Evaluation of bioelectrical impedance analysis and Fulton's condition factor as nonlethal techniques for estimating short-term responses in postsmolt Atlantic salmon (*Salmo salar*) to food availability

Abstract—We evaluated measures of bioelectrical impedance analysis (BIA) and Fulton's condition factor (*K*) as potential nonlethal indices for detecting short-term changes in nutritional condition of postsmolt Atlantic salmon (*Salmo salar*). Fish reared in the laboratory for 27 days were fed, fasted, or fasted and then refed. Growth rates and proximate body composition (protein, fat, water) were measured in each fish to evaluate nutritional status and condition. Growth rates of fish responded rapidly to the absence or reintroduction of food, whereas body composition (% wet weight) remained relatively stable owing to isometric growth in fed fish and little loss of body constituents in fasted fish, resulting in nonsignificant differences in body composition among feeding treatments. The utility of BIA and Fulton's *K* as condition indices requires differences in body composition. In our study, BIA measures were not significantly different among the three feeding treatments, and only on the final day of sampling was *K* of fasted vs. fed fish significantly different. BIA measures were correlated with body composition content; however, wet weight was a better predictor of body composition on both a content and concentration (% wet weight) basis. Because fish were growing isometrically, neither BIA nor *K* was well correlated with growth rate. For immature fish, where growth rate, rather than energy reserves, is a more important indicator of fish condition, a nonlethal index that reflects short-term changes in growth rate or the potential for growth would be more suitable as a condition index than either BIA measures or Fulton's *K*.

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Populations of Atlantic salmon (*Salmo salar*) are broadly distributed across the North Atlantic Ocean, reproducing in coastal rivers of Iceland, Europe, northwestern Russia, and northeastern North America. Historically, native Atlantic salmon ranged throughout New England waters, but by the late 1880s, they were extirpated from many of the rivers. Currently, native populations exist only in central and southeast Maine (Northeast Fisheries Science Center, http://www.nefsc.noaa.gov/sos, accessed July 2011). A significant decline in these populations during the 1990s resulted in the listing of the Gulf of Maine Distinct Population Segment (DPS) as endangered under the United States Endangered Species Act (Federal Register, 2009). Hatchery-based restoration of salmon to this area began in 1970 and continues today. Although all life-stages have been released to the rivers, recent emphasis has focused on releasing fry and smolts produced in the hatchery from field-caught adults from the DPS region. Once smolts reach the sea, they must adapt to an environment and food source radically different from their freshwater habitats. To evaluate the success of these restoration efforts, managers need tools to assess whether hatchery-reared fish are thriving in the natural environment and to assess condition of the postsmolt Atlantic salmon population as a whole.

Growth rate and fat content are key measures of condition in fish. During a fish's early life-history stage, rapid growth rates increase the probability of survival and recruitment, primarily through decreased vulnerability to predation and starvation (see review by Fonseca and Cabral, 2007). Fat, the primary energy repository in marine fish, increases when food intake exceeds metabolic needs and decreases during food-limited times when it provides energy for maintenance, growth, and reproduction (Shulman and Love, 1999; Jobling, 2001). Growth rates of fish caught in the wild are often estimated by using nucleic acid analysis (the ratio of RNA to DNA) or otolith microstructure analysis (Chambers and Miller,

1995). The former requires removal of a plug of muscle tissue (MacLean et al., 2008), and the latter requires removal of an otolith, a lethal procedure. Direct measurement of fat content is also lethal, requiring chemical analysis of a sacrificed fish. Identifying a minimally invasive, nonlethal index that could estimate growth rate or body composition in postsmolt salmon would allow restoration managers to evaluate the condition of field-captured fish.

We chose to evaluate two nonlethal techniques which had the potential to reflect fish condition: bioelectrical impedance analysis (BIA) and Fulton's condition factor (hereafter, also called Fulton's K). BIA is a technique which has been applied to humans and other mammals as a means to estimate nutritional status and body composition (Baumgartner et al., 1988; Marchello and Slanger, 1994; Schwenk et al., 2000; Barbosa-Silva et al., 2003). Recently, BIA has been used to estimate body composition content in fish (Cox and Hartman, 2005; Pothoven et al., 2008; Hanson et al., 2010). For BIA, a small, portable, battery-operated instrument is used to generate a mild alternating current between two sets of electrodes that have been placed on the subject. The resulting voltage drop is recorded as resistance (R) and capacitive reactance (Xc) in series. When an alternating current passes around a cell, R can be affected by extracellular water (good conductor) and fat (poor conductor). When a constant signal frequency is applied, a geometrical system can be modeled as a cylinder (conductor volume = $\rho L^2/R$, where L is length) (Lukaski et al., 1985). With this conductor volume approach, predictive equations have been constructed to estimate water content and fat-free mass in humans (Lukaski et al., 1985; Chumlea et al., 2002), and water-, fat-, protein-, and ash-content in fish (Cox and Hartman, 2005; Pothoven et al., 2008; Hanson et al., 2010).

Impedance values have also been used to calculate a condition index (phase angle, arctangent Xc/R converted to degrees) in both humans and fish (Schwenk et al., 2000; Barbosa-Silva et al., 2003; Cox and Heintz, 2009). In an organism, Xc is a measure of the phase shift that results from an electrical charge being momentarily stored in the double phospholipid layer of a cell membrane. When cells die, Xc drops to zero; phase angles thus range from zero (zero Xc, all cells dead) to 90° (zero R). In humans, lower phase angle values have been associated with conditions such as reduced survival in HIV-infected patients, and malnutrition (Schwenk et al., 2000; Barbosa-Silva et al., 2003). In fish, significant decreases in phase angles have been observed in juvenile rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) after three weeks of fasting, and in juvenile Chinook salmon (*O. tshawytscha*) after eight weeks of fasting (Cox and Heintz, 2009). In those studies, fish were repeatedly measured over a period of weeks with no mortalities associated with the BIA procedure—an attribute that made it attractive for our application.

Fulton's K (weight/length^3) (Ricker, 1975) is a widely used fish condition index based on morphometrics (e.g.,

Anderson and Gutreuter, 1983; Stevenson and Woods Jr., 2006), measurements that can be obtained easily in the field. This index is based on the assumption that within a cohort, individuals with higher K values (more rotund fish) contain more energy reserves (fat and protein), and thus are in better condition than those with lower K values.

The response time of a condition index can be affected by factors such as water temperature, life-stage, season, and species (Busacker et al., 1990). Cox and Heintz (2009) measured phase angles in food-deprived rainbow trout and brook trout on a weekly basis, and in food-deprived Chinook salmon intermittently for 13 weeks. Because our field recaptures of hatchery-reared postsmolts occur two to three weeks after release, we measured response of BIA measures and K to varying food availability every 3–4 days throughout a 3-week time period. Thus the objectives of our study were 1) to assess and validate the relations between two nonlethal condition indices (BIA measures and Fulton's condition factor) and two measures of nutritional condition (growth rate and body composition); and 2) to determine the short-term response time (days to a few weeks) of these measures to varying food availability.

Materials and methods

Smolts used in this study were progeny of field-caught Atlantic salmon from the Penobscot River, Maine, which had been spawned at Craig Brook National Fish Hatchery, East Orland, Maine, and reared at the Green Lake National Fish Hatchery, Ellsworth, Maine, for 13–15 months. Randomly selected smolts (52–113 g, 16–21 cm) were anesthetized in buffered tricaine methane sulfonate (MS-222, 150 mg/L) and implanted intramuscularly with a passive integrated transponder tag (PIT tag, Biomark, Boise, ID[1]) to permit identification of individuals. The smolts were then returned to the hatchery tank to allow time for full recovery, resumption of feeding, and removal of any tagging-related mortalities. Twenty-five days later the fish were transported to the University of Rhode Island's Blount Aquarium facility in Narragansett, Rhode Island, where they were randomly placed into two aerated, flow-through tanks (360-L capacity) initially filled with freshwater trucked from the hatchery. Over a period of five to six hours, freshwater was gradually replaced with sand-filtered seawater (10°C, 30 ppt). During the subsequent three weeks, while the fish were recovering from the transfer and acclimating to seawater, the water temperature was gradually raised to 12°C. During this period fish were fed to satiation twice per day with a commercial feed (Corey Optimum Hatchery Feed for Salmonids, Corey Nutrition Co., Fredericton, NB, Canada) supplemented with freeze-dried krill (*Euphausia pacifica*, Aquatic

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1

Sampling schedule for Atlantic salmon (*Salmo salar*) postsmolts reared at 12°C under three feeding regimens (fed; fasted; fasted then refed) in order to obtain a range of nutritional condition and growth rates. Nonlethal condition indices (Fulton's condition factor [*K*] and bioelectrical impedance analysis [BIA] measures) and two measures of nutritional condition (wet-weight based growth rate and proximate body composition) were determined for each fish. The refed group was fasted for 11 days and then fed. Numbers listed are number of fish sampled.

| | Sampling day and feeding regimen | | | | | | | | | | | | |
| | Day 0 | | | | Day 3 | | | Day 7 | | | Day 11 | | |
	Base-line	Fed	Fasted	Refed	Fed	Fasted	Refed	Fed	Fasted	Refed	Fed	Fasted	Refed
Weight, length (Fulton's *K*)	5	24	24	22	4	4	0	4	4	0	4	4	22
BIA measures	5	24	24	0	4	4	0	4	4	0	4	4	0
Proximate body composition	5	0	0	0	4	4	0	4	4	0	4	4	0

| | Sampling day and feeding regimen | | | | | | | | | | | |
| | Day 15 | | | Day 19 | | | Day 23 | | | Day 27 | | |
	Fed	Fasted	Refed	Fed	Fasted	Refed	Fed	Fasted	Refed	Fed	Fasted	Refed
Weight, length (Fulton's *K*)	4	4	5	4	4	5	4	4	5	0	0	7
BIA measures	4	4	5	4	4	5	4	4	5	0	0	7
Proximate body composition	4	4	5	4	4	5	4	4	5	0	0	7

Eco-Systems, Inc., Apopka, FL). Twenty-five days after the initial seawater transfer, when the now postsmolts appeared to be acclimated and feeding well, the experiment commenced (day 0).

Throughout the experiment, water temperature in each tank was recorded hourly with a HOBO® data logger (Onset Computer Corp., Bourne, MA), and ammonia levels and salinity were tested weekly. Water temperatures averaged 12.0°C, standard deviation (SD)=0.2; salinity averaged 31 ppt, SD=1; and the photoperiod was 15 hours of light to 9 hours of dark. Two-thirds of each tank surface was covered with black plastic to provide a low-light refuge, and the remaining third was exposed to overhead fluorescent lighting covered with red plastic. All experiments were conducted in accordance with guidelines established by the Institutional Animal Care and Use Committee (IACUC) at the University of Rhode Island.

Sampling protocols

Day 0 sampling On day 0, five fish were randomly selected and sacrificed to provide baseline body composition data. To obtain a range of nutritional condition levels, all remaining postsmolts were subdivided into three different feeding treatments (tanks): fed, fasted, and fasted then refed. The fed treatment (*n*=24) was continually fed, the fasted treatment (*n*=24) received no food, and the fasted, then refed treatment (*n*=22)

received no food for 11 days followed by feeding for 16 days. Individuals were anesthetized with buffered MS-222 (150 mg/L) in chilled (12°C) seawater, blotted dry, measured for initial weight (wet weight, *WW,* nearest 0.1 g) and fork length (FL, nearest 0.1 cm), and the PIT tag number and any gross external abnormalities were noted. BIA measurements were taken on all fish assigned to the fed and fasted treatments. Fish assigned to the fasted, then refed treatment had their fork length and wet weight recorded at the start of their fasting (day 0), and again on day 11 when they were fed for the remainder of the experiment. Total handling time per fish for wet weight, fork length, and BIA measurements was no more than 30 seconds.

During daylight hours, fish in the fed treatment and refed group were provided freeze-dried krill *ad libitum* from a belt feeder. On days fish were sampled, no food was provided until after sampling was completed (~1200).

Days 3–27 sampling In order to determine the response time of BIA measures and *K* to the three feeding treatments, and to construct predictive equations for body composition, fish were sampled and sacrificed every three to four days over a 23–27 day period. On day 3 and every 4 days thereafter, 4 fish each from the fed and fasted treatments were sacrificed. On day 15 and every 4 days thereafter, 5 fish from the refed group were sacrificed and sampled, except on the final day when 7 fish were sacrificed (Table 1).

All fish were killed by an overdose of buffered MS-222 (300 mg/L) in chilled (12°C) seawater. The fish were immediately blotted dry, measurements of wet weight, fork length, and BIA (in that order) were taken, and PIT tag numbers and external abnormalities were noted. Internal temperatures (muscle and stomach) were determined by inserting an instant-read digital thermometer into the dorsal musculature of the fish, and down the esophagus into the stomach. Total sampling time for each fish was ~1.5 min. Each fish was then dissected, its liver removed, its gut evacuated, and sex and maturity status was determined. Livers and carcasses were wrapped separately in aluminum foil before being vacuum-sealed in plastic bags and stored frozen at –80°C until subsequent analysis.

Body composition analysis Liver and carcass wet weights were determined to the nearest 0.1 mg before being freeze-dried to a constant weight and reweighed. Each dried sample was ground in a Foss Tecator® Cyclotec 1093 sample mill (FOSS, Hilerød, Denmark) and stored at –20°C in glass scintillation vials under nitrogen gas until further analysis. Total water (*TWa*) was calculated by subtracting total dry weight (liver dry weight plus carcass dry weight, *DW*) from total wet weight.

Freeze-dried carcasses were analyzed for proximate body composition (protein, fat, ash) by an independent laboratory (A&L Great Lakes Laboratory, Fort Wayne, IN) by using Association of Official Analytical Chemists international certified methods and were reported to us on a percent DW basis. Nitrogen was determined by using a LECO nitrogen combustion analyzer (LECO Corp., St. Joseph, MI; Dumas method), protein was calculated by multiplying nitrogen values by 6.25 (Jones, 1931), fat was obtained with a 4-hr ether reflux extraction, and ash was determined after combustion at 600°C for 2–4 hr. Body composition (g) (total amount of each proximate body constituent) were calculated from percent dry weight concentrations by dividing the independent laboratory values by 100 and multiplying by the total dry weight. To conform to the format most often reported in the literature, percent dry-weight-based concentrations were converted to a percent wet-weight-based concentration by dividing body composition (g) by wet weight and multiplying by 100 (body composition [%WW]).

Liver lipids, which are often mobilized first during fasting (Love, 1970; Black and Love, 1986), were measured separately in order to detect changes more easily. Liver lipid content was determined in-house by using a modification of Folch et al. (1957). Entire freeze-dried livers were first extracted by ultrasonic homogenization with 2:1 methylene chloride:methanol solvent (20 mL/g tissue), then back extracted with aqueous 0.1 M KCl and centrifuged to remove water, methanol, and water-soluble and water-insoluble tissue components by phase separation. The remaining nonaqueous fractions were evaporated to remove methylene chloride, and the non-volatile lipid residue was weighed on a Mettler® AE240 balance (nearest mg, Mettler-Toledo, Inc., Columbus, OH). Individual livers were not analyzed for protein or ash because of their small size; therefore body composition values were obtained for carcass water, liver water, total water (liver water plus carcass water), carcass fat, liver fat, total fat (liver fat plus carcass fat), carcass protein, and carcass ash.

Growth-rate calculations

Individual instantaneous wet-weight based growth rates were calculated with the following formula (Ricker, 1979):

$$growth\ rate \text{ (per d)} = (\ln WW_{t2} - \ln WW_{t1})/(t_2 - t_1), \quad (1)$$

where WW = the wet weight of an individual at time t (day).

Growth rates for fish in the fed and fasted treatments were calculated from day 0 (t_1) until the day they were sacrificed (t_2). For the fasted portion of the fasted, then refed treatment, growth rates were calculated from day 0 (t_1) until day 11 (t_2); for the refed portion, growth rates were calculated from the first day of refeeding (day 11, t_1) until the day they were sacrificed (t_2). Growth rates calculated over intervals of less than five days were excluded from our analyses because minimal weight changes over those short time intervals, combined with the inherent variability of measuring wet weight, resulted in inaccurate growth rate estimates.

BIA measurement protocol and BIA measures

BIA measurements were determined with a Quantum-X® (RJL Systems, Point Heron, MI) four-electrode single frequency (800 μA, 50 KHz) analyzer. Needle electrode probes were constructed in-house according to Cox and Hartman (2005). For each probe, two 12-mm×28-gauge electroencephalographic (EEG) needles (Grass Telefactor, West Warwick, RI) were mounted in balsam wood 1 cm apart and with 0.5 cm of the needle exposed. The fish were placed on their right sides on a nonconductive board. The detector electrode of the anterior probe was inserted midway between the posterior edge of the operculum and the leading edge of the dorsal fin, and midway between the base of the dorsal fin and the lateral line. The signal electrode of the posterior probe was inserted at the leading edge of the adipose fin, and midway between the base of the adipose fin and the lateral line. Serial R, serial Xc, and the distance between the inside (detector) electrodes, were recorded for each fish.

All BIA measures were initially calculated by using both their series and parallel forms. Results of statistical analyses indicated that the parallel forms were more highly correlated to the independent variables. For this reason, as well as the instrument manufacturer's recommendation that the parallel forms most

closely approximate the real electrical values of biological tissue (RJL Systems, http://www.rjlsystems.com/docs/bia_info/principles/, accessed April 2008), only the parallel forms of the BIA measures are discussed and reported.

Serial R and Xc values were transformed to their parallel equivalents with the following formulas:

$$R_{par}(\Omega) = R + Xc^2/R, \qquad (2)$$

$$Xc_{par}(\Omega) = Xc + R^2/Xc . \qquad (3)$$

Because R and Xc are dependent upon the distance the current must travel (D, distance between the electrodes in cm), the BIA instrument manufacturer advises that when these variables are used in prediction equations, the effect of this distance must be accounted for (RJL Systems, http://www.rjlsystems.com/docs/bia_info/principles/, accessed April 2008). We therefore also calculated standardized R_{par} and Xc_{par} values by dividing R_{par} and Xc_{par} by D (R_{par}/D, Xc_{par}/D).

Conductor volumes were calculated by using the following formulas:

$$R_{par} \ conductor \ volume = D^2/R_{par} , \qquad (4)$$

$$Xc_{par} \ conductor \ volume = D^2/Xc_{par} . \qquad (5)$$

Capacitance (a measure of the electrical storage capacity) and impedance (a measure of the opposition to the flow of electrical current) were calculated using the following formulas:

$$capacitance \ (pF) = 1 \times 10^{12}/(2\pi \bullet 50000 \bullet Xc_{par}), \qquad (6)$$
$$impedance \ (\Omega) = sqrt((R_{par}^2) + (Xc_{par}^2)) , \qquad (7)$$

where 50,000 is the frequency applied by the BIA instrument in Hertz.

In order to conform to values previously reported in the literature, phase angles were calculated with Xc and R in their series form:

$$phase \ angle \ (°) = arctan \ (Xc /R) \bullet 180/\pi, \qquad (8)$$

where Xc and R are the vertical and horizontal axis, respectively. The arctangent of the ratio will yield the angle of the impedance vector in radians, which is then converted to degrees by multiplying by $180/\pi$. A series-based phase angle is equal to 90° minus the parallel-based phase angle.

Impedance measurements are negatively related to temperature (van Marken Lichtenbelt, 2001). Water temperature, room temperature, and internal fish temperatures (mean muscle temperature=13.4°C, SD=0.9; mean stomach temperature=12.6°C, SD=1.0) were constant in our study and therefore not included as factors in our analyses. Unless otherwise noted, the term "BIA measures" refers collectively to R_{par}, Xc_{par}, R_{par}/D, Xc_{par}/D, *phase angle*, R_{par} and Xc_{par} *conductor volumes*, *capacitance*, and *impedance*.

Fulton's *K*

Fulton's condition factor (K) was calculated with the following formula (Ricker, 1975):

$$K = 100 \bullet WW/FL^3 , \qquad (9)$$

where WW (in g) and FL (in cm) are values from the day the fish was sacrificed.

Data analysis

Within treatments A Dunnett two-tailed *t*-test with final FL as a covariate was used to detect changes in body composition (% WW), BIA measures, and K, within the fed and fasted treatments and the refed group. Baseline values (day 0) were specified as the control for all variables except BIA measures, where day-3 fed values were the specified controls.

Between treatments A two-way multivariate analysis of covariance (MANCOVA) for unbalanced design was used to compare body composition (%WW) (total fat concentration, $TF\%$; total water concentration, $TWa\%$; carcass protein concentration, $CP\%$), BIA measures, growth rate, and K between the three feeding treatments (fed; fasted; fasted, then refed) and sampling times, with final FL as the covariate. When interactions were significant, feeding treatment was nested in day and follow-up comparisons were examined by using Tukey's HSD multiple range tests. Because we found no significant differences in percent liver fat between any of the treatments or days, and liver fats comprised <1% of total fats (range: 0.39–0.99%), only total fat values were used in all analyses.

Prediction models Prediction models for body composition expressed as both content (total fat, TF; TWa; carcass protein, CP), and concentration ($CP\%$, $TF\%$, $TWa\%$), and growth rate were developed. We used an information-theoretic approach for small sample sizes (Akaike's information criterion, AICc) to select the "best-fit" models (Wagenmakers and Farrell, 2004). Because we had no prior knowledge of the variables or combination of variables that would be the best predictors of the dependent variables, all nine BIA measures plus the interaction of R_{par}, and Xc_{par} with D were tested, along with WW, FL, and K. Testing 14 independent variables generated a large number of models for each dependent variable, with many models significant at the $P<0.0001$ level. For brevity, only the top three most parsimonious models (as indicated by the smallest AICc values) are reported and discussed.

Correlations Pearson product-moment correlations were used to investigate the relations between body composition (both g and %WW), and WW, BIA measures, K, and growth rate. All statistical analyses were carried out with SAS software vers. 9.1 (SAS Inst., Inc., Cary,

Table 2

Proximate body composition (% whole-body wet weight), capacitance, and % change in wet weight of Atlantic salmon (*Salmo salar*) postsmolts reared at 12°C under 3 feeding regimens (fed; fasted; fasted then refed). The refed group was fasted for 11 days and then fed. *TF%*=total fat concentration; *TWa%*=total water concentration; *CP%*=carcass protein concentration. *Capacitance* (pF) is a measure of the electrical storage capacity of cells. Percent change in wet weight was calculated by subtracting the initial wet weight of a fish on day 0 (fed and fasted treatments) or day 11 (fasted then refed treatment, day they were fed) from its wet weight on the day it was sacrificed, expressed as a percent of its initial wet weight. Asterisks (*) in the body composition columns indicate a significant difference from baseline values, and in the capacitance column they indicate a significant difference from day 3 fed values (Dunnett two-tailed *t*-test, $P \leq 0.05$). Values are means with standard deviation (SD) in parentheses. Mean initial wet weight of all fish was 76.0 g (SD=12.6, [no. of fish sampled=74]). NA=not available. *n*=number of fish sampled.

Feeding regimen	Sampling day	*n*	*TF%*	*TWa%*	*CP%*	*Capacitance*	% change in wet weight
Baseline	0	5	7.1 (1.1)	73.2 (1.2)	17.6 (0.5)	NA	0
Fed	3	4	7.4 (1.8)	73.9 (1.5)	17.0 (0.4)	2275 (322)	NA
	7	4	6.3 (1.4)	74.1 (1.3)	17.4 (0.7)	2057 (66)	1.2 (1.9)
	11	4	5.9 (0.9)	74.9 (1.3)	16.9 (0.7)	2012 (333)	9.0 (2.3)
	15	4	5.5 (0.8)	74.9 (1.0)	17.4 (0.4)	2144 (150)	15.5 (6.5)
	19	4	6.2 (1.1)	73.8 (1.3)	17.8 (0.3)	2024 (113)	25.9 (5.0)
	23	4	6.7 (2.0)	73.9 (2.6)	17.6 (0.7)	2024 (126)	20.7 (7.2)
Fasted	3	4	7.9 (1.2)	72.6 (1.1)	17.0 (1.0)	2112 (268)	NA
	7	4	6.4 (1.3)	74.8 (1.6)	16.6 (0.4)	2042 (156)	−2.7 (0.8)
	11	4	6.0 (1.0)	74.5 (1.2)	16.9 (0.2)	1861 (180)*	−4.2 (0.9)
	15	4	5.2 (0.6)*	75.9 (1.1)*	17.0 (1.0)	1919 (152)*	−4.6 (1.4)
	19	3	5.3 (1.6)	75.8 (1.6)*	17.3 (0.2)	1988 (175)	−7.3 (2.5)
	23	4	4.9 (1.1)*	76.5 (1.5)*	16.2 (1.1)	1744 (297)*	−7.3 (2.5)
Fasted, then refed	15	5	6.5 (2.1)	74.8 (2.8)	17.3 (0.9)	2005 (228)	NA
	19	5	5.7 (0.8)	75.7 (1.2)	16.8 (0.7)	1876 (151)	4.0 (4.5)
	23	5	5.8 (0.8)	74.7 (0.9)	17.4 (0.7)	1978 (136)	17.5 (2.7)
	27	7	5.8 (0.8)	75.1 (0.7)	17.2 (0.5)	1953 (74)	15.5 (5.9)

NC). Unless otherwise stated, the level of significance was set at $P \leq 0.05$.

Results

General observations

At the start of the experiment we observed frayed or eroded dorsal fins in ~78% of the fish, and fraying of the pelvic fin in ~12% of the fish. During the experiment there was no change in the frequency of these abnormalities and there were no mortalities; however, one severely emaciated, moribund fish was excluded from the data set. None of the fish had well developed gonads and therefore all were considered to be immature. Because sex did not emerge as a significant factor in any of the statistical analyses, it was not included in the data set.

Within two weeks after their transfer to seawater, the postsmolts were actively feeding and appeared to be acclimated to the seawater and their surroundings. On day 0, wet weight ranged from 43 to 132 g, and fork length from 18 to 23 cm; size distributions of fish were not significantly different between feeding treatments (fed mean=76 g, SD=12; fasted mean=75 g, SD=13; fasted then refed mean=80 g, SD=4).

On day 0 we encountered instability problems with our handmade probes, which were resolved by the end of the day. Because we were not completely confident in our BIA measurements that day, we have not included these initial BIA values in any of our analyses. Currently, BIA needle-electrodes are not manufactured and must be made in-house. Standardized manufactured probes would be necessary if BIA is to be routinely used.

Within-treatment effects

Over the course of the experiment, changes in fish weight and body composition (%WW) were observed within a treatment. On the final day of the experiment, fish in the fed treatment had increased in weight by 14–28%, whereas fasted fish had lost 5–10% of their weight (Table 2). Fish in the fasted, then refed treatment lost an average of 4% in weight during their 11 days without food, and gained 10–21% after 16 days of refeeding. Within each of the 3 feeding treatments, *CP%* (Table 2) and *CA%* (not shown) remained fairly constant. Changes in *TF%* (decreasing) and *TWa%* (increasing) occurred in fasted fish only, and differences from baseline values became significant after 15 d of fasting (Table 2). Within a treatment and sampling day, *TF%* was the most variable of the body components, averaging an

18% coefficient of variation (CV) compared to 3.7% for *CP%* and 1.9% for *TWa%*. In continually fasted fish only, changes in two BIA measures from day-3 fed values were observed: capacitance significantly decreased beginning on day 11 (Table 2) and impedance increased on day 23 only (not shown).

Between-treatment effects

Results of the MANCOVA indicated that of the variables tested (body composition [%WW], BIA measures, growth rate, *K*), only growth rate (*P*<0.0001, df=13) and *K* (*P*=0.0009, df=16) revealed significant differences due to feeding treatment. Results of Tukey's HSD multiple range tests indicated that beginning on day 11 and continuing until the end of the experiment, growth rates (negative) of the continually fasted fish were statistically significantly slower than the fed treatment (Fig. 1A). Eight days after refeeding (day 19), growth rates of the refed group were significantly faster than those of the continually fasted treatment. On day 19 the fed treatment also had faster growth rates than those of the refed group, whereas the relation was reversed on day 23. On day 23, *K* values were significantly smaller in the continually fasted fish than those of the fed fish (Fig. 1B). There was a trend of higher mean phase angle values in the fed treatment than those in the fasted treatment (Fig. 1C), but the values between the feeding treatments were not statistically different owing to high variability within a day's sample.

Prediction models

Body composition (g), R_{par}, and Xc_{par} measured in the postsmolts encompassed a range of values (Table 3). The best predictor models for body composition (g) all contained *WW* and *FL* as independent variables, with some models also including BIA measures or *K* (Table 4). In these models, *K* can be viewed as an interaction term between *WW* and *FL* (i.e., a size-related variable). The models for *TWa* and *CP* had high predictive capabilities (coefficient of determination (r^2)>0.98),whereas the model for *TF* was less so (r^2 range: 0.74–0.76). Adding any of the BIA measures to models containing only size or size-related independent variables (size-based-only models) increased the explanatory capabilities by <1.5%.

The best predictor models for body composition (%WW) also contained size-related variables (*WW* or *FL*, and often *K*) (Table 4). The models for *CP%* additionally contained two or three BIA measures. Adding the BIA measures to a size-based-only *CP%* model increased the explanatory capabilities by <3.4%.

The best predictor models for growth rate included size-related variables plus two BIA measures (Table 4). Adding the two BIA measures to size-based-only models increased the predictive capability of the growth equations by 18–22%.

Models with ΔAICc values ≤2 are considered to be equally probable to the "best fit" model (Burnham and Anderson, 2002). Based on the ΔAICc and r^2 values,
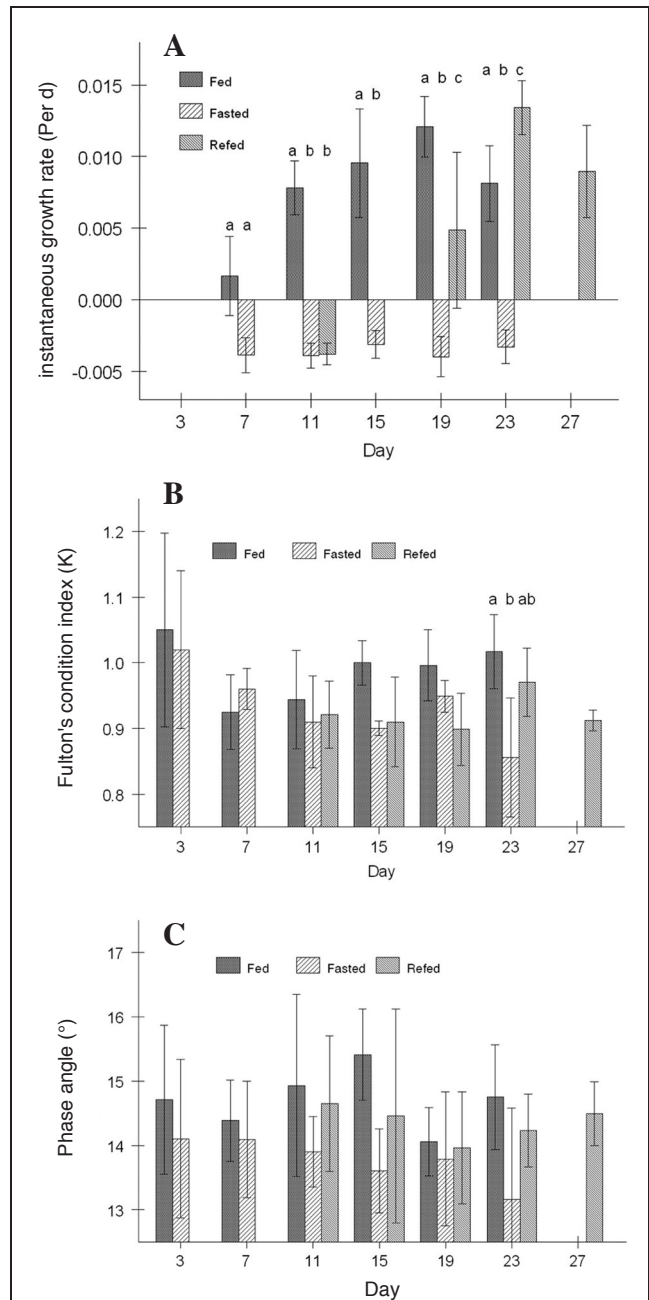

Figure 1

(**A**) Instantaneous wet-weight-based growth rate (per d); (**B**) Fulton's condition factor (*K*= 100•final wet weight/ fork length³); and (**C**) phase angle (arctangent reactance/ resistance converted to degrees) of laboratory-reared Atlantic salmon (*Salmo salar*) postsmolts measured over 27 days. Values are mean (±standard deviation [SD]) for each sampling day. Fish were either fed *ad libitum*, fasted, or fasted until day 11 and then fed for the remainder of the experiment (refed). Within a sampling day, food treatments sharing a common superscript or without superscripts do not differ significantly (Tukey's HSD multiple range tests). For fed and fasted fish *n*=4 for each sampling day. For refed fish *n*=22 for day 11, *n*=5 for days 15, 19, and 23, and *n*=7 for day 27.

little difference was evident between the top three models for each dependent variable measured. Statistical significance of all models was high ($P<0.0001$); however, the predictive capability of the body composition content (g) models (74–99%) was much higher than the concentration (%WW) and growth rate models (all <50%), with the $TF\%$ models having the lowest explanatory capability (<33%).

Correlations

Body composition (g) values were most highly correlated with WW (Table 5), although CP and TWa were also well correlated with Xc_{par} conductor volume (Table 5). CP and TWa were highly positively correlated with each other, and TF and TWa were less so; thus the majority of the changes in WW would be from the relation of water to protein, not water to fat. The ratio of water to protein averaged 4.4 (SD=0.2), which is very similar to the value reported by Breck (2008) from other studies.

Variables most highly correlated with $TF\%$ (positively) and $TWa\%$ (negatively) were WW and K (Table 5), whereas $CP\%$ and growth rate were most correlated with WW. $TF\%$, $TWa\%$, and $CP\%$ had no or low correlation with growth rate, indicating that the fish were growing isometrically and not storing fat (Table 5). Overall, correlations of body composition (%WW) were much lower than correlations of body composition (g) with WW, BIA measures, and K.

Discussion

Treatment effects

Feeding treatment (fed; fasted; fasted then refed) clearly impacted weight-based growth rates of postsmolt salmon. Growth rates of fed fish (mean=0.0078/d; range: −0.0018 to 0.0139/d) were significantly faster than those of fasted fish (mean=−0.0036/d; range: −0.0020 to −0.0053/d) and fell within ranges reported for other laboratory studies using similarly aged Atlantic salmon postsmolts (Handeland et al., 2000; Jobling et al., 2002; Bendiksen et al., 2003; Sissener et al., 2009). Growth rates of fish responded quickly to the absence or reintroduction of food; decreasing after seven days of fasting, and increasing eight days after refeeding. Wilkinson et al. (2006) reported a similar response time for growth rates of Atlantic salmon smolts after 15 days of fasting (their first sampling date) and seven days of refeeding. Somatic growth rate in immature fish is an important index of condition because faster growers are considered to have a higher probability of survival (Lundvall et al., 1999; Craig et al., 2006; Fonseca and Cabral, 2007). Rapid growth rate, which results in larger individuals, is also thought to be critical for over-winter survival (Beamish and Mahnken, 2001). The widespread importance of rapid growth rates during early life history stages is demonstrated in a meta-analysis of 40 fish studies (Perez and Munch, 2010) where 77% of estimated

Table 3

Mean, standard deviation (SD) in parentheses, and range of body composition (g) and bioelectrical impedance analysis measures (BIA; parallel resistance [R_{par}] and reactance [Xc_{par}]) of postsmolt salmon (*Salmo salar*) reared at 12°C and three feeding regimens in order to obtain a range of nutritional condition. Fish weight ranged from 43 to 132g and length from 18 to 23 cm (no. of fish sampled=74).

Variable	Mean	Range
Total water content (TWa) (g)	60.0 (11)	33.7–92.4
Carcass protein content (CP) (g)	13.7 (3.1)	6.4–24.3
Total fat content (TF) (g)	5.1 (1.8)	1.8–12.7
Carcass ash content (CA) (g)	1.9 (0.4)	1.0–3.0
R_{par} (Ω)	408 (24)	340–480
Xc_{par} (Ω)	1613 (178)	1204–2437

selection differentials favored a larger fish size during this stage.

During our study there was little impact of feeding treatment on the body composition (%WW) of postsmolt salmon. The reason for this relative constancy among feeding treatments was two-fold: 1) isometric growth in fed fish resulted in stable body composition (%WW) throughout a range of growth rates; and 2) there was little loss of proximate body constituents in fasting fish. When excess energy in young fish is directed primarily toward isometric growth, differences in growth rate cannot be discerned from body composition (%WW). For this reason, indices based on body composition (%WW) may not be the best metrics for assigning nutritional status and condition in immature fish.

Generally, fish are well adapted to fluctuations in the food supply—a scenario they encounter often in the wild (Jobling, 2001). Atlantic salmon and other fish can respond to instances of food deprivation by reducing oxygen consumption, resulting in a decreased rate of fat and protein catabolism (Beamish, 1964; Metcalfe, 1998; O'Connor et al., 2000). Our results indicate that fasted postsmolts used only a small portion of fat for their metabolic needs during the 3-week experiment. The combination of isometric growth in the fed fish, and short-term food deprivation period in the fasting fish, resulted in nonsignificant differences in body composition (%WW) among the feeding treatments.

The utility of BIA as a condition index is based on differences in proportions of body constituents translating into measurable differences in impedance when an electric current is applied. Because body composition (%WW) of the postsmolts did not differ among the feeding treatments, it was not surprising that none of the nine BIA measures differed among the feeding treatments. Within the fasted treatment, capacitance values did decrease with increasing time fasted, possibly reflecting the decline in fat concentration in this

Table 4

Coefficients and Akaike's second-order information criterion for small sample sizes (AIC_c) for the top 3 most parsimonious regression models for proximate body composition (expressed as g and % wet weight) and growth rate of postsmolt Atlantic salmon (*Salmo salar*) reared at 12°C under 3 feeding regimens in order to obtain a range of nutritional condition and growth rates. WW=wet weight (g); FL=fork length (cm); R_{par}=resistance in parallel (Ω); Xc_{par}=reactance in parallel (Ω); D=distance between bioelectric impedance detector electrodes (cm); R_{par} conductor volume=D^2/R_{par}; K=Fulton's K ($100 \cdot WW/FL^3$); *capacitance* (pF) is a measure of the electrical storage capacity of cells; *impedance* (Ω) is a measure of the opposition to the flow of electrical current; $R_{par}/D=R_{par}$ standardized for D; *phase angle* (°)=arctangent of Xc/R converted to degrees; Δ$AICc$=difference in $AICc$ values with respect to the most parsimonious model. For all models $P<0.0001$. r^2 = coefficient of determination.

Dependent variable	n	Model	r^2	AICc	ΔAICc
Total fat content (*TF*)(g)	60	$0.733 + 0.172(WW) - 0.696(FL) + 0.090(R_{par}/D)$	0.755	−6.72	0
		$8.326 + 0.150(WW) - 0.755(FL)$	0.742	−5.71	1.0
		$6.573 + 0.173(WW) - 0.648(FL) -$			
		$54.90(R_{par}$ conductor volume$)$	0.750	−5.49	1.2
Carcass protein content (*CP*)(g)	65	$13.794 + 0.260(WW) - 0.825(FL) - 4.453(K)$	0.985	−119.18	0
		$28.269 + 0.285(WW) - 1.131(FL) - 0.002(capacitance) -$			
		$0.002(impedance) - 6.62(K)$	0.986	−117.97	1.2
		$2.261 + 0.215(WW) - 0.234(FL) - 0.0005(capacitance)$	0.985	−117.16	2.0
Total water content (*TWa*)(g)	67	$-12.187 + 0.616(WW) + 1.109(FL)$	0.993	−3.20	0
		$-30.19 + 0.541(WW) + 2.002(FL) + 6.210(K)$	0.993	−2.93	0.3
		$-7.791 + 603(WW) + 1.087(FL) - 0.055(R_{par}/D)$	0.993	−2.35	0.8
Total fat concentration (*TF%*)	60	$15.272 + 0.099(WW) - 0.842(FL)$	0.326	9.81	0
		$-1.568 + 0.28(WW) + 5.70(K)$	0.324	10.02	0.2
		$8.086 + 0.330(FL) + 7.910(K)$	0.318	10.55	0.7
Carcass protein concentration (*CP%*)	65	$32.185 + 0.044(WW) - 0.218(FL) -$			
		$0.004(capacitance) - 0.004(impedance)$	0.503	−83.17	0
		$27.318 + 0.026(WW) - 0.003(capacitance) -$			
		$0.004(impedance)$	0.483	−82.96	0.2
		$28.090 + 0.036(WW) + 0.041(R_{par}/D) -$			
		$0.004(capacitance) - 0.005(impedance)$	0.498	−82.52	0.6
Total water concentration (*TWa%*)	67	$86.901 - 0.044(WW) - 9.180(K)$	0.499	20.00	0
		$97.183 - 0.522(FL) - 12.553(K)$	0.496	20.35	0.3
		$60.435 - 0.154(WW) - 1.310(FL)$	0.485	21.86	1.9
Instantaneous wet-weight based growth rate (per day)	56	$-0.6866 - 0.0016(WW) + 0.0219(FL) +$			
		$0.3882(R_{par}$ conductor volume$) +$			
		$0.0025(R_{par}/D) + 0.1931(K)$	0.481	−582.29	0
		$-0.1087 + 0.0033(FL) - 0.00003(capacitance)$			
		$+ 0.00374(phase angle) + 0.0560(K)$	0.453	−581.85	0.4
		$-0.2156 + 0.0034(FL) + 0.00003(impedance) +$			
		$0.0031(phase angle) + 0.0578(K)$	0.444	−580.98	1.3

group. However, when the whole data set was examined, capacitance and fat concentration (range: 4–10%) were only somewhat correlated (coefficient of correlation [r]=0.41, $P<0.005$).

Isometric growth is assumed for Fulton's K, and differences in the weight–length relation are interpreted as an indication of stored energy. Because the postsmolts were growing isometrically with little energy storage, Fulton's K was unable to distinguish between fast and slow growers within the fed treatment, and K values of fed fish were significantly higher than those of fasted fish only on the final day of sampling (day 23). Generally, Fulton's K tends to have a long temporal response (weeks to months) (Busacker et al., 1990). The relations among a fish's wet weight, water weight,

protein weight, and fat weight may explain this lag time. The wet weight of a fish is highly related to water weight (as was observed by Sutton et al. [2000] in Atlantic salmon parr), and water weight is much more strongly associated with protein weight than fat weight (20–40× more) (Breck, 2008). Therefore during the early stages of fasting, when fat stores are used first (Shulman and Love 1999, Jobling 2001), changes in a fish's wet weight may be fairly subtle, but once a fish begins to use protein for energy, water loss (and thus wet weight loss) would accelerate. In our study, mean fat concentration in fasted fish decreased slightly with time while mean protein concentration remained constant. Within the fasted treatment there was a decreasing trend in mean K values, but owing to high variability

Table 5

Pearson product-moment correlations (r) between (A) proximate body composition (g) or (B) wet-weight-based proximate body composition or instantaneous wet-weight-based growth rate of Atlantic salmon (*Salmo salar*) post-smolts, and wet weight, bioelectrical impedance analysis (BIA) measures and Fulton's K. R_{par}=resistance in parallel; Xc_{par}=reactance in parallel; D=distance between BIA detector electrodes; R_{par} and Xc_{par} *conductor volume*=D^2/R_{par} and D^2/Xc_{par}, respectively; *phase angle* (°)=arctangent of Xc/R converted to degrees; *capacitance* is a measure of the electrical storage capacity of cells; *impedance* is a measure of the opposition to the flow of electrical current; Fulton's K=(100•wet weight/fork length³). Boldface type highlights highest correlation between body composition or growth rate, and wet weight, BIA measures, and Fulton's K. * $P\leq0.050$, ** $P<0.005$, *** $P<0.0001$. n=number of fish sampled.

A

Variable	WW	CP	TF	TWa	
n	69	66	61	68	
Wet weight (*WW*)	1	**0.99*****	**0.84*****	**0.99*****	
Carcass protein content (*CP*)	0.99***	1	0.84***	0.98***	
Total fat content (*TF*)	0.84***	0.74***	1	0.78***	
Total water content (*TWa*)	0.99***	0.98***	0.78***	1	
R_{par} conductor volume	0.92***	0.91***	0.71***	0.93***	
Xc_{par} conductor volume	0.94***	0.93***	0.74***	0.95***	
Phase angle	0.62***	0.60***	0.53***	0.62***	
Capacitance	0.56***	0.54***	0.54***	0.54***	
Impedance	−0.58***	−0.57***	−0.54***	−0.57***	
Fulton's K	0.28*	0.31*	0.46**	0.24*	

B

Variable	WW	CP%	TF%	TWa%	Growth rate
n	69	66	61	68	56
Wet weight (*WW*)	1	**0.66*****	**0.48*****	**−0.57*****	**0.53*****
Carcass protein concentration (*CP%*)	0.66***	1	0.32*	−0.44**	0.38*
Total fat concentration (*TF%*)	0.48***	0.32*	1	−0.93***	0.09
Total water concentration (*TWa%*)	−0.57***	−0.44**	−0.93***	1	−0.23
R_{par}/D	0.92***	0.58***	0.34*	−0.44**	0.50***
Xc_{par}/D	0.94***	0.60***	0.38**	−0.48***	0.51***
Phase angle	0.62***	0.44**	0.37**	−0.46**	0.37*
Capacitance	0.56***	0.39**	0.41**	−0.51***	0.22
Impedance	−0.58***	−0.45**	−0.41**	0.53***	−0.22
Fulton's K	0.28*	0.31*	0.47**	**−0.57*****	0.32**

within a single day's sample, the values were not significantly different from day 0 (Fig. 1B). Contrary to our findings, Wilkinson et al. (2006) observed significantly lower mean K in 14-month-old Atlantic salmon smolts after 15 days of fasting (when compared to continually fed fish values), and significantly higher mean K 7 days after being refed (when compared to continually fasted fish values). By the end of our experiment (16 days after refeeding), K values for refed vs. fasted fish were not significantly different. Differences in metabolic rate or initial fat content may explain the dissimilarity in response time of K values to fasting and refeeding observed between these studies.

A significant correlation between K and total fat ($r=0.74$) was observed during an 18-day study of food-deprived juvenile steelhead trout (*Oncorhynchus mykiss*) (total fat range 0.4 to 3.6g) (Hanson et al., 2010). K and total fat were less correlated in our study ($r=0.46$) which encompassed a larger range of total fat values

(1.8–12.7g). In our postsmolt Atlantic salmon, K values were not highly correlated with body composition (g or %WW) or growth rate (all $r<|0.6|$)

Estimates of conductor volume

Equations based on conductor volume have been developed to predict water-, fat- and protein-content of five species of fish (Cox and Hartman, 2005; Pothoven et al., 2008; Hanson et al., 2010). For simple cylindrical shapes, when R is measured at a constant temperature and frequency, a conductor volume (V) calculated with Ohm's law ($V=\rho L^2/R$) should approximate a physical volume (Lukaski et al., 1985). Both Cox and Hartman (2005) (brook trout) and Hanson et al. (2010) (juvenile steelhead) observed strong linear relations between conductor volume ($r^2\geq0.95$) and total water and protein content in their fish. The relation of total fat to conductor volume was equally high in the brook trout ($r^2=0.96$; Cox and

Hartman, 2005) but was not significant in the steelhead (r^2=0.02; Hanson et al., 2010). Pothoven et al. (2008) observed significant relations between conductor volume and total fat or dry mass in yellow perch (*Perca flavescens*), walleye (*Sander vitreus*), and lake whitefish (*Coregonus clupeaformis*), with r^2 values ranging from 0.62 to 0.93. In our study, we also found conductor volume to be highly correlated with carcass protein content and total water (r=0.93 and 0.95, respectively) but more weakly correlated with total fat (r=0.74). Pothoven et al. (2008) suggested that significant correlation between published conductor volumes and body composition (g) is most likely due to biases imposed by the distance between the electrodes. The consistent placement of BIA electrodes on each fish results in the numerator of the conductor volume equation becoming a proxy for the size of the fish, and fish size is highly correlated with body contents (i.e., the larger the animal, the greater its total fat, water, protein, and ash content). In both our study (protein, fat, water) and Pothoven et al. (2008; yellow perch, fat and dry weight; walleye, fat), wet weight had a stronger relation to body composition (g) than did conductor volume. Neither Cox and Hartman (2005) nor Hanson et al. (2010) reported correlation results between wet weight and body composition (g). It would be valuable to know whether wet weight could estimate total fat, protein, and water content equally well or better than BIA conductor volume in their studies as well.

Prediction models

In our study, the most parsimonious models for predicting body composition (g) all contained wet weight and fork length, and frequently a weight-length interaction term (Fulton's *K*). Adding any combination of the nine BIA measures to size-based-only models increased the explanatory capabilities by less than 2%. Bosworth and Wolters (2001) estimated carcass fat content in channel catfish (*Ictalurus punctatus*) using wet weight, *R*, and *Xc* as the predictor variables, resulting in an r^2=0.75. They determined that adding the BIA measures to a model containing wet weight only increased the predictive capability by 71%; however, *R* and *Xc* in their model had not been corrected for the distance between the electrodes. Because these impedance measurements are highly dependent upon the distance the current must travel between the electrodes, they must be standardized to distance when used in prediction equations (RJL Systems, http://www.rjlsystems.com/docs/bia_info/principles/, accessed April 2008) or they simply become proxies for length. Therefore the Bosworth and Wolters (2001) carcass fat content model essentially contains size-related only variables—and size is highly correlated with content values.

When body constituents are expressed as concentrations (e.g., percent wet weight), the values are less size-dependent. Pothoven et al. (2008) examined the capability of 4 models (3 of which contained BIA measures) to predict percent total fat in 3 fish species. Interestingly, all of the variables used in their models were size re-

lated (body mass, total length, conductor volume, R_{par} and Xc_{par} uncorrected for distance between electrodes). In their study, fat concentration within a species encompassed a range of values (yellow perch: 2.7–8.7%; walleye: 6.0–18.2%; lake whitefish: 2.4–14.7%), but overall predictive capability of the most parsimonious model for each species was low (r^2 range: 0.17–0.53).

In our study, all of the body composition (%WW) models also had low predictive capabilities, with less than 50% of the variability explained by any of the top 3 models. Our most parsimonious models for *TF%* and *TWa%* contained only size variables (Table 4), whereas the *CP%* and growth rate models contained both size and either 2 or 3 BIA measures (Table 4). In the *CP%* models there was little added value of BIA measures to models containing only size variables (explanatory capabilities increased by <3.4%), and in the growth-rate models, adding two BIA measures did increase the r^2 of size-based-only models by ~20%. However, the overall predictive capability of the growth-rate models was still less than 50%.

In a recent study by Hartman et al. (2011), highly predictive models for estimating percent dry weight in coastal bluefish (*Pomatomus saltatrix*) were constructed by using BIA measures. The most highly predictive models for their fish (15°C, r^2=0.86; 27°C, r^2=0.91) contained either phase angle or capacitance, plus *R/D* and *Xc/D*. Ideally, standardizing *R* and *Xc* by the distance between the electrodes (*D*) would eliminate the effect of size on the impedance values; however, in our study we determined that even after standardization, R_{par}/D and Xc_{par}/D were still highly correlated with size (wet weight, r>0.91, Table 5B). Correlations between size (wet weight) and candidate BIA predictor variables were not reported in the coastal bluefish study, and size (wet weight or length) was not tested as a predictor variable in any of the models.

In our study, size continually emerged as a significant variable in all of the body composition models. To our knowledge, no experiment has been conducted which controls the effect of size well enough to determine the actual contribution of BIA measures to the estimation of body composition independent of size. Until this is better understood, it will remain unclear to what extent BIA measures can improve upon size-only estimates of body composition.

Phase angle

The use of BIA-estimated phase angle as a measure of fish condition has been proposed by Cox and Heintz (2009). They concluded that in the 5 species they studied, fish with phase angles >15° were in better condition than those with phase angles <15°. Caution should be used before universally applying this 15°cut-off value. In their own study, Cox and Heintz (2009) observed 12° phase angles in field-caught Pacific herring (*Clupea pallasii*) with mass-specific energy contents of 7.15 kJ/g, and 15° phase angles in Pacific herring caught four months later with energy contents of 5.02 kJ/g. They also

reported phase angles >15° in large juvenile rainbow trout which had experienced a 9% loss of body weight after four weeks of food deprivation (Cox and Heintz, 2009). In our study, we found a low correlation ($r=0.37$) between growth rate and phase angle. On the basis of our results and those of Cox and Heintz (2009), we feel it is unlikely that a single phase angle value can correctly distinguish good from poor condition fish in all applications, and that the cut-off value will be influenced by factors such as life-stage, age, reproductive status, species, and temperature.

In the Cox and Heintz (2009) study, the time frame necessary to clearly differentiate fasted from fed laboratory fish by phase angle varied by species and size. Significant differences between feeding treatments were observed in small juvenile rainbow trout after 14 days of fasting, in large juvenile rainbow trout after 21 days, in juvenile brook trout after 28 days, and in juvenile Chinook salmon after >77 days. This large range of response times may reflect differences in fat reserves in the test animals. If fat reserves do affect response time, then more than just a decline in fat content would be necessary to elicit a decrease in phase angle. A decrease in protein content (with its concomitant water loss) may also be necessary for a significant decline in phase angle. If this is true, then the response time of K to food deprivation may be similar to (or possibly better than) that of phase angle. On the final sampling day of our study, K values, not phase angle, were significantly different between the fasted and fed fish. It would be interesting to know what the response time of K values were in the Cox and Heintz (2009) food-deprivation study.

Conclusions

The use of BIA as a proximate body composition estimator or fish condition index is relatively new. As with any condition index, its validity must be established for the specific application in which it will be used. Field personnel are seeking a nonlethal index that can reflect the condition of Atlantic salmon postsmolts 2 to 3 weeks after the fish are released from the hatchery, when the majority of postsmolts are captured on targeted trawl surveys. We designed an experiment to evaluate the utility of BIA and Fulton's condition factor as indices of condition during that time-frame. Results of our study indicated that 1) growth rates of postsmolts responded rapidly to the withholding and re-introduction of food; 2) fed postsmolts grew isometrically; and 3) 3 weeks of withholding food is not sufficient time to elicit significant declines in proximate body constituents in fasting postsmolts. This combination of isometric growth in fed fish and short-term starvation period in fasting fish resulted in nonsignificant differences in body composition (%WW) among the feeding treatments (fed; fasted; fasted, then refed). The utility of BIA and Fulton's K as condition indices depends upon detecting differences in proportions of body constituents. During our study, BIA measures were not significantly different among the 3 feeding treatments, and only on the final day of sampling was K in fasted fish significantly less than in fed fish. Our study has demonstrated that neither BIA nor Fulton's K would be an appropriate choice of index 1) to reflect short-term changes (weeks rather than months) in postsmolt condition, or 2) to monitor fish condition during a life-stage where excess energy is primarily directed toward isometric growth rather than energy storage. We propose that a methodology that measures growth rate (directly or indirectly) would be a more suitable condition index for isometric growth life-stages. We will be reporting on the utility of two potential growth-rate indices in Atlantic salmon postsmolts in a later publication.

Results from our study also supported conclusions by Pothoven et al. (2008) that simple measures of size were better predictors of body composition than BIA measures. Additionally, we observed that Fulton's K responded more quickly to food deprivation than BIA measures, and that a single cut-off value for phase angle, as a distinction between good and poor condition fish, should be used with caution. BIA is an emerging technique in fishery biology, and as such, its application will require more research to identify its appropriate use.

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