Development of Bioelectrical Impedance Analysis (BIA) for Rapid Assessment of Fish Condition

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Overview

This project focuses on the further development of an innovative technique, bioelectrical impedance analysis (BIA), for the rapid, cost-effective, accurate, and non-lethal measure of proximate body composition (i.e. lipids, energy density) of fish collected in the field or laboratory. BIA instantaneously measures the resistance and reactance (capacitance) along the length of the fish, which then can be used to quantify proximate body composition. The study will focus on generating necessary calibration equations for three commercially important Great Lakes fishes, yellow perch, lake whitefish, and walleye, which differ in terms of mass-specific lipid content, energy density, and total body size. Ultimately, this study will enhance efforts to assess the condition (health) of these species in a rapid, cost-effective manner.

Scientific Rationale

Estimating the proximate body composition (e.g., lipids, proteins, water, energy density) of fish can be useful from numerous standpoints. Such estimates can provide insight into the health or condition of individuals, and can help understand causal mechanisms of differential growth and mortality (e.g., Ludsin et al. 1997). By assessing condition of individuals in a population over a period of time, this information can help assess the current state of an ecosystem for that particular organism. For example, recent observations of reduced lake whitefish (Pothoven et al. 2000) and alewife (Madenjian et al. 2003) condition in Lake Michigan, relative to preceding periods, indicate that Lake Michigan likely cannot support these species in the same way that it did historically, potentially owing to dreissenid-driven reductions in *Diporeia* prey. Additionally, estimates of proximate body composition can be valuable for modeling purposes. Bioenergetics-based modeling, which has been used extensively to predict growth or consumption of both freshwater and marine fishes, uses energy density as is its “currency” for tracking energy flows into and out fish. Other applications may include forecasting alewife overwinter mortality from fall measures of condition (e.g., Norcross et al. 2001).

Bioelectrical Impedance Analysis on a Yellow Perch

The development of alternative, rapid, and non-lethal approaches for estimating proximate body composition would be of great value to fishery scientists and management agencies. Quantifying the proximate body composition of fish may not always be feasible. Current laboratory methods are time-consuming, and costly, which limits the number of individuals that can be sampled in a single study. This poses problems in large-scale investigations that require numerous individuals from multiple populations to be sampled. In situations in which time-series measurements of fish condition are required on the same individual, or in which individuals cannot be sacrificed because they belong to a threatened population, measuring proximate body composition is impossible because current laboratory techniques require that individuals be sacrificed. Researchers may be forced to use potentially less reliable, non-lethal techniques to measure fish health/condition, or estimate proximate body composition using surrogate species, which may induce error.

Recently, a rapid (‹ 30 s), non-invasive, non-lethal technique commonly used to assess proximate body composition (i.e., water and fat content) in humans, Bioelectrical Impedance Analysis (BIA), has been used to quantify proximate body composition of other mammals and fish. This approach instantaneously measures the resistance and reactance (capacitance) along the length of the fish. These measures can be correlated with known measures of proximate body composition to develop calibration equations, which then can be used to predict proximate body composition on individuals upon which BIA was conducted. Key to the use of BIA in ecological research is the ability to demonstrate strong predictive relationships between BIA measures and body composition attributes (e.g., water content, lipid levels, caloric content). To date, we only know of two published studies that have attempted to assess the use of BIA for quantifying proximate body composition of fish:

1. Bosworth and Wolters (2001) documented significant predictive relationships between resistance and reactance, as determined by BIA, and both the fat content ($r^2 = 0.75$) and water content ($r^2 = 0.65$) of live channel catfish *Ictalurus punctatus*

2. Cox and Hartman (2005) demonstrated strong, positive relationships between calculated (predicted from developed BIA equations) and observed values of energy density (r^2 = 0.94), fat mass (r^2 = 0.92), protein mass (r^2 = 0.98), dry mass (r^2 = 0.98), water mass (r^2 = 0.98), and ash mass (r^2 = 0.96) for live brook trout.

Plans

1. Refine BIA hardware and software for application to Great Lakes fishes.
2. Develop predictive relationships between BIA output and a) total lipid content, b) neutral lipid content (i.e., triglycerides, including individual triglyceride classes), c) water content, d) caloric content (including energy density), and e) fish condition (Fulton's K) of yellow perch, lake whitefish, and walleye., using fish collected from Lake Erie and Lake Michigan.

Methods

Field Collections

Fish for this project will be collected from both Lake Erie and Lake Michigan. Lake Erie walleye (n = 30 to help refine our extant predictive relationships; n = 10 to serve as an independent test set to test our predictive relationships) will be collected by Jason Van Tassell, a PhD student at OSU, during March through mid-April 2006. Lake Erie yellow perch (n = 10 to help refine our extant predictive relationships; n = 10 to serve as an independent test set to test our predictive relationships) will be collected by Paris Collingsworth, a PhD student at The Ohio State University (OSU), during mid-April through June 2006. Lake whitefish (n = 40 to help develop predictive relationships; n = 10 to serve as an independent test set to test our predictive relationships) will be collected via gillnetting and bottom trawling off of Muskegon during June-November 2006. (No lake whitefish were collected during 2005 because none were encountered in the central basin of Lake Erie, while conducting IFYLE program research.). BIA measures will be made on all fish at the time of collection, using GLERL's Quantum II Bioelectrical Body Composition Analyzer (RJL Systems) and established protocols from 2005. Lengths, wet weights, sex, and spawning condition also will be recorded and these individuals will be wrapped in foil and stored frozen individually in labeled plastic bags for later laboratory analysis.

Energy and Lipid Measurements

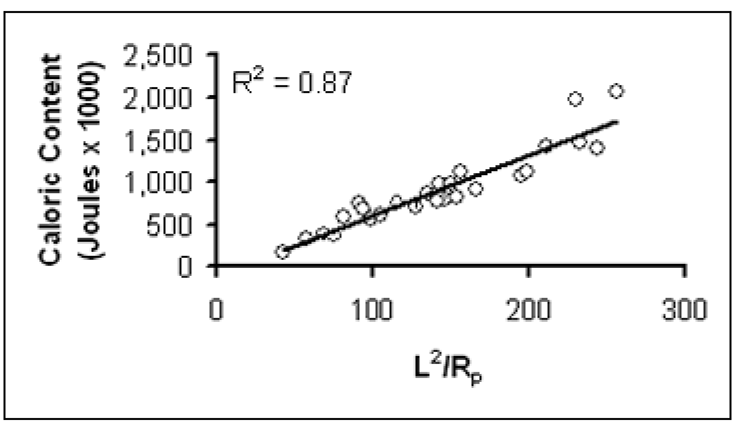
In the laboratory, analyses of proximate body composition will be made on all individuals. Individuals will be homogenized (frozen) and split into two subsamples, one for determination of energy density and the other for determination of lipid content. Total energy content and mass-specific energy density will be determined using bomb calorimetry, a technique commonly used in fisheries research. Walleyes and yellow perch collected by our OSU collaborators will be processed using their bomb calorimeter, whereas lake whitefish will be processed using GLERL's bomb calorimeter. To test for potential instrumentation biases, we will quantify energy content of a subset of individuals (n = 10), using both bomb calorimeters.

We will use two methods to quantify lipid content of our fish. Lipids will be extracted using a GLERL's soxhlet extraction setup. Total lipid content will be quantified by summing individual lipid classes (sterol esters, triglycerides, free fatty acids, sterols, phospholipids), as determined from GLERL's Chromorod-Iatroscan TLC-FID (Thin Layer Chromatography-Flame Ionization Detector). We also will generate two measures of neutral lipid (i.e., triglyceride) content to correlate with BIA measures, given that triglycerides are an important energy source during periods of stress, whereas the other types of lipids are tissue components with slow mobilization rates that likely do not reflect an organism's condition (see Ludsin and DeVries 1997). One measure will be generated with GLERL's Iatroscan TLC-FID. This method has been shown to provide reliable estimates of triglycerides (and other lipid classes) for both invertebrates and vertebrates, including fish (Ludsin and DeVries 1997). Triglycerides also will be quantified using a second thin-layer chromatography technique (Haugen et al. 2003, Muñoz-Garcia and Williams 2005). In addition to providing an independent measurement of total triglycerides, this second technique can provide measurement of individual triglyceride classes, which might provide an even better measure of condition (Muñoz-Garcia and Williams 2005).

Once measurements of BIA, length and wet weight (from which Fulton's K condition factor can be determined), energy content (total caloric content and energy density), and lipid content (total lipids, total triglycerides, triglyceride classes) have been made, we will use a non-linear multiple-regression modeling approach to develop relationships among these variables for each species. Of primary importance will be developing predictive relationships between BIA measurements and measures of proximate body composition.

Accomplishments

In 2005, we collected 41 yellow perch and 10 walleye from Lake Erie for BIA analysis. Preliminary results from 2005 indicate strong relationships between BIA resistance measures for yellow perch relative to total calories (r2 = 0.87) and water content (r2 = 0.88) (Fig 1). Given these positive findings, BIA holds great potential for Great Lakes researchers and agencies that desire the ability to rapidly and accurately quantify fish condition and health.



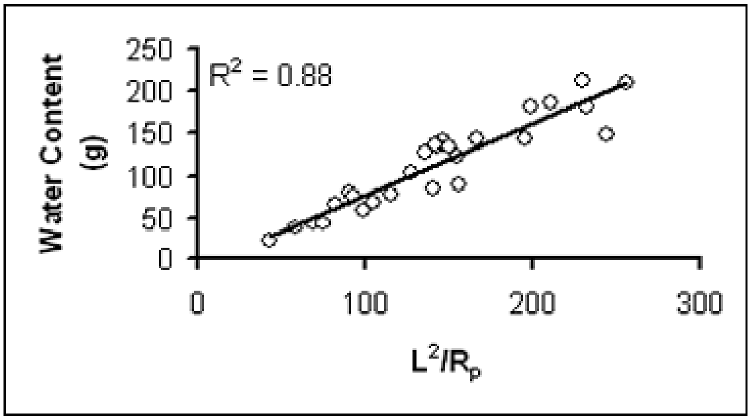


***Figure 1**: Total energy content (top panel) and total body water (bottom panel) of age 1+ yellow perch relative to parallel reactance measures (L^2/R_p, where L = total length (mm) and R_p = Reactance) obtained from BIA.*

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