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ARTICLE

Does Bioelectrical Impedance Analysis Accurately Estimate the Physiological Condition of Threatened and Endangered Desert Fish Species?

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Abstract

Bioelectrical impedance analysis (BIA) is a nonlethal tool with which to estimate the physiological condition of animals that has potential value in research on endangered species. However, the effectiveness of BIA varies by species, the methodology continues to be refined, and incidental mortality rates are unknown. Under laboratory conditions we tested the value of using BIA in addition to morphological measurements such as total length and wet mass to estimate proximate composition (lipid, protein, ash, water, dry mass, energy density) in the endangered Humpback Chub *Gila cypha* and Bonytail *G. elegans* and the species of concern Roundtail Chub *G. robusta* and conducted separate trials to estimate the mortality rates of these sensitive species. Although Humpback and Roundtail Chub exhibited no or low mortality in response to taking BIA measurements versus handling for length and wet-mass measurements, Bonytails exhibited 14% and 47% mortality in the BIA and handling experiments, respectively, indicating that survival following stress is species specific. Derived BIA measurements were included in the best models for most proximate components; however, the added value of BIA as a predictor was marginal except in the absence of accurate wet-mass data. Bioelectrical impedance analysis improved the R^2 of the best percentage-based models by no more than 4% relative to models based on morphology. Simulated field conditions indicated that BIA models became increasingly better than morphometric models at estimating proximate composition as the observation error around wet-mass measurements increased. However, since the overall proportion of variance explained by percentage-based models was low and BIA was mostly a redundant predictor, we caution against the use of BIA in field applications for these sensitive fish species.

Understanding changes in fish population abundance and distribution can often be informed by knowledge of the health of individual animals. Condition indices are commonly used to assess the health and energetic status of individual fish and can give insight into the effects of resource management on fish populations (Stevenson and Woods 2006). In addition, condition can be a sensitive metric for measuring organismal responses to environmental conditions that influence short-term growth and reproduction, which have implications for survival over longer time scales.

The condition of fish is often estimated using total length and wet mass to calculate condition indices, under the assumption that morphological changes in body shape reflect changes in fish condition (Le Cren 1951; Anderson et al. 1996). Length- and mass-based condition indices are widely used in fisheries research because they can be derived using standard field equipment and do not require sacrificing fish, as is needed for more quantitative estimates of lipid, protein, water, ash, and carbohydrate (proximate composition analysis) and derived energy density metrics. However, field-based wet-mass measurements can be inaccurate because of wind, uneven surfaces, residual water, or the movement of fish while on the scale, which render condition estimates unreliable. Since lipid (the primary form of energy in fish; Adams 1999) can be metabolized at a faster rate than water loss (Sutton et al. 2000), condition estimates can also be inflated

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when fish are starving and lipids are replaced by water (Shearer 1994). Wet mass can also be inflated when fish are reproductively active (contain eggs) or heavily parasitized, which can render condition estimates unreliable (Dibble and Meyerson 2012).

Accurate, nonlethal condition estimates are often very important for assessing the status of and managing imperiled fish populations, such as those protected under the U.S. Endangered Species Act of 1973, creating the need to develop alternative, nonlethal tools to estimate fish condition. One such tool, bioelectrical impedance analysis (BIA), is based on the concept that lipid and water offer different levels of resistance (extracellular) and reactance (intracellular) to low-level currents passed through fish (Cox and Hartman 2005; Cox and Heintz 2009). Since percent lipid and percent water are inversely related (Shearer 1994; Hartman and Margraf 2008), higher resistance and reactance levels denote higher lipid content and thus better physiological condition. This technology has been used to measure condition in mammals for decades (Lukaski et al. 1985; Lukaski 1987) but has only recently been extended to fish populations (Bosworth and Wolters 2001; Cox and Hartman 2005).

Bioelectrical impedance analysis methods are typically developed in the laboratory on a species basis, whereby specimens of various sizes are fed a range of diet treatments to elucidate physiological responses that affect condition factor. The fish are then sacrificed, measures of proximate composition are regressed against resistance and reactance, and equations are developed for field application (Cox and Hartman 2005). Refinements to BIA methodology based on multiple studies have occurred over the past decade as this tool has become more popular for use in fisheries science. For example, multiple BIA electrode placement locations are selected to provide a clearer picture of whole-body fish condition (Duncan et al. 2007; Pothoven et al. 2008; Cox et al. 2010), and resistance and reactance are corrected for body temperature prior to the calculation of BIA metrics (Cox and Heintz 2009; Hartman et al. 2011; Hafs and Hartman 2015). Response variables have varied and include proximate composition as a proportion of wet mass (%WM; Pothoven 2008; Stolarski et al. 2014; Hafs and Hartman 2015) or dry mass (%DM; Garner et al. 2012), or as total body mass (TBM [g]; Cox and Hartman 2005; Duncan 2007; Cox et al. 2010; Hanson et al. 2010). However, determining whether BIA is successful in estimating fish condition may rely on the sample size, the range in percent dry-mass lipid contained in fish in the study (Hartman et al. 2015), and whether the results are reported on a percentage or TBM basis (Pothoven 2008; Caldarone et al. 2012; Garner et al. 2012).

It is often stated that BIA is a tool particularly suited for use in endangered species research because it provides a nonlethal way of assessing fish condition (Cox and Hartman 2005; Hartman and Margraf 2008; Pothoven 2008), but only one study has tested the effectiveness of BIA on an endangered fish species, Atlantic Salmon *Salmo salar* (Caldarone et al. 2012). In the Colorado River basin native species that were once abundant or widely distributed are now listed as endangered (Humpback Chub *Gila cypha* and Bonytail *G. elegans*) under the Endangered Species Act or as species of concern by the state of Arizona (Roundtail Chub *G. robusta*). Declines in these species resulted from predam water diversions and the introduction of nonnative fish species (e.g., Common Carp *Cyprinus carpio* and Channel Catfish *Ictalurus punctatus*), followed by the construction of large dams on the main-stem Colorado River (e.g., Hoover, Glen Canyon, and Mohave; Mueller and Marsh 2002). Although Bonytail are largely extirpated from the upper and lower basins (Gloss and Coggins 2005), Roundtail Chub are still common in the upper basin (but at a reduced population size) and in the lower basin (where they occur as distinct population segments; Mueller and Marsh 2002; USFWS 2015). There are six extant populations of Humpback Chub, the largest one residing near the Little Colorado River (Mueller and Marsh 2002). State and federal agencies monitor the growth and abundance of these populations (Makinster et al. 2010; Yackulic et al. 2014; Dzul et al. 2017); however, wet-mass data have not always been collected because of concerns that the data would be too inaccurate to warrant the extra handling of the fish (Meretsky et al. 2000; Persons et al. 2013).

In this study, we evaluated the effectiveness of BIA as a tool with which to estimate the condition of Humpback Chub, Bonytails, and Roundtail Chub in a series of laboratory experiments. We asked the following five research questions: (1) What is the added predictive power of including BIA measurements with total length (TL) and wet mass to estimate proximate composition in these three species? (2) In the absence of accurate wet-mass measurements, can BIA alone be used to estimate proximate composition in these species? (3) If the observation error around wet-mass measurements increases, do BIA models become more accurate at predicting proximate composition than models based on morphology? (4) Which type of resistance/reactance measurement (dorsal or lateral) is a better indicator of proximate composition? and (5) What is the added risk of mortality of taking BIA measurements in addition to TL and wet mass?

METHODS

We conducted BIA and mortality experiments in a temperature-controlled greenhouse equipped with recirculating 568-L tanks at the U.S. Forest Service's Rocky Mountain Research Station in Flagstaff, Arizona. Each tank contained an Aqua Logic 1/3-hp (1 hp = 746 W) drop-in chiller designed to maintain a consistent water temperature. We used hatchery-raised Humpback Chub and Bonytails and wild-captured Roundtail Chub from Fossil Creek, Arizona, as experimental species. The Humpback Chub originated from the U.S. Fish and Wildlife Service's Dexter National Fish Hatchery and Technology

Center (Dexter, New Mexico) and the Bonytails from the state of Utah's Wahweap Fish Hatchery (Big Water). Fish specimens were held in separate tanks and acclimated to laboratory conditions by being fed sinking commercial pellet feed (2.5 g of 1.5 mm, 50% protein; Pentair Aquatics) for several months prior to each trial. The designs of the BIA and mortality trials are detailed in Table 1 as well as Suplementary Figure S.A.1 in Supplement A available in the online version of this article.

Laboratory-based BIA experiments.—Laboratory trials were conducted for 6 weeks to enable differences in body condition to develop from a range of feeding conditions that fish may encounter in the field. We conducted separate trials for each species, designed as split-plot experiments with six replicate tanks per trial. The details of each experiment are provided in Table 1. Within each tank were four enclosed mesh baskets (800-µm mesh), two size-classes of fish (small and large, the exact lengths varying by species), and two diet treatments (ad libitum and starvation), such that each basket contained a unique size-class–diet treatment (small, ad libitum; small, starvation; large, ad libitum; and large, starvation). Because of concerns that high densities of large fish would reduce their growth potential and mask treatment effects, each basket contained 3 large fish or 10 small fish (Table 1). We provided 2.5 g of feed pellets on weekdays to the fish fed ad libitum and withheld feed from those in the starvation treatment. We set tank temperature to 20°C across all six tanks to promote fish growth; however, a few days prior to the BIA analysis we decreased the temperature in three tanks to 10°C to develop temperature-correction equations that could be applied to fish specimens in the field. These temperatures were chosen to simulate those in the main stem (~10°C) and tributaries (~20°C) of the Colorado River where the study species are typically sampled. Since all three species evolved in warm rivers where temperatures reach or exceed 20°C during the spring and summer growing seasons, 20°C in laboratory tanks was sufficient to promote growth.

We used a Quantum IV Body Composition Analyzer (RJL Systems) retrofitted to an external battery to measure resistance (r) and reactance (x) in fish specimens. We interfaced the BIA unit to software developed at the U.S. Geological Survey (G. Bennett, personal communication) that simultaneously captured 3–6 replicates of resistance and reactance at 10-s time intervals. The sensing and detecting electrode wires from the original BIA unit were soldered to 22–27-gauge hypodermic needles and attached to nonconducting plastic Vernier calipers (22.8 × 0.76 cm) using epoxy. This ensured fixed distances between the two electrodes (10 mm), consistent needle penetration depths and angles, and accurate measurements of detector length (DL; Figures 1, 2, S.A.2, S.A.3). Needle penetration depths and gauges were kept consistent for each species to ensure uniform BIA measurements in model development. We conducted BIA calibration tests between the sensing and detecting electrode pairs prior to fish measurement to ensure unit precision and accuracy.

Fish were removed from tanks in small batches and anesthetized for 1.5–2 min in oxygenated water of similar temperature containing tricaine methanesulfonate (MS-222; 150 mg/L). We measured TL (mm), wet mass (to the nearest 0.1 g; Sartorius Signum Model SIWADCP-V6), and initial cavity temperature (°C; measured with a 4-mm wire inserted into the anus along the interior ventral surface of the fish [Fisher Scientific, Traceable Digital Thermometer]). We placed fish on their right sides on a nonconductive board and wore nitrile gloves to avoid electrical interference if the fish were touched during measurement. Calipers were adjusted to one of two electrode placement locations: dorsal (anterior signal detector in line with the operculum, posterior detector in line with the posterior edge of the anal fin, needles inserted into the sagittal plane) and lateral (midpoint between the dorsal plane and the lateral line, anterior signal detector in line with the operculum, posterior detector in line with the posterior edge of the anal fin, needles inserted into the frontal plane). We recorded detector length for each electrode location, took 3–6 replicates of resistance and reactance spaced 10-s apart, and then measured the ending cavity temperature (Table 1). If the fish moved while measuring resistance and reactance, we repeated the BIA measurements. Fish were euthanized via decapitation while anesthetized, placed in a chest freezer or on dry ice, and transported to an ultra-low (–80°C) freezer for storage prior to proximate composition analysis.

Analysis of proximate body composition.—Proximate analysis is the determination of the percentages of wet or dry mass (%WM; %DM) or total body mass (TBM [g]) of lipid, protein, water, ash, and carbohydrate in fish samples. We prepared samples for analysis by grinding partially frozen, small whole-body Humpback Chub and Bonytails

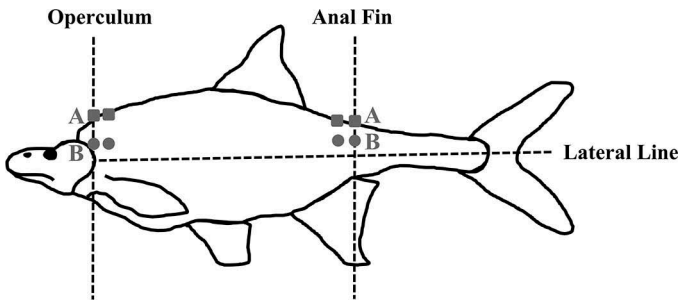

FIGURE 1. Diagram showing the dorsal and lateral electrode placement locations and the sagittal and frontal insertion points of the needles for bioelectrical impedance analysis (BIA). In the dorsal placement (A–A), the anterior signal detector is in line with the operculum and the posterior signal detector is in line with the posterior edge of the anal fin; the needles are inserted into the sagittal plane. In the lateral placement (midway between the dorsal edge and the lateral line [B–B]), the anterior signal detector is again in line with the operculum and the posterior signal detector in line with the posterior edge of the anal fin, but the needles are inserted into the frontal plane. See Figure S.A.3 for photographs of calipers inserted into the dorsal and lateral planes of a large adult Roundtail Chub.

TABLE 1. Details of the bioelectrical impedance analysis (BIA) and mortality experiments, by species.

Experiment	Humpback Chub	Bonytail	Roundtail Chub
BIA experiment			
Size-classes (mm)	70–90, 100–130	50–70, 80–110	120–200, 201–310
No. fish/basket	10	10	3
Total fish (before mortalities)	240	240	72
Trial dates (no. days in parentheses)	Dec 15, 2014–Jan 28, 2015 (44)	May 1–Jun 12, 2014 (42)	Jun 22–Aug 5, 2015 (43)
Fish temperature (°C; means in parentheses)	12.4—21.3 (16.0)	14.3—26.1 (19.5)	10.7—23.5 (17.2)
Lipid (% dry mass; means in parentheses)	20.8—61.7 (40.9)	4.6—73.6 (69.0)	17.0—51.6 (34.6)
Mortality experiment			
Size-classes (mm)	80–100, 101–120	70–90, 91–120	120–200, 201–310
No. fish/basket	10	10	3
Total fish (before mortalities)	60	60	72
Trial dates (no. days in parentheses)	Aug 10–17, 2015 (7)	Aug 10–17, 2015 (7)	Jul 28–Aug 4, 2015 (7)

using a laboratory-grade Scilogex D160/10 homogenizer for several minutes to ensure complete homogenization of tissue samples. Because of their larger size, Roundtail Chub were homogenized using dry ice and a commercial food blender (Fleetwood Skyfood Model LI-1.5) and transferred to a –20°C freezer for sublimation overnight. To clarify our terminology, the term "wet mass" is used to describe the total mass of the intact fish weighed when wet; it was used in condition factor estimates and as the denominator in proximate composition models based on percent wet mass. The terms "water (%)" and "water (g)" refer to the aqueous fraction of the tissue included in the proximate composition analysis, as determined by dehydrating minced tissue in a drying oven per the methods described below. We also calculated "dry mass" to remove the potentially confounding effects of water on the proximate composition results; this was used as the denominator in %DM models.

We extracted the lipid fraction of fish tissue using standard lipid extraction protocols (Bligh and Dyer 1959 [AOAC 983.23] as modified by Phillips et al. 1997). Since subsampling is standard in proximate composition analysis (Phillips 1997; Wuenschel et al. 2013; Stolarski et al. 2014) and the quantity of sample needed for analysis represented a large proportion of each fish, we weighed a subsample of 1.0 ± 0.05 g of fish tissue into a 50-mL conical centrifuge tube and added 5.7 mL of sodium acetate, 8 mL of chloroform, and 16 mL of methanol. Closed tubes were mixed at 300 rpm for 2 h (LabGenius Digital Orbital Shaker EF9796), after which we added 8 mL of chloroform, sonicated the tubes for 10 min (ColeParmer 8891), added 8 mL of deionized water, mixed the tubes for 20 min, and centrifuged the tubes at 2,900 × *g* for 10 min (Eppendorf 5702). We extracted 10 mL of the chloroform–lipid layer using a Hamilton gas-tight syringe, evaporated the chloroform layer in a preweighed glass vial under nitrogen gas, reweighed the vial after oven drying and desiccation, and determined the lipid fraction of the fish tissue from the following equation (Phillips 1997):

$$\text{Total lipid}(\text{g}/100\ \text{g wet mass}) = [(W_2 - W_1)(V_C) \cdot 100]/(V_A \cdot S_W),$$

where W_2 is the mass of the glass vial + the dried lipid extract (g), W_1 is the mass of the empty glass vial (g), V_C is the total volume of chloroform (16 mL), V_A is the volume of chloroform extract dried (10 mL), and S_W is the wet mass of the extracted fish tissue (1.0 ± 0.05 g).

Percent water was determined by adding 1.0 ± 0.05 g fish tissue to a previously weighed aluminum foil tray, drying it at 105°C in a drying oven for 24 h (VWR 1327 F), and recording the postdesiccation mass. Aluminum trays containing dried fish tissue were transferred to a muffle furnace (Thermo Scientific F30400) and heated to 550°C for 3 h to determine percent ash. Since carbohydrate is known to be negligible in fish tissue (<1%; Black 1958; Brett et al. 1969; Weatherley and Gill 1983; Cooke and Philipp 2009), we estimated percent protein by subtracting the percentages of lipid, water, and ash from 100. Therefore, the percent "protein" estimate in this analysis is actually the estimated percentage of protein plus a small fraction of carbohydrate—an estimate that has been used successfully in other studies (Van Pelt et al. 1997; Berg and Bremset 1998; Anthony et al. 2000; Berg et al. 2011).

The proportions of each proximate component were calculated on a %WM or %DM basis relative to fish mass by including or excluding water mass. In addition, we estimated the TBM (g) of lipid, protein, water, and ash using fish wet mass (g) and the proportion of each proximate component. We calculated energy density (kJ/g) and energy content (kJ) using

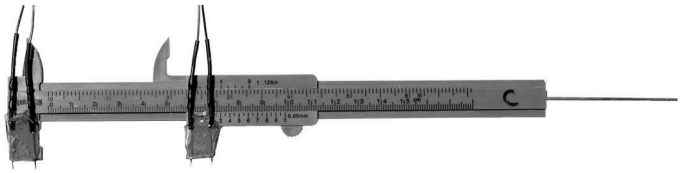

FIGURE 2. The BIA caliper unit designed for this study.

standard nutrition equations as applied to fishery studies (e.g., Anthony et al. 2000; Wuenschel et al. 2006; O'Neill et al. 2014), which employed the energy equivalents of metabolizable teleost fish lipid (8.66 kcal/g or 36.2 kJ/g) and protein (4.8 kcal/g or 20.1 kJ/g; Brett and Groves 1979). Carbohydrate is often omitted from energy density analysis because its contribution to proximate composition is negligible; nevertheless, since the energy equivalents of protein and carbohydrate are similar our estimates include all potential energy sources:

$$\text{Energy density (kJ/g)} = [(\%\,\text{lipid}/100) \times 36.2\,\text{kJ/g}] + [(\%\,\text{protein}/100) \times 20.1\,\text{kJ/g}]$$

$$\text{Energy content (kJ)} = [\text{lipid (g)} \times 36.2\,\text{kJ/g}] + [\text{protein (g)} \times 20.1\text{kJ/g}].$$

Laboratory-based mortality experiments.—In this experiment we assessed bacterial and fungal infections following puncture by BIA needles and calculated mortality rates to determine the short-term effects of BIA on fish. We also included a control treatment that tested the influence of handling (i.e., measuring TL and wet mass) on fish mortality. We used the same set of recirculating 568-L tanks used in the BIA experiments to assess mortality. For Humpback Chub and Bonytails we conducted a combined, independent mortality experiment after their respective BIA experiments. Our study design included three tanks with four baskets per tank. The baskets contained unique combinations of the two species and treatments (Humpback Chub, control and BIA; Bonytail, control and BIA). Within each basket were 10 fish—5 small fish and 5 large fish, corresponding to approximately the same size-classes in the BIA trial (Table 1). Tank temperatures were set at 20°C, and fish were fed ad libitum to separate the effects of feeding and temperature from those of BIA. Fish were acclimated for 5 d prior to commencing the mortality experiment. For Roundtail Chub, we incorporated the mortality experiment into the BIA experiment because of the small sample size available for large-bodied specimens. One week prior to the end of the BIA experiment (but before temperatures were lowered to 10ºC), we conducted the mortality trial as described below on all 72 fish; we used mortality rates during the previous month as the control.

All fish subjected to the BIA treatment in the mortality trials were removed from the 20°C tanks, anesthetized in oxygenated water containing MS-222 for 1.5–2 min, measured (mm), and weighed (g). We measured the starting cavity temperature (ºC), took three replicates of lateral and dorsal BIA measurements every 10 s as described above, and measured the ending cavity temperature. Fish were transferred to an oxygenated freshwater bucket for recovery and then returned to their original baskets and tanks. The Humpback Chub and Bonytails in the control treatment were subjected to the same procedures as in the BIA treatment (minus the BIA), thereby allowing for the assessment of handling mortality. The fish were removed from the tanks, anesthetized in oxygenated water containing MS-222 for 1.5–2 min, measured, and weighed. Since the process used to measure the starting temperature, conduct BIA, and measure the ending temperature took an average of 2 min, fish were left on a damp nonconductive board for approximately that time, transferred to an oxygenated freshwater bucket for recovery, and then returned to their original baskets and tanks. Infection at BIA puncture sites was monitored daily by looking for the presence of bacterial and/or fungal infections, and mortality rates were quantified after 7 d post-BIA and handling for all species.

Statistical analyses.—We compared the effects of our diet treatment on fish using Welch's two-sample *t*-tests. We estimated the added predictive power of including BIA measurements in addition to TL and wet mass data using generalized linear mixed models (GLMMs), which included a normal (Gaussian) error distribution and basket as a random effect. We initially treated tank and basket as nested random effects, but the variance associated with tank was negligible and therefore removed. The GLMM response variables (in separate individual models) included %WM, %DM, and TBM estimates of lipid, protein, ash, water, dry mass, and energy density/content. Predictors included the TL, fish condition, and BIA metrics. We estimated fish condition using residuals from a $\log_e$–$\log_e$ TL and wet-mass linear regression and divided observed wet mass by predicted wet mass.

As correction of the mean resistance and reactance values for temperature was necessary prior to analysis (Hartman et al. 2011; Hafs and Hartman 2015), we calculated the mean cavity temperature from the BIA starting and ending temperatures (Table 1) and the mean resistance and reactance from the 3–6 replicates per electrode location. We then used simple linear regression (SLR) to model the relationships between mean cavity temperature and mean resistance and reactance for each species and electrode

location resulting from exposure to 10°C and 20°C water temperatures. Since 20°C was within the range of the mean cavity temperatures measured in the laboratory (Table 1) and is a temperature that fish commonly encounter in rivers in the Colorado River basin, we corrected all BIA metrics to 20°C. We chose not correct BIA metrics to the grand mean of the Humpback Chub, Bonytail, and Roundtail Chub cavity temperatures (16.0, 19.5, and 17.2°C, respectively; Table 1) because a sensitivity analysis revealed that the majority of the best models resulted from using a 20°C rather than a 15°C or no temperature correction (Supplement B). The interpretation of our results did not change between the three temperature correction options, so we only applied the 20°C correction to the BIA metrics. We used the slope of each linear regression combined with the temperature correction equations (see Table 1 in Hafs and Hartman 2015) to correct the lateral and dorsal resistance and reactance measurements to 20°C, then developed nine BIA metrics commonly used as model predictors for the lateral and dorsal electrode locations (18 predictors in all; Table 2; Cox and Hartman 2005; Hafs and Hartman 2014).

Preliminary analysis indicated that the 18 temperature-corrected BIA metrics were highly correlated ($r > 0.6$) and could therefore not be input as separate parameters in a GLMM due to collinearity (Dormann et al. 2013), so we used principal component analysis (PCA) to develop an independent set of predictors for the GLMM models based on the highest axis loadings. The PCA biplots revealed that lateral and dorsal measurements loaded closely together across species, indicating that both electrode locations produced similar results. For all species the highest loadings fell on the first and second axes (PC1, PC2; Supplement C). The majority ($n = 16$) of the dorsal and lateral BIA metrics representing resistance, reactance, capacitance, and impedance in series and in parallel loaded on the first axis, accounting for 86, 91, and 87% of the total variance for Humpback Chub, Bonytails, and Roundtail Chub, respectively. The remaining two BIA metrics (phase angle—dorsal and lateral locations) loaded highly on the second axis and accounted for 7, 5, and 8% additional variance, cumulatively explaining 92, 97, and 95% of the variance, respectively. The proportion of variance explained

TABLE 2. Bioelectrical impedance analysis equations used to develop metrics for use in the GLMM models (via principal component analysis; see Supplement C). The slopes of the temperature-correction equations for r_t and x_t were modeled after Hafs and Hartman (2015) using lateral and dorsal measurements and individual fish cavity temperatures. Abbreviations are as follows: HBC = Humpback Chub, BTC = Bonytail, RTC = Roundtail Chub, T_m = mean cavity temperature, and DL = detector length (mm).

Parameter	Symbol	Units	Equation
Resistance	r	Ohms	Measured by Quantum IV for dorsal (r_{dor}) and lateral (r_{lat}) electrode locations
Reactance	x	Ohms	Measured by Quantum IV for dorsal (x_{dor}) and lateral (x_{lat}) electrode locations
Temperature-corrected resistance (20°C, using slope of linear regression in equations)	r_t	Ohms	HBC dorsal: $[-38.93 \cdot (20 - T_m)] + r_{dor}$ HBC lateral: $[-36.06 \cdot (20 - T_m)] + r_{lat}$ BTC dorsal: $[-15.32 \cdot (20 - T_m)] + r_{dor}$ BTC lateral: $[-10.07 \cdot (20 - T_m)] + r_{lat}$ RTC dorsal: $[-8.30 \cdot (20 - T_m)] + r_{dor}$ RTC lateral: $[-7.75 \cdot (20 - T_m)] + r_{lat}$
Temperature-corrected reactance (20°C, using slope of linear regression in equations)	x_t	Ohms	HBC dorsal: $[-6.97 \cdot (20 - T_m)] + x_{dor}$ HBC lateral: $[-5.49 \cdot (20 - T_m)] + x_{lat}$ BTC dorsal: $[-3.93 \cdot (20 - T_m)] + x_{dor}$ BTC lateral: $[-3.44 \cdot (20 - T_m)] + x_{lat}$ RTC dorsal: $[-1.27 \cdot (20 - T_m)] + x_{dor}$ RTC lateral: $[-0.90 \cdot (20 - T_m)] + x_{lat}$
Resistance in series[a]	R_s	Ohms	DL^2 / r_t
Reactance in series[a]	X_c	Ohms	DL^2 / x_t
Resistance in parallel[a]	R_p	Ohms	$DL^2 / [r_t + (x_t^2 / r_t)]$
Reactance in parallel[a]	X_{cp}	Ohms	$DL^2 / [x_t + (r_t^2 / x_t)]$
Capacitance[a]	C_{pf}	Picofarads	$DL^2 / [1/(2 \cdot \pi \cdot 50{,}000 r_t)] \cdot 10^{12}$
Impedance in series[a]	Z_s	Ohms	$DL^2 / (r_t^2 + x_t^2)^{\circ.5}$
Impedance in parallel[a]	Z_p	Ohms	$DL^2 / [(r_t \cdot x_t) / (r_t^2 + x_t^2)^{\circ.5}]$
Phase angle[a]	PA	Degrees	$\arctan (x_t / r_t) \cdot (180/\pi)$
Standardized phase angle[a]	DLPA	Degrees	$DL \cdot [\arctan (x_t / r_t) \cdot (180/\pi)]$

[a]Parameter included in PCA; dorsal and lateral BIA metrics combined into a single PCA for each species.

by the third axis (PC3) was minimal and the PC3 eigenvalues for all three species fell below 1.0, so we only included PC1 and PC2 as representatives of the 18 BIA metrics in the GLMM models.

For each species and response variable, we compared six models [TL, TL + condition (i.e., "base"), TL + BIA, BIA, TL + condition + BIA, and condition + BIA; Table 3; Supplement D] using the second-order Akaike information criterion (AIC_c). The best BIA and condition + BIA models included PC1 or a combination of PC1 and PC2. Because of the high correlation between TL and PC1 across species (r = 0.94–0.97), the TL + condition + BIA and TL + BIA models only contained PC2. However, TL or PC1 was included as a covariate in every model to account for the influence of fish length on the response variables. To facilitate interpretation, all continuous predictors were centered on their means and standardized by their standard deviations prior to analysis. We extracted predicted values (fixed effects) from each GLMM model, used SLR to quantify the relationship between the predicted and observed proximate component values, and compared the adjusted R^2 of each model with the adjusted R^2 of the morphometric "base" model (TL + condition) to assess the added value of including BIA measurements. We also compared the proportion of variance explained between the morphometric and BIA-only models to assess the value of BIA if reliable wet-mass data were not available.

Wet-mass data can be associated with high error rates due to environmental conditions, such that it may not be measured in the field (Meretsky 2000; Persons et al. 2013). We ran a simulation experiment to quantify how erroneous the wet-mass data would have to be before the BIA models had a lower AIC_c value than the morphometric (TL + condition) models. We sequentially increased the observation error (standard deviation) around precise laboratory wet-mass measurements from 0.01 to 0.20 in 0.01 increments and ran 100 simulations that tested whether the BIA or morphometric model was better at estimating fish condition for each response variable and species. For example, if the expected mean wet mass of a fish was 5.0 g but the estimated field error associated with the measurements (because of wind or equipment failure) was 0.20, approximately 99% of the observations would range between 4.4 and 5.6 g. These error estimates are similar to those estimated in the field using mark–recapture data (M. Dzul, Grand Canyon Monitoring and Research Center, personal communication). We evaluated the critical point at which 50% or more of the simulations (at each increment) indicated that models including BIA measurements were better than those based on morphology for estimating fish condition. We fit GLMM models using the lme4 package and ran SLR models, PCA, and wet-mass simulations in the software package R (version 3.1.3; R Core Team 2015).

RESULTS

Experimental Design

Diet treatment had varying effects on proximate composition, but for all three species the fish in the starvation treatment had significantly less lipid than those in the ad libitum treatment (Humpback Chub: t = 6.84, df = 232.5, $P < 0.001$; Bonytails: t = 19.67, df = 210.6, $P < 0.001$; Roundtail Chub: t = 6.82, df = 68.8, $P < 0.001$). A recent power analysis has suggested that BIA will only be successful in estimating fish condition in populations with at least a 29% range in lipids (% dry mass) and a sample size of at least 60 specimens, yielding an R^2 of 0.80 (Hartman et al. 2015). For fish that survived the trial, our ranges in percent dry-weight lipids and sample sizes exceeded those recommendations (Humpback Chub: 40.9%, n = 236; Bonytails: 69.0%, n = 210; and Roundtail Chub: 34.6%, n = 71; Table 1). Therefore, our laboratory trials were run for an adequate amount of time to produce a range of fish conditions to be successful for estimation with BIA per the results of Hartman et al. (2015).

Laboratory-Based BIA Experiments and Analysis of Proximate Body Composition

Because BIA studies have reported results from models based on %WM, %DM, and TBM, we compared these approaches. All model results are reported in Supplement D. Here, we focus on percentage-based models, as mass-based models only have high R^2 values because fish size explains a high proportion of the variance. Further, since the wet- and dry-mass models yielded similar results, we concentrate on the %WM models.

Overall, our model results indicate that using BIA in addition to TL and wet mass does not significantly improve proximate composition estimates. Linear regressions of observed versus predicted proximate components revealed weak relationships for the best Humpback Chub, Bonytail, and Roundtail Chub models based on %WM (Tables 3–4; Figures 3–5; Supplement D) and %DM (Supplement D). Adding BIA metrics to the Humpback Chub and Bonytail morphometric models did not increase the proportion of explained variance by more than 2%, but in most cases the improvement was negligible (<1%; Tables 3–4; Supplement D). Similarly, the inclusion of BIA metrics in Roundtail Chub wet- and dry-mas models increased the proportion of explained variance by no more than 3% and 4%, respectively, relative to the morphometric model (Tables 3–4; Supplement D).

As expected, modeling Humpback Chub, Bonytail, and Roundtail Chub response variables on a TBM basis improved the overall model R^2 across all measures of proximate composition (Supplement D). However, BIA metrics increased the proportion of explained variance by no more than 1% in the Humpback Chub models and in some cases decreased it by 4% relative to the morphometric model. The addition of BIA metrics to Bonytail and Roundtail Chub models only increased

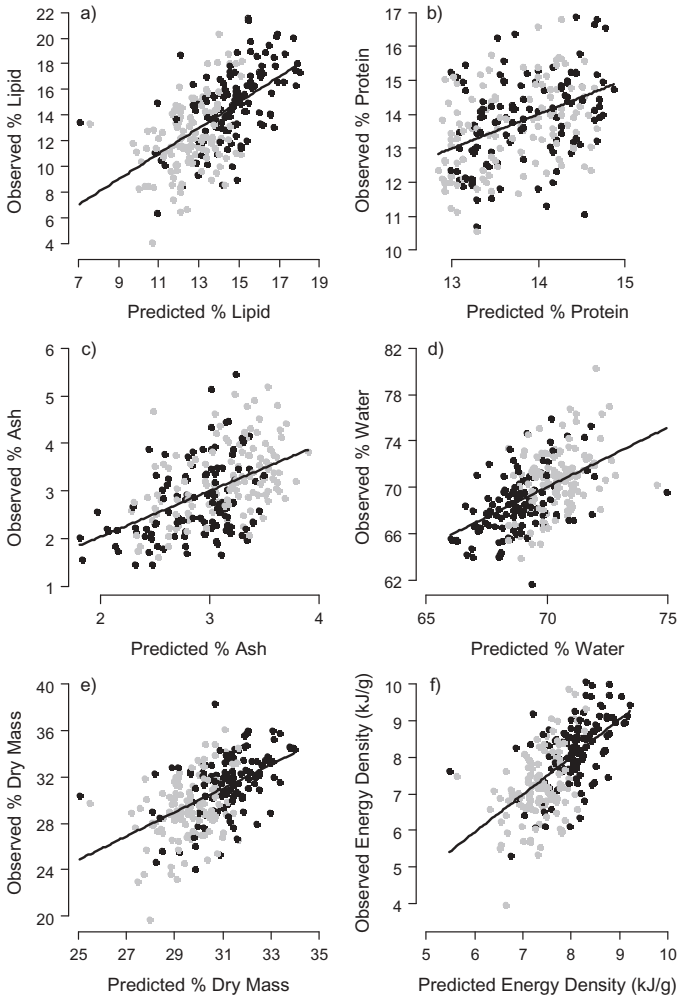

FIGURE 3. Results from the Humpback Chub GLMM models used to predict proximate composition on a %WM basis for **(a)** lipid, **(b)** protein, **(c)** ash, **(d)** water, and **(e)** dry mass as well as **(f)** energy density (kJ/g). Black dots denote values for the ad libitum treatment, gray dots values for the starvation treatment. The *x*- and *y*-axes have different scales owing to the GLMM model's underprediction relative to the observed values.

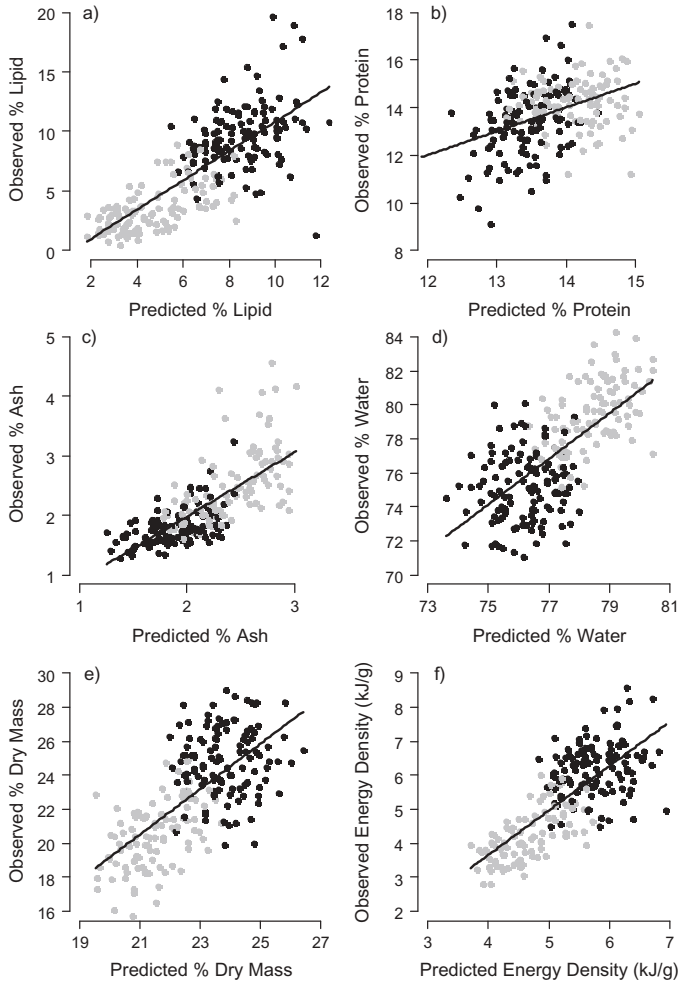

FIGURE 4. Results from the Bonytail GLMM models used to predict proximate composition on a %WM basis for **(a)** lipid, **(b)** protein, **(c)** ash, **(d)** water, and **(e)** dry mass as well as **(f)** energy density (kJ/g). See Figure 3 for additional details.

the proportion of explained variance by 1–3% and 3–6%, respectively, relative to morphometric models (Supplement D).

The results of simulations that incrementally increased the observation error around laboratory wet-mass measurements were variable across species and response variables (Figure 6; Supplement E). All models reached a critical error at which the BIA model had a lower AIC_c value than morphometric models in more than 50% of the simulations, but we only report results for errors up to 0.20. In general, the Humpback and Roundtail Chub models were associated with high observation error, such that BIA only became a better predictor than TL + condition when the wet-mass measurements exhibited high error rates (0.11–0.20; Figure 6). However, the majority of Bonytail models were associated with low observation error, such that BIA became a better predictor than TL + condition in estimating proximate composition at low levels of variability in the wet-mass data (0.01–0.10; Figure 6).

Laboratory-Based Mortality Experiments

Humpback Chub exhibited 0% mortality in both the BIA (0/29) and handling-only (control) trials (0/30) and exhibited no signs of bacterial or fungal infection. Fish in the Bonytail BIA trial exhibited a 14% mortality rate (4/28) across the three baskets (0, 20, and 20%, respectively), while fish in the Bonytail handling trial exhibited a 47% mortality rate (16/30; 20, 30, and 90%). The proportions of Bonytail mortalities were roughly equal in the 70–90-mm and 91–120-mm size ranges (n = 8 and 10, respectively), and all mortalities exhibited evidence of bacterial and fungal infection in the caudal peduncle. Roundtail Chub lacked infection and exhibited 1% mortality (1/72) in the BIA trial and 0% mortality in the handling trial (0/72, i.e., no mortality in the previous month).

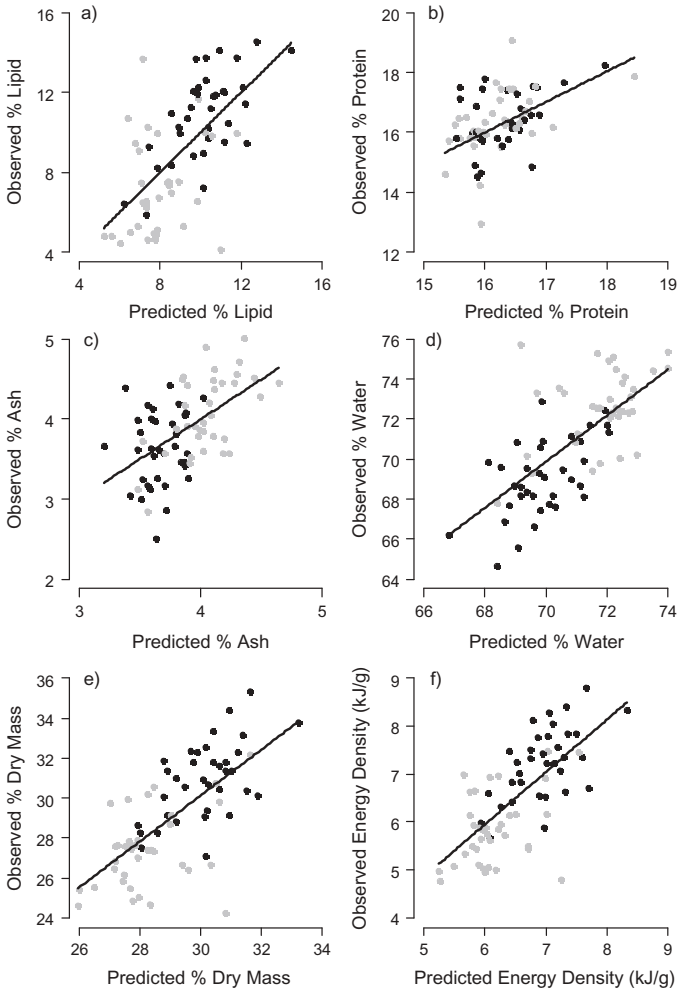

FIGURE 5. Results from the Roundtail Chub GLMM models used to predict proximate composition on a %WM basis for **(a)** lipid, **(b)** protein, **(c)** ash, **(d)** water, and **(e)** dry mass as well as **(f)** energy density (kJ/g). See Figure 3 for additional details.

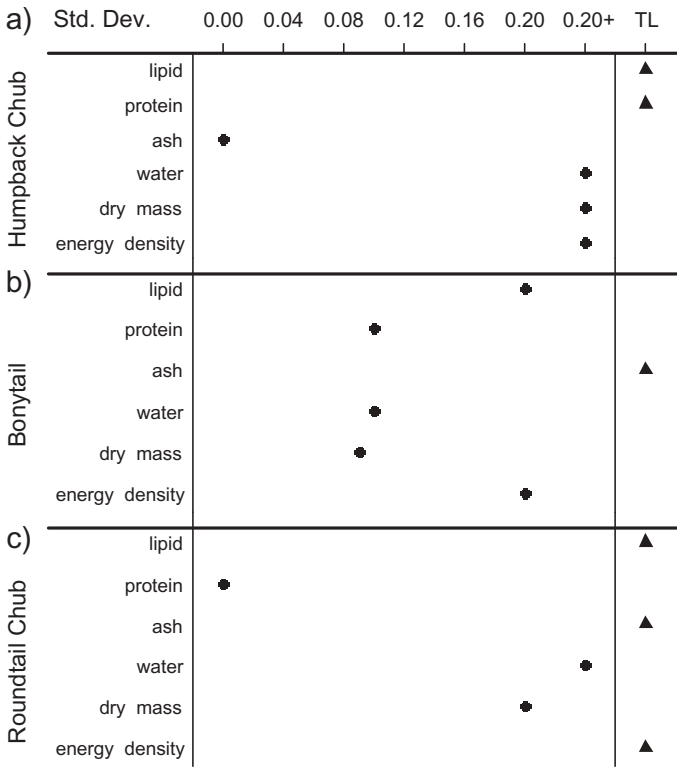

FIGURE 6. Results of simulations that incrementally increased the observation error (standard deviation) of wet-mass data in %WM models for **(a)** Humpback Chub, **(b)** Bonytails, and **(c)** Roundtail Chub from 0.01 to 0.20 to determine the error at which 50% or more of the BIA (or BIA + TL) models performed better (i.e., exhibited lower AIC_c values) than the morphometric (TL + condition) model. In this figure the term "TL" indicates models for which length alone was a better predictor of proximate composition than the predictors in the BIA and morphometric models. Zero observation error was associated with models in which BIA measurements were always better predictors than morphology, such that increasing the observation error did not influence the results. For all models there was an observation error at which the BIA model performed better than the morphology model, but those that failed to reach the 50% cutoff at SD = 0.20 were lumped into the 0.20+ category.

DISCUSSION

Overall, our results indicate that BIA is not a useful tool with which to estimate proximate composition in the three desert fish species in this study. Standard morphological field measurements of fish condition only explained up to 37, 61, and 46% of the variance in Humpback Chub, Bonytail, and Roundtail Chub %WM proximate composition models, respectively. The addition of BIA metrics to these morphometric models increased the proportion of explained variance by no more than 2, 2, and 3% for these species. Model fit declined further when BIA alone was used to estimate proximate composition, explaining no more than 19, 22, and 15% of the variance for these species. As expected, morphometric models based on TBM explained high proportions of the variance (as much as 96, 96, and 95% for Humpback Chub, Bonytails, and Roundtail Chub, respectively), but the addition of BIA metrics only increased the proportion of explained variance by a small amount (up to 1, 3, and 6%). This was the case even with high simulated field error in the wet-mass measurements, such that error estimates would have to reach 0.20 (SD) for BIA to be a better predictor of proximate composition than fish morphology. Therefore, this study supports the inclusion of wet-mass measurements in standard field monitoring protocols to estimate fish condition for endangered fish species. If the observation error exceeds 0.20 and wet-mass data are inaccurate by several grams, managers may consider BIA as an alternative nonlethal tool with which to measure fish condition—but only if those species exhibit low mortality in response to BIA measurement and handling in laboratory trials.

Estimating Fish Condition: Considerations for Future Studies

Our study is not the first to find that percentage-based models of proximate composition exhibit low R^2 values

TABLE 3. Best generalized linear mixed models (lowest AIC_c values) and linear models (LMs) by proximate composition response and species. Results are only reported for the highest-ranking models based on %WM; full results are provided in Supplement D. Abbreviations are as follows: *C* = condition, PC1, PC2 = principal component axes 1 and 2, and pred = predicted proximate composition (fixed effects) from the GLMM models, regressed against the observed proximate composition.

Predictor(s)	Response variable	GLMM equation	LM equation	LM R^2	Base R^{2a}	ΔR^2	ΔAIC_c[b]	*n*
		Humpback Chub						
C, PC1	Lipid	$(1.82 \cdot C) - (0.35 \cdot PC1) + 13.59$	$(1.00 \cdot pred) + 0$	0.34	0.33	0.01	2.85	236
TL	Protein	$(0.55 \cdot TL) + 13.78$	$(1.00 \cdot pred) - 0.05$	0.13	0.13	<0.01	1.70	236
C, PC1	Ash	$(-0.14 \cdot C) - (0.36 \cdot PC1) + 3.01$	$(0.97 \cdot pred) + 0.09$	0.23	0.21	0.02	7.88	236
C, PC1	Water	$(-1.49 \cdot C) + (0.30 \cdot PC1) + 69.62$	$(1.03 \cdot pred) - 2.22$	0.28	0.28	<0.01	2.17	236
C, PC1	Dry mass	$(1.49 \cdot C) - (0.30 \cdot PC1) + 30.38$	$(1.03 \cdot pred) - 0.97$	0.28	0.28	<0.01	2.17	236
C, PC1	Energy density	$(0.62 \cdot C) - (0.05 \cdot PC1) + 7.70$	$(1.03 \cdot pred) - 0.22$	0.37	0.37	<0.01	0.38	236
		Bonytail						
C, TL	Lipid	$(2.04 \cdot C) + (1.46 \cdot TL) + 6.60$	$(1.23 \cdot pred) - 1.51$	0.61	0.61	Base	0.00	232
C, PC1, PC2	Protein	$(-0.43 \cdot C) - (0.31 \cdot PC1) + (0.26 \cdot PC2) + 13.72$	$(1.00 \cdot pred) + 0.01$	0.12	0.10	0.02	4.38	210
C, TL	Ash	$(-0.29 \cdot C) - (0.32 \cdot TL) + 2.14$	$(1.07 \cdot pred) - 0.17$	0.53	0.53	Base	0.00	210
C, TL, PC2	Water	$(-1.24 \cdot C) - (0.82 \cdot TL) - (0.48 \cdot PC2) + 77.24$	$(1.34 \cdot pred) - 26.38$	0.46	0.44	0.02	8.84	210
C, TL, PC2	Dry mass	$(1.24 \cdot C) + (0.82 \cdot TL) + (0.48 \cdot PC2) + 22.76$	$(1.34 \cdot pred) - 7.64$	0.46	0.44	0.02	8.84	210
C, TL, PC2	Energy density	$(0.62 \cdot C) + (0.45 \cdot TL) + (0.11 \cdot PC2) + 5.26$	$(1.30 \cdot pred) - 1.56$	0.59	0.57	0.02	1.84	210
		Roundtail Chub						
C, PC1, PC2	Lipid	$(1.99 \cdot C) - (0.22 \cdot PC1) - (0.49 \cdot PC2) + 9.04$	$(1.00 \cdot pred) - 0.01$	0.42	0.39	0.03	0.94	71
PC1	Protein	$(0.55 \cdot PC1) + 16.24$	$(1.02 \cdot pred) - 0.31$	0.15	0.15	<0.01	1.79	71
C, TL	Ash	$(-0.28 \cdot C) - (0.10 \cdot TL) + 3.85$	$(1.00 \cdot pred) + 0$	0.29	0.29	Base	0.00	71
C, TL, PC2	Water	$(-1.66 \cdot C) - (0.24 \cdot TL) + (0.47 \cdot PC2) + 70.86$	$(1.15 \cdot pred) - 10.79$	0.47	0.44	0.03	1.71	71
C, TL, PC2	Dry mass	$(1.66 \cdot C) + (0.24 \cdot TL) - (0.47 \cdot PC2) + 29.14$	$(1.15 \cdot pred) - 4.44$	0.47	0.44	0.03	1.71	71
C, TL, PC2	Energy density	$(0.70 \cdot C) + (0.05 \cdot TL) - (0.17 \cdot PC2) + 6.54$	$(1.09 \cdot pred) - 0.60$	0.49	0.46	0.03	0.85	71

[a] R^2 from the morphometric (TL + condition; "Base") model.

[b] AIC_c of morphometric (TL + condition) model − AIC_c of best model.

TABLE 4. Proportion of explained variance (R^2) from linear regressions of observed versus predicted values of proximate body composition based on %WM for the three study species across all GLMM models. The results show the variance for the morphometric "base" model relative to one of the two models with the highest R^2 in the morphometric + BIA and BIA categories (Supplement D). A model including only TL as a predictor is included for comparison purposes. Values in bold italics indicate the categories with the best models based on AIC_c selection.

Species	Response variable	TL	BIA	Morphometric ("base")	Morphometric + BIA
Humpback Chub	Lipid	0.00	0.01	0.33	***0.34***
	Protein	***0.13***	0.13	0.13	0.14
	Ash	0.14	0.19	0.21	***0.23***
	Water	0.00	0.00	0.28	***0.28***
	Dry mass	0.00	0.00	0.28	***0.28***
	Energy density	0.00	0.00	0.37	***0.37***
Bonytail	Lipid	0.17	0.20	***0.61***	0.61
	Protein	0.02	0.05	0.10	***0.12***
	Ash	0.21	0.22	***0.53***	0.53
	Water	0.06	0.13	0.44	***0.46***
	Dry mass	0.06	0.13	0.44	***0.46***
	Energy density	0.10	0.16	0.57	***0.59***
Roundtail Chub	Lipid	0.00	0.00	0.39	***0.42***
	Protein	0.13	***0.15***	0.15	0.17
	Ash	0.01	0.06	***0.29***	0.29
	Water	–0.01	–0.01	0.44	***0.47***
	Dry mass	–0.01	–0.01	0.44	***0.47***
	Energy density	–0.01	–0.01	0.46	***0.49***

relative to models based on TBM. Caldarone et al. (2012) compared lipid, protein, and water on %WM and TBM bases using endangered Atlantic Salmon and found that the proportion of explained variance in the best models for total body lipid (g), protein (g), and water (g) reached 76, 99, and 99%, respectively, while models based on the percentages of lipid, protein, and water explained no more than half the variance (33, 50, and 50%). Likewise, Pothoven et al. (2008) compared models of lipid content for Yellow Perch *Perca flavescens*, Walleye *Sander vitreus*, and Lake Whitefish *Coregonus clupeaformis* and found that the best TBM models explained 76, 92, and 88% of the variance for those species, compared with 17, 25, and 53% for percentage-based models. However, the large differences in explained variance are simply due to the fact that percentage-based models already account for fish size in the response. BIA metrics attempt to correct for this and the cylindrical shape of a fish's body through volumetric conversions of detector length in BIA equations based on Ohm's Law (Lukaski et al. 1985; Lukaski 1987; Pothoven 2008; Hafs and Hartman 2014). However, the standardized and consistent placement of BIA electrodes results in detector length becoming a proxy for fish size. Since response variables based on TBM increase allometrically with fish length, comparison with BIA predictors alone or in combination with TL and condition will naturally yield higher R^2 values for models based on TBM (Caldarone et al. 2012). Therefore, future studies should move beyond reporting proximate composition on a TBM basis and compare percentage-based models of morphology with those including BIA metrics to gauge the actual benefit of using this tool in field operations.

Many studies to date have included morphometric and BIA (or just BIA) metrics as candidate predictors in models without including a morphometric-only model by which to evaluate the added utility of BIA (Cox and Hartman 2005; Duncan 2007; Webster and Hartman 2007; Hanson et al. 2010). The addition of BIA metrics to our percentage-based morphometric models only increased the proportion of explained variance marginally (as much as 4% across species), such that the effort of taking BIA measurements in addition to length and wet-mass measurements is likely not worth the gain in predictive power. Similarly, Caldarone et al. (2012) found that adding BIA metrics to morphometric models of lipid, protein, and water for endangered Atlantic Salmon increased the proportion of explained variance by no more than 2%. Therefore, we suggest that future studies include a morphometric model containing combinations of length, wet mass, and condition factor for comparison purposes but also urge the inclusion of length as a covariate with condition factor in models. We found that models with condition factor alone explained ~20% less variability than TL + condition models. Therefore, if models with only one condition factor were compared to BIA models, investigators might conclude that BIA was a useful measurement to add to field protocols when use of both length and condition factor was warranted to standardize fat content at length by fish.

Models that predicted percent lipid and energy density exhibited higher predictive power than models that predicted percent protein, ash, water, and dry mass; nevertheless, the total variance explained by these models was low, and no model was able to predict body composition with sufficient accuracy for practical application. Humpback Chub, Bonytails, and Roundtail Chub are members of the family Cyprinidae, many of which are known to deposit excess lipid reserves into mesenteries (Shul'man 1974; Adams 1999; Sykes 2011) rather than muscle or liver tissue. We were unable to include a dorsal-to-ventral cross section because of short body depths and BIA caliper constraints for the majority of the fish in this experiment, so our electrode placement may have estimated lipid in just muscle tissue rather than the additional visceral tissue, possibly resulting in an underestimation of energy components. This has been a common issue in BIA research (Duncan 2007; Pothoven 2008; Hanson et al. 2010; Caldarone et al. 2012; Garner et al. 2012), so future studies should incorporate multiple dorsal, ventral, and dorsal-to-ventral cross sections to include all potential fat sources in BIA estimates (Cox et al. 2010; Hafs and Hartman 2011; Hartman et al. 2011). Physical limitations related to large caliper size and small fish size when working with fish <130 mm may require development of a smaller BIA caliper unit that would allow for the benefits mentioned above but also provide the opportunity to measure shorter (<10-mm) dorsal-to-ventral cross sections. In addition, proximate composition estimates themselves are not without measurement error, so future studies should consider averaging multiple subsamples of homogenized fish tissue to provide the best estimate of proximate composition possible for use in models.

A flaw of BIA studies to date is the inclusion of highly correlated BIA metrics as predictor variables in linear and GLMM models without discussion of how those correlations may distort model estimation and prediction (Dormann 2013). Collinearity can result in the retention of predictors that have little effect on the overall results and but that influence the direction of the relationship between the response and predictor variables. Principal component analysis can be used in future studies to develop a set of uncorrelated predictor variables. It offers the additional benefit that biplots can elucidate whether electrode locations produce similar resistance and reactance readings. For example, the biplots in our study indicated that dorsal and lateral electrode locations yield similar results, so that studies of endangered species can reduce stress and decrease handling time by 50% by combining similar electrode locations. Nevertheless, future studies should address the potential effects of collinearity on model results if highly correlated predictors are included in the same model.

BIA and Mortality: Is It Safe?

In our study, Humpback Chub exhibited no mortality and Roundtail Chub low mortality from BIA and handling, but 14% and 47% of the Bonytails died within 1 week of the

BIA and handling experiments, respectively. All Bonytails succumbed to bacterial and fungal infection, likely because of stress from the experiment since there was high mortality in both Bonytail treatments while the Humpback Chub in the same tanks were not infected. As further evidence, Bonytails exhibited a more negative physiological response to the starvation treatment in our independent BIA trial than did the Humpback and Roundtail Chub (Figures 3–5); therefore, Bonytails may respond more negatively to anthropogenic stressors and this may be why the species is largely extirpated from the Colorado River basin (Mueller 2003; Gloss and Coggins 2005).

Our study is not the first to find that, for certain species, BIA may result in low risk to specimens in controlled laboratory experiments. Several studies have subjected fish to repeated measures of BIA, with reports of post-BIA bruising but no significant infection rates (Cox and Hartman 2005; Garner et al. 2012; Wuenschel et al. 2013). Since Humpback and Roundtail Chub exhibited no or low morality in our laboratory trials, BIA may be cautiously extended to endangered population segments in the field. However, resource managers may wish to weigh the added benefit of BIA relative to other metrics of condition (including wet mass), particularly with regard to the expected error rates resulting from unstable environmental field conditions.

Conclusions and Management Implications

In this study we expanded upon previous research using two endangered species and one species of concern and improved upon current BIA methodology by (1) comparing BIA models with a morphometric model to assess the added utility of including BIA; (2) simulating observation error in measurements of wet mass to assess BIA's utility in field applications; (3) using PCA to develop a set of uncorrelated BIA predictor variables for use in GLMM models; (4) developing a BIA caliper unit that standardizes sensing and detecting electrode distances, takes accurate measurements of detector length, and provides for consistent needle penetration depth and angles; (5) developing software that simultaneously captures replicates of resistance and reactance at specified time intervals; and (6) assessing the added risk of mortality from BIA versus traditional fish handling techniques that measure TL and wet mass.

Our results suggest that mortality following BIA is species specific but that BIA can be used to safely estimate the condition of Humpback and Roundtail Chub in a laboratory setting. A combination of TL and condition factor can be used to predict proximate composition without BIA, but only with accurate estimates of wet mass. Increasing the error in wet-mass measurements to simulate unstable field conditions produced variable results, but in general BIA became a more effective tool for estimating condition as observation error increased. However, the proportion of explained variance in morphometric percentage-based models was low, and the addition of BIA only increased the explained variability modestly (up to 4%), such that BIA was mostly a redundant predictor across models. Therefore, other nondestructive methods of measuring body composition that can be utilized in the field should be explored to estimate condition in Humpback Chub, Bonytails, and Roundtail Chub in the Colorado River basin.

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