Abstract—In this study, phase angle (the ratio of resistance and reactance of tissue to applied electrical current) is presented as a possible new method to measure fish condition. Condition indices for fish have historically been based on simple weight-at-length relationships, or on costly and time-consuming laboratory procedures that measure specific physiological parameters. Phase angle is introduced to combine the simplicity of a quick field-based measurement with the specificity of laboratory analysis by directly measuring extra- and intracellular water distribution within an organism, which is indicative of its condition. Phase angle, which can be measured in the field or laboratory in the time it takes to measure length and weight, was measured in six species of fish at different states (e.g., fed vs. fasted, and postmortem) and under different environmental treatments (wild vs. hatchery, winter vs. spring). Phase angle reflected different states of condition. Phase angles <15° indicated fish in poor condition, and phase angles >15° indicated fish that were in better condition. Phase angle was slightly affected by temperatures (slope=−0.19) in the 0–8°C range and did not change in fish placed on ice for <12 hours. Phase angle also decreased over time in postmortem fish because of cell membrane degradation and subsequent water movement from intra- to extracellular (interstitial) spaces. Phase angle also reflected condition of specific anatomical locations within the fish.

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Electrical phase angle as a new method to measure fish condition

M. Keith Cox (contact author)
Ron Heintz

Email address for contact author: Keith.Cox@noaa.gov

NOAA-National Marine Fisheries Service
Alaska Fisheries Science Center – Auke Bay Laboratories
11305 Glacier Hwy
Juneau, Alaska 99801

For nearly a century, fisheries biologists have struggled to develop a way to simply and accurately assess body composition and condition of fish (Adams et al., 1993; Shearer et al., 1994). Attempts at assessing body composition or creating a condition index have focused on simple relationships between length and weight of fish (Le Cren, 1951; Anderson and Neumann, 1996). These early methods were later replaced by formulations of length and weight information such as relative weight (*Wr*) by Wege and Anderson (1978) and Fulton's condition factor by Murphy et al. (1990), which are easily obtained, but lack sensitivity specific to an individual's body composition. In contrast, more difficult and technical approaches involving necropsy, histology, or pathology (NHP) can provide more detail, but these approaches can not conducted easily in the field (Strange, 1996). The more technical approaches of NHP can provide detailed information about individual fish, but the cost and technical expertise required to conduct them has restricted field biologists' ability to effectively apply these methods on broad scales.

A bridge is needed between simple, cost-effective, and robust length-weight regressions and complex, expensive, yet sensitive laboratory methods. Recently, common ground has been found by Cox and Hartman (2005) in body composition estimates with the use of bioelectrical impedance analysis (BIA). This method still depends on chemical analysis of subsets of fish in order to develop calibration curves that relate resistance (R) and reactance (Xc) to body composition, but analytical costs are reduced because after a curve is created, there is no longer a need for body composition analysis. Use of BIA relies on correlations between the electrical conductivity of fish tissues and body composition. Thus, BIA is an indirect measure of total body water (*TBW*), dry weight (*DW*), fat-free mass (*FFM*), total body protein (*TBP*), total body ash (*TBA*), total body fat (*TBF*), or mass-specific energy density (*ED*). Another BIA method currently used in human health studies involves the phase angle (ratio of Xc and R) as a direct measure of nutritional condition (Barbosa-Silva and Barros, 2005). In these studies, phase angle indicates cell membrane potential and water distribution between the intra- and extracellular spaces and is used widely in human medicine as a means to measure nutritional status, but it has never been applied to fish or lower vertebrates.

Phase angle represents the relationship between the two vector components R and Xc that represent impedance. Specifically, phase angle is defined as

$$\textit{Phase angle} = \arctan(X_c/R)180°/\Pi, \qquad (1)$$

where R and Xc are measured in ohms.

Phase angle ranges between 0° and 90°; 0° if the circuit is only resistive (as in a system with no or degraded cell membranes), and 90º if the circuit is only capacitive (all membranes have no extracellular fluid). In either fish or human health studies, 45° phase

angles reflect circuits that consist of equal amounts of capacitive reactance and resistance (Meeuwsen et al., 2001). Lower phase angles appear to be consistent with low reactance and either cell death or a breakdown in the selective permeability of the cell membrane (Schwenk et al., 2000a, 2000b). Higher phase angles are consistent with high reactance and large quantities of intact cell membranes and body cell mass (Abu Khaled et al., 1988; Foster and Lukaski, 1996). The use of phase angle as a human health indicator is becoming more common in medical fields (Dejmek and Miyawaki, 2002; Damez et al., 2007). Even when other anthropometric evaluation methods such as body mass index (BMI) and skin-fold tests are not accurate or sensitive to tissue change, phase angle has been found to be accurate. Furthermore, phase angle is considered to be a possible global marker of health and evaluation of cell membrane function, and consequently serves as a prognostic tool for human disease (Barbosa-Silva and Barros, 2005).

We demonstrate that phase angle can be used to measure the nutritional condition of fish under laboratory and field conditions. Our objective was to determine whether fish known to have decreasing nutritional status have lower phase angles than those fish with high nutritional status. We made these measurements on six species of fish: brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*O. tshawytscha*), chum salmon (*O. keta*), pink salmon (*O. gorbuscha*), and Pacific herring (*Clupea pallasii*) under laboratory conditions and on fish collected from the field. In addition, we examined postmortem changes in phase angle in fish to illustrate how phase angle reflects membrane integrity and water balance.

Materials and methods

We tested the hypothesis that phase angle changes with the nutritional status of fish by conducting three laboratory experiments and three field studies using live or freshly killed fish. Throughout this study, we assume that malnourished, over-wintering, and migrating anadromous fish are in diminished nutritional condition compared to their nourished, prewinter, and premigratory counterparts. This assumption has been thoroughly evaluated in the literature where condition declines have been found to be synonymous with depletion of stored fat, protein, and carbohydrates (Adams et al., 1982; Weatherup and McCracken, 1999). We also monitored phase angle in postmortem adult salmon to measure how phase angle changes in response to cell degradation and water movements from intra- to extracellular spaces. In all cases, phase angles were calculated from impedance measures (R and Xc) of fish that were sampled according to the methods in Cox and Hartman (2005). In this study, two sets of needle electrodes (stainless 28 gauge, Grass Telefactor, West Warwick, R.I.) each consisting of a signal and detecting electrode were inserted to a depth of 1 cm. One set was placed towards the caudle peduncle and the second set was placed in the nape region of the fish. Variables that could introduce error in R and Xc measures (e.g. varying the depth and gauge of needles, placement of fish on different conductive surfaces) were standardized to negate any bias, whereas temperature and time effects are explained experimentally.

Temperature and time

To test for significant effects of temperature on impedance measurements from dead fish, regression analysis was used to test whether slopes and intercepts differed from zero on regressions of temperature and response measures (phase angles). Three adult pink salmon (520–550 mm fork length) were killed and connected to a BIA Quantum-II Desktop System (RJL Systems, Point Heron, MI) using standard needle electrodes and orientations as described by Cox and Hartman (2005). The Quantum-II was set to record impedance every five minutes for 12 hours. An ibutton thermometer (Maxim Integrated Products Inc., Sunnyvale, CA) was placed 3 cm inside the dorsal musculature of the fish and was set to record temperatures every 5 minutes. Both impedance measurements and thermometers were synchronized before the experiment. Each fish was brought directly from the water, killed, and placed on a 4-inch wire stand (to allow air flow around the fish) in the empty freezer compartment of a standard freezer. Initial fish temperatures were equal to ambient water temperature of 8°C. After 12 hours, the fish was removed from the freezer and impedance measures and temperature data were downloaded onto a computer. For regression analysis, only impedance measures taken when the fish temperature was between 0°C and 8°C were used. Impedance measures of R and Xc were used to calculate phase angle measures. Significance tests to test for nonzero slopes were done on each fish by using a standardized major axis (SMA) test and between fish by using the Bartlett-corrected likelihood ratio (LR) test for differences in the slopes. An analysis of covariance was used to test effects of temperature and individual fish on phase angle.

We examined the effect of time after death on response measures (phase angles) to determine time effects on phase angle. Juvenile coho salmon (n=30, mean=10 g, standard deviation [SD]=2.72) from the Sheldon Jackson Hatchery, Sitka, AK, were killed and groups of six fish were randomly placed in plastic bags and placed on ice. At 0-, 3-, 6-, 9-, and 12-hour intervals, a bag was removed and six fish were measured for length, weight, and impedance. Impedance measures followed standardized procedures found in Cox and Hartman (2005). Impedance measures were then used to calculate phase angle. Linear-effects mixed models were used to test for effects of time on phase angles.

Laboratory study 1: fasted and fed brook trout

To determine the effects of malnutrition on phase angle in brook trout in fresh water, phase angle was repeatedly measured in fed and fasted juvenile brook trout over a period of nine weeks (December 2002–March 2003).

We obtained brook trout (n=16, mean weight=15.29 g, SD=1.21) from the Bowden State Fish Hatchery, Bowden, WV, and transferred them to West Virginia University. Before transfer, fish were fed standard hatchery trout pellets *ad libitum* approximately three times per day. After transfer, fish were randomly selected to be in one of two groups (n=8): fasted or fed. Each fish was housed in individual 33L aquaria at 14–16°C. Fish in the fed group were offered *ad libitum* rations of fly larvae (*Sarcophaga bullata*) (Grubco Inc., Hamilton, OH). Each week for nine weeks, all fish were anesthetized with a solution of 1 g tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Inc., Redmond, WA) to 9 L water, and measured for length, weight, and impedance (i.e., eight replicates for each of two groups every week for nine weeks) according to the methods in Cox and Hartman (2005).

Laboratory study 2: fasted and fed rainbow trout

The effect of malnutrition on phase angle measures was measured in rainbow trout in fresh water by repeatedly measuring the phase angle in fed and fasted juvenile rainbow trout over a period of four weeks starting in December 2004. Rainbow trout used in this study were raised and housed at the U.S. Department of Agriculture, Agricultural Research Service, National Center for Cool and Cold Water Aquaculture (USAD/ARC NCCWA) in Leetown, WV. Two weeks prior to the experiment, 11 small (mean weight=68.76 g, SD=6.02) and 12 large (mean weight=193.03 g, SD=15.72) fish were individually placed in 2.75-L and 9.0-L aquaria and fed standard hatchery pellets *ad libitum*. Two days before the start of the experiment, all fish were fed standard hatchery pellets at a rate of 2% body weight per day. At the start of the experiment each size class of fish was randomly split into two groups: fasted (n=11) or fed (n=12). Fed fish were offered hatchery pellets at a rate of 2% body weight per day and all holding tanks were kept at 15ºC for the duration of the experiment (four weeks). Every week for four weeks, each fish was anaesthetized with MS-222 (1g/9L water) and measured for impedance.

Laboratory study 3: fasted and fed Chinook salmon

We tested the effect of malnutrition on phase angle measures in Chinook salmon in salt water. Impedance was repeatedly measured in fed and fasted juvenile Chinook salmon over a period of 13 weeks (mean weight=10.25 g, SD=2.23). In November 2007, fish reared at the National Marine Fisheries Service (NMFS) research hatchery at Little Port Walter, AK, were transferred to the Auke Bay Laboratories, Juneau, AK. Fish were acclimated for approximately one month during which time salinity was increased from 0 to 32 and were then randomly assigned to fasted (n=7) or fed (n=12) groups. Pairs of individually marked fish (fin clipped) were placed into 8-L aquaria and held at 4°C. Bioelectrical impedance was measured in individual fish on weeks 1, 8, 11, and 13, and different numbers of fasted and fed fish were measured each week. The number of fed and fasted fish measured in each sampling week were as follows: week 1 (n=12 and 7); week 8 (n=4 and 7); week 11 (n=8 and 5); and week 13 (n=5 and 5).

Differences in phase angle in the laboratory studies were analyzed by a repeated measures analysis of variance (ANOVA). The analysis was used to test for 1) significance differences in phase angle means between fed and fasted groups, 2) a time effect on group means, and 3) interactions between time and group means. A linear mixed-effects (LME) model for repeated measures was used to compare phase angles from the start of the experiment with those measured after the start.

Field study 1: wild and hatchery brook trout

To determine if phase angles differed between hatchery and wild fish, phase angles were compared between wild fish foraging under natural conditions and captive fish fed *ad libitum*. In July 2002, phase angle was measured in 56 brook trout, 34 from headwater streams (mean weight=21.21 g, SD=12.85) and in 11 brook trout from Bowden State Fish Hatchery, Bowden, WV (mean weight=43.17 g, SD=4.76). Before they were measured, hatchery-reared trout were fed standard trout pellets *ad libitum* three times each day according to standard hatchery procedures. Wild fish were captured by electrofishing from unidentified streams located within the Middle Fork watershed in Randolph County, WV. After capture, impedance was immediately measured in both groups. A two-sample t-test was used to compare calculated phase angles between hatchery and wild fish. Internal fish temperature was not measured, but water temperatures measured in both areas were similar (mean=13.36°C, SD=0.94).

Field study 2: phase angle in migrating chum salmon

The effect of migration on phase angle was compared in migrating adult chum salmon. On 4 August 2004, a total of 47 chum salmon (mean=3438.29 g, SD=511.41) were collected by gillnet from a downstream site in the Yukon River near Emmonak, AK. During 27–29 September 2004, 40 chum salmon (mean weight=2927.50 g, SD=470.13) from an upstream site in the Yukon River near Delta, AK (approximately 3200 km upstream from Emmonak), were collected by gillnet. Impedance measures on both the dorsal and ventral (gonadal) areas of each fish were measured within one hour of capture. Internal temperatures were taken by a digital thermometer on both groups of fish in the anal vent (downstream mean temperature=16.44°C, SD=1.22; and upstream mean temperature=3.00°C, SD=0.48). A two-sample t-test was used to compare phase angles of upstream and downstream salmon.

Field study 3: overwintering Pacific herring

We monitored phase angle in Pacific herring during winter to determine if decreases in fish condition due

to season could be detected. During the winter of 2006, Pacific herring were sampled in January (n=68, mean weight=111.26 g, SD=41.00), February (n=70, mean weight=8.98 g, SD=1.86), March (n=68, mean weight=35.92 g, SD=56.37), and April (n=23, mean weight=166.14 g, SD=36.30) from Sitka Sound, AK. Fish were collected by cast net. Impedance was measured within 12 hours of capture and fish were held on ice or snow between capture and measuring. After measuring, a subset of the fish (January n=20, March n=14, April n=18) was sent to the Auke Bay Laboratory, Juneau, AK, for bomb calorimetry. A linear mixed-effects (LME) model was used to test for differences in phase angles between months.

Phase angle and water balance in postmortem fish

Adult pink salmon were measured to characterize changes in phase angle in postmortem fish to better understand how changes in cell integrity influence phase angle. Three adult pink salmon (520 mm–550 mm) were individually killed and connected to the Quantum-II and placed in a standard ice chest without ice and held at <11°C. The Quantum-II was set to record impedance every five minutes for five days. A temperature data logger placed inside the ice chest recorded temperature every five minutes. Each fish was removed from the ice chest 4–5 days later and impedance measures and temperature data were downloaded for analysis. Changes in phase angle of postmortem fish over time were analyzed by regression analysis. Significance tests were done on each fish to test for nonzero slopes by using a standardized major axis (SMA) test and by reporting the Bartlett-corrected likelihood ratio test (LR) for differences in the slopes between the different fish.

Results

Temperature and time

Phase angles measured on dead fish depended on temperature and decreased as temperatures increased (Fig. 1A). Slopes of phase angles (–0.19) were not different among fish (LR=5.87, P=0.05). There was an interaction between fish and phase angle ($F_{2,\ 46}$= 20.92, P=0.01). Time did not affect phase angles in fish that were placed on ice for up to 12 hours. Phase angles measured in groups of dead fish placed on ice were not significantly different between times of less than 12 hours, (LME $t_{30,\ 30}$<2, P>0.08) (Fig. 1B). At 12 hours, there was a significant effect of time on phase angle (LME $t_{30,\ 30}$>5, P<0.05).

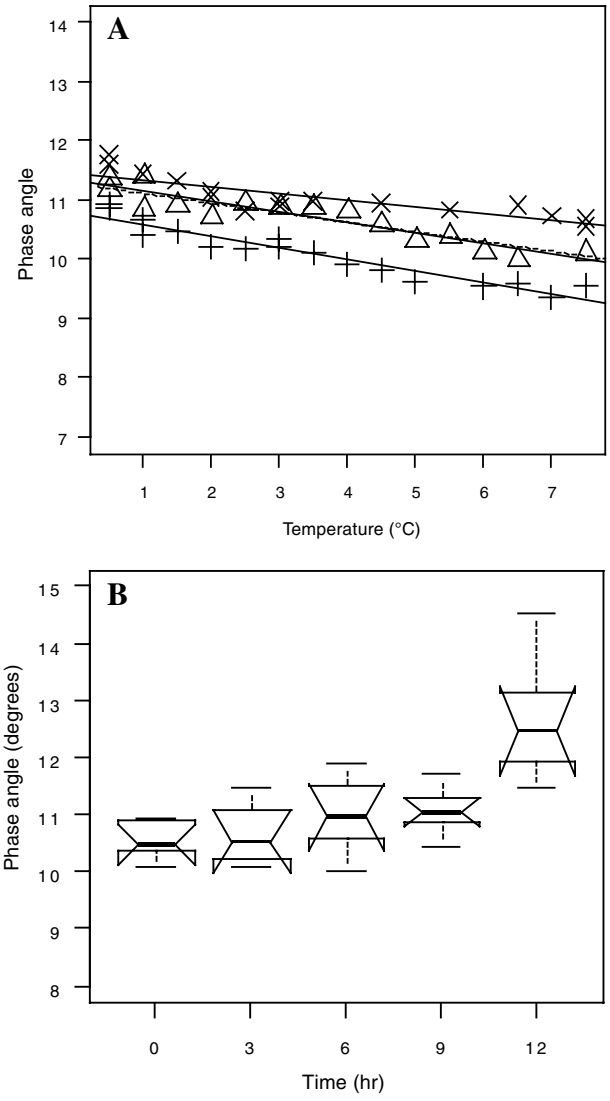

Figure 1

(**A**) Repeated measures of phase angles for three adult pink salmon (*Oncorhynchus gorbuscha*) that were brought from 8°C to freezing. Time required for freezing was <1 hour. Each of the three symbols and their corresponding line represents one of the three fish. (**B**) Phase angles of group means for juvenile coho salmon (n=6 per group) that were killed and placed on ice. Every 3 hours, a group was measured for impedance, and the phase angle means per group were calculated. Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals, horns indicate that the interval is larger than the interquartile range (as seen in the bottom half of each of the boxplots).

Laboratory studies 1–3

In each of the laboratory studies, phase angle decreased in fasted fish and not in fed fish. In the brook trout experiment there was a significant interaction in phase angle between feeding group and time (ANOVA, P<0.05, df=8). The interaction resulted in a temporal change in phase angle among the fasted fish—a change that was not observed among fed fish. Phase angles in the fasted brook trout in weeks 3–9 were significantly lower than

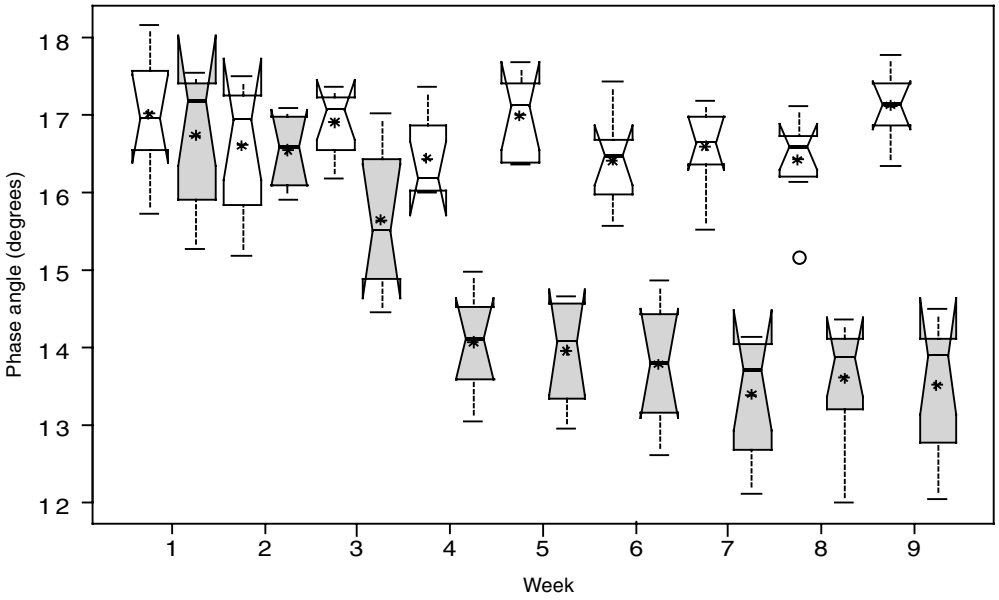

Figure 2

Notched boxplots and means (*) of phase angles measured weekly for nine weeks from brook trout (*Salvelinus fontinalis*) that were fed ad libitum (white boxes, n=8) or fasted (gray boxes, n=8). Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals; horns indicate that the interval is larger than the interquartile range. Open circle (○) represents outlier determined by a Grubbs test.

those in week 1 (LME, P<0.001, df=63). In contrast, there were no differences in phase angle between weeks 2 and 9 and week 1 in the fed fish (LME, P>0.05, df=63) (Fig. 2). The fed group increased in size (mean=15.5 g to 68.5 g) over the same time period, whereas the fasted group lost mass (mean=15.0 g to 11.9 g) (Cox, 2004).

In rainbow trout, ANOVA results were similar for both small and larger fish and interactions of phase angle between feeding group and time were significant (ANOVA, P<0.05, df=3) (Fig. 3, A and B). In the fasted group for both small and large fish, phase angle was significantly lower in weeks 3 and 4 than in week 1 (LME, P<0.03, df=40). At the start of the experiment one fish from the fed group died; however, no changes in phase angle were detected between weeks 2–4 and week 1 in the fed group (LME, P>0.09, df=43). Small fish in the fed group grew in size from a mean of 66.4 g to 98.7 g, and large fed fish grew from an average of 197.2 g to 297.7 g. Likewise, small fish in the fasted group lost weight from a mean of 87.8 g to 80.5 g, and larger fasted fish decreased in weight from an average of 188.7 g to 172.0 g.

Results of the Chinook salmon experiment were similar to those of the brook and rainbow trout experiments, but phase angles in the saltwater fish were generally lower than those of the freshwater fish. The interaction between feeding group and time was significant (ANOVA, P<0.001, df=3) as phase angle decreased with time in the fasted group (LME, P<0.009, df=20), whereas it increased in the fed group (LME, P<0.04, df=20) (Fig. 4). Phase angle changes were consistent with changes in wet mass. The fasted fish lost an average 0.2% of their wet mass per day. In contrast, the fed group gained an average 0.2% of their wet mass per day. Phase angles were lower in Chinook salmon than in rainbow or brook trout in both fed and fasted groups. Phase angles for Chinook salmon and rainbow, and brook trout in the fed groups averaged 15.6°, 16.2°, and 16.5°, respectively, and 13.4°, 15.0°, and 14.5°, respectively, for the fasted groups.

Field studies 1–3

Phase angle reflected changes in the presumed nutritional status of wild fish in each of the field studies. In comparisons between hatchery and wild trout, mean phase angle was significantly higher in the hatchery trout (two-sample t, P=0.04, t=2.03, df=53). Mean values for hatchery and wild brook trout were 19.43° and 18.27°, respectively (Fig. 5). Similarly, comparison of adult chum salmon sampled in the Yukon River indicated that upstream fish had significantly lower phase angles (two-sample t, P<0.001, t=16.5, df=72) from the dorsal measures, but not from the ventral measures (two-sample t, P=0.15, t=–1.4, df=65). Downstream and upstream phase angles averaged 23.98° and 17.53°, respectively,

for dorsal measures, and 10.30° and 11.15°, respectively, for ventral measures (Fig. 6). Phase angle also decreased during winter months in Pacific herring collected from Sitka Sound (LME, $P<0.003$, df=225). Phase angle decreased from 12° to 10° from January to March (Fig. 7), whereas mass-specific energy content declined from 7.15 kJ/g to 4.79 kJ/g. Phase angle increased to 15° in April, and mass specific energy increased to 5.02 kJ/g.

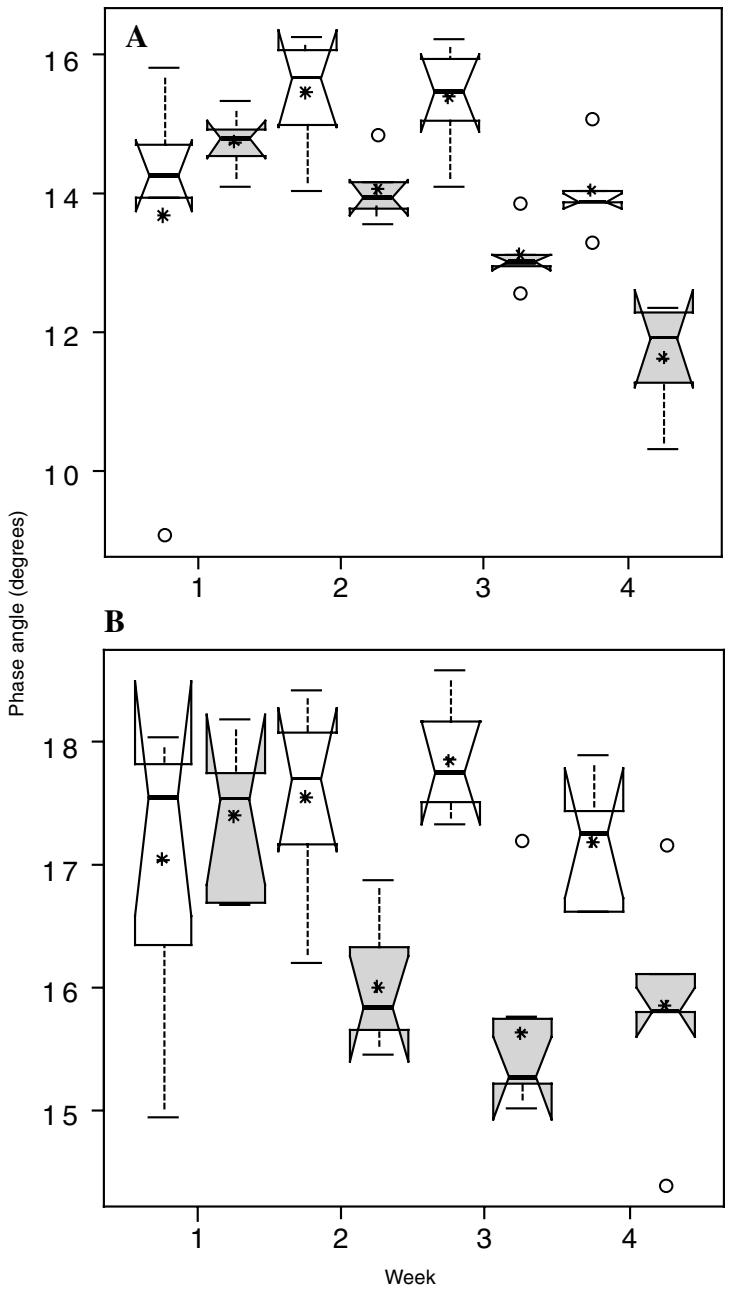

Figure 3

Notched boxplots and means (*) of phase angles measured weekly for 4 weeks from (**A**) small (<100 mm) and (**B**) large (100 mm) rainbow trout (*Oncorhynchus mykiss*) that were fed *ad libitum* (white boxes, n=12) or fasted (gray boxes, n=11). Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals. Open circles (○) represent outliers determined by a Grubbs test.

Phase angle and water balance in postmortem fish

Phase angles over time in postmortem fish reflected changes in cell integrity and subsequent water movement from intra- to extracellular spaces, reflected by the two components (R and Xc) which are used to calculate phase angle. In all three dead fish, slopes were initially positive reflecting an increase in Xc and a concomitant increase in R. Within 12 hours of death, this process reversed and slopes became negative as phase angle decreased (Fig. 8). There was not enough evidence to indicate that slopes were different between fish (LR [likelihood ratio]=110.7, $P<0.001$, slope=−4.2).

Discussion

In this study, in both laboratory and field settings, phase angle was used to compare the relative nutritional condition of salmonids and clupeids in fresh and saltwater while each was in a different condition. In each case where fish were expected to be in poor condition, phase angle was lower than in fish in good condition. The range of phase angle values was also greater in the larger rainbow trout than in the smaller rainbow trout. Phase angle reflects changes in condition by directly measuring the R and Xc of the body tissue, and more specifically, the ratio of these two values directly represents changes in intra- and extracellular water distributions (i.e., intracellular dehydration and extracellular hydration) (Schwenk et al., 2000b). Water distribution (namely, the movement from intra- to extracellular spaces) can be attributed to use of energy stores as indicated by the herring study in field study 3. In humans, phase angle can reflect loss of body protein during starvation as well as presence of infection, both of which decrease the condition of the organism (Plank et al., 1998; Schwenk et al., 2000a). Changes in phase angle in fish are therefore likely to reflect the general health of fish in addition to their nutritional status. Consequently, phase angle should be considered a reliable independent marker of fish condition.

Phase angle changes with nutrient levels in fish. When fish fast, nutrient inputs of

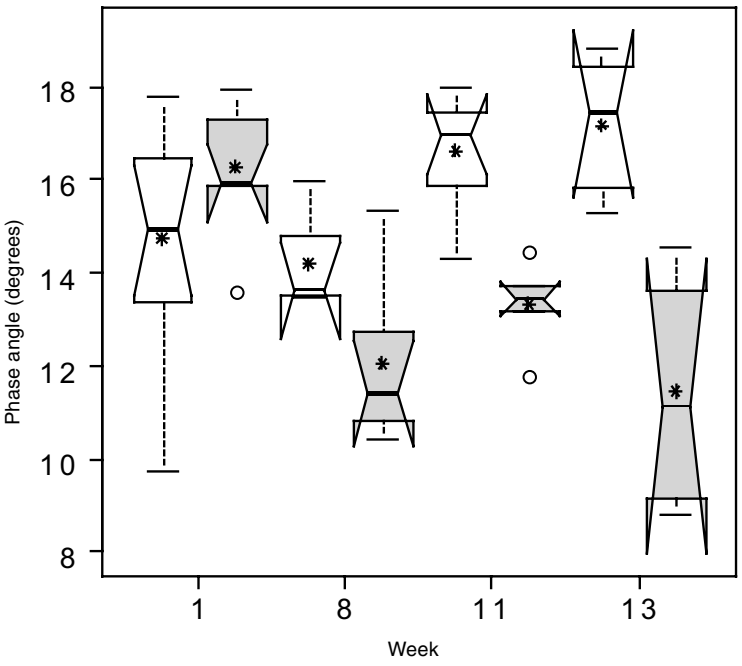

Figure 4

Notched boxplots and means (*) of phase angles measured during weeks 1, 8, 11, and 13 from Chinook salmon (*Oncorhynchus tshawytscha*) that were fed *ad libitum* (white boxes, n=12) or fasted (gray boxes, n=7). Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals. Open circles (○) represent outliers determined by a Grubbs test.

proteins, carbohydrates, and fats are lacking, which forces the use of stored nutrients to meet energetic demands (Moyle and Cech, 2004). Consistent with this use of stored nutrients are shifts in intra- and extracellular water, and findings by Finn et al. (1996) show that loss of body protein parallel a loss of intracellular water causing subsequent cell shrinkage and progressive cellular dehydration. As fish fasted, cells likely became more and more dehydrated and phase angles decreased. This has also been observed in humans with anorexia nervosa, where low phase angles reflect decreased nourishment (Mika et al., 2004). As organisms continue to starve, phase angles continue to decrease as stored cofactors such as vitamins, which are needed for metabolic conversions, are depleted and cause a further decline in condition. Typical symptoms of vitamin deficiencies include muscle and cellular atrophy, poor growth, and anemia, all of which would lower phase angles. As phase angle changes with the nutritional status of laboratory-fasted fish, it is apparent that phase angle can be used to determine if a fish has been subjected to a food-limited scenario.

When we compared hatchery fish to wild fish, we found that phase angle was lower in the wild fish, indicating that the condition of wild fish was lower. It can be assumed that food is not a limiting factor in hatchery fish, nor is there a need to forage. The opposite is true in wild fish, where food is usually limited and variable, and where there is almost always a need to forage. In the optimal foraging theory it is assumed that when food is limited, all energetic functions are not fulfilled and energy must be allocated to different different physiological parameters to maximize survival of the animal (Molles, 2005). This conclusion is supported by Berg and Bremset (1998) who found that there are seasonal changes in the body composition of juvenile salmonids that are due to changing energy allocations. In hatchery fish, foraging costs are reduced, risk of predation is minimized, and food is abundant. Furthermore, the weights and condition of wild foraging fish would naturally be more variable (as seen in these data) because both of these parameters are dictated by numerous variables. Any excess energy consumed by hatchery fish is allocated towards growth and storage and concurrently provides the fish with proper vitamins and minerals to maximize growth. Phase angle was lower in wild fish where they may have had to use energy for foraging and storage.

The lower phase angles observed in upstream adult salmon were due to their presumed diminished condition resulting from consumption of endogenous energy stores and to increased extracellular water volume. Like

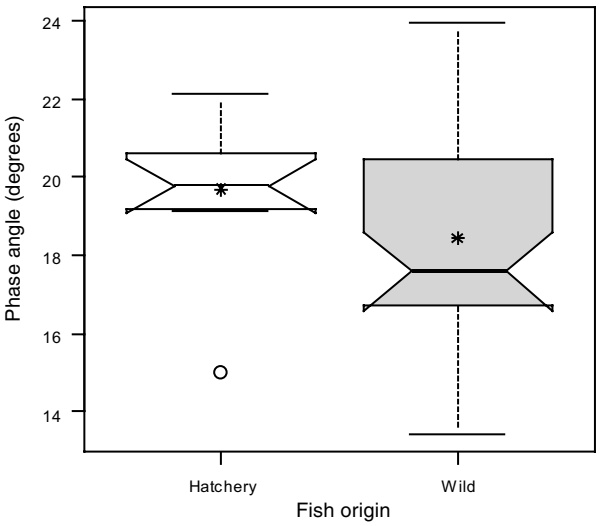

Figure 5

Notched boxplots of phase angles and means (*) from brook trout (*Salvelinus fontinalis*) from a hatchery (n=21) and from the wild (n=35). Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals. Open circle (○) represents outlier determined by a Grubbs test.

the fasted fish in the laboratory studies, anadromous salmon fast as they migrate upstream to spawn and must rely on stored fat and protein as energy sources during their journey. Jonsson et al. (1997) found that during upstream migrations, percent somatic and visceral lipid content can decrease from 12% to 2%, and 11% to 1%, respectively, and total stored energy losses can total 60%. Both cellular degradation and extracellular hydration would result in decreasing phase angles from the dorsal tissue.

Lower phase angles were observed in the ventral musculature, confirming that phase angle measures are location-specific. Phase angles calculated from ventral tissue revealed that ventral tissues do not degrade at the same rate as dorsal musculature. Conservation of the cell integrity in the musculature surrounding the gonads is consistent with observations that gonad quality is conserved during migration. Jonsson et al. (1997) found that although migrating salmon experience a marked decrease in energy content of somatic and visceral tissues during upstream migration and spawning, energy content in ventral gonadal tissue remained high throughout migration. It is noteworthy that phase angle was sensitive enough to indicate tissue-specific differences in condition and therefore could have potential use in quantifying reproductive readiness.

The sensitivity of phase angle to nutritional status was further indicated among Pacific herring between March and April. Whereas mass-specific energy content increased by approximately 5% between March and

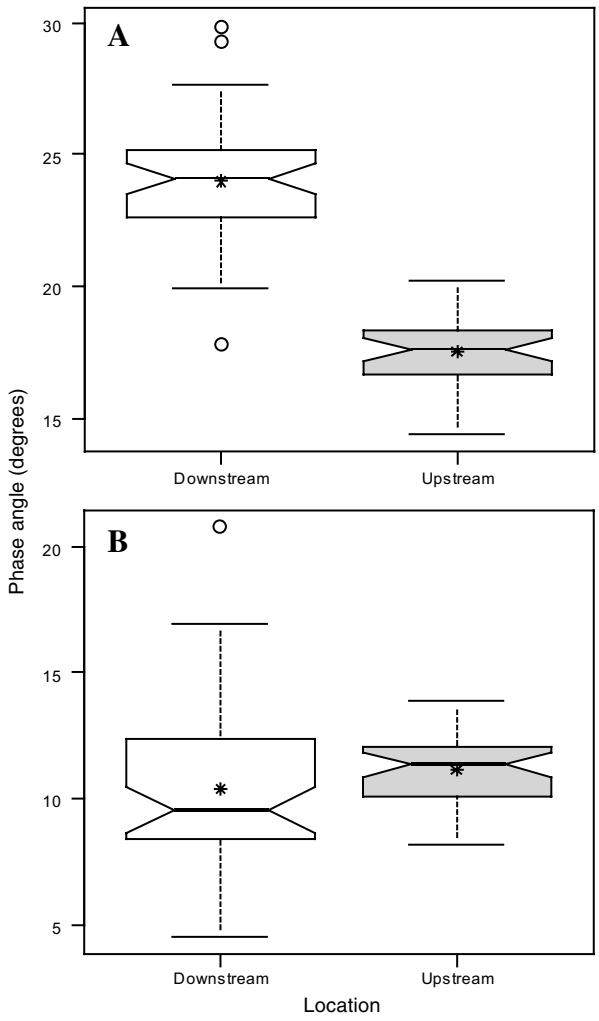

Figure 6

Notched boxplots of phase angles and means (*) calculated from bioelectrical impedance analysis measurements taken on the dorsal (**A**) and ventral (**B**) sides of chum salmon (*Oncorhynchus keta*) at both the mouth of the Yukon (downstream) (n=47) and 2000 km upstream (n=40). Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals. Open circles (○) represent outliers determined by a Grubbs test.

April, phase angle increased by 50%. Multiple studies on Pacific herring indicate reductions in energy and lipid content, and growth rates during over-wintering (Arrhenius and Hansson, 1996; Pangle et al., 2004). As energy stores become diminished from lack of food, physiological condition of the fish declines in a manner similar to the aforementioned "starving" fish. Vollenweider (2005) found that diminished mass-specific energy content in Pacific herring rebounded after spring algal blooms. This rebound occurs after spawning and likely reflects the reallocation of energy from gonad maturation to somatic growth and the replenishment of energy

stores. However, changes in mass-specific energy content can only be detected in tissues when there has been a sufficient change in the amount of lipid in relation to protein. In contrast, phase angle apparently changes much faster, reflecting local alterations in membrane potential and water balance.

Measuring changing phase angles in dead fish provides a useful model for illustrating BIA performance in live fish. Phase angle changes with time after death owing to rigor mortis, cellular breakdown, and water movement from intra- to extracellular spaces. Although timeframes for the onset and resolution of rigor mortis depends on species, condition of fish at time of death, environmental stress, and temperature (Martinsen et al., 2000; Damez et al., 2007), rigor mortis usually occurs within the first 18 hours of death, when muscles remain contracted until resolution. This period is reflected in our data as an increase in phase angle with time. Human studies involving muscle contractions and impedance indicate that muscle contractions result in an increase in R and Xc (Kashuri et al., 2007). Our study was consistent with Kashuri et al. (2007) in that Xc increased during rigor mortis (contraction). Their study indicated that increases in Xc were due to cell membrane and intracellular changes (possibly metabolites) and not to volumetric or morphological changes. In our study, decreases in impedance upon resolution (relaxation) were most likely due to the physical breakdown of cell membranes and subsequent autolysis and nucleotide catabolism. The breakdown of cell membranes and muscle hydrolysis causes the release of electrolytes and water into extracellular space, therefore decreasing the phase angle (area of decreasing slope in Fig. 8). In a study of postmortem changes in dielectric properties of haddock (*Melanogrammus aeglefinus*) muscle, Martinsen et al. (2000) found that the onset and resolution of rigor mortis affected R levels at multiple frequencies and they associated the increase with a response to edema. This makes the use of phase angle applicable to measuring changes during the postmortem period in fish after the resolution of rigor mortis, therefore allowing phase angle to determine time of death in fish where ambient temperature is known.

It is important to know that phase angles were only slightly affected by temperature and not affected by time as long as the fish were placed on ice and measured within 12 hours of capture. Slopes were −0.19, or approximately a 1° drop in phase angle, for every 5°C increase in temperature. There was no effect of time on phase angle on fish iced for less than 12 hours. At about 12 hours, juvenile coho salmon enter rigor mortis and phase angle measures begin to increase. These are important findings because fishery biologists using BIA can minimize error caused by temperature effects by icing fish or by trying to keep temperature fluctuations to a minimum before BIA measurements.

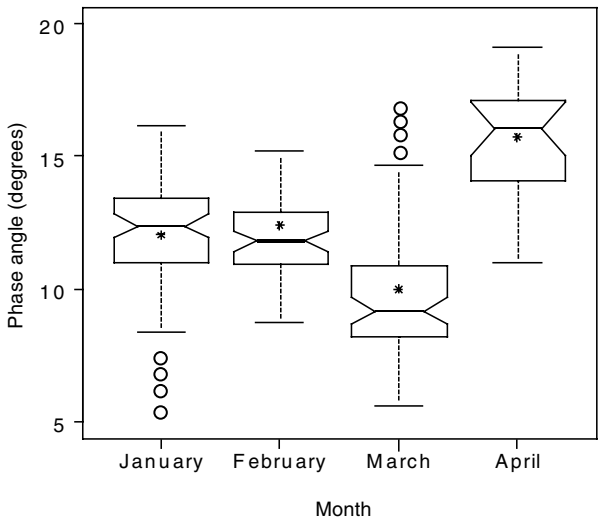

Figure 7

Notched boxplots of phase angle means (*) for Pacific herring (*Clupea pallasii*) (n=229) captured during January, February, March, and April of 2007 in Sitka Sound, Alaska. Open circles (○) represent outliers determined by a Grubbs test.

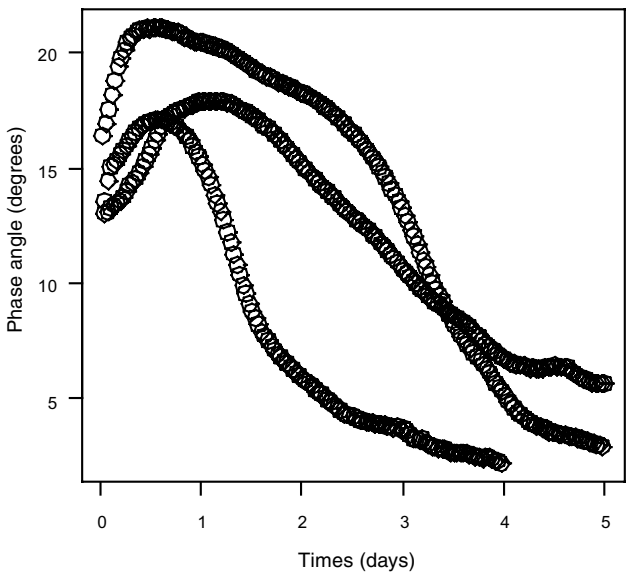

Figure 8

Phase angles for postmortem adult pink salmon (*Oncoryhnchus gorbuscha*) (n=3) measured every 10 minutes for 5 days while stored at temperatures <11°C.

In summary, phase angle reflected the nutritional status of six species of fish in fresh and saltwater after three to four weeks of starvation and reflected the presumed nutritional status of field-caught fish. Re-

gardless of the environment, phase angles decreased with impaired fish condition. The use of phase angle as a direct measure of nutritional status and possibly general health can bridge the gap between indirect nonspecific measures such as length-weight indices and direct specific measures of physiological parameters that are obtained through laboratory analysis. Furthermore, phase angle allows real-time direct measurements of condition in fish in the field without the need to sacrifice them. The low cost and rudimentary technical expertise required to conduct phase angle measurements will allow field biologists and technicians to effectively measure the condition of fish and apply these measurements on broad ecological scales. Although variables were controlled as much as possible in this study, further research should be undertaken to investigate additional potential sources of error that may affect these measures and should include temperature and time of measurement after death as well as the configuration, gauge, and depth of needles used during measurements.

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