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ARTICLE

Seasonal Changes in Condition of Appalachian Brook Trout

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Abstract
Reliable fish condition estimates help managers better understand ecosystem population dynamics. Therefore, our study objectives were to measure seasonal changes in percent dry weight and energy density (physiological-based measures of condition) of stream-dwelling Appalachian Brook Trout *Salvelinus fontinalis*, field-validate bioelectrical impedance analysis (BIA) models, and compare reliability of BIA and morphometric-based estimates of condition. Percent dry weight was highly correlated to energy density (R^2 = 0.93; J/g wet weight = −1,803.5 + 286.43 · [percent dry weight]), and the relationship was clearly different from those published previously for other salmonids. Significant seasonal changes in adult Brook Trout condition were observed and likely related to energy depletion from reproduction and changes in terrestrial invertebrate consumption. Adult percent dry weight peaked in early September and was lowest in February. Age-0 fish did not have large changes in measured condition between summer and winter. Bioelectrical impedance analysis was able to estimate adult mean monthly percent dry weight reliably; however, it did not appear to outperform results from relative weight (W_r). Neither W_r nor predicted percent dry weight from BIA was a reliable estimator of condition for individual adult fish. The BIA model for age-0 fish was unable to provide reliable predictions for either individual fish or monthly mean estimates, due in large part to the small range in measured condition. The BIA estimated monthly mean energy density more reliably than did W_r. Overall results of this study indicate that BIA did not perform appreciably better than W_r, which required much less effort to collect. Potential BIA model improvements may be possible by accounting for changes in skin temperature. Until improvements in the Brook Trout BIA models occur, their use should be limited to estimating mean energy density.

Researchers and fisheries biologists often seek reliable estimates of fish condition. Detailed information about fish condition can provide insight to fisheries researchers and managers about the overall health of an ecosystem (Karr 1981). Fish condition can be correlated to environmental variables such as flow (Weisberg and Burton 1993), sedimentation (Sullivan and Watzin 2010), pH (Suns and Hitchin 1990), dissolved oxygen level (Benejam et al. 2008), and concentration of heavy metals (Suns and Hitchin 1990; Clements and Rees 1997). Because changes in these important variables often occur due to environmental degradation, reliable estimates of fish condition can be used to monitor and ultimately protect aquatic ecosystems and, in many circumstances, the human populations using them.

Fish condition also varies naturally over time with changes in food abundance (Jackson et al. 2002; Yamamura et al. 2002), temperature (McClendon and Rabeni 1987), flow (Oliva-Paterna et al. 2003), and reproductive cycles (Kortet et al. 2003). Cunjak and Power (1986) demonstrated that lipid levels in Brook Trout *Salvelinus fontinalis* decreased and water content increased over winter in a subarctic river system. Decreases in the condition of stream-dwelling Brook Trout from summer to winter appear to be related to both reproduction and

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insufficient energy intake in relation to metabolic costs (Cunjak et al. 1987). Survival, reproduction, and overall abundance will likely be influenced in stream systems where large seasonal changes in Brook Trout condition occur. Therefore, by closely monitoring condition we can better explain changes in the population dynamics of Brook Trout and their role in the ecological function of aquatic ecosystems. With this detailed knowledge we can make better management decisions that will help improve damaged aquatic ecosystems and prevent degradation to the healthy ones.

Fish condition has historically been estimated using morphometric-based indices such as Fulton's condition factor (see Nash et al. 2006), relative condition (Le Cren 1951), or relative weight (Wege and Anderson 1978). In addition to these morphometric-based approaches, several studies have recently been published where bioelectrical impedance analysis (BIA) was used to estimate fish condition (see Hartman et al. 2015). Although techniques such as BIA have been used frequently over the past decade, it is still unclear how well resulting estimates of condition perform compared with traditional morphometric-based approaches (Hartman et al. 2015).

The potential field applications of BIA range from providing estimates of fish condition to estimating seasonal changes in energy density in a cost-effective manner without having to euthanize the fish. However, BIA field studies are needed to validate previously developed BIA models by collecting data that are completely independent from model development and are also from a large temporal and temperature range. Because taking BIA measures in the field is more time consuming than measuring length and weight alone, a direct comparison of BIA and morphometric-based estimates of condition is warranted.

Bioelectrical impedance analysis models and temperature corrections established in the laboratory are currently available for Brook Trout (Hafs and Hartman 2015) and provide an excellent starting point for a BIA field validation study. Furthermore, Utz and Hartman (2009) sampled Brook Trout at nine different sites that spanned a wide range of stream sizes and Brook Trout densities. They were able to provide evidence of density dependence for both age-0 and adult Brook Trout in Appalachian streams. By sampling the same sites previously looked at by Utz and Hartman (2009) over a large temporal scale, we should be able to sample Brook Trout over a large range of body conditions and water temperatures, thereby creating a favorable setting for a BIA field validation study. Based on results of previous research, the objectives of this study were to (1) accurately measure seasonal changes in percent dry weight and energy density (physiological-based measures of fish condition) of stream-dwelling Appalachian Brook Trout, (2) field-validate previous BIA Brook Trout models, and (3) provide a direct comparison of BIA and morphometric-based estimates of Brook Trout condition.

METHODS

Fish sampling.—Brook Trout were collected at nine different sample sites on six different Appalachian headwater streams (Strahler order 1 or 2), all within the Middle Fork River watershed, West Virginia. Kittle Creek contained three sites; Rocky Run had two sites; and Light Run, Brush Run, Sugar Drain, and Mitchell Lick Fork each contained one site. When a stream contained multiple sites, the sites were separated by ≥2 km. Basin area at sample sites ranged from 0.83 to 15.38 km^2, while mean riffle depth and pool depth of sample sites ranged from 7.46 to 15.21 cm and from 15.53 to 32.52 cm, respectively (Utz and Hartman 2009). Previous research (Utz and Hartman 2009) indicated that these nine sites had a wide range of Brook Trout densities (0.028–0.237 $fish/m^2$); therefore, fish from these sites were also considered to have been in a wide range of condition and thus suitable for BIA model validation. Each 200-m-long site was sampled on the first weekend of every month from May 2010 to April 2011, except in February. Snow-covered roads prevented us sampling in Mitchell Lick Fork, upper Kittle Creek, Brush Run, and middle Kittle Creek during that month. Two passes with backpack electrofishing equipment were done at each site to capture trout. Upon capture, trout were anesthetized with tricaine methanesulfonate (MS-222) and BIA analysis was done.

Data collection for BIA.—Resistance and reactance were measured on all adult Brook Trout (>100 mm TL; Hakala 2000; Sweka 2003), with both external rods and subdermal needle electrodes using a Quantum II bioelectrical body composition analyzer (RJL Systems, Clinton Township, Michigan). Only external rod electrodes were used for age-0 trout because age-0 Brook Trout are more sensitive and we wanted to limit sampling time. Electrode specifications were the same as those in Hafs and Hartman (2011) for adult fish and Hafs and Hartman (2014) for age-0 Brook Trout. Measurements for BIA were taken at two locations for both adult and age-0 trout based on the recommendations of Hafs and Hartman (2011, 2014). For adult Brook Trout, the first measurement was taken by placing the electrodes along the dorsal midline of the fish, with one electrode located immediately posterior to the head while the other electrode was positioned immediately anterior to the adipose fin (DML). For age-0 trout, the first measurement was taken by placing one electrode parallel to the lateral line halfway between the lateral line and the dorsal midline directly above where the lateral line intersects the opercular flap. The other electrode was placed halfway between the lateral line and the dorsal midline directly below the adipose fin (DTL). The second set of measurements for both adult and age-0 trout were taken by placing one electrode along the dorsal midline just anterior to the dorsal fin while the other electrode was placed along the ventral midline below the other electrode (DTV). Electrode placements are shown in Figure 1. Following the directions of Hafs and Hartman (2011), the distance between each of the

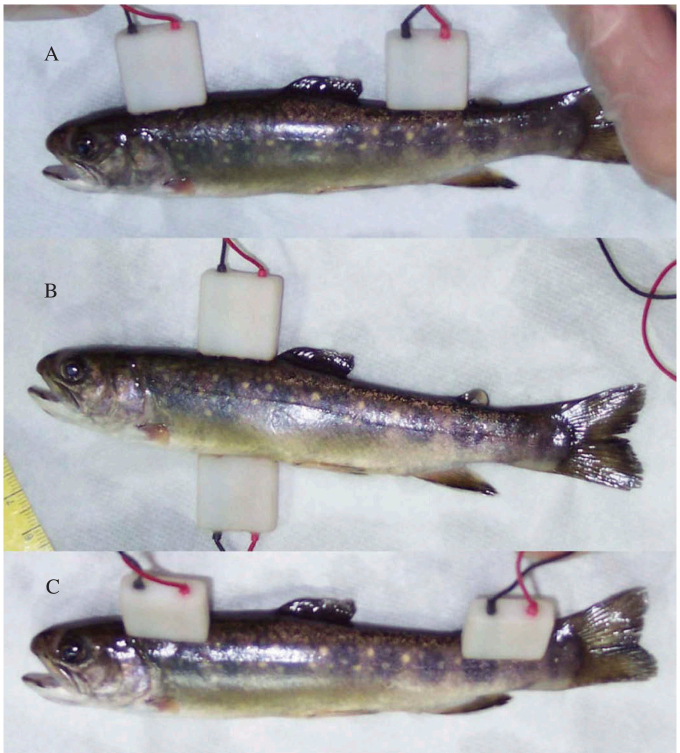

FIGURE 1. Electrode locations: dorsal midline (DML; upper photo), dorsal to ventral ahead of dorsal fin (DTV; middle photo), and dorsal total length (DTL; lower photo). The DML and DTV electrode locations were used to take BIA measurements on adult Brook Trout while the DTL and DTV locations were used for age-0 fish.

two electrodes (detector rod–needle combinations) was measured. For adult fish, core body temperatures were measured using a standard meat thermometer inserted down the esophagus into the stomach. Because core temperatures could not be taken on small, age-0 fish, water temperature was measured instead. These temperature measurements were later used to correct BIA measures to 12.5°C following the suggestion of Hafs and Hartman (2015). Upon completion of BIA, wet weight (WW; g), TL (mm), and FL (mm) of each fish was measured.

Each month, every 10th adult fish and every 10th age-0 fish collected from each site were euthanized with an overdose of MS-222. These fish were brought back to the laboratory and oven dried at 80°C to a constant weight, and percent dry weight (dry weight/WW × 100) was calculated and used as a direct measure of body condition. At the end of the study, 32 of the sacrificed adult trout that represented a wide range of percent dry weights were sent to Dominion Environmental, the environmental quality subsidiary of Dominion Resources, Richmond, Virginia. There, estimates of energy density were calculated by means of bomb calorimetry. Three 1-g pellets were analyzed from each fish, and the resulting energy density estimates were averaged to increase accuracy. Only adult fish with dry weights greater than 3 g were sampled for energy density, so the use of a microbomb calorimeter was not required.

Data analysis.—Measured percent dry weight values of fish harvested for validation were used to determine whether condition was significantly different by month. Tests for significant differences were done using ANOVA with a Tukey's honestly significantly different (HSD) test for pairwise comparisons. As needed, data were normalized by applying the Box–Cox transformation, a procedure that selects the best power transformation to normality (Sokal and Rohlf 1995).

The BIA measurements were corrected to 12.5°C using the temperature corrections (Table 1) from Hafs and Hartman (2015). Corrected resistance and reactance were then entered into the BIA models (Table 2) from Hafs and Hartman (2015) to provide estimates of percent dry weight. Calculations for all

TABLE 1. Temperature (T) correction equations from Hafs and Hartman (2015) for both resistance (r) and reactance (x) at both dorsal total length (DTL) and dorsal to ventral (DTV) locations for age-0 Brook Trout and for the dorsal midline (DML) and DTV locations for adult fish. Equations are provided that correct BIA measures to 12.5°C, the water temperature at which BIA models were originally developed at in the laboratory.

Model	Measurement	Electrode	Equation
Age-0	DTLr	Rods	$(12.5 - T) \cdot -24.8632 + \text{DTLr}$
	DTLx		$(12.5 - T) \cdot -8.7328 + \text{DTLx}$
	DTVr		$(12.5 - T) \cdot -12.3012 + \text{DTVr}$
	DTVx		$(12.5 - T) \cdot -10.3659 + \text{DTVx}$
Adult	DMLr	Needles	$(12.5 - T) \cdot -9.4040 + \text{DMLr}$
	DMLx		$(12.5 - T) \cdot -2.0361 + \text{DMLx}$
	DTVr		$(12.5 - T) \cdot -3.4464 + \text{DTVr}$
	DTVx		$(12.5 - T) \cdot -0.8849 + \text{DTVx}$
Adult	DMLr	Rods	$(12.5 - T) \cdot -9.4296 + \text{DMLr}$
	DMLx		$(12.5 - T) \cdot -2.2484 + \text{DMLx}$
	DTVr		$(12.5 - T) \cdot -3.3057 + \text{DTVr}$
	DTVx		$(12.5 - T) \cdot -2.0677 + \text{DTVx}$

TABLE 2. Regression coefficients for BIA models used to predict percent dry weight of age-0 or adult Brook Trout (Hafs and Hartman 2015). Subdermal needle and external rod electrodes were used for adult fish, but only rods for age-0 trout. The parameter column indicates which location's resistance and reactance measurements should be used to calculate the electrical parameter in parentheses. Calculations for parameters are listed in Table 3. DML = midline; DTL = dorsal total length; DTVpre = dorsal to ventral predorsal fin.

	Adults		Age-0
Parameter	Needles (DML DTVpre)	Rods (DML DTVpre)	Rods (DTL DTVpre)
Intercept	17.3855	26.6039	14.6193
FL	–0.0794	–0.1000	0.0888
WW	0.0558	0.0663	
DTL(r)			–0.0019
DTL(Xc)			36.0957
DTL(Xcp)			
DTL(Rp)			7.7824
DTL(Zp)			–37.1532
DTL(DLPA)			
DML(Rs)		–6.4609	
DML(Rp)	7.7144		
DML(Zs)	–7.7714	6.4777	
DML (DLPA)	0.0126	0.0108	
DTVpre(r)	0.0268		
DTVpre(x)		0.0654	0.0165
DTV(Rp)		–0.4466	
DTVpre(PA)		–0.3584	
DTVpre (DLPA)		0.0057	
Residual			0.6473

parameters used in the BIA models are provided in Table 3. The BIA models developed by Hafs and Hartman (2015) were then validated by comparing predicted percent dry weight values to actual measured values from the harvested validation fish. Root mean square error (RMSE) and R^2 estimates were calculated and used to determine the reliability of BIA under variable field conditions.

Relative weight W_r was estimated for all harvested validation fish. Standard weight W_S (g) for Brook Trout was calculated using the equation from Hyatt and Hubert (2001):

$$\text{Log}_{10}W_S = -5.186 + 3.103 \cdot \log_{10}\text{TL},$$

where TL represents fish total length (mm). The W_r was then calculated as WW/W_S × 100, where WW is wet weight (g). To determine how closely W_r was related to actual measured condition, W_r was regressed against both percent dry weight and energy density independently; R^2 estimates were calculated and compared with BIA results.

The relationship between energy density (J/g wet weight) and percent dry weight was used to develop regression equations following the procedures in Hartman and Brandt (1995). Measured energy density was then compared with the predicted energy density calculated when BIA-estimated percent dry weight was entered into the equation. Estimates of R^2 and RMSE were then used to compare predicted and measured energy density estimates. All statistical analyses were performed in program R (R Development Core Team 2009).

RESULTS

Over the 12-month study, BIA measurements were taken and percent dry weight was estimated for 938 adult Brook Trout and 1,383 age-0 fish. A total of 115 age-0 and 98 adult fish were harvested for validation of percent dry weight estimates over the course of the study. Ranges of fish core temperatures, water temperatures, and air temperatures were 0.1–22.6°C, 0.2–21.1°C, and −6.1–39.4°C, respectively, demonstrating that a wide range of environmental conditions were present during sampling events.

There were significant differences in body condition (based on actual percent dry weight of validation fish) by month for both adult ($F_{11,\ 86}$ = 6.61, P < 0.01) and age-0 ($F_{10,\ 104}$ = 5.46, P < 0.01) Brook Trout. For adult Brook Trout, Tukey test results from month-by-month comparisons indicated that body condition was significantly higher throughout the summer months but declined sharply after peaking the first weekend of September at a mean percent dry weight of 24.46%. Condition was then significantly lower during late fall and winter reaching a minimum in early February at a mean percent dry weight of 19.29% (Figure 2). Trends in body condition of age-0 trout were not as clear. Body condition, represented by mean percent dry weight, was significantly lower in July (16.57%) than in most months; then, from August (19.41%) through November (18.93%), condition improved and was significantly greater than body condition in July. However, in December, body condition again decreased enough to be significantly lower (17.22%) than in both August and the spring when body condition had recovered (Figure 3).

When comparing percent dry weight estimates from sacrificed validation fish to actual measured percent dry weight, the subdermal needle electrode model produced mean percent dry weight estimates that fell within the 95% confidence intervals of actual mean percent dry weight values in 7 of the 12 months (Figure 2). The RMSE for these 98 fish was 1.67 and the R^2 was 0.35. However, when comparing the predicted monthly mean percent dry weight values from these same 98 fish with the actual monthly means, RMSE and R^2 estimates were 1.20 and 0.71, respectively (Figure 4).

Results from measurements taken using external rod electrodes were similar to those produced by subdermal needle

TABLE 3. List of parameters that are included in the BIA models used in this study. For the residual from the length–weight equation, the length–weight equation is provided, and the residual is calculated by subtracting the predicted from the observed wet weight (WW). DL = detector length, Ω = ohms, ° = degrees.

Parameter	Symbol	Units	Calculation
Resistance	r	Ω	measured by Quantum II
Reactance	x	Ω	measured by Quantum II
Resistance in series	Rs	Ω	DL^2/r
Reactance in series	Xc	Ω	DL^2/x
Resistance in parallel	Rp	Ω	$DL^2/[r + (x^2/r)]$
Reactance in parallel	Xcp	Ω	$DL^2/[x + (r^2/x)]$
Impedance in series	Zs	Ω	$DL^2/(r^2 + x^2)^{0.5}$
Impedance in parallel	Zp	Ω	$DL^2/[r{\cdot}x/(r^2 + x^2)^{0.5}]$
Phase angle	PA	°	$\text{atan}(x/r){\cdot}180/\pi$
Standardized phase angle	DLPA	°	$DL{\cdot}[\text{atan}(x/r){\cdot}180/\pi]$
Residual from length–weight equation	Resid		$WW = 0.0000072 \cdot TL^{3.0056}$

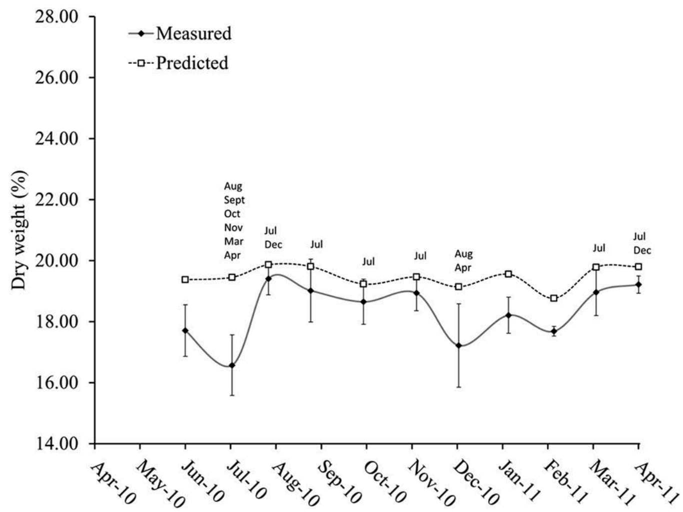

FIGURE 3. Predicted (open squares) monthly mean percent dry weight of age-0 validation Brook Trout (*n* = 115) compared with actual measured mean values (black diamonds). Black bars represent 95% confidence intervals. Months listed on top of data points indicated measured monthly means (black diamonds) that are significantly different from the month indicated on the *x*-axis (given as month-year).

electrodes. For adult Brook Trout, the predicted mean percent dry weight estimate was within 95% confidence intervals of actual percent dry weight measured 8 of the 12 months (Figure 3). For the 98 validation fish, RMSE and R^2 estimates were 1.76 and 0.38, respectively. When comparing monthly mean predicted percent dry weight to actual monthly mean percent dry weight calculated using only the 98 validation fish, RMSE (1.27) and R^2 (0.70) estimates improved and were similar to the estimates produced by subdermal needle electrodes (Figure 4). Although the models for subdermal needles and external rods were corrected for temperature, analysis of residuals demonstrates model error increased as body temperatures approached or exceeded 20°C and dropped below 5°C for adult Brook Trout (Figure 5). Model error also increased substantially for age-0 fish when water temperature exceeded 15°C.

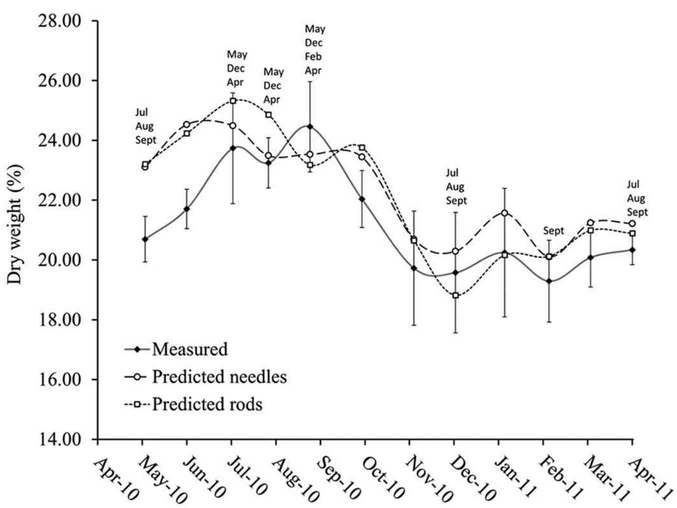

FIGURE 2. Predicted mean percent dry weight estimates for adult validation Brook Trout (*n* = 98) for both subdermal needle (open circles) and external rod (open squares) models compared with actual measured mean values (black diamonds). Black bars represent 95% confidence intervals. Months listed on top of data points indicated measured monthly means (black diamonds) that are significantly different from the month indicated on the *x*-axis (given as month-year).

For age-0 Brook Trout, external rod electrode models produced predicted mean percent dry weight estimates that were within 95% confidence intervals of actual percent dry weight measured in only 5 of 11 months that age-0 fish were captured (Figure 3). When comparing predicted percent dry weight from the 115 validation fish to measured percent dry weight, RMSE and R^2 estimates were 1.28 and 0.27, respectively. When comparing monthly mean percent dry weight to monthly mean values from validation fish, RMSE was 1.15 and R^2 was 0.40 (Figure 4).

There was a significant relationship between the percent dry weight of individual adult validation fish and W_r; however, similar to the results of BIA, a large portion of the variation in actual percent dry weight was unaccounted for ($P < 0.01$, $R^2 = 0.16$; Figure 6). The relationship between monthly mean W_r and monthly mean percent dry weight resulted in a $R^2 = 0.71$, a value identical to the highest R^2 produced using BIA.

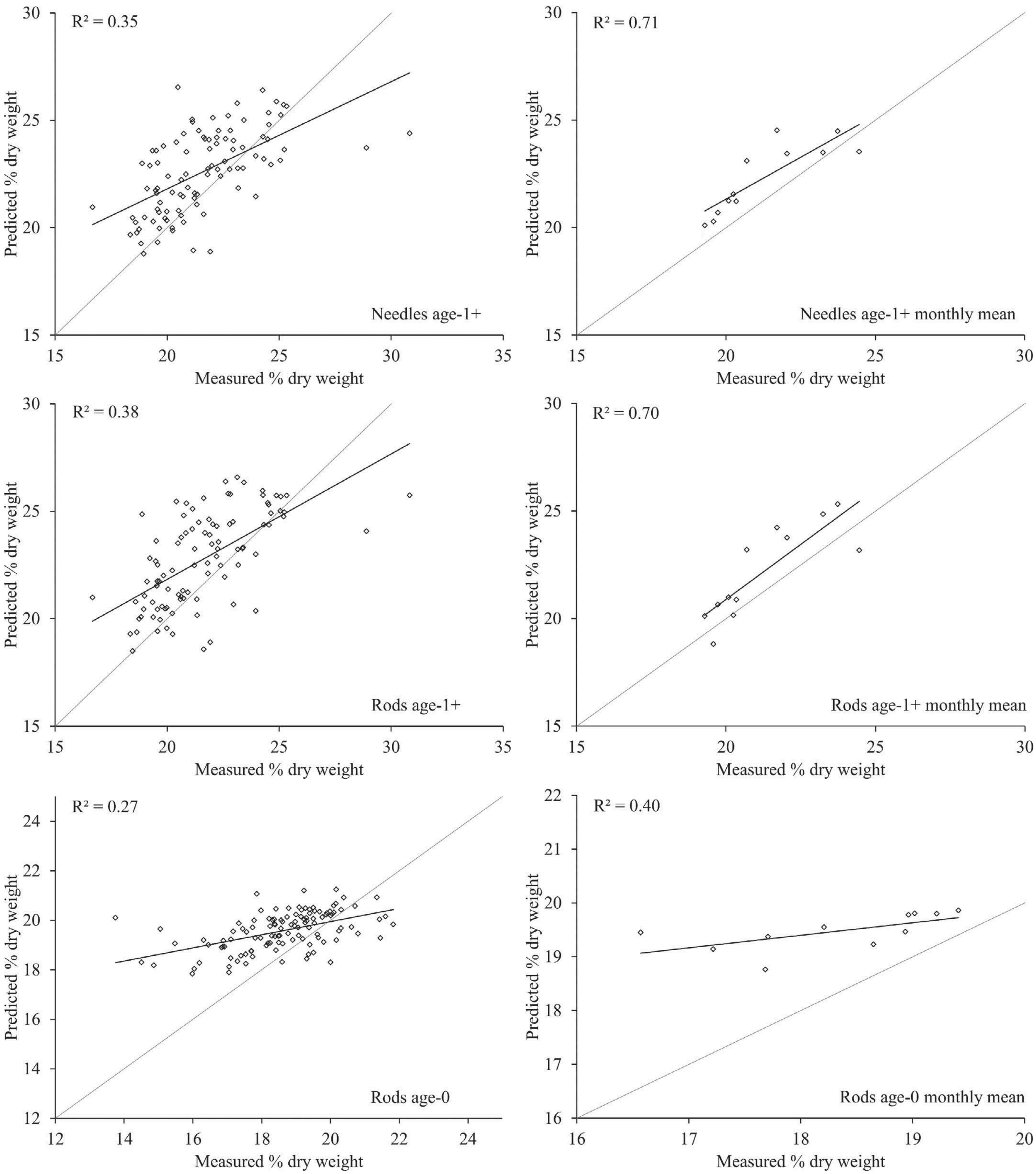

FIGURE 4. Predicted versus measured percent dry weight for the BIA models that had BIA measures corrected to 12.5°C. The light grey line in each graph represents what the trend line (dark black line) would look like if there was a 1:1 relationship between measured and predicted percent dry weight values.

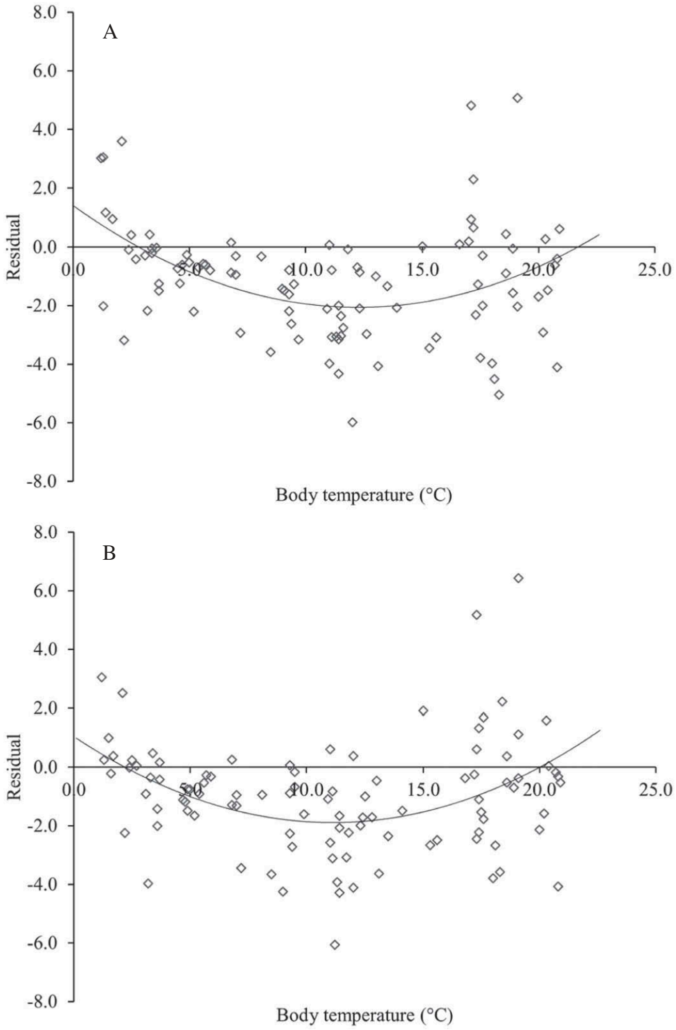

FIGURE 5. Residuals from both the external rod (upper panel) and subdermal needle (lower panel) BIA models that predict percent dry weight of adult Brook Trout, plotted against fish body temperature.

The relationship between percent dry weight and energy density (R^2 = 0.93) resulted in the following equation (Figure 7):

$$\text{Energy density}(\text{J/gWW}) = -1,803.5 + 286.43 \cdot (\text{percent dry weight}).$$

When the BIA-predicted percent dry weight values from the subdermal needle and external rod electrode model were entered into this equation and compared with actual measured energy densities, the R^2 values were 0.39 and 0.56, respectively. The RMSE estimates were 604.8 and 596.3 for subdermal needle and external rod electrode models, respectively. There were 6 months in which two or more samples were taken for energy density estimates. When monthly mean predicted energy density was compared with actual mean energy density the R^2 value was 0.91 for the subdermal needle model and 0.98 for the external rod model. For monthly mean estimates RMSE was 315.5 and 426.3 when subdermal needle and external rod electrode models were used, respectively. Because energy density is a function of percent dry weight, monthly trends in energy density closely followed patterns described earlier for percent dry weight. The relationship between W_r and energy density resulted in a R^2 = 0.07. The relationship between monthly means for W_r and energy density resulted in an R^2 = 0.52.

DISCUSSION

Brook Trout Body Condition

Based on the streams sampled during the course of this study, it is clear that adult Brook Trout populations present in headwater streams of the Appalachian Mountains undergo significant changes in body condition over the course of a normal year. Similar to the findings of Cunjak et al. (1987), changes in percent dry weight seem to follow a pattern similar to a sine wave with condition peaking in late August to early September. Brook Trout body condition, represented by percent dry weight, then declines sharply during the month of October. Brook Trout spawning in West Virginia typically begins in early October and often continues through early December (Hakala and Hartman 2004; Petty et al. 2005). During this time period, a substantial portion of the lipid reserves are used for reproduction and can often lead to high mortality rates during winter (Hutchings 1994). Because percent dry weight is correlated to percent fat (Hartman and Margraf 2008), the significant decline in body condition witnessed in this study can be explained by the losses in lipids that occur during reproduction (Hutchings 1994). From November through early February, body condition of adult Brook Trout continues to decline. During these months, terrestrial invertebrates are no longer available to Brook Trout, eliminating a major source of energy required for positive growth and increased body condition (Allan 1981; Utz and Hartman 2007; Sweka and Hartman 2008). Utz and Hartman (2007) determined that Brook Trout consumption of terrestrial invertebrates was the major factor in determining whether Brook Trout fed above a maintenance ration in the Middle Fork River watershed. The temporal trends in terrestrial invertebrate consumption by Brook Trout measured by Utz and Hartman (2007) closely resemble the trends in body condition reported in this study. Therefore, it is likely that terrestrial invertebrate consumption and Brook Trout reproductive cycles are the two major factors controlling the trends in adult Brook Trout condition.

The changes in percent dry weight for age-0 fish were not as pronounced as the trends in condition witnessed for adult fish. Two interesting patterns in age-0 trout condition were the drop in July condition and the lack of the substantial drop in winter condition that was prevalent with adult Brook Trout. The drop in July condition is most likely explained by the fact

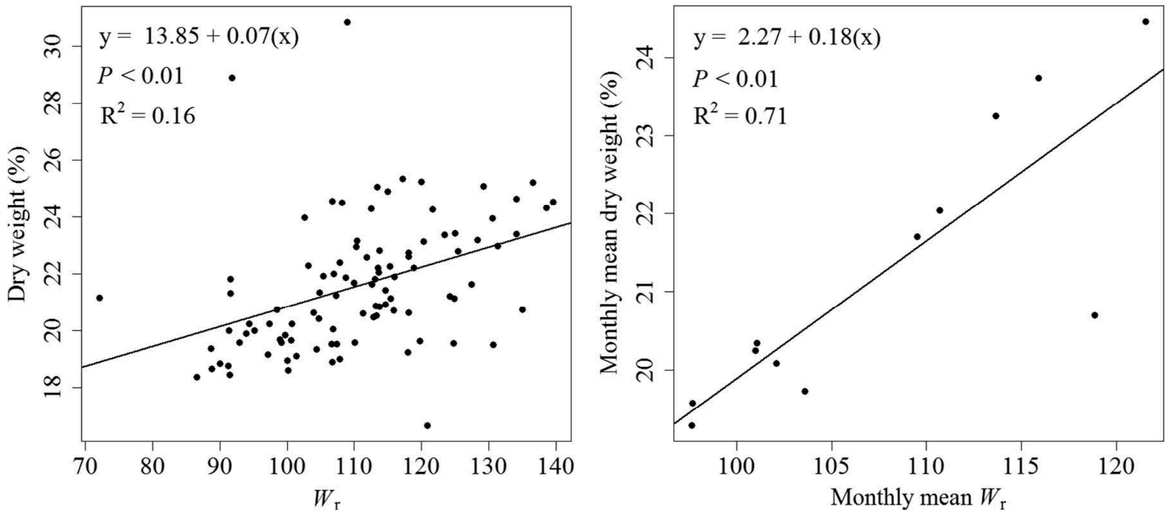

FIGURE 6. Relationship between percent dry weight and relative weight W_r (left panel) and monthly mean percent dry weight and monthly mean relative weight W_r (right panel) for Appalachian Brook Trout sampled from tributaries of the Middle Fork River, West Virginia, between May 2010 and April 2011.

that the average water temperature of 18.0°C in July was the highest measured during the study, and water levels were also lower than any of the other sample periods (mean = 12 ft^3/s; USGS 2011, gauging station 03052000). It is possible that during this time period of low flow, aquatic invertebrate numbers would have been diminished (Schlosser and Ebel 1989), and with limited surface area, possibly fewer terrestrial invertebrates would have fallen into the streams. The combination of reduced food availability and the increased metabolism resulting from elevated water temperature is a likely explanation for the decreased condition of age-0 fish in July. This pattern was not evident for adult fish, which was probably the result of larger adult fish outcompeting age-0 fish for limited remaining preferred habitat locations (Young 2004).

The other interesting trend in age-0 Brook Trout condition was the lack of the substantial drop in condition from October to February noticed in adults. There are likely two contributing factors that would explain this. First, age-0 fish did not have the energy losses associated with reproduction. Second, the sum of percent fat, protein, and ash components, when based on wet weights, will equal the percent dry weight of the fish. Protein and ash components of age-0 Brook Trout typically add up to about 13.5% of the fish based on their wet weights (Phillips et al. 1960). The results of this study indicate that mean percent dry weight of age-0 fish in December and January was lowest at approximately 17%. This means that the average fish has about 3.5% fat based on the wet weight of the fish; this value agrees well with those published in Phillips et al. (1960). When the percent fat drops below 1%, increased mortality can occur (Biro et al. 2004). Therefore, fish with percent dry weight estimates much lower than 17% will be rarely captured due to their increased chance of mortality resulting from low lipid reserves.

Brook Trout Energy Density

This is the first paper to publish an energy density equation specifically designed for Brook Trout. The equation provided in this paper demonstrates the relationship between energy density and percent dry weight for Brook Trout captured in the Middle Fork watershed is different than relationships for other salmonids published in Hartman and Brandt (1995). As the percent dry weight of Brook Trout increases, energy density increases at a slower rate (slope = 283.4) than that reported for the general salmonid relationship published previously (slope = 386.7; Hartman and Brandt 1995). As a result, at a low percent dry weight value (17%) energy density estimates produced by the general salmonid model from Hartman and Brandt (1995) and those from our Brook Trout model differed by only 4.0%, while at a high percent dry weight value (31%) energy density values differed by 15.3%. Energy density estimates can be one of the most sensitive parameters included in bioenergetics models (Beauchamp et al. 1989), and although the energy density relationship from our research should be independently verified for Brook Trout from different locations, bioenergetics predictions for Brook Trout should be improved if the equation provided in this paper is used in place of the more general salmonid model currently used.

BIA Field Application

Validation of BIA models is a necessary step before the models can be used in the field. This study tested the reliability of Brook Trout BIA models over a wide range of field conditions including air temperatures ranging from −6.1°C to 39.4°C, wind, snow, and rain. This rigorous field validation study has allowed us to determine the strengths and weaknesses of BIA, as well as determine the limits of the models

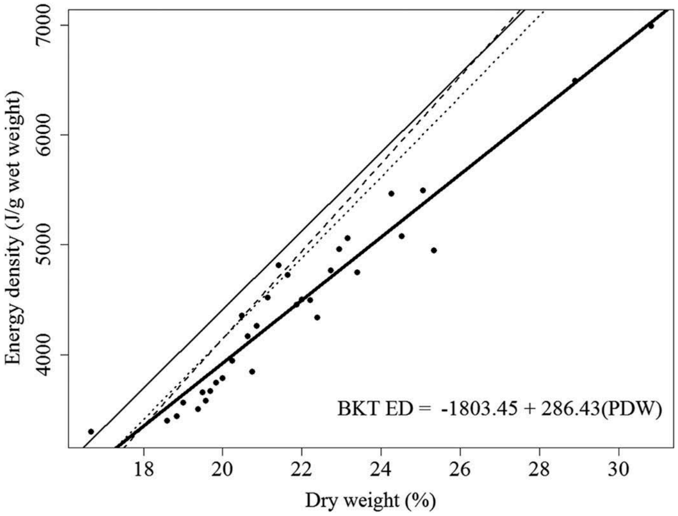

FIGURE 7. Relationship of energy density (BKT ED; J/g WW) to percent dry weight (PDW) for adult Appalachian Brook Trout sampled from tributaries of the Middle Fork River, West Virgina, between May 2010 and April 2011 (R^2 = 0.93, $y = -1803.45 + 286.43(x)$, thick black line). Similar relationships for Rainbow Trout *Oncorhynchus mykiss* ($y = -2735 + 357.5(x)$; thin black line), Lake Trout *Salvelinus namaycush* ($y = -3809 + 397.9(x)$; dashed line), and Coho Salmon *O. kisutch* ($y = -3207 + 367.8(x)$; dotted line) from Hartman and Brandt (1995) are included for comparison.

being tested. For example, BIA models for subdermal needle electrodes were able to predict monthly mean body condition of adult Brook Trout, represented via percent dry weight, with good results. Estimates of RMSE and R^2 were 1.20 and 0.71, respectively, for the 12 months of the study. This means that if the goal is to predict the average condition of adult Brook Trout in an aquatic ecosystem using BIA with subdermal needle electrodes and following the methods and models provided by Hafs and Hartman (2014, 2015), mean body condition of adult Brook Trout can be predicted successfully in a wide range of field conditions.

However, field conditions clearly had an influence on the ability of BIA to predict percent dry weight for individual fish. Measurements were taken under tarpaulins on multiple occasions due to rain and snow. Wind affected the accuracy of electronic scales, and even sunlight was a major factor during the summer causing elevated air temperatures. The combination of these factors resulted in unexplained measurement error that, in general, caused estimates of percent dry weight for individual fish to be less reliable than those produced previously in laboratory studies. For example, estimates of both RMSE and R^2 using subdermal needles for individual adult fish in this study were 1.67 and 0.35, respectively. These values were worse than average RMSE (0.90) and R^2 (0.91) estimates from Hafs and Hartman (2015) under controlled laboratory conditions over a wide range of temperatures. However, fisheries biologists and managers are most often more interested in trends of the population as a whole than the status of individual fish. When using BIA to calculate monthly mean percent dry weight, BIA did a much better job and produced very reliable estimates, especially for adult fish with either subdermal needle or external rod electrodes. The most likely explanation for this result is that the models are unbiased so measurement error on the individual fish can be high at times, but when averaged over many fish, the method produces estimates that are very close to actual body condition of fish in the population. Although BIA has shown some potential and the fundamental concepts behind the methods are sound, it should be used with caution because monthly averages of W_r appeared to track the average percent dry weight of adult Brook Trout just as well as BIA measures. Because W_r can be measured with much less effort, the use of BIA as a tool for Brook Trout management should be done with caution until more progress on the use of BIA in variable field condition can be made.

Analysis of residuals clearly indicated that both external rod and subdermal needle electrode models performed the worst as body temperatures approached or exceeded 20°C. In our opinion, the reason for a large amount of unexplained error as temperatures approached or exceeded 20°C was that during the summer when the fish's body temperature was near 20°C, the air temperatures were often much warmer and the sun was out. This probably caused the fish's skin temperature to be much warmer than the internal body temperature of the fish we measured. Skin temperature of humans can significantly influence BIA measures (Gudivaka et al. 1996), and it is likely that elevated skin temperatures during the summer introduced a large amount of unexplained variation during this study. It is possible that if skin temperature was measured, model performance could be improved. In our opinion, BIA measurements taken during warm summer months should be in a shaded area out of direct sunlight to avoid the fish's skin temperature to be increased in comparison with the internal body temperature. In this study, model residuals also indicated BIA model performance decreased as body temperatures dropped below 5°C. The temperature correction equations were developed at temperatures ranging from 5°C to 20°C (Hafs and Hartman 2015), so this model error is likely the result of extrapolating outside the range of the data set.

A major problem with using BIA on age-0 fish is that the range of percent dry weights measured in the field is very low (16.57–19.41%), and even an RMSE as low as 1.15 results in a R^2 of only 0.40. To achieve a higher R^2, one of two things must occur: either the range of percent dry weights sampled must be increased, which may not be possible for wild populations of age-0 fish, or more work needs to be done to improve age-0 models and decrease RMSE estimates. The age-0 model used in this study was developed by Hafs and Hartman (2015) in a laboratory setting where measurements were taken on each fish with two electrode types and at three different temperatures in an effort to develop temperature corrections. It is possible the BIA model for age-0 Brook Trout could be improved by taking BIA measurements at only the two best locations (DTL and DTV) that span a wide range of body conditions. This could minimize any error associated with repeated BIA measures or temperature-related influences caused by contact with the researcher.

Using predictions of percent dry weight from BIA models resulted in mean energy density estimates that were very close ($R^2 \geq 0.91$) to measured monthly mean estimates provided by the bomb calorimeter. This was one area where BIA seemed to outperform W_r, although, again, caution is urged because the number of data points used to generate these relationships was low (n = 6). Still, this is exciting because it means future researchers should be able to use the BIA models and temperature corrections provided by Hafs and Hartman (2015) to produce reliable estimates of mean energy density in situations where small population size may limit the ability to sacrifice fish or where cost of laboratory analysis is a limiting factor. Furthermore, any future improvements in BIA models that predict percent dry weight will likely strengthen the ability of BIA to predict energy density for use in bioenergetics models.

Conclusions

Adult Brook Trout have large changes in body condition throughout the year that closely resemble reproductive patterns and trends in their consumption rates of terrestrial invertebrates. Age-0 trout do not have a large change in measured condition between summer and winter. This is most likely because they do not incur energy depletion due to reproduction, energy is not reserved during the summer as an adult would do but is instead used for growth to avoid predation, and fish in poor condition experience higher mortality rates and are rarely sampled, essentially putting a limit on how low measured percent dry weight can get during winter.

The general trend for BIA prediction in this study was that average body condition for the population could be predicted with reliable results, but predictions were not as good for the individual fish. Predictions using subdermal needle electrodes were similar to those of external rod electrodes for adult Brook Trout. The BIA predictions for age-0 fish resulted in a low RMSE; however, the range of percent dry weights measured in the field was very narrow, so R^2 values were also low. More work is needed to improve BIA models as current models did not appear to outperform a traditional morphometric-based approach. Researchers could start by attempting to minimize error associated with the rapid changes in temperature that occur on the surface of the fish.

Energy density relationships for Brook Trout resulting from this study are clearly different from other salmonid models presented in previous literature and will help improve the accuracy of bioenergetics models for future research. However, because energy density relationships can differ by location, independent verification is needed. Finally, BIA predictions of percent dry weight appear to allow for the cheap, nonlethal estimation of mean energy density for Brook Trout populations.

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