (Figure 2).

The BIA instrument measures the network of resistors and capacitors within the subject as a series circuit (resistance and reactance, respectively; Table 1). A series measure treats the whole subject as one measurement of resistance or reactance and can be transformed to their parallel equivalence. We use BIA in fish to predict parameters that are related to composition, health, or condition. These models are not mechanistic and thus include both series (equations E1 and E3; Table 1) and parallel calculations (E2, E4, and E6; Table 1) as candidate variables (Cox and Hartman 2005; Hafs and Hartman 2011; Hartman et al. 2011).

Equations based upon resistance (*R*) should relate to fat, which is nonconductive: the higher the lipid content, the higher the resistance. Reactance (X_c) is sensitive to cell volume and hence should relate to the volume of total healthy cells (Pethig 1979; Lukaski et al. 1985; Kyle et al. 2004). The low-frequency current is not strong enough to penetrate cell membranes and therefore is carried by electrolytic ions located within interstitial spaces (Kyle et al. 2004). *R* is sensitive to interstitial volumes by Ohm's law:

$$I = \Delta V * R^{-1},$$

where *I* is the current (A), *V* is voltage (V), and *R* is resistance (Ω). Our method of bioimpedance holds the current (*I*) steady and measures the changes in voltage (*V*) to calculate resistance (*R*). Theoretically, *R* and *V* are negatively correlated, so an increase in *R* represents a decrease in interstitial volume. *R* changes with length (*L*) of the circuit and area (*A*) through which the current passes from the equation:

$$R = (\rho * L)/A,$$

where ρ is a density constant. By multiplying *L* by the numerator and dominator, we end up with volume (*A***L*) in the denominator,

$$R = [(\rho * L) * L]/(A * L),$$

and L^2 in the numerator. By exchanging *R* and volume, the volume equation is

$$\text{Volume} = (\rho * L^2)/R.$$

Interstitial volume changes when fish gain or lose fat as water is moved from interstitial spaces into cells.

Reactance (X_c) is reflective of how much cell membrane material is present. Cell membranes are composed of a phosolipid bilayer, which is nonconductive (dielectric). Dielectrics do not carry a charge but briefly hold a charge before releasing it. The more dielectric material, the more charges can be held, and the higher the X_c. With organism growth, dielectric material and X_c increase. With decreases in growth (or condition), the resultant X_c values will decrease. The two measured vectors of current, *R* and X_c, are used to derive other

Figure 1. The BIA analyzer consists of a solid state instrument (here, RJL Systems Quantum II) with two pairs of signal and detection electrodes. Electrodes (inset) are often needle electrodes that penetrate just below the skin of the fish. Rod electrodes, which measure along the surface of the skin, are shown for comparison (from Hafs 2011), but these surface electrodes are more likely to have contact issues, particularly for scaled fishes. Photo credit: K.J. Hartman.

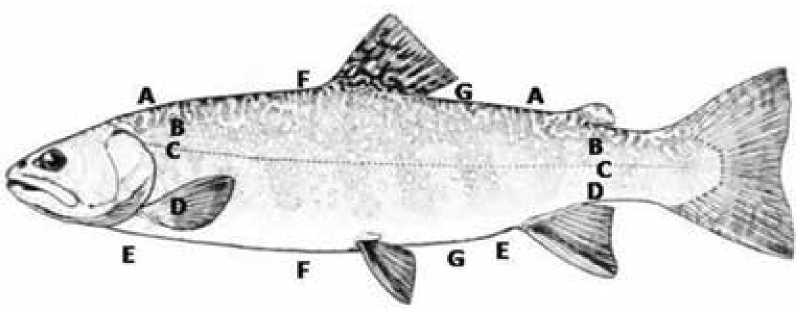


Figure 2. Diagram of a Brook Trout *Salvelinus fontinalis* showing the electrode locations utilized by Hafs and Hartman (2011) to determine the best location(s) for developing BIA models: (A) dorsal midline (DML), (B) dorsal total length (DTL), (C) lateral line (LL), (D) ventral total length (VTL), (E) ventral midline (VML), (F) dorsal to ventral predorsal fin (DTVpre), and (G) dorsal to ventral postdorsal fin (DTVpost).

TABLE 1. Electrical variables for AC series and parallel circuits used as candidate predictor variables in BIA models of fish condition. Variables are calculated for each BIA measurement location.

Electrical variable	Abbreviation	Units	Measure or Equation
Detector length	DL	mm	Linear measure between electrodes
Resistance in series	R	Ohms	Measured directly by Quantum II
Reactance in series	X_c	Ohms	Measured directly by Quantum II
Resistance index	E1	Ohms	DL^2/R
Parallel resistance index	E2	Ohms	DL^2/LR_p, where $LR_p = R + (X_c^2/R)$
Reactance index	E3	Ohms	DL^2/X_c
Parallel reactance index	E4	Ohms	DL^2/LX_{cp}, where $LX_{cp} = X_c + (R^2/X_c)$
Parallel capacitance index	E5	picofarads	DL^2/LC_{pf}, where $LC_{pf} = (\pi E7)/X_c$
Impedance index	E6	Ohms	DL^2/LZ, where $LZ = (R^2 + X_c^2)^{0.5}$
Phase angle	E7	degrees	$\mathrm{atan}(X_c/R)$
Standardized resistance	E8	Ohms/mm	R/DL
Standardized reactance	E9	Ohms/mm	X_c/DL

electrical property equations (Hartman et al. 2011) representing different aspects of how current flows through the body (Table 1). These derived electrical equations represent a set of candidate variables that can predict water, fat, and protein, or energy content of the body by using standard linear modeling methods to calibrate BIA. Because our goal is prediction, we are not concerned with independence of the variables.

Detector length (DL) has been criticized for its use in many of the electrical properties listed in Table 1 because DL is correlated with fish size, leading some to argue it is length that is providing BIAs predictive power. However, by Ohm's law, resistance increases with length of the circuit; therefore, DL is used to standardize and eliminate size bias in resistance-based measures.

HISTORY OF BIA

The history of BIA and its evolution from use in humans to use in lower vertebrates was rapid. It was first applied to humans in 1981 when W. Mills, M.D., studied hydration status of soldiers in high-altitude, cold-weather environments. By 1985, Lukaski et al. published the first paper on estimation of fat-free mass in humans using BIA. In the 1990s and 2000s, BIA technology was widely found in journal articles dealing with body-water mass in pregnant women (Lukaski et al. 1994; Morita et al. 1999; McCarthy et al. 2004) and in articles dealing with body composition of domestic livestock (Marchello and Slanger 1994; Slanger et al. 1994; Daza et al. 2006). Although with limited success, Bosworth and Wolters (2001) were the first to publish an application of BIA to body fillet yield and composition in Channel Catfish *Ictalurus punctatus*. Cox and Hartman (2005) reported the first successful use of BIA to estimate body composition in Brook Trout *Salvelinus fontinalis*. Between 2007 and 2012, at least 15 articles were published using BIA in a fisheries application (online bibliography of BIA in fish: faculty.bemidjistate.edu/ahafs/research/bia.)

Increasing use of BIA in fisheries management and science led to a special symposium at the American Fisheries Society Annual Meeting in 2013, which brought together BIA practitioners and others interested in the tool (Harrell 2013).

DOES BIA HARM THE FISH?

Very little work has assessed injuries or mortality of fish following BIA measures with needle electrodes. In small Brook Trout (110–220 mm total length [TL]), repeated measures of BIA weekly for 3 weeks resulted in no mortality, no changes in swimming or feeding, and only slight bruising on a few individuals (Cox and Hartman 2005). These procedures have not been tested on smaller (<110 mm TL) fish, more fragile species, or under stressful environmental conditions (e.g., high temperatures, low dissolved oxygen, etc.).

SUCCESSES AND FAILURES

The literature on BIA use in fish is divided between examples considered successful and those declared failures (Table 2). Though successes provide hope that the method will be widely applicable for fisheries management use, failures give reason for pause. We considered studies with models yielding R^2 values ≥ 0.8 to be successful, whereas those less than 0.8 likely lacked the ability to accurately predict body composition.

DATA NEEDS FOR A GOOD BIA CALIBRATION MODEL—A CASE STUDY

As a means of identifying sample requirements to develop a good calibration model, we present a data set looking at BIA measures on field-caught adult Chum Salmon *Oncorhynchus keta* from the Yukon River drainage in Alaska (Margraf, Hartman, and Cox, unpublished report to the U.S. Fish and Wildlife Service). Because we used only adults, the size range was limited (545–668 mm), minimizing impacts of length. The analysis was robust with a sample size of 86 (47 from within the

TABLE 2. Attributes of databases for different species for which BIA models have been attempted. We define success as BIA models yielding R^2 values ≥ 0.8, except for Rasmussen et al. (2012), who declared their models successful with lower R^2 values. Range refers to the range in percentage dry lipids in the source data set. Studies are split between those using BIA to predict mass-based body composition and those estimating percentage-based composition. Mass-based approaches yield artificially high R^2 values due to relations between detector lengths (correlated with fish length) and total mass. A question mark for range indicates that we were unable to determine the range of percentage dry lipids from the source.

Species	Success	*n*	Range	Source	Possible reasons for lack of success
Mass-based					
Brook Trout *Salvelinus fontinalis*	Yes	30	9	Cox and Hartman (2005)	N/A
Cobia *Rachycentron canadum*	Yes	60	?	Duncan et al. (2007)	N/A
Steelhead *Oncorhynchus mykiss* (anadromous Rainbow Trout)	Mixed	30	?	Hanson et al. (2010)	Did not use multiple regression. Low sample size—only 15 of 30 fish used for model development.
Rainbow Trout *Oncorhynchus mykiss*	Yes	216	?	Bourdages (2011)	N/A
Percentage-based					
Bluefish *Pomatomus saltatrix*	Yes	96	45	Hartman et al. (2011)	N/A
Brook Trout *Salvelinus fontinalis*	Yes	32	~30	Rasmussen et al. (2012)	N/A
Brook Trout *Salvelinus fontinalis*	Yes	139	32	Hafs and Hartman (2011)	N/A
Chum Salmon *Oncorhynchus keta*	Yes	86	45	Our data	N/A
Dolly Varden Char *Salvelinus malma*	Yes	192	50	Stolarski et al. (2014)	N/A
Atlantic Croaker *Micropogonias undulatus*	No	130	?	Garner et al. (2012)	Used only phase angle and E3 in analysis. Limited range of lipid levels in juvenile fish.
Atlantic Salmon *Salmo salar*	No	60	?	Calderone et al. (2012)	Low percentage fat range (means of 4.9% to 7.9%).
Black Sea Bass *Centropristis striata*	No	41	?	Wuenschel et al. (2013)	Low sample size, used only one (E7 from Table 1) of potential BIA properties to predict.
Common Carp *Cyprinus carpio*	No	22	~17	Klefoth et al. (2013)	Small sample size with limited dry percentage fat.
European Eel *Anguilla anguilla*	No	40	~25	Klefoth et al. (2013)	Small sample size.
Lake Whitefish *Coregonus clupeiformis*	No	34	?	Pothoven et al. (2008)	Small sample size, lack of temperature consideration, likely low range in percentage dry fat. Only resistance and reactance used in models.
Yellow Perch *Perca flavescens*	No	38	?	Pothoven et al. (2008)	Small sample size, lack of temperature consideration, likely low range in percentage dry fat. Only resistance and reactance used in models.
Walleye *Sander vitreus*	No	30	?	Pothoven et al. (2008)	Small sample size, lack of temperature consideration, likely low range in percentage dry fat. Only resistance and reactance used in models.

mouth of the river and 39 from near spawning areas about 1,600 km upstream; 41 males, 45 females) and fat content ranging between 3% and 48% (as percentage wet weight: 0.4–19.4%). We chose to use percentage dry weight fat because this was the value obtained from the laboratory analysis, and we did not want to confound results by converting fat values to percentage wet weight. When percentage fat content estimated using BIA was regressed against values determined by laboratory analysis, the resulting coefficient of determination (R^2) was 0.89 (using temperature along with the suite of properties in Table 1, the best model selected based on the lowest corrected Akaike information criterion). Studies that have estimated body composition mass using BIA have noted strong correlation with fish length, weight, or simple condition factors. However, in the Chum Salmon data set, we found that length, weight, and Fulton's *K* explained only 52% of the variability in percentage dry fat, and inclusion of BIA variables increased the R^2 to 0.89.

The first question gleaned from this robust data set was, "What affect does sample size (maintaining the full range in fat content) have on the resulting ability to predict percentage dry weight of fat?" To estimate the effect of sample size, we took an average of three runs of 1,000 simulations at each possible sample size ($n = 86$ to $n = 24$). The resulting power analysis indicated that to achieve an R^2 of 0.8, a sample size of 54 fish was needed (Figure 3). A sample size of 42 resulted in an R^2 value of 0.7. At a sample size of 32 fish, the predictive ability fell to near 0.5 and dropped precipitously from there.

The data set boasted a very broad range in fat content. Thus, the second question addressed was, "What is the influence of the range of percentage dry weight of fat on predictive ability?"

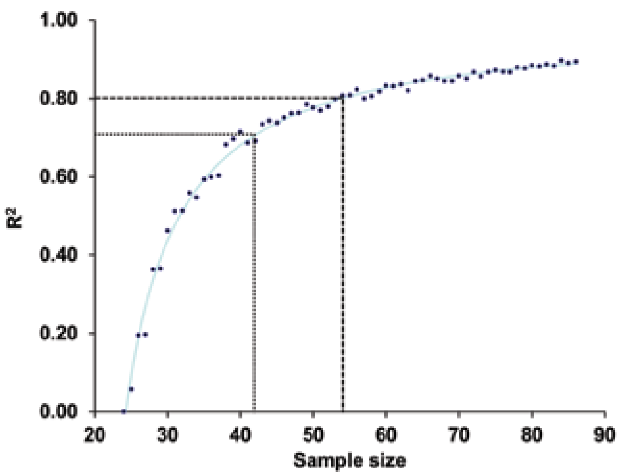


Figure 3. Power analysis of the sample size of Chum Salmon on the ability of BIA to predict percentage dry weight of fat at a constant range of 3% to 48%.

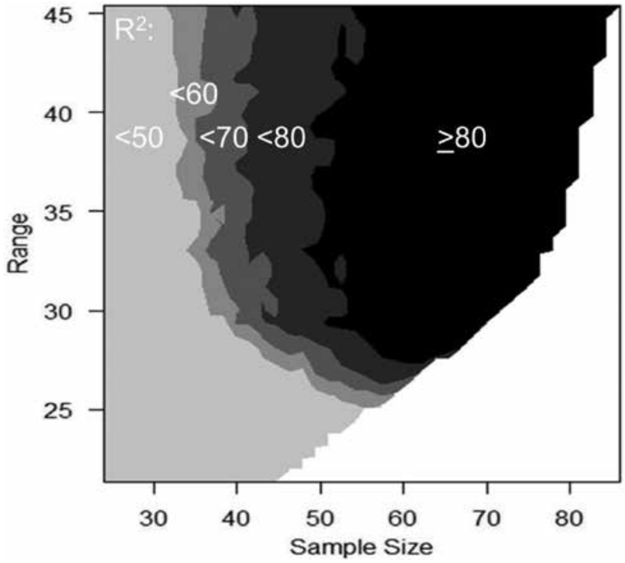


Figure 4. Response surface of the predictive ability of BIA across the range (RANGE = highest minus lowest) in percentage dry weight of fat and sample size for Chum Salmon.

This question was answered similarly to above except for each simulation we removed the fish with the highest or lowest percentage fat value until R^2 fell to near zero. Because reduction in the fat range also resulted in a concomitant reduction in sample size, the result was a power analysis response surface (Figure 4). To attain $R^2 \geq 0.08$, this analysis suggested that a sample of 60 or greater with a dry fat range of about 27% was required (range here meaning the spread between the highest percentage fat and the lowest percentage fat). At a range of about 29% dry fat, a sample size of at least 50 was required to attain $R^2 \geq 0.80$. Above this range the sample size required to yield the same statistical power did not change. Thus, we recommend a minimum sample size of 60 fish and a minimum fat range of 29% to obtain an arbitrarily chosen $R^2 \geq 0.80$.

An interesting question is what happens when these sample size and fat range criteria are applied to examples from the literature? Do they help explain why some attempts to use BIA were successful and others not (Table 2)? Applying these criteria to known examples of where BIA has been used, all of the successful examples fit the criteria, except where only fat mass was evaluated (Cox and Hartman 2005) or lower R^2 was considered successful (Rasmussen et al. 2012). Of the unsuccessful attempts, all but one (Atlantic Croaker *Micropogonias undulatus*) clearly fell well below the minimum recommended values. Though the unsuccessful Atlantic Croaker example had a large sample size $n = 130$, the range in fat (not reported) was likely low because these were juvenile fish, and only phase angle and "compositional index" (E3 in our Table 1) were used to calibrate the instrument (one of several possible electrical properties to use in BIA calibration modeling; Table 1).

IMPROVEMENTS IN THE USE OF BIA

Many early studies using BIA in fish focused on estimates of the mass of proximate components (water, protein, fat, ash; Cox and Hartman 2005; Duncan et al. 2007). Although these studies were often successful, the value within itself can be ambiguous. Percentage-based estimates are preferred for several reasons. Percentage-based estimates are less dependent on the length of the fish. Therefore, percentage-based estimates, particularly percentage fat and percentage water (often represented by percentage dry weight), can be used as indices of condition. For example, depending on the size of the fish, 5 g of fat could suggest a fish with substantial energy reserves or a fish close to death from starvation (Hutchings et al. 1999), because additional information about fish length is needed to assess the condition. Yet, indicating that a stream-dwelling Brook Trout is composed of 10% fat immediately indicates that the fish is in good condition and well-suited to survive the harsh winter months (Cunjak and Power 1986).

Another benefit of percentage-based BIA estimates, in particular percentage dry weight, are the published studies relating percentage dry weight to estimates of proximate composition (Hartman and Margraf 2008; Luo et al. 2013) or energy density (Hartman and Brandt 1995; Pedersen and Hislop 2001; Morley et al. 2012). Proximate composition analysis is relatively expensive to estimate from both cost and time perspectives, but percentage dry weight can be measured in the lab at relatively low cost, so using BIA to predict percentage dry weight is a cost-effective alternative to expensive proximate composition or energy density measurements. As a result, recent research with BIA has attempted to predict percentage-based estimates, which we suggest should continue in the future.

Research with fish BIA is often done in field settings where a subset of fish is used to develop a calibration model that is subsequently used to estimate some aspect of proximate composition or condition for the rest of the population (Cox and Hartman 2005; Pothoven et al. 2008; Rasmussen et al. 2012). However, it is known that temperature can influence BIA measures (Figure 5). Therefore, it is crucial that BIA field studies are corrected for temperature differences, or the BIA data should be collected at the same temperature at which the calibration model was developed. Temperature correction equations can be developed in the laboratory (Hafs and Hartman 2015), preferably during experiments used to develop the calibration models.

Temperature corrections (Hartman et al. 2011; Hafs and Hartman 2015) have been made by taking BIA measures on individuals at several temperatures spanning the range of environmental temperatures. Experimental fish were successively measured at multiple temperatures starting with the warmest and ending with the coldest, allowing time for the fish's

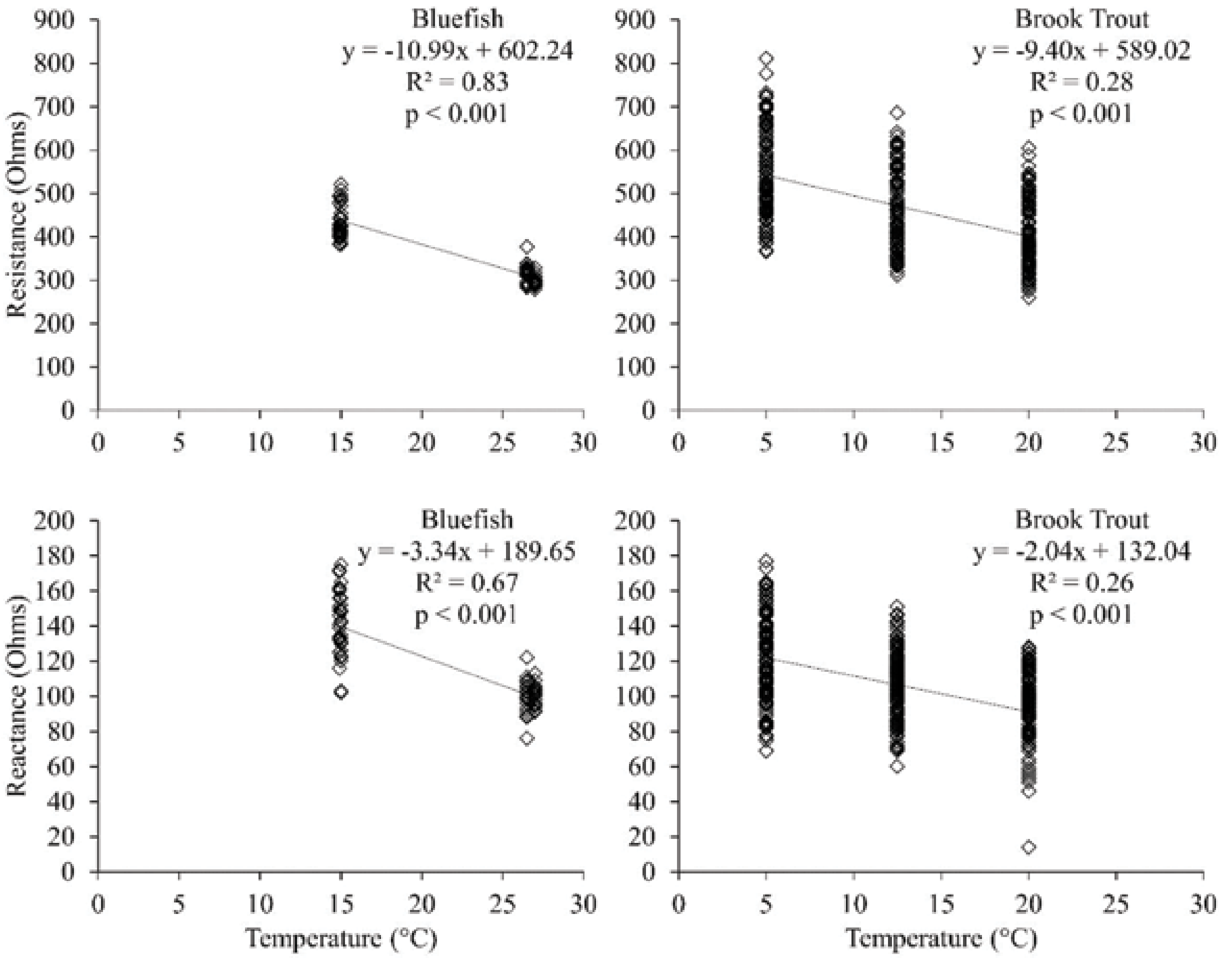


Figure 5. Trends in measured resistance and reactance at 15°C and 27°C for Bluefish (data from Hartman et al. 2011) and across a range of temperatures for adult Brook Trout using subdermal needle electrodes placed along the dorsal midline (data from Hafs 2011).

body temperature to reach equilibrium (Hartman et al. 2011; Hafs and Hartman 2015). Times to reach equilibrium range from about 8 h for fish about 100 g to over 18 h for fish about 3,000 g for temperature differentials of about 12°C (K. J. Hartman, personal observation). Thus, it may take 2–3 days to complete measures at all temperatures before appropriately euthanizing the fish for proximate analysis. This method assumes that changes in proximate or energy content are negligible over that time. More studies are needed to determine whether temperature corrections can be generalized across taxa or life stage or whether species-specific relationships are needed.

Laboratory studies also provide an opportunity to develop standardized methods needed for universal application of BIA. Without such standardization, BIA researchers continue to use varying electrode locations on fish even though research has indicated that, at least for salmonids, results can be improved by testing which locations provide the most accurate predictions (Hafs and Hartman 2011). It is also likely that there is an optimal needle gauge and penetration depth for each species and size of fish, but these protocols still need to be developed. These additional protocols related to needle gauge, penetration depth of the electrodes, and electrode placement should become part of the BIA standardization procedure to improve model accuracy.

OTHER FACTORS AFFECTING ACCURACY

Based on our experience, several other factors can influence accuracy of BIA measurements. As with any electrical circuit, a requirement when applying BIA to a fish is to establish a solid connection. With needle electrodes, this means that the electrodes must penetrate the skin and scale layers (scales may need to be removed in the contact area for some species) and make good contact with the underlying tissue. A good connection is denoted by a stable reading of resistance or reactance from the analyzer when firm pressure is applied to the electrodes. Readings that jump around suggest poor connection or may indicate that needle electrodes are damaged and require replacement. They may also suggest that not enough pressure is being applied to the electrodes (think of this as a loose electrical connection). We recommend firm, steady pressure on the electrodes. Testing of the circuits between the electrodes and the analyzer unit is more straightforward and should be done with a known value resistor before every BIA sampling event.

Changes in temperature of the fish during handling can also affect BIA measurement. As differences between air and water temperature increase, or the sun is shining directly on the fish, the surface of the fish can change temperature rapidly. Skin temperature was shown to significantly influence BIA measures in humans (Gudivaka et al. 1996). Bioelectrical impedance analysis procedures should minimize the opportunity for temperature changes in the fish during measurement by making rapid measures and minimizing conditions that allow the fish's temperature to change during measurement.

Related to temperature and connection errors discussed above, user experience is also a major factor (Cox et al. 2011). More experienced users will be better able to detect issues with connections and be able to take measurements faster, minimizing the possibility of temperature changes. If fish are sacrificed before BIA measures are taken, another possible source of error is time after death (Cox et al. 2011). Cells begin to lyse and break down quickly postmortem, altering BIA measures. Cox et al. (2011) found that resistance remained stable for 24 h or more after death, but reactance values began deviating from live values within a few hours for Coho Salmon *O. kisutch*; therefore, fish should be alive or recently dead at the time of BIA assessment.

Often during field-based BIA studies, fish are captured via gillnets (Pothoven et al. 2008) or electrofishing (Hafs 2011; Rasmussen et al. 2012). Physiological changes resulting from stress during capture events and the influence those changes have on BIA measures is an area where research is currently lacking. Often hatchery fish, or wild fish reared in laboratory conditions for extended periods, are used in the calibration model development stage. There are physiological differences between hatchery and wild Rainbow Trout *O. mykiss* (Woodward and Strange 1987), but very limited research has been done to determine whether these differences are substantial enough to alter BIA measures.

Reproductive status (maturity and gonad development) holds the potential to influence BIA measures as fish experience large changes in fat content during reproductive periods. The Chum Salmon data set and the Brook Trout data set (Hafs 2011) are direct examples of this occurring in nature. Morphometric-based condition estimates have a difficult time dealing with making comparisons between fish undergoing these types of changes (Hanson and Nate 2005). Bioelectrical impedance analysis is not influenced by relationships between length and weight and relies on changes of internal composition. Therefore, BIA should have less trouble dealing with issues related to reproductive development than traditional morphometric-based measures.

COSTS

The advantage BIA holds over laboratory measures of body composition to assess condition are the relative cost and time savings. The Quantum II Bioelectrical Impedance Analyzer (RJL Systems, Clinton Township, MI) cost is US$2,590 (September 2014). In addition to the analyzer, electrodes must be constructed, typically at a cost of less than $50. Although drying or proximate analysis measures are required to develop a BIA calibration model, once established for a species, it can be applied without additional cost. Fish or sample acquisition costs will be the same whether using BIA or retaining fish for proximate analysis. However, in proximate analysis, fish must be homogenized and samples sent to analytical laboratories for analysis. Proximate analysis measures are on small subsamples of tissue and, hence, are subject to their own sample and measurement errors and bias. Typical proximate analysis costs range from $35 to $150 (2014) per sample depending on the laboratory. Such costs limit the widespread use of proximate analysis to assess fish condition.

CONCLUSIONS AND RECOMMENDATIONS FOR STANDARDIZATION

Our analysis of a robust data set provides guidelines for the range of body conditions and sample sizes needed to produce successful BIA calibration models. Although these guidelines may vary for other species, Figure 4 provides a reference for sample size and condition ranges needed to achieve a given level of accuracy. If a calibration model R^2 of 0.8 is the target, we recommend a minimum sample size of 60 and a minimum range of about 29% in percentage dry weight of fat. In circumstances where this range of percentage dry fat cannot be achieved, having a large sample size during model development is crucial.

Temperature influences resistance and reactance and, therefore, must be accounted for in developing and applying BIA calibration models. Recent studies by Hartman et al. (2011), Hafs and Hartman (2015), and Stolarski et al. (2014) provide excellent examples of how temperature can be accommodated in calibration models.

Electrodes necessarily vary with the size and species of fish under study. However, standardization should be maintained between the electrode design used to develop BIA calibration models for a species and those used in later applications. Fish possessing large, thick scales may necessitate scale removal in the vicinity of electrode placement to ensure sound electrical contact. Needle electrodes need only penetrate the skin and bilipid layer and do not need to be deep in the muscle. Therefore, the penetrating length of needle (see Figure 1 inset) can vary with the thickness of the scales and skin layer on a fish. It may be necessary to have several electrode sizes to match the size of fish studied. For fish up to a meter in length, electrode pairs with a 10-mm distance between signal and detecting needles and 2, 3, or 5-mm penetration depth have been used (Cox and Hartman 2005; Hanson et al. 2010; Calderone et al. 2012; Rasmussen et al. 2012), whereas for fish greater than 100 mm in length, electrodes with a 5-mm distance between needles and 1.5-mm penetration depth have been used (Hafs and Hartman 2014). Given differences in needle electrodes or needle penetration depth, as well as differences in lipid storage with fish size, it may be necessary to construct different calibration models for different sizes of fish within a species (see Hafs 2011 and Hartman et al. 2011 for how this was done). Whenever different electrode configurations are used, a separate calibration model should be developed for each configuration.

Different species store lipids in different parts of the body, necessitating the study of the best measurement locations for BIA to most accurately assess condition (Jacobs et al. 2008). Within taxonomic groupings, this may not be necessary if it is fully evaluated for one species. For example, Hafs (2011) took impedance measures on seven different locations in Brook Trout (see Figure 2), ultimately concluding that two locations (one along the lateral length of the fish and one spanning the dorsal to ventral axis in front of the dorsal fin) provided the best calibration. Given the extensive analysis by Hafs (2011) for Brook Trout and the similarity among other salmonid species, it may not be necessary to evaluate measurement locations for other salmonid species. However, for BIA calibrations with new taxa, we recommend considering multiple electrode locations that cover potential differences in body composition and lipid storage laterally and vertically along the fish's body. Correlations between BIA models developed for Brook Trout and applied to eight unrelated species (Table 3 in Cox and Hartman 2005) suggest the possibility of recalibrating models for a related species (e.g., for rare species) with fewer observations than suggested by our Chum Salmon analysis.

Bioelectrical impedance analysis calibration models should be validated with independent data. Cox and Hartman (2005) validated their Brook Trout model using 20 fish not used in the modeling exercise and found strong correlations between observed and predicted body compositional masses. Hartman et al. (2011) used a combination of field and lab fish in validations with Bluefish *Pomatomus saltatrix* and found that the model explained 78–86% of the variability in observed percentage dry weight. We found similar results with our Chum Salmon data set. Models developed by Hafs (2011) in the lab were able to accurately predict ($R^2 = 0.71$) the monthly average percentage dry weight of Brook Trout captured over the course of a yearlong field validation study. More work is needed to validate BIA models with independent data to ensure transferability. Additional validation exercises are needed as calibration models for new species are developed. Bioelectrical impedance analysis is a promising tool in fisheries management and biology, but more research is needed (Table 3).

THE FUTURE OF BIA

Bioelectrical impedance analysis promises to provide a nonlethal, low-cost, rapid means to assess condition of fish populations. Following guidelines and principles presented here and future improvements, we foresee an increasing use of BIA in fisheries management where it can provide better resolution of fish condition, body composition, and energetic levels than was previously practical. In particular, we envision BIA being extensively used by fisheries managers and researchers in situations where fish change condition rapidly or where more accurate assessments are needed than can be attained using standard length–weight measures. Its use is likely to become routine in bioenergetics assessments, because accurate measurement of energy content is much needed on a relatively large scale. Assessment of rare or sensitive species, where measurements that do not result in death are imperative, will benefit greatly from the broad use of BIA. In commercial aquaculture, BIA would be useful to assess fish fat levels to achieve an optimal product. Models of BIA and their applications are likely to be more successful in situations where fish are capable of a wide range of body condition (e.g., percentage fat), suggesting that results with adult fish will be better than for juveniles of the same species.

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TABLE 3. Improvements in the use of BIA in fisheries will rely on continued research. Below we list some of the areas where further research is needed and, where applicable, direct readers to studies to date that may provide some guidance into such research.

Area of BIA research	Relevant literature
Influence of BIA measurement on fish (injuries, growth, mortality)	Cox and Hartman (2005)
Standardization of measurement locations, needle electrode configurations, and penetration depths	Hafs and Hartman (2011)
Identification of best measurement locations for a given species	Hafs and Hartman (2011)
What is the influence of needle penetration depth on BIA measures and model performance?	None
Influence of stress physiological changes on BIA measures	None
Do differences between hatchery and wild fish influence model development vs. application?	Hafs (2011)
Calibration of models with independent data	Hafs (2011)
Can models developed for one species be calibrated to apply to rare species?	None
How best to correct for temperature effects on BIA measures	Hartman et al. (2011); Hafs and Hartman (2015); Stolarski et al. (2014)
How long after death can BIA measures be taken before introducing errors in model predictions?	Cox et al. (2011)
Can BIA be used to nonlethally estimate energy content or disease?	Bourdages (2011)—energy density

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