2014

Effects of Salinity and Temperature on Bioelectrical Impedance Analysis of Gulf killifish, Fundulus grandis

Shea A. Miller
Louisiana State University and Agricultural and Mechanical College

EFFECTS OF SALINITY AND TEMPERATURE ON BIOELECTRICAL IMPEDANCE ANALYSIS OF GULF KILLIFISH, FUNDULUS GRANDIS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical Collage
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Renewable Natural Resources

by
Shea Miller
B.S., University of Louisiana at Lafayette, 2005
August 2014

ACKNOWLEDGEMENTS

I would like to thank my wife, Lindsay, for her endless support. Thanks also to the many friends, cohorts, and faculty, for the help, advice, and use of their resources along the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Bioelectrical impedance analysis (BIA) has been proposed as an easy and accurate technique for a non-lethal index of condition in fish; however, factors affecting its accuracy have yet to be properly identified. The technique uses the resistance (R) and reactance (Xc) of water and lipids in contiguous tissues to estimate lipid content. Studies have investigated the use of BIA on fish, but with mixed results, possibly caused by unrecognized sources of influences. Our study explored possible sources of variance for BIA measurements in gulf killifish, *Fundulus grandis,* through laboratory feeding and fasting experiments, and experimental exposures to acute changes in temperature and salinity. In our feeding and fasting experiments, wild caught *F. grandis* were held in recirculating tanks and either fed ad libitum or withheld food for 28 days and sampled every 7 days. In the fed and fasted experiments, BIA measurements, plasma osmolarity, Lecren's Kn, and % organ lipid were significantly different by day with most variables showing a gradual change over time (ANOVA, $p < .05$). However, in the fasted experiment BIA measurements did not follow the trends of declining condition indicated by Kn and % organ lipid In our experiments with environmental factors, fish acclimated to 24^0C and 12 ppt salinity where exposed to tanks randomly assigned treatments of salinities 2, 12, 24 and temperatures 2, 12, 24^0C. Reactance was consistently significantly higher at salinity 2 ppt, and R significantly decreased as temperature increased (ANOVA,Tukey-Kramer $p < .05$). Our results indicate additional possible sources of variance including sex, size, temperature and changes in body chemistry. Some of the mixed results from other studies may be explained by these sources of variance. If these sources of variance are shown to be easily correctable, BIA may prove to be an effective index of condition.

CHAPTER 1. LITERATURE REVIEW & RESEARCH GOALS

1.1 Introduction

Effective monitoring techniques provide critical data to inform management and protection of natural resources. In vulnerable and rapidly changing environments, such as coastal Louisiana, the ability to accurately and effectively monitor changes in natural resources is of utmost importance. The coastal waters of Louisiana support a valuable cultural heritage, and are critically important for the local economy and our national seafood production. In 2011, the Louisiana coast produced nearly 32 percent of the contiguous United States' seafood by weight, valued at 339 million dollars (NOAA 2011). Marine sport fishing in Louisiana has generated an economic output near $750,000,000 some years (ASA 2002). Much of the unique cuisine in Louisiana is based on local seafood. This culturally and economically valuable resource has come under serious threat in recent years from natural and anthropogenic events. Most recently, for example, was the 2010 Deep Water Horizon oil spill, which released more than 4.9 million barrels of oil into the Gulf of Mexico (McNutt et al. 2012). The effects of such an event are hard to measure, especially in estuarine systems as variable as the Louisiana Gulf coast. Since environmental health is a difficult concept to define, the condition of biological indicators, such as fish, are used to quantify environment health. Presently there are few options for sensitive and accurate measurements of condition of an organism that can be conducted in the field (Adams et al. 1994). Broadly speaking, this project examines the use of a rapid, non-lethal, field-based technique (bioelectrical impulse analysis, BIA) for assessing fish condition as a potential tool for biological monitoring in coastal ecosystems.

1.2 Measuring Biological Response

Identifying change has become the essence of modern ecology, and effective indices of change in an ecosystem are the responses of the inhabiting organisms (Adams 2005). The idea is not a new one; at the beginning of the 20th century the use of canaries to monitor air quality in mines was common practice (Burrell and Siebert 1916). After the Clean Water Act of 1972 called for the protection of the biological integrity of the nation's waters, biological monitoring became common practice in aquatic environments. Biota responses are ideal environmental indicators, as they integrate both direct and indirect stressors, and directly reveal the effects in their fitness, population, and community (Adams 2005).

Biological responses imparted by the changes in an ecosystem can be separated into four levels (Van Gestel and Van Brummelen 1996). First, there is a physiological response within an individual organism, such as tissue damage or bioaccumulation. Second, there is a physical response by the individual, such as changes in survival, growth, and reproduction. Third, there is a population level response with altered genetics, age structure, and abundance. Finally there is the ecosystem level response with changes in species diversity and community dynamics. The responses at the individual, population, and ecosystem levels that provide information on the status of a system are known as biocriteria, responses below the individual level are commonly called biomarkers (NRC 1987; Adams 2005). A good investigation of ecological integrity of a system will often use biomarkers to monitor for change, while using biocriteria like abundance to collect information on overall conditions (Schiemer 2000).

A classic example of a bioindicator early warning is the decline in raptors following the use of dichlorodiphenyltrichloroethane (DDT) in the 1950s (Ratcliff 1970). Though general levels found in the environment were low and did not indicate a problem, the response of biocriteria in the ecosystem indicated a change not be evident at the environmental level (Evans et al. 1991). DDT was accumulating in higher predators, and as DDT concentration in raptor eggs increased to 15 ppm, raptor egg mortality approached 100% (Wiemeyer et al. 1984). Due

to the biocriteria response indicating a problem, steps were taken to limit the use of DDT and allow ecosystems to recover (Grier 1992). Bioindicators are useful tools for bringing clarity to a complex problem, and allowing policymakers to identify key aspects and determine appropriate action (Niemeijier 2002).

An important caveat in biological monitoring is the need to use more than one indicator. Responses can be complex and interact with one another, and for an integrated evaluation of response, multiple indices should be measured (Adams et al. 1994). For example, measuring a limited range of responses at an individual level may lead to incorrect assumptions about the condition of the organism (Copeland et al. 2008). In a study of largemouth bass, several biochemical markers of stress were compared to a morphological fish health index. Biochemical markers found to indicate stress, hepatic cytochrome P450 IA (CYP IA), ethoxyresorufin-O-deethylase (EROD), heme-oxygenase (HSP30) and metallothionein (MT) were measured in the liver tissues of each individual (Schlenk et al. 1996). Only CYP IA and HSP30 were found to correlate with fish health indicating stress, despite the fact that the other biochemical markers measured had been shown in other studies to be indicators of stress. If only one biochemical indicator had been used exclusively, it may have led to incorrect conclusions.

In aquatic environments, a range of organisms can be used as indicators, including plants, micro-invertebrates, mollusks, and fish (Mazor et al. 2006; Germian and Pinte 1990; Adams et al., 1999). Fish have proven particularly good indicators due to their relatively long life, ease of collection, simple identification, and usually known life history (Plafkin et al. 1989). Biomarkers of fish condition provide integrative measures of the effects of biotic and abiotic environmental influences on fish (Searcy et al. 2007; Zapfe and Rakocinski 2008). Whether a fish puts its energy into homeostasis, growth, fecundity, or storage depends on factors such as food availability, environmental factors, seasons, or pollution (Diana 1995). These factors make fish a good candidate for use as a bioindicator.

Numerous lab studies have demonstrated the effects of various factors on fish condition. The majority of a fish's energy budget is spent on maintaining homeostasis, growth, and reproduction (Febry and Lutz 1987; Nicieza and Metcalfe 1997; Lambert and Dutil 2000). If the caloric needs of survival become too large, a fish's condition can be negatively affected (Nicieza and Metcalfe 1997). Food availability may have the most profound effect on fish energetics; lack of food resources can negatively affect body weight, growth rates and alter hormones in some species (Fox et al. 2006; Picha et al. 2005). Additionally, fish growth has been shown to be affected not just by caloric input, but also nutritional value of food (Bowen et al. 1995). Low lipid content in a diet can lead to low reproduction and offspring survival, while increased protein can lead to increased growth and earlier maturation (Gunasekera et al. 1995; Izquierdo et al. 2001). Exertion can also affect fish physiology, where fish exposed to a more physically demanding swim regime have shown increased mortality and metabolism (Bagatto et al. 2001). Environmental characteristics such as temperature, dissolved oxygen, and salinity have been shown to alter growth and energy allocation in fish (Brown et al. 2011; Martinez et al. 2006; Sogard and Spencer 2003). Water temperature has a significant effect on metabolism of fish, and fish growth can be stunted by deviations from a nominal range (Elliott 1976; Bowen et al. 2013). Environments contaminated with heavy metals and petroleum have been shown to negatively affect fish growth and physiology (McKinley et al. 2012; Hicken et al. 2011; Gillers et al. 2006). The more energy a fish must allocate to factors that threaten its survival, the less energy there is to use to grow (Febry and Lutz 1987; Nicieza and Metcalfe 1997). As such, numerous studies have used fish condition to compare habitat quality (Wierner and Hannenar 1982; Booth and Shipley 2012), examine fish response to pollution (Larose et al. 2008; Bervoets and Blust 2003; Kermabrun et al. 2012), compare nursery grounds (Vaxconcelos et al. 2009; Ribeiro et al. 2004), and assess restored habitats (Jackson et al. 2002; Dibble and Meyerson 2012).

Several morphometric and biochemical indicators of fish condition have been developed in the last century. Morphometric approaches are often used because they are cheap, easy and field-friendly (Stevenson and Woods 2006). One example, the Fulton condition factor, based on the ratio of body length to weight, was developed in the early 1900s (Blackwell et al. 2000). More recently, other scientists developed slightly different measures expanding on Fulton's factor, termed relative condition factor and relative weight (Lecren 1951; Blackwell et al. 2000). These techniques use predetermined condition values to formulate condition indexes (Lecren 1951; Blackwell et al. 2000). These measures rely on ratios of fish length and weight to estimate the fat or energy content of the fish as a condition index, with the general assumption that a heavier organism of the same length is in better condition (Lecren 1951; Blackwell et al. 2000; Stevenson and Woods 2006).

Recent calculations of body condition use an ordinary least square regression of mass to length to remove the effects of size variation (Reist 1985). This calculation has a set of assumptions that need to be met: mass increases linearly, a fish's health is independent of the length and mass ratio, the length and mass ratio is a measure of true size, and there is no correlation between size and other structures (Green 2001). Few studies take these assumptions into consideration when making calculations of body condition but the technique has still been shown to be accurate when the assumptions are met (Green 2001; Schulte-Hostedde et al. 2005). An alternative is the TL method which also removes the size effects through scaling (Lleonart et al. 2000). The method begins with a plot of mass and length to remove any outliers. Then a standardized major axis regression is performed on the plot of the natural log transformed data. The slope of this line can be used in the following formula:

$$\hat{M}_i = M_i \left[\frac{L_0}{L_i} \right]^{b_{SMA}}$$

Where M_i is the body mass, L_i is the body length, b_{SMA} is the slope, and L_0 is the mean length for the study (Peig and Green 2009). This technique has been shown to be more accurate than the ordinary least square regression (Peig and Green 2009). Though these methods are easy and inexpensive, their index of condition can be a poor measure of actual health and lacks sensitivity to fish with non-isometric growth or fat storage and multiple indices are still needed (Cone 1989; Adams et al 1999; Jakob et al. 1996; Copeland et al. 2008).

Other techniques use biomarkers as indicators of fish condition. Biomarkers are measures of specific parts of an organism that indicate the effects of environmental contamination (Peakall 1992). Common biomarkers used in fish studies are gill lesions, hepatic lesions, and gene expression, though there are many others (Myers et al. 1994; Spies et al. 1996; dos Anjos et al. 2011). Another biomarker can be proximate measures of condition. Proximate measure present more direct measures of fish condition and have been used extensively particularly in pollution studies. These methods tend to be more complicated and are lethal to the fish. For example, direct measurements of fish body composition (i.e., protein, water, fat, ash) can be measured with proximate body analysis (Brown and Murphy 1991; Herbinger and Friars 1991). With proximate body analysis, a fish is sacrificed and processed for its lipid content, which is a strong indicator of fish health as it is a measure of energy content (Brown and Murphy 1991). Each of these methods has strengths, but all are difficult to apply in the field, expensive, time consuming or lethal to the fish and require technical expertise. With indirect methods lacking accuracy, and direct methods time consuming, expensive, and difficult to conduct in the field, there is a still a need to develop better field friendly indices.

1.3 Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) presents a potentially accurate, rapid and non-lethal method for routine evaluation of fish condition in the field. The technique has been used

for condition measurements in humans since the 1970s (Lukaski et al. 1985; Segal et al. 1991; Kyle et al. 2004). Recently this technique been explored for use on fish in both laboratory and field studies (Bosworth and Wolters 2001; Cox and Hartman 2005; Cox et al 2011). Specifically, the BIA method has been proposed as a means to rapidly acquire non-lethal measures of fish condition and body composition using the conductive properties of water and lipid content in contiguous tissues masses (Lukaski et al. 1985; Cox and Heintz 2009). The technique consists of taking two electrical measurements of resistance and reactance of the biological tissues with 425 micro-amps at ~0 and 50 khz. Two probes consisting of pairs of needles are inserted into the fish's tissues usually using physical features for consistent orientation of placement. One probe is lined up near base pectoral fin, and one probe was lined up with the front edge of the anal fin (Figure 1). One measurement is made at a low frequency, which will not penetrate cell membranes (Thomasset 1963; Pethig 1979; Kyle et al. 2004); this is the resistance of the extracellular fluids. Electrical resistance is the opposition to electrical charge passing through a medium. The other measurement is made at a higher frequency, at which cell membranes become capacitive and allow electric current to flow through them (Thomasset 1963; Pethig 1979). This higher frequency measurement is the reactance of the tissues and is the opposition to change in electrical current; it is theoretically cause by the presence of lipids. These two measurements can be used to calculate different indices of fish condition including phase angle, or reactance and series measurements adjusted for parallel systems, or adjusted for individual fish length, as researchers attempt to find the correct approach to accurately track fish condition (Cox et al. 2011). Results have been mixed, and the technique is still being refined for fish (Cox et al. 2011; Table 1). In tests with larger fish, such as catfish, brook trout, and cobia, BIA correlated strongly with many proximate measures of total body protein, water, fat, and dry mass (Bosworth and Wolters 2001; Cox and Hartman 2005; Duncan et al. 2007). In similar tests with fish species such as steelhead, Atlantic salmon, walleye, BIA failed to correlate with lipid content relative to size, or lacked accuracy (Pothoven et al. 2008*;* Hanson et al. 2010;

Table 1. Year, authors, title, and short summary of paper using BIA on fish.

Year	Author	Title	Results Summary
2001	Bosworth and Wolters	Evaluation of bioelectric impedance to predict carcass yield, carcass composition, and fillet composition in farm-raised catfish	Did not use whole fish for analysis but R^2 values 0.7-0.8
2005	Cox and Hartman	Nonlethal estimation of proximate composition in fish	$R^2 > 0.96$ for water, protein, fat, fat-free, and dry masses
2007	Duncan et al	Bioimpedance assessment of body composition in cobia Rachycentron canadum (L. 1766)	Lipid $R^2 = 0.3337$, water, protein, fat-free, ash, and dry mass $R^2 > 0.85$
2008	Hartman and Margraf	Common relationships among proximate composition components in fishes	Used to estimate dry, protein, total fat, fat-free mass
2008	Pothoven et al	Reliability of Bioelectrical Impedance Analysis for Estimating Whole-Fish Energy Density and Percent Lipids	Significant predictor, only slightly better than body mass alone
2008	Willis and Hobday	Application of bioelectrical impedance analysis as a method for estimating composition and metabolic condition of southern bluefin tuna (Thunnus maccoyii) during conventional tagging	Weight composition index to BIA $R^2 > 0.78$
2009	Keith Cox	Electrical phase angle as a new method to measure fish condition	Phase angle significantly different between starved fish
2010	Hanson et al	Comparison and Validation of Nonlethal Techniques for Estimating Condition in Juvenile Salmonids	Water and protein $R^2 > 0.74$, Poor predictor of lipid
2011	Cox et al.	Measurements of resistance and reactance in fish with the use of bioelectrical impedance analysis: sources of error	Sources of error in technique paper
2011	Hafs and Hartman	Influence of Electrode Type and Location upon Bioelectrical Impedance Analysis Measurements of Brook Trout	Techniques paper, BIA to dry weight $R^2 = 0.85$
2011	Hartman et al	Temperature Effects on Bioelectrical Impedance Analysis (BIA) Used to Estimate Dry Weight as a Condition Proxy in Coastal Bluefish	Temperature significant effect
2012	Calderone et al	Evaluation of bioelectrical impedance analysis and Fulton's condition factor as nonlethal techniques for estimating short-term responses in postsmolt Atlantic salmon (Salmo solar) to food availability	Wet weight better predictor of body composition
2012	Garner et al	Use of Bioelectric Impedance Analysis to Assess Total-Body Condition and Predict Energy Density in Juvenile Atlantic Croaker	Lower predictive power than relative condition
2012	Rasmussin et al	Empirical relationships between body tissue composition and bioelectrical impedance of brook trout Salvelinus fontinalis from a Rocky Mountain Stream	Low correlation with lipid
2013	Klefoth et al	Reliability of non-lethal assessment methods of body composition and energetic status exemplified by applications to eel (Anguilla anguilla) and carp (Cyprinus carpio)	BIA poor indicator of dry mass and significant temperature effect

Caldarone et al. 2012). With such mixed results of BIA, there are many aspects of the technique that could use further study.

1.4 The Effect of Changing Environmental Variables on Measuring Fish Condition

The organisms that inhabit an environment are often the best source of information on changes within that environment (Adams 2005). Organisms used as environmental indicators are useful tools for bringing clarity to a complex problem, providing policymakers with critical information regarding environmental change which allows them to determine appropriate action (Niemeijier 2002). In aquatic environments, there are a range of organisms that can be used as indicators, but fish are often used as indicators due to their long life, ease of collection, known life history and sensitivity to the environment (Plafkin et al. 1989; Hicken et al. 2011; Bowen et al. 2013).

The health of individual fish in a population has been shown to be an important indicator of the quality of the systems in which they exist (Adams 2005). In order to categorize or index the health of an individual many different techniques have been developed over the last century. Some indices are based on easily attainable information such as length and weight ratios, while others require fish mortality, and more detailed analyses including proximate analysis of blood or tissues (Brown and Murphy 1991; Blackwell et al. 2000). Regardless of ease of use or impact on the individual fish, the goal of all of these efforts is to provide a measurement of condition that can be used to monitor and identify change in condition in response to environmental quality. The ideal technique would be easy to apply in the field, accurate, and nonlethal to fish.

Bioelectrical impedance analysis (BIA) presents a potentially accurate, rapid and non-lethal method for routine evaluation of fish condition in the field and lab. Specifically, the BIA method has been proposed as a means to rapidly acquire non-lethal measures of fish condition

and body composition using the conductive properties of water and lipid content in contiguous tissues masses (Lukaski et al. 1985; Cox and Heintx 2009). Recent studies of the accuracy of BIA use on fish have shown the technique to be sensitive to changes in fish condition, but poor at creating an index of condition for comparing between fish with often widely varying results (Cox and Hartman 2005; Pothoven et al. 2008; Klefoth et al. 2013). Understanding the causes of variance in the conductance of tissues is complex, and researchers are still trying to identify and understand these sources and their effects on BIA measurements on fish (Pethig 1979; Cox et al. 2011; Hartman et al. 2011; Klefoth et al. 2013).

Fish adaptions to aquatic environments allow them to exhibit changes in their body physiology that can be expected to affect BIA measurements (Light 1984; Brill et al. 1994; Zadunaisky et al. 1995). When sampling in estuarine environments, this is an important factor to consider given that salinity and temperature are known to have high variability within and between years. For example, because fish are ectotherms, any change in water temperature will result in a change in their tissue conductance (Brill et al. 1994; Crockett and Londraville 2005; Gray 2004). If the sources of variance in BIA measurement in fish could be better understood, it could prove a valuable tool in using fish as environmental indicators.

The first aspect of the technique that needs to be addressed is the influence of altered extra and intra-cellular fluids. BIA measurements are based on conductance, which in a solution is affected by the presence and concentration of ions (Gray 2004). Changes in these characteristics within tissue fluids could be a source of variance when making BIA measurements on fish. While teleost fish inhabiting environments with ionic composition and osmolarity different from their maintained internal conditions are proficient at maintaining these differences, fish adapting to new salinities have altered plasma ion levels (Foskett et al. 1983; Zandunaisky et al. 1995). In euryhaline fish, regulation of ion transport happens in two steps, an initial immediate response and a slower adaptation (Zadunaisky et al. 1975). For instance, when *F. heteroclitus* is exposed to a higher salinity environment, there is a rapid increase in the

gill Cl^- permeability (Maetz and Bornancin 1975; Zadunaisky et al. 1995). The increased salinity causes the fish's chloride cells to begin to shrink from fluid loss, and this shrinking activates a cascade of transport molecules opening Cl^- channels (Zadunaisky et al. 1995). By tracking the blood plasma osmolarity, it can be shown that after an initial spike, osmolarity levels slowly return to normal (Figure 2). With longer exposure there is a gradual adaption of the epithelium cells, changes in electrical resistance, increases in chloride cells and transport enzymes (Sardet et al. 1979; Karnaky et al. 1976). Complete adaptation can take several days and until then plasma ion levels remains altered (Zandunaisky et al. 1995). As the conductance of extracellular fluids is fundamental to BIA measurements, two identical fish with different plasma osmolarity may give different BIA readings. Identifying this variance could clarify why BIA results are variable, and would be vital to the successful use of BIA in estuaries where fish experience variable ion flux.

The second component of BIA that needs investigation is influence of body temperature. The conductance of materials is strongly affected by its temperature (Gray 2004). Due in part to fish oxygen requirements, and variable oxygen content of water, along with high heat capacity and thermal exchange rate, most fish do not physiologically regulate their body temperature relative to their environment (Brill et al. 1994; Crockett and Londraville 2005). Water's conductivity can be affected by temperature by 1 – 5% / ºC depending on its purity (Hayashi 2004). Thus, fish measured with BIA from environments with differing temperatures may have altered results from the differing tissue temperatures. There could also be long term temperature adaptations that effect BIA. Temperature affects the cell membrane viscosity, with cooler temperatures affecting the rigidity of acyl chains of constituent phospholipids (Farkas et al 2001). To compensate for this change some fish alter the structure and composition of membrane phospholipids (Hazel and Prosser 1974; Wodtke 1978; Wallaert and Babin 1994). These alterations can have secondary effects on water permeability, where membranes that increased cholesterol to combat loss of fluidity decrease cell membrane water permeability

(Robertson and Hazel 1999). Temperature also alters the ion and water balance in fish by shifting the energy needed for protein-mediated ion transport across membranes (Metz et al 2003). In addition, fish have adopted a myriad of strategies to cope with fluctuating body temperatures, ranging from antifreeze to heat shock proteins (Devries and Wohlschlag 1969; Iwama et al 1998; Stebbing et al 2002). These changes may also affect BIA measurements, and need to be better understood if BIA is to be used in field settings.

While some studies have documented effects of temperature on BIA measures, these studies have yet to document effects across the range of temperatures that fish along the Gulf Coast might be exposed to, and while a temperature correction for BIA measures has been suggested, but not developed (Hartman et al. 2011). Similarly, events such as physical stress, pollutants, and acute changes in salinity can affect osmoregulation and the composition of tissues and body fluids (Lowe et al. 1993; Zadunaisky et al. 1995; Souza-Bastos et al. 2011) which has been suggested to affect BIA measurements (Light 1984; Gray 2004). Before BIA can be suggested for use on assessing condition of wild populations, better understanding of how environmental factors affect BIA outcomes is critical.

1.5 Research Goals

Broadly speaking, this study was designed to investigate the relationship between BIA and changes in the condition status of an estuarine fish, as well as further examine the influence that salinity and temperature may have on that relationship. Specific objectives of this work were to (1) quantify the relationship between BIA measurements and changes in lipid content and plasma osmolality in fasted and fed fish in the lab, (2) identify the relationship between BIA measurements, temperatures and salinities in the lab. Two major studies were completed. First, to test the ability of BIA to differentiate between fish subjected to a range of conditions, we developed a set of experiments to quantify the relationship between BIA measurements, tissue

ion content, and plasma osmolality in recently caught wild fish and recently caught wild starved fish, over a thirty day period. Second, to evaluate potential use of the method in the field, where organisms are exposed to water quality changes, we tested the short-term effects of acute salinity and temperature changes on BIA measurements, tissue ion content, and plasma osmolality, using recently caught wild fish, and lab-conditioned fed fish. From these two studies, we were able to develop a comprehensive overview on the suitability of BIA in measuring fish condition.

CHAPTER 2. EXPERIMENTAL DESIGN

2.1 Experimental Fish

Fundulus grandis, a ubiquitous estuarine prey species, was selected for this study. *F. grandis* are fast growing, small fish with maximal sizes reaching 18 cm (tail length), *F. grandis* are found from the St. Johns River, FL to Laguna De Tamiahua, Veracruz, Mexico (Lee et al. 1980), inhabiting a wide range of salinities, from fresh to 76 ppt (Simpson and Gunter 1956; Tabb and Manning 1961). Wild-caught *F. grandis* were purchased from a local bait dealer (Terry's Live Bait, Golden Meadow, LA) for use in this study. On June 5 and July 3 2013, approximately 500 fish were transported to the Louisiana State University AgCenter Aquaculture Research Station in multiple aerated 114 L coolers. Fish purchased on June 5 were used in the condition experiments (Study 1, &2 below); fish purchases on July 3 were used in the exposure experiments (Study 3 & 4 below).

2.2 Experimental Systems

F. grandis transported to LSU AgCenter Aquaculture Research Station were placed in a 13,700-L holding tank, with a recirculating system consisting of a 325-L sump, bead filter, and ultra-violet filter. The system was maintained at ambient temperature (20 - 32ºC) and a salinity of 12 ppt which is approximately isosmotic for similar species, allowing passive ion flow (Fritz and Garside 1974). Salinity and oxygen were monitored daily. The salinity of the tanks was maintained by the addition of Instant Ocean. An automatic feeder dispensed approximately 300 mg of Cargill Aquaxcel fish marine starter food (2.2 mm, 50% protein, 14% fat) twice a day. Fish were maintained under these conditions until selected for experimental studies, detailed below. Fish used in the four studies outlined below are categorized as non-acclimated fish (wild) which were used in experiments within 5 days of transportation to LSU, and lab-conditioned fish, which were held at LSU and fed as described above for a minimum of 60 days.

The experiments were all conducted in separate tanks system consisting of a sump, inline cooling system, bead filter, aeration, and ultra-violet filter. Throughout the experiments, salinity, temperature, and dissolved oxygen were checked daily using an YSI-556. Ammonia, nitrites/nitrates were measured bi-weekly.

2.3 Study Design

Four studies were conducted with the goal of better understanding the relationships between BIA, fish condition, and changing environmental variables. The first two studies aimed to quantify the relationship between BIA measurements, lipid content, and plasma osmolality in recently caught wild fish. To ensure a range of fish conditions, two experiments were run: (1) fish were fed throughout the 30 day experiment, while condition and BIA measurements were recorded, and (2) fish were deprived of food throughout the 30 day experiment, while fish condition and BIA measurements were recorded. All metrics used for experiments are listed and described in Table 2.

In June 2013, within 24 hours of placement of experimental fish in the large holding tank at LSU, 128 male and 128 female fish were randomly selected and transferred into temperature controlled tanks maintained at 24ºC and a salinity of 12 ppt for acclimation for 96 hours. Fish were fed ad libitum twice a day using Cargill Aquaxcel fish marine starter food (2.2 mm, 50% protein, 14% fat). After 72 hours, food was withheld prior to sampling in order to ensure gut content would not influence BIA measurements (Cox et al., 2011). At 96 hours, 18 female and 18 male fish were randomly selected and processed as described below (Methods 3.3.1) to provide a baseline of fish condition, and Day 0 data for both the fed and the starved experiments

2.3.1 Study 1: Wild-caught, Fed Condition Experiment

On Day 0, forty male and forty female fish were placed in experimental tanks maintained at a salinity of 12 ppt and a temperature of 24ºC (mean TL: 97.3 ± 1.8 mm, mean % organ lipid: 30.4 ± 0.3). Fish where fed Cargill Aquaxcel fish marine starter food (2.2 mm) ad libitum twice a day. . For each sample event, food was withheld for 24 hours prior to sampling in order to ensure gut content would not influence BIA measurements (Cox et al., 2011). On days 7, 14, 21, and 28, 10 males and 10 females were randomly selected and processed for condition and related metrics (Table 2) as described in Methods 3.3.1, below. Fish condition was expected to remain constant or increase under fed lab conditions.

2.3.2 Study 2: Wild-caught, Fasting Condition Experiment

On the same Day 0 as the fed experiment, 72 male and 72 female fish were placed in tanks maintained at a salinity of 12 ppt and a temperature of 24ºC (mean TL: 94.3 ± 1.6 mm, mean % organ lipid: 30.4 ± 0.3 %). To prevent possible cannibalization, fish were held in separate sub-compartments within each tank. The tanks were checked daily for mortality, and any dead fish were removed. The higher number of fish compared to Study 1 was selected due to the possibility of mortality from fasting. On days 7, 14, 21, and 28, 10 males and 10 females were randomly selected and processed for condition and associated metrics as described in Procedures, below. Fish condition was expected to decrease with time under fasting conditions.

2.3.3 Study 3: Wild-caught, Acute Salinity x Temperature Exposure Experiment

A split-plot fully crossed experiment was conducted to examine the effects of acute exposure to 4 temperatures (2, 12, 24, 36°C) and 4 salinities (2, 12, 24, 36 ppt) on BIA measurements, length, weight, condition, lipid content, osmolarity and plasma and muscle Na^+

concentrations. The experiment was conducted on non-acclimated (wild caught) fish to represent fish in conditions found in the field. Within 24 hours of fish being transported to LSU tanks, 64 fish (mean TL: 85.1 ± 1.5 mm, mean % organ lipid: 50.0 ± 2.3 %) were randomly selected and placed in a temperature (24°C) and salinity (12 ppt) controlled tank consisting recirculating system with a sump, bead filter, aeration and ultra-violet filter. Fish were fed Cargill Aquaxcel fish marine starter food (2.2 mm) ad libitum twice a day while they acclimated to the tank, and to ensure no mortality from handling. After 72 hours, fish were withheld food for 24 hours to ensure that there was no food in the digestive track during sampling. At 96 hours, fish were randomly selected and placed into salinity and temperature treatment tanks (4 salinities x 4 temperatures x 4 fish) where they were held for 6 hours. A 6 hour exposure was shown in other studies to be long enough to cause a change in cellular fluids, but short enough that the fish would not osmoregulate changes back to homeostasis (Zadunaisky et al. 1995). When 6 hours had passed, all fish were removed and immediately processed for BIA, lipid, plasma osmolality and other metrics as outlined in Methods 3.3.1, below. Fish condition was not expected to change under this short term acute exposure.

2.3.4 Study 4: Lab-Conditioned, Acute Salinity x Temperature Exposure Experiment

A split-plot fully crossed experiment was conducted to examine the effects of acute exposure to 4 temperatures (2, 12, 24, 36°C) and 4 salinities (2, 12, 24, 36 ppt) on BIA measurements, length, weight, condition, lipid content, osmolarity and plasma and muscle Na+ concentrations. The experiment was conducted on lab-conditioned fish (tank fed for 60 days). After being maintained in salinity controlled tanks (salinity = 12 ppt), under ambient temperatures for 60 days at LSU, and fed Cargill Aquaxcel fish marine starter food (2.2 mm) ad libitum twice a day, 64 fish (mean TL: 81.5 ± 0.5 mm, mean % organ lipid: 71.1 ± 4.2 %, Aug 2013) were randomly selected and placed in a temperature (24°C) and salinity (12 ppt) controlled tank consisting of a recirculating system with a sump, bead filter, aeration and ultra-

violet filter. Fish were fed Cargill Aquaxcel fish marine starter food (2.2 mm) ad libitum twice a day while they acclimated to the tank, and to ensure no mortality from handling. After 72 hours, fish were withheld food to ensure that there was no food in the digestive track during sampling. At 96 hours, fish were randomly selected and placed into salinity and temperature treatment tanks where they were held for 6 hours (4 salinities x 4 temperatures x 4 fish). When 6 hours had passed, all fish were removed and immediately processed for BIA, lipid, plasma osmolality and other metrics, as outlined in Procedures, below. Fish condition is not expected to change under this short term acute exposure.

CHAPTER 3. METHODS

3.1 Lab Procedures

All experimental fish were processed using the same protocols, listed below. All metrics collected are listed in Table 2, with their symbols, formulas, and units. At sampling, all fish were immediately placed in solution of 1.0 grams of tricaine methanesulfonate (MS222) in 1 liter of tank water, buffered to neutral pH with sodium bicarbonate, and kept in the solution until fully anesthetized and ceased moving. Fish were removed from the MS-222 solution, patted dry, and blotted weight (g) and total length (mm) were recorded. Length and weight were used to calculate a relative fish condition using Lecren's (Kn) condition index (Lecren 1951). BIA measurements (resistance, reactance), and blood and tissue for osmolarity, ion content, and lipid content were taken following procedures listed below.

3.1.1 BIA Measurements

Resistance (R) and reactance (Xc) for BIA were measured using a Quantum X impedance analyzer (RJL Systems, Point Heron, MI) following standard methods (Cox and Hartman 2005; Cox, Hertz and Hartman 2011). Each of the meter's two probes was assembled by attaching a pair of 28 gauge needle electrodes (Grass Technologies) to a 0.5 cm wide sliver of PVC, allowing for 0.5 cm of exposed needle beyond the sliver for penetration. The needle electrodes were held in place with a dot of thermoplastic adhesive to ensure consistent probe separation and depth. Before measurements on fish were collected, the Quantum X meter was calibrated using a 500 ohms resistor. To maintain consistent probe insertion across varied fish sizes, probe placement was based on morphological landmarks. The anterior probe location

Table 2. List of symbols, formulas, and units used to quantify metrics, and a word description of the metrics used in this investigation. All metrics were examined as response variables to treatments in all four studies of this work.

Metric	Symbol	Formula and Measurement	Description
Length	L	total length (mm)	Length from rostrum to the end of the tail
Weight	W	body weight (g)	Patted dry weight
Lecren's Condition	Kn	$Kn = Wt / a(L)^b$ where a and b are constants from Length and Weight trendline	Ratio of actual weight compared to a hypothetical ideal weight
Organ Lipid %		(lipid content of body cavity / weight of the organs of the body cavity)*100	Index of energy content of fish
Body Lipid %		(lipid content of body cavity / W)*100	Index of energy content of fish relative to length
Plasma Osmolarity		micro-moles / kilogram of plasma, (mmol/kg)	Particles per kilogram of solution
Plasma Na^+		micro-moles of sodium / ml plasma, (mmol/ml)	Particles of sodium per kiloliter of solution
Muscle Na^+		micro-moles of sodium / gram of muscle, (mmol/g)	Particles of sodium per gram of solution
Resistance Series	R	ohms (Ω)	Conductance of extra-cellular fluids at low frequency (0 kilohertz)
Reactance Series	Xc	ohms (Ω)	Conductance of extra-cellular and intra-cellular fluids at high frequency (50 kilohertz)
Phase Angle Series	PA	(atan(R / Xc)) * (180/ (pi)), (^{0})	Index of condition based on ratio of R and Xc
Resistance Parallel	R_{para}	(Ω) - R + ((Xc)^2)/ R)	R adjusted for an electrical system arranged in parallel
Reactance Parallel	Xc_{para}	Xc + ((R)^2)/ Xc), ohms (Ω)	Xc adjusted for an electrical system arranged in parallel
Phase Angle Parallel	PA_{para}	(atan(R_{para} / Xc_{para})) * (180/ (pi)), (^{0})	PA adjusted for an electrical system arranged in parallel
Resistance Length	R_{length}	(Length^2)/ R_{para}, ohms (Ω)	R_{para} adjusted for the length of the fish
Reactance Length	Xc_{length}	(Length^2)/ Xc_{para}, ohms (Ω)	Xc_{para} adjusted for the length of the fish
Phase Angle Length	PA_{length}	(atan(R_{length} / Xc_{length})) * (180/ (pi)), (^{0})	PA_{para} adjusted for the length of the fish
Resistance Probe	R_{probe}	(Probe seperation)^2)/ R_{para}, ohms (Ω)	R_{para} adjusted for the probe seperation distance
Reactance Probe	Xc_{probe}	(Probe Seperation^2)/ Xc_{para}, ohms (Ω)	Xc_{para} adjusted for the probe seperation distance
Phase Angle Probe	PA_{probe}	(atan(R_{probe} / Xc_{probe})) * (180/ (pi)), (^{0})	PA_{para} adjusted for the probe seperation distance

was above the lateral line on the antero-posterior (AP) axis lined up with base of the pectoral fin and on the dorso-ventral (DV) axis lined up with the top of the operculum (Figure 1). The posterior probe location was above the lateral line, on the AP axis lined up with beginning of the dorsal fin and on the DV axis lined up with the top of the operculum (Figure 1). The two needles on each probe where inserted parallel to the AP axis. The order of the needles on the probes was positive, negative on the anterior probe, and negative, positive on the posterior probe. The distance between the probes was recorded (mm) and measurements were taken on both the left and right side of each fish. The fish carcass was foil wrapped, placed in a whirlpak, and frozen at -20^{0}C for subsequent organ and muscle tissue processing.

Figure 1. Photos showing positioning of probes for bioelectrical impedance analysis on *F. grandis*. Positive probe is lined up near base pectoral fin even with the top of the operculum, and negative probe was lined up with the front edge of the dorsal fin and the top of the operculum

The BIA measurements taken are resistance (R) and reactance (Xc) and assume the conductance of tissue behaves as an electrical system arranged in series (Cox and Hartman 2005). A third BIA measurement phase angle (PA) is calculated from ratio of R and Xc (Cox and Heintx 2009). R, Xc, and PA are adjusted into three other indices. R_{para}, Xc_{para}, and PA_{Para} are adjusted assuming tissues behave as an electrical system arranged in parallel (Pethig 1984; Cox and Hartman. 2005). R_{para}, Xc_{para}, and PA_{Para} are adjusted for fish length (mm) into R_{length}, Xc_{length}, and PA_{length} and adjusted for probe distances (mm) into R_{probe}, Xc_{probe}, and PA_{probe} (Cox and Hartman 2005; Garner el at. 2012). Metric definitions and equations are listed in Table 2.

3.1.2 Plasma Osmolarity and Plasma Ion Analyses

Blood was collected in order to measure plasma osmolarity (mmol /kg), plasma Na^+ (µmol ml). After measurements with BIA, the tail of the fish was severed posterior to the anal fin and blood was taken in a hematocrit tube from the caudal vein until blood ceases flowing. The scales were removed from the caudal peduncle region and the tail was severed with a scalpel. The blood was collected from the caudal vein with an ammonium heparinized glass capillary tube (Chase Scientific Glass). After blood flow ceased, the tube was closed with Chā-seal tube sealing compound. The tubes were then centrifuged in an IEC Clinical Centrifuge for 10 minutes at 200 *g.* Tubes were stored at 3ºC and processed within 24 hours. For processing, the end of the tube was snapped off and the plasma portion of the blood was extracted with a 100 µl Hamilton gas chromatography syringe. Plasma samples were measured on a Vapro Vapor Pressure Osmometer (Wescor Inc) for osmolarity. Any extra plasma was stored in a 1.5 micro-centrifuge tubes at -20ºC for later ion analysis. Once defrosted, samples were diluted for ion analysis. A small volume (0.5 µl) was pipetted into a 2 ml micro-centrifuge tube containing 2 ml of Millipore water with 5% nitric acid. The samples were analyzed on a Fast Sequential Atomic Absorption Spectrometer (Varian) for Na^+ content.

3.1.3 Lipid Content

Lipid extraction was conducted on muscle tissue and organs from the frozen fish to calculate % organ lipids and % body lipid. Extraction was conducted using a method described by Folch et al. (1957). Muscle tissue for lipid extraction was sampled from the area on the fish between the probe insertions and approximately 1 cm wide. The skin was removed from the area, and the muscle tissue was removed down to caudal vertebrate. All of the organs from the body cavity, from the esophagus to the vent, were removed as well. Tissues dissected from the thawed fish were transferred to clean and pre weighed 50 ml falcon centrifuge tubes and

weighed on a digital micro-scale (mg). An addition of 20 vols (ml /g) of a solution of 2:1 chloroform /methanol was made to each tube. The sample was then homogenized and allowed to set for 1 hour. After setting, 0.2 vols (ml /g) of 0.9% KCL (90 mg KCL /ml deionized H_20) was added to the sample. The samples were thoroughly homogenized and centrifuged for 30 minutes at 100 *g.* A 1 ml sample of the bottom layer of chloroform was removed with a pipette and added to a pre-weighed glass 2 dram vial. The sample was then evaporated under nitrogen, vacuum desiccated overnight, and re-weighed for lipid mass.

3.1.4 Tissue Ion Content

A muscle tissue sample was collected for ion analysis. The sample was dried in a small aluminum tin for 24 hours at 60ºC and dry weight determined (mg). The tissue was then dissolved in 5 ml of nanopure water containing 50% nitric acid at 100ºC for 30 minutes. The sample was then diluted to 10 ml, and a 100 µl portion of the solution was pipetted into a 2 ml micro-centrifuge tubes containing 1.9 ml of Millipore water containing 5% nitric acid. The samples were run on a Fast Sequential Atomic Absorption Spectrometer (Varian) and sodium concentration measured (µmol /g).

3.2 Statistical Analyses

All statistical analysis where conducted in SAS v9.3 (SAS Institute, 2002). Data were checked for normality and outliers using PROC UNIVARIATE. If a data point's studentized residuals exceeded ± 2.2, it was removed from analysis. All reported means and standard errors were calculated using PROC MEANS. A p-value of 0.05 was used as a significance threshold. All variables listed in Table 2 were used for all analyses, unless indicated otherwise. For all studies, Pearson's correlation analysis was run on all 12 BIA measures. When measures were highly correlated (R^2> 0.9), only one measure was retained in further analyses.

Study 1 & 2 – Each study was run separately following the same statistical code. A two-factor ANOVA (sex, day, sex*day), blocking on fish was run to examine differences in all dependent variables (Table 2) using a GLMM (Proc Glimmix) followed by LSMEANS Tukey-Kramer grouping when significant differences were found. To examine the ability to predict significant measures by day, simple linear regressions were run on day with PROC REG for each dependent variable that was significant in the GLMM.

Study 3 & 4 – Each study was run separately following the same statistical code. The temperature and salinity exposure experiments were evaluated as a two-factor (temperature, salinity, temperature*salinity) split plot design, blocking on fish, and tank. High fish mortality in 32ºC /0.2 ppt salinity and 4ºC /34 ppt salinity had the potential to unbalance the results of the experiment, thus, the highest temperature (32ºC) and the highest salinity (34 ppt) were dropped from the analyses. All response variables (Table 2) were examined separately using PROC GLIMMIX with a LSMEANS Tukey-Kramer grouping when significant differences were found.

CHAPTER 4. RESULTS

For all studies, correlation analysis indicated that BIA measurements R, Xc, and PA were highly correlated with their parallel adjusted values, R_{para}, Xc_{para}, and PA_{para} ($R^2 > 0.9$). Only results from R, Xc and PA were reported. For the salinity and temperature exposure studies, Study 2 Wild Caught and Study 3 Lab Conditioned, the goal was to test the sensitivity of BIA's base measurements, R, Xc, and PA, no variables adjusted for parallel, length, or probe separation will be reported.

4.1 Study 1: Wild-caught, Fed Condition Experiment

There was no significant interaction by sex and day, but there were significant differences for single effects of sex and day (Table 3, Figure 2). BIA measures of R, Xc, R_{probe}, Xc_{probe} and PAprobe differed significantly by sex with females in all cases having higher values as compared to males. No other measured variable showed a significant difference between sexes. In contrast, almost all measured metrics showed a significant difference by day; only length, weight, and PAlength failed to demonstrate a significant difference between days. Kn, % organ and body lipid, and Xc and PA increased throughout the experiment; remaining variables decreased over time (Figure 2).

4.2 Study 2: Wild-caught, Fasted Condition Experiment

There was no significant interaction by sex and day, and there were no significant differences between sexes for any measured variables. By day, almost all variables differed

Table 3. ANOVA of fish condition metrics and BIA measurements by day for fed experiment. Numerators, denominators, F-values, and p-values are given for the model with Sex and Day interaction, then Sex and Day with interaction removed. Significant p-values are bold; units for each metric are reported in Table 2. Percent organ and body lipid were only measured on fish on Day 0 and 28.

	Sex and Day Interaction				By Sex				By Day			
Metric	Num.	Den.	F Value	Pr > F	Num.	Den.	F Value	Pr > F	Num.	Den.	F Value	Pr > F
L	4	95.0	0.95	0.44	1	99.0	2.87	0.09	4	99.0	0.64	0.63
W	4	95.0	0.97	0.43	1	99.0	2.69	0.10	4	99.0	0.6	0.67
Kn	4	24.7	0.71	0.59	1	99.0	0.13	0.72	4	99.0	27.18	**<.01**
OrganLipid %	1	2.2	4.59	0.15	1	5.2	3.41	0.12	1	5.2	45.12	**<.01**
Body Lipid %	1	4.3	2.33	0.20	1	5.1	3.13	0.14	1	5.1	59.14	**<.01**
Osmolarity	4	7.6	0.39	0.81	1	4.7	0.02	0.89	4	29.9	5.62	**<.01**
R	4	40.9	0.73	0.58	1	5.5	7.23	**0.04**	4	41.6	3.14	**0.02**
Xc	4	8.3	1.44	0.30	1	12.8	13.93	**<.01**	4	13.2	44.54	**<.01**
PA	4	93.6	2.43	0.05	1	1.3	3.41	0.27	4	5.9	116.96	**<.01**
R_{para}	4	41.2	0.82	0.52	1	99.0	11.85	**<.01**	4	99.0	2.47	**<.05**
Xc_{para}	4	93.9	1.14	0.34	1	5.2	0.76	0.42	4	34.9	40.45	**<.01**
PA_{para}	4	93.6	2.43	0.05	1	1.3	3.41	0.27	4	5.9	116.96	**<.01**
R_{length}	4	38.3	2.22	0.09	1	10.5	3.54	0.09	4	11.4	16.02	**<.01**
Xc_{length}	4	7.2	1.71	0.25	1	11.9	15.38	**<.01**	4	10.1	40.98	**<.01**
PA_{length}	4	95.0	0.68	0.61	1	99.0	0.62	0.43	4	99.0	23.6	**<.01**
R_{probe}	4	7.9	1.82	0.22	1	11.8	0.62	0.45	4	12.3	5.12	**0.01**
Xc_{probe}	4	8.3	1.44	0.30	1	12.8	13.93	**<.01**	4	13.2	44.54	**<.01**
PA_{probe}	4	95.0	0.57	0.68	1	99.0	0.46	0.50	4	99.0	3.04	**0.02**

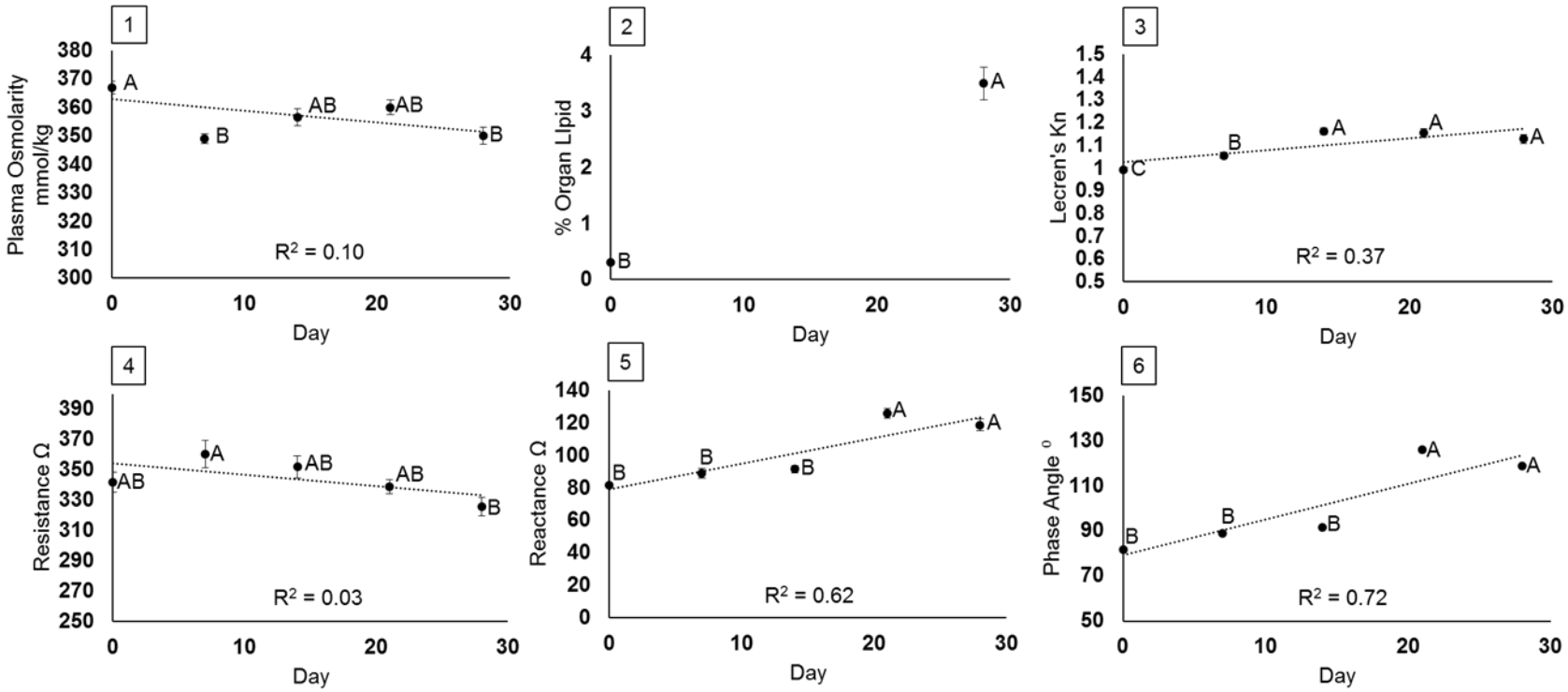

Figure 2. Results of change in (1) plasma osmolarity (mOsm), (2) % organ lipid, (3) Lecren's Kn, (4) resistance (Ω), (5) reactance (Ω) and (6) phase angle (^{0}) by day for Study 1, measuring the change in condition in Fundulus grandis while fed twice daily held in temperature controlled (24 ^{0}C) and salinity controlled (12 ppt) tanks for 28 days. Points are means with standard error bars. Within each individual graph, different letters indicate significant differences indicated by generalized linear models by day. Linear trend lines are placed on graphs which had a significant linear regression with the calculated R^2 for the line located on the graph. Regression was not run on % lipid because only two data points were taken.

significantly, with most variables, weight, Lecren's condition, % organ lipid, and % body lipid decreasing Osmolarity was significantly higher on Day 1 as compared to all subsequent days (Figures 3).throughout the experiment (Table 4). BIA measures of Xc, PA, Rlength, Rprobe, and Xcprobe were significantly different with a decrease on day 14, where R had a significant peak (Figure 3).

4.3 Study 3: Wild-caught, Acute Salinity x Temperature Exposure Experiment

Muscle ion concentrations had a significant interaction for temperature and salinity, but no other measured variables showed interaction (Table 5). There were significant temperature and salinity single effects for R, Xc, and PA. R and Xc decreased with increasing salinity, while PA increased with increasing salinity (Figure 4). For temperature, R and PA differed significantly, decreasing with increasing temperature (Figure 5). Osmolarity is not reported due to low sample collection numbers.

4.4 Study 4: Lab-Conditioned, Acute Salinity x Temperature Exposure Experiment

There was no significant temperature by salinity interaction. Plasma osmolarity and R differed significantly by salinity. The lowest salinity, 2, affected both variables, with an increase in plasma osmolarity and a decrease in R (Table 6, Figure 6). R differed significantly by temperature and was significantly lower at the highest temperature treatment as compared to all other temperatures (Figure 7). No other variables differed significantly between treatments.

Table 4. ANOVA of fish condition metrics and BIA measurements by day for fasted experiment. Numerators, denominators, F values, and p-values are given for the model with Sex and Day interaction, then Sex and Day with interaction removed. Significant p-values are bold; units for each metric are reported in Table 2. Percent organ and body lipid were only measured on fish on Day 0 and Day 28.

	Sex and Day Interaction				**By Sex**				**By Day**			
Metric	**Num.**	**Den.**	**F Value**	**Pr > F**	**Num.**	**Den.**	**F Value**	**Pr > F**	**Num.**	**Den.**	**F Value**	**Pr > F**
L	4	7.8	1.54	0.28	1	8.8	3.07	0.11	4	8.8	1.87	0.20
W	4	7.2	1.71	0.25	1	8.2	0.95	0.36	4	8.3	4.2	**0.04**
Kn	4	6.9	0.08	0.99	1	2.5	3.74	0.17	4	8.1	13.99	**<.01**
OrganLipid %	1	3.4	3.16	0.16	1	4.3	0.7	0.44	1	3.9	26.46	**0.01**
Body Lipid %	1	3.5	0.18	0.69	1	4.7	1.17	0.33	1	4.3	6.39	0.06
Osmolarity	4	6.6	0.37	0.82	1	11.9	0.39	0.54	4	10.2	31.83	**<.01**
R	4	126.0	0.47	0.76	1	2.2	2.06	0.28	4	126.2	5.21	**<.01**
Xc	4	131.4	0.61	0.65	1	2.4	0.15	0.73	4	133.1	5.98	**<.01**
PA	4	5.4	1.69	0.28	1	11.9	1.63	0.23	4	8.5	23.11	**<.01**
R_{length}	4	126.8	0.24	0.92	1	2.3	0.5	0.54	4	129.7	1.14	0.34
Xc_{length}	4	127.8	2.42	0.05	1	2.0	0.11	0.77	4	9.3	12.67	**<.01**
PA_{length}	4	8.2	1.7	0.24	1	9.0	2.58	0.14	4	9.1	2.19	0.15
R_{probe}	4	126.6	0.15	0.96	1	1.0	0.68	0.56	4	110.3	2.69	**0.03**
Xc_{probe}	4	131.4	0.61	0.65	1	2.4	0.15	0.73	4	133.1	5.98	**<.01**
PA_{probe}	4	6.3	0.49	0.75	1	22.9	0.89	0.35	4	148.8	0.31	0.87

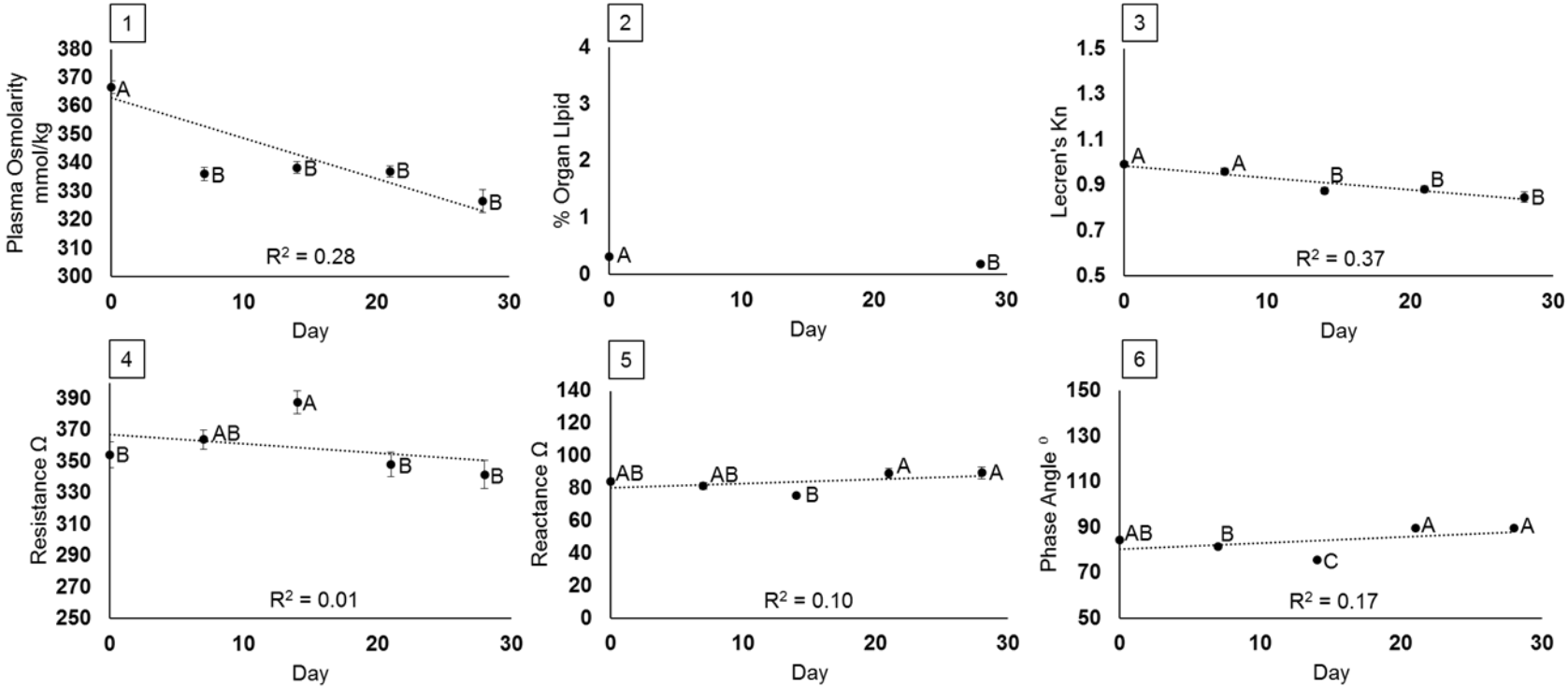

Figure 3. Results of change in (1) plasma osmolarity (mOsm), (2) % organ lipid, (3) Lecren's Kn, (4) resistance (Ω), (5) reactance (Ω) and (6) phase angle (^{0}) by day for Study 2, measuring the change in condition in Fundulus grandis while fasted and held in temperature controlled (24 ^{0}C) and salinity controlled (12 ppt) tanks for 28 days. Points are means with standard error bars. Within each individual graph, different letters indicate significant differences indicated by generalized linear models by day. Linear trend lines are placed on graphs which had a significant linear regression with the calculated R^2 for the line located on the graph. Regression was not run on % lipid because only two data points were taken.

Table 5. ANOVA of fish condition metrics and BIA measurements by salinity and temperature for Study 3, wild-caught exposure. Numerators, denominators, F-values, and p-values are given for the model with temperature and salinity interaction, then salinity and temperature with interaction removed. Significant p-values are bold; units for each metric are reported in Table 2. Muscle Na was showed significant interaction with temperature and salinity, and was not modeled further. Plasma osmolarity and Na was not analyzed due to low sample numbers

	Temp. and Sal. Interaction				**Salinity 2,12,24**				**Temperature 4,12,24**			
Metric	**Num**	**Den.**	**F Valu**	**Pr > F**	**Num**	**Den.**	**F Valu**	**Pr > F**	**Num**	**Den.**	**F Valu**	**Pr > F**
L	4	27.0	0.39	0.81	2	31.0	0.87	0.43	2	31.0	0.06	0.94
W	4	27.0	0.61	0.66	2	31.0	1.09	0.35	2	31.0	0	1.00
Kn	4	8.6	1.33	0.33	2	12.7	0.17	0.85	2	12.7	2.36	0.13
OrganLipid %	4	9.0	1.04	0.44	2	13.1	0.45	0.65	2	13.1	1.85	0.20
Body Lipid %	4	8.7	0.92	0.49	2	13.1	1	0.39	2	13.1	1.8	0.20
Muscle Na^+	4	27.0	4.07	**0.01**								
R	4	8.6	1.73	0.23	2	11.5	6.67	**0.01**	2	11.5	6.11	**0.02**
Xc	4	8.9	2.48	0.12	2	12.6	7.36	**0.01**	2	12.6	0.4	0.68
PA	4	8.8	2.27	0.14	2	13.0	4.65	**0.03**	2	13.0	3.8	0.05

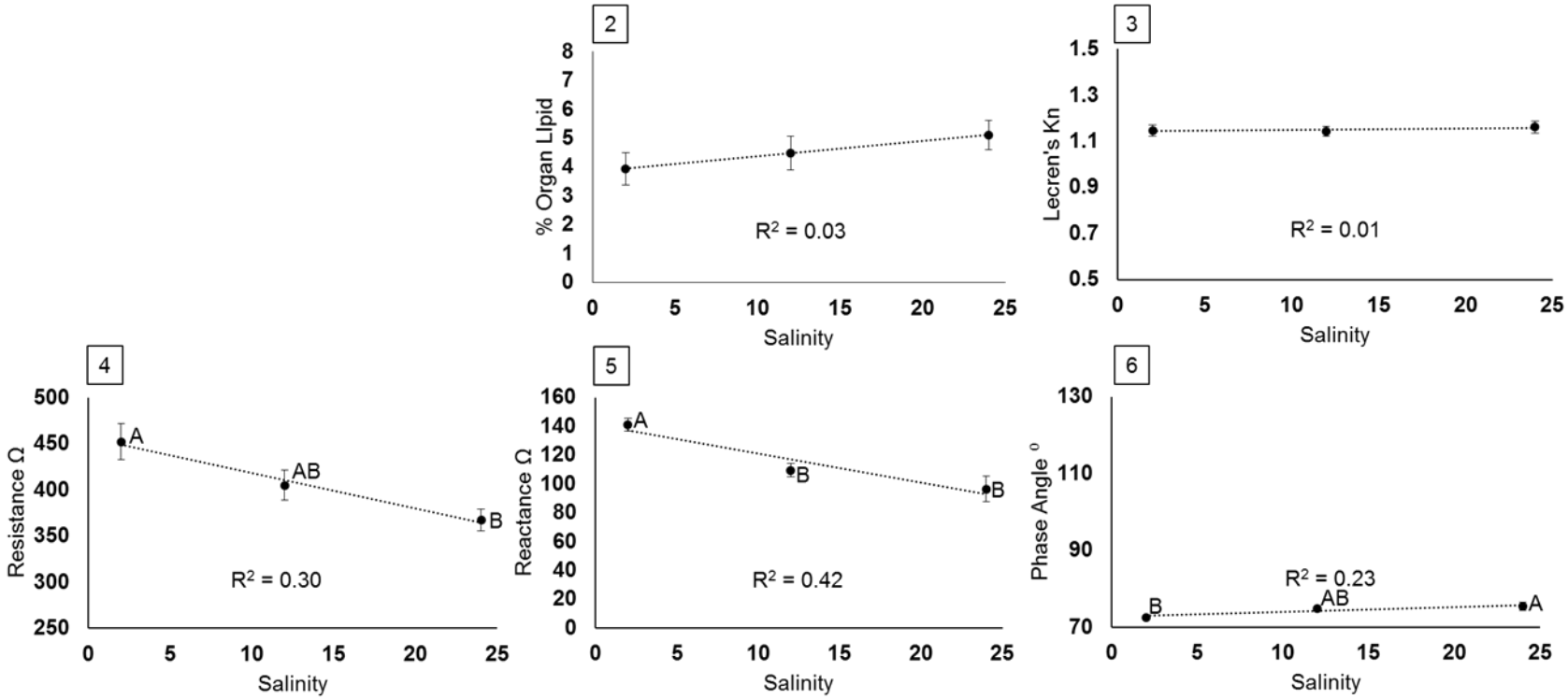

Figure 4. Results of change in (2) % organ lipid, (3) Lecren's Kn, (4) resistance (Ω), (5) reactance (Ω) and (6) phase angle (^{0}) by salinity (ppt) for Study 3 wild-caught exposure, measuring the effects of acute exposure to changes in temperature and salinity. Points are means with standard error bars. Within each individual graph, different letters indicate significant differences indicated by generalized linear models by salinity. Linear trend lines are placed on graphs which had a significant linear regression with the calculated R^2 for the line located on the graph. There were too few plasma samples collected in this experiment to analyze plasma osmolarity and Na^2.

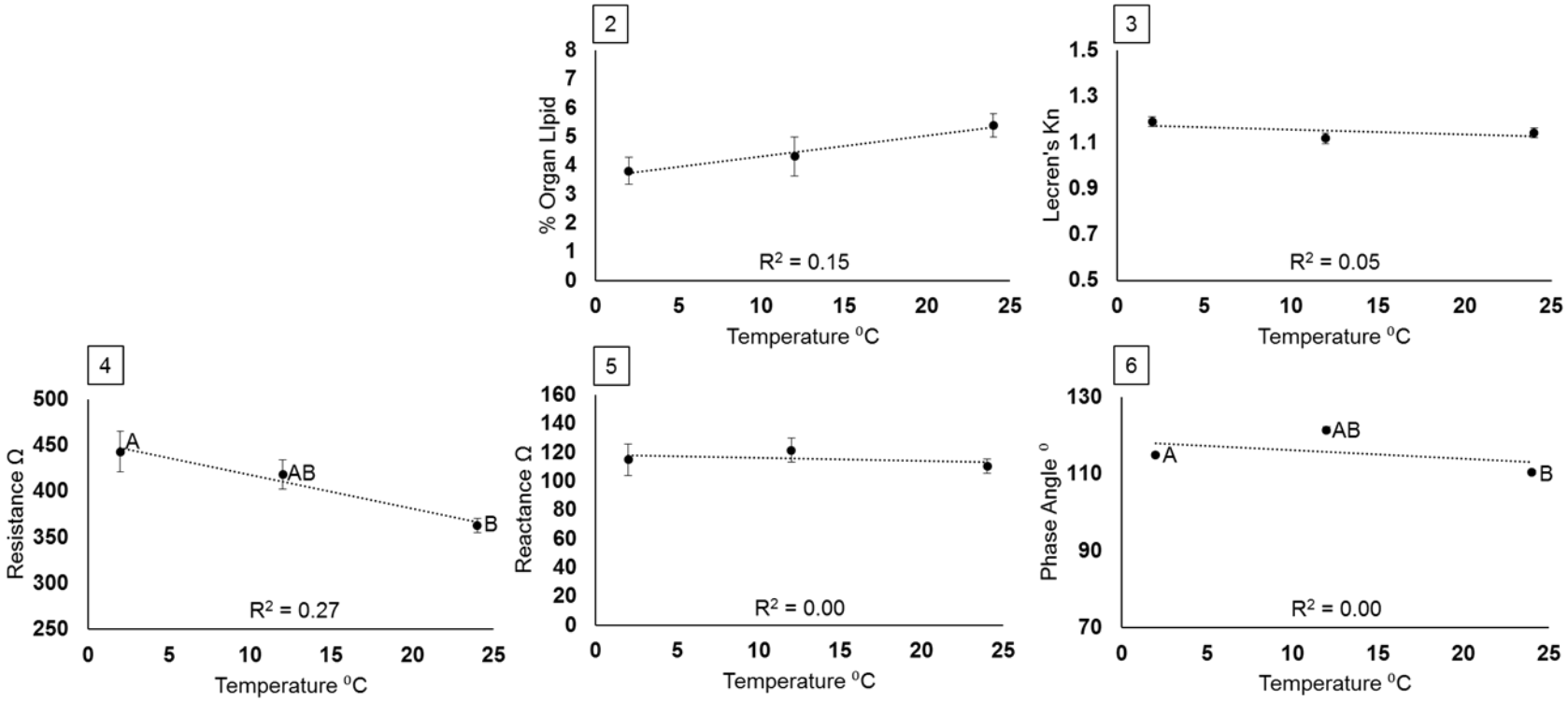

Figure 5. Results of change in (2) % organ lipid, (3) Lecren's Kn, (4) resistance (Ω), (5) reactance (Ω) and (6) phase angle (^{0}) by temperature for Study 3 wild-caught exposure, measuring the effects of acute exposure to changes in temperature and salinity. Points are means with standard error bars. Within each individual graph, different letters indicate significant differences indicated by generalized linear models by temperature. Linear trend lines are placed on graphs which had a significant linear regression with the calculated R^2 for the line located on the graph. Plasma osmolarity was not analyzed due to low sample numbers.

Table 6. ANOVA of fish condition metrics and BIA measurements by salinity and temperature for Study 4, lab-conditioned exposure. Numerators, denominators, F-values, and p-values are given for the model with temperature and salinity interaction, then salinity and temperature with interaction removed. Significant p-values are bold; units for each metric are reported in Table 2.

	Temp. and Sal. Interaction				**Salinity 2,12,24**				**Temperature 4,12,24**			
Metric	**Num**	**Den.**	**F Valu**	**Pr > F**	**Num**	**Den.**	**F Valu**	**Pr > F**	**Num**	**Den.**	**F Valu**	**Pr > F**
L	4	27	0.9	0.48	2	31	0.39	0.68	2	31	0.23	0.79
W	4	27	1.22	0.33	2	31	0.98	0.39	2	31	0.11	0.90
Kn	4	27	0.51	0.73	2	31	0.83	0.45	2	31	0.04	0.96
OrganLipid %	4	27	0.5	0.73	2	31	0.53	0.59	2	31	0.05	0.95
Body Lipid %	4	9	0.1	0.98	2	13	0.75	0.49	2	13	1.25	0.32
Osmolarity	4	21	0.33	0.86	2	25	7.71	**<.01**	2	25	1.2	0.32
Plasma Na^+	3	4.2	0.77	0.57	2	8.0	0.98	0.42	2	9.3	0.14	0.87
Muscle Na^+	4	27	1.68	0.18	2	13	2.4	0.13	2	13	2.21	0.15
R	4	9	1.34	0.33	2	13	3.21	0.07	2	13	10.4	**<.01**
Xc	4	9	1.18	0.38	2	13	5.5	**0.02**	2	13	1.1	0.36
PA	4	9	0.65	0.64	2	13	3.62	0.06	2	13	0.37	0.69

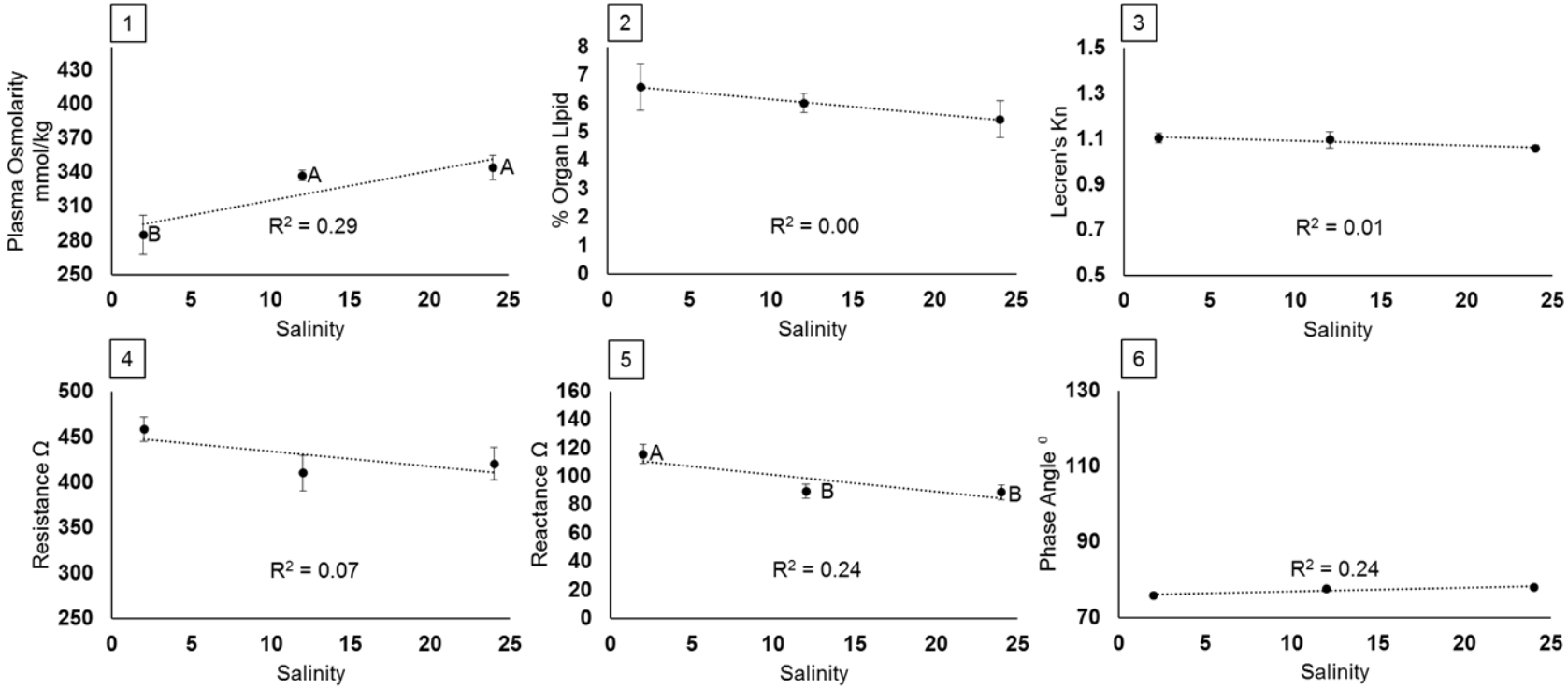

Figure 6. Results of change in (1) plasma osmolarity (mOsm), (2) % organ lipid, (3) Lecren's Kn, (4) resistance (Ω), (5) reactance (Ω) and (6) phase angle (^{0}) by salinity (ppt) for Study 4 lab-conditioned exposure, measuring the effects of acute exposure to changes in temperature and salinity. Points are means with standard error bars. Within each individual graph, different letters indicate significant differences indicated by generalized linear models by salinity. Linear trend lines are placed on graphs which had a significant linear regression with the calculated R^2 for the line located on the graph.

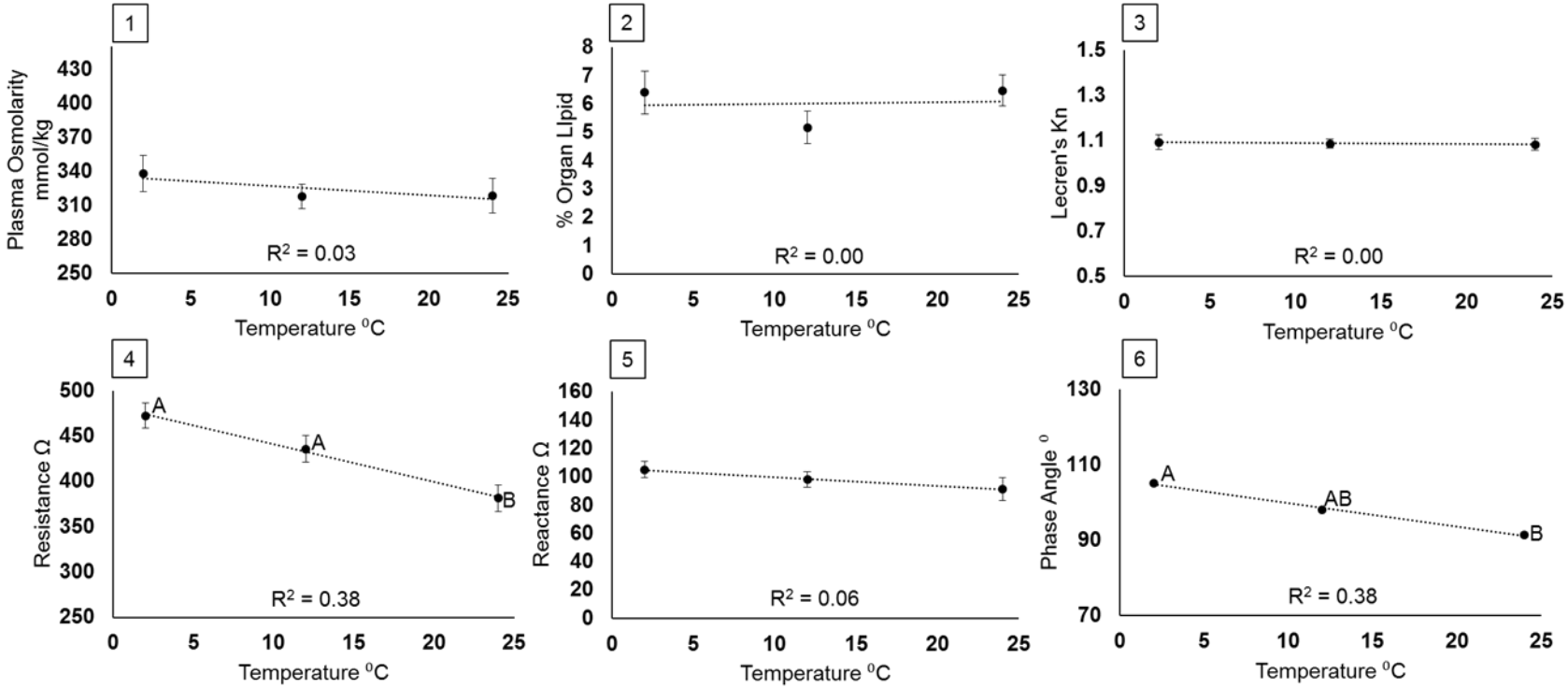

Figure 7. Results of change in (1) plasma osmolarity (mOsm), (2) % organ lipid, (3) Lecren's Kn, (4) resistance (Ω), (5) reactance (Ω) and (6) phase angle (^{0}) by temperature for Study 4 lab-conditioned exposure, measuring the effects of acute exposure to changes in temperature and salinity. Points are means with standard error bars. Within each individual graph, different letters indicate significant differences indicated by generalized linear models by temperature. Linear trend lines are placed on graphs which had a significant linear regression with the calculated R^2 for the line located on the graph.

CHAPTER 5. DISCUSSION AND CONCLUSION

BIA has potential to be an effective index of condition, but the technique's sources of variance need to be better understood (Cox et al. 2011). This study examined how initial condition of wild-caught fish could influence BIA results, as well as how environmental variation common to estuarine environments, of salinity and temperature might influence outcomes. Overall, BIA measures appeared to accurately track large changes in condition, but may be sensitive in some cases to large differences in individual fish size, lipid composition and plasma osmolarity. Furthermore, the acute salinity and temperature experiments indicated that BIA measures may be sensitive to changes in salinity and temperature. Plasma osmolarity, which may adjust to both environmental salinity and fish stress (Barton et al., 1986; Zadunaisky et al., 1995; Kammerer et al., 2010), may be one source of variance that needs to be better understood. Ultimately, the goal for a tool such as BIA is to ensure its accuracy and ability to track meaningful changes in condition of wild fish; if these sources of variance are shown to be easily correctable BIA may prove to be an effective non-lethal and rapid means to index condition of wild-caught individuals, and contribute to environmental assessments.

Wild-caught fish reflect the local environment in which they are caught; to promote BIA as a potential tool for monitoring changes in the local environment, BIA should be sensitive across the range of environments and conditions in which the fish may be found. In the fed and fasted experiments, BIA measurements reflected large changes in condition that could be expected in the field, but were not sensitive enough to register small changes. Specifically, while the fed experiment showed clear effects of increasing condition over time as Lecren's Kn, % body lipid, % organ lipid, and BIA measurements all increased through the experiment, and phase angle and reactance were highly correlated to % lipid measurements. In contrast, the fasted experiment, in which a decline in condition was expected, both Lecren's Kn and % organ

lipid indicated a decrease in condition, however BIA measures changed only minimally and failed to indicate a significant decline in condition (R = -15; Xc=+8; PA= +4).

The sensitivity of BIA to changes in condition when fish are at lower conditions may be a critical aspect related to the ability of BIA to be used on wild-caught organisms. The major difference between the fed and fasted experiments was the amount of change. Fish which were exposed to fasting experienced a 34% decrease in % organ lipid over 28 days, while fed saw an 1103% increase in % organ lipid over 28 days. While an increase in condition of wild-caught fish fed twice a day could be detected, further declines in fish condition due to environmental degradation may be difficult to detect. Although a lack of long-term environmental monitoring within the estuary where the fish were collected makes it difficult to say with certainty, there is no reason to suspect that the fish collected and used in these experiments were in poor condition or highly stressed.

The sex of a fish is often considered a possible source of variance in condition, due to females growing larger and faster than males in some species and allocating energy to egg production (Poole and Reynolds 1996; Henderson et al. 2003; Morita et al. 2012; Hendry et al. 1999). Sex was a variable in two of our experiments (fed, fasted), and while only significant in the fed experiment, this was the only experiment that showed a meaningful change in condition with BIA measures. As the length, weight, Lecren's and % lipids between the sexes in the fed experiment were similar, a potential explanation of the difference in BIA measures between the two sexes could be potentially attributed to the reproductive status of the females, but this was not determined. No other study of BIA accounted for the differences between sexes, though many studies report using salmonids which exhibit sex based morphological differences (Quinn and Foote 1994; Young 2005). To better understand the variables effecting BIA, sex must be accounted for in fish studies, and to use BIA in the field would require the selection of indicator fish species where sex can be easily determined.

Traditional condition measurements such as Lecren's Kn adjust for size to create an index of condition that is comparable for fish of different sizes (Blackwell et al., 2000). Many studies have suggested that fish size (length, weight) may need to be corrected for in BIA measures (Cox and Hartmann 2005, Hartman et al. 2011). The size of a conductor does affect some of its electrical properties, and fish size could influence BIA measures through the same principles (Lukaski et al., 1986; Cox et al., 2011). Previous studies have included multiple calibrating calculations using length or probe separation in order to correct for this influence and better fit data (Cox and Hartman 2005; Hafs and Hartman 2011; Cox et al., 2011). These and other studies have suggested that calibrations for size could contribute to BIA's high predictive power for proximate body composition measurements such as total lipid or total protein which are not adjusted for the size of the subject (Bosworth and Wolters 2001; Pothoven et al. 2008; Klefoth et al., 2013). However, from all four studies reported here, correcting for probe length or fish total length failed to provide any better measure of condition than the series measurements. Interestingly, when data collected for our two condition experiments were separated by size into large (fish > mean TL) and small (fish < mean TL) categories, BIA measures, R, Xc, and PA were all significantly higher for small fish, despite no significant difference for % organ or body lipid (Appendix E). Furthermore, BIA measurements corrected for size, R_{length}, Xc_{length}, PA_{length} and R_{probe}, Xc_{probe}, PA_{probe}, still differed significantly between these size classes, despite supposedly having adjusted for length. Similar to our investigation, studies that investigated BIA's predictive power for body composition measurements relative to size such as % dry weight, % lipid, and energy density (J/g) showed BIA to be less reliable (Pothoven et al. 2008; Garner et al. 2012; Klefoth et al. 2013).

While fish condition, size, sex are all important considerations for BIA, in estuarine environments in particular, local salinity and temperature fluctuations are common and their potential effects on BIA measures need to be understood. For example, if fish are exposed to a freshet event (large drop in salinity over a short period of time), or a storm front increasing

salinity rapidly, immediately prior to sampling, will this effect BIA measures? This is important as it might guide timing of sampling. Acute exposure to changes in temperature and salinity exposure in the lab found BIA sensitive to environmental variables, indicating the need for a better understanding of sources of variance and correction factors for BIA.

In our exposures there were mortalities for the salinity 2 ppt and temperature 34 ºC and the salinity 36 ppt and temperature 4ºC for both the wild and lab conditioned fish. The salinity and temperature treatments separately are within the ranges seen in habitats in which *F. gran*dis survives (Simpson and Gunter 1956). Acclimating to 36 ppt should not cause mortality in *F. grandis*, but a temperature of 4ºC can inhabit osmoregulation and combine with changing salinity to cause death (Kidder et al. 2006). Mortality at the low salinity and high temperature we assume was caused by the energy budget of the fish being exceeded. Acclimating to 2 ppt is more taxing for *F. grandis* and coping with higher temperatures is energetically expensive, (Lankford and Targett 1994; Patterson et al. 2012). No mortality was seen in the other combinations of treatments.

Temperature had a negative effect on BIA measurements, decreasing values, similar to other acute temperature exposure studies (Hartman et al. 2011; Klefoth et al. 2013). Corrections for temperature's effect on BIA have been suggested in the range of approximately -2.5% per 1ºC, but were estimated from only two temperatures which fail to cover the range of temperatures commonly experienced in Gulf Coast estuaries (Hartman et al., 2011). Two notable difference in our study compared to other studies was that (1) we exposed the fish to a range of temperatures, and (2) the temperature effect on BIA measurements were only seen in the series measurements of R. Taking the same approach as Hartman et al. (2011), our estimated correction factor would be a linear decrease in R with temperature by 0.85) – 0.92 % per 1ºC. Differences between our findings and Hartman et al. (2011) may be due reflect several variables, including (1) species differences, (2) fish size, or (3) range of temperatures tested;

size might be a factor as resistance is assumed to be affected by the volume of the conductor (Lukaski et al. 1986).

Additionally, previous temperature studies used adjustments to BIA measurements to better fit the theoretical parallel and not series electrical arrangement of tissues (Cox and Hartman., 2005; Cox and Heintx 2009; Hartman et al. 2011; Klefoth et al. 2013). The adjustment for parallel arrangement uses the Xc value in the adjustment of R and vice versa (Cox and Hartman. 2005). This may be why both R and Xc were significantly affected by temperature in other studies and could lead to improper corrections for temperature. Our study suggests temperature has a moderate negative effect on R measurements, and corrections should occur before parallel adjustments, where only R is affected.

BIA measures were also found to differ significantly by salinity exposure; this is extremely important when considering the use of BIA on estuarine fish with a salinity tolerance form 0 to 70 ppt. Specifically, an increase in salinity negatively affected R and Xc, and positively affected PA and plasma osmolarity. A study of a species similar to *F. grandis* has shown that when exposed to a change in salinity there is a short period of homeostasis deviation before osmoregulation begins (Zandunaisky et al. 1995). For sampling of estuarine fish where salinity is variable, this might indicate that either a correction factor needs to be developed for sampling across salinities, or sampling needs to be always timed within a period of similar and steady salinity regimes.

Plasma osmolarity may indicate a source of variance for BIA. Studies of humans have shown BIA measurements to be sensitive to changes in body fluids (Shanholtzer and Patterson 2003; Sengun et al. 2012). The conductance of fluids in a body has been shown to be sensitive to status changes such as dehydration, pregnancy, and illness (Shime et al. 2002; Schanholtzer and Patterson 2003; Rezac 2008). It has been shown that concentrations of amino acids, proteins, and ions in blood plasma can affect the fluid's conductance (Pethig

1979; Cha et al., 1994; Singh et al. 1998). In our fed, fasted, and lab conditioned salinity experiments, plasma osmolarity was significantly different by each treatment. No other BIA studies of fish monitored for changes in body fluid content or concentrations. As changes in plasma osmolarity and blood chemistry can be triggered by a range of events, including stress and environmental cues (Barton et al. 1996; Ciereszko et al 1997; Bjornsson 1997), it is a variable that should be more carefully monitored when assessing BIA results.

The results of this study indicate the sensitivity of BIA to a range of variables. Environmental factors and changes in actual body condition exert a strong influence over the sensitivity of BIA measures. Assessing organism condition is not a simple task and the use of multiple indexes is often suggested for accurate work (Gilliers et al. 2004; Adams 2005; Piazza and La Peyre 2010). BIA may prove to be a powerful tool particularly given that it is cheap, non-lethal and easy; however, to ensure its accuracy, all sources of variance for BIA must be identified. Its sensitivity to temperature and salinity may not make BIA ideal for work in estuaries due to fluctuations in these water characteristics in those systems. Future studies should focus on the physiological details that are affecting BIA measurements.

LITERATURE CITED

Adams, S. M. 2005 Using multiple response bioindicators ot assess the health of estuarine exosystems: an operational frame work. In Estuarine Indicators Bortone, Stephen A (ed.) pg. 5-16

Adams, S. M., M. S. Bevelhimer, J. M. S. Greeley, D. A. Levine, and S. J. Teh. 1999. Ecological risk assessment in a large river--reservoir: 6. Bioindicators of fish population health. Environmental Toxicology & Chemistry **18**:628.

American Sportsfishing Association. 2002. Sportfishing in America, Values of our Tradional Pasttime. http://asafishing.org

Bagatto. B., B. Pelster, and W. W. Burggren. 2001. Growth and metabolism of larval zebrafish: effects of swim training. Journal of Experimental Biology **204**: 4335-4343

Barton, B. A., C. B. Schreck, and L. A. Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Transactions of the American Fisheries Society **115**:245-251.

Bervoets, L. and R. Blust. 2003. Metal concentrations in water, sediment and gudgeon (Gobio gobio) from a pollution gradient: relationship with fish condition factor. Environmental Pollution **126**:9-19.

Bjornsson, B. T. 1997. The biology of salmon growth hormone: from daylight to dominance. Fish Physiology and Biochemistry **17**:9-24.

Blackwell, B. G., M. L. Brown, and D. W. Willis. 2000. Relative weight (Wr) status and current use in Fisheries assessment and management. Reviews in Fisheries Science **8:1-44**.

Booth, M. T. and A. J. Shipley. 2012. Spatial dynamics and growth of two native species of catostomids: are movements restricted? Southwestern Naturalist **57**:248-256.

Bosworth, B. G. and W. R. Wolters (2001). "Evaluation of Bioelectric Impedance to Predict Carcass Yield, Carcass Composition, and Fillet Composition an Farm-Raised Catfish." Journal of the World Aquaculture Society **32**(1): 72-78.

Bowen, Jenna N.; Jian G. Qin; David A. J. Stone. 2013. Protein, lipid and energy requirements of cultured marine fish in cold, temperate and warm water. Reviews in Aquaculture **5**:10-32.

Brill, R. W., H. Dewar, and J. B. Graham. 1994. Basic concepts relevant to heat-transfer in fishes, and their use in measuring the physiological thermoregulatory abilities of tunas. Environmental Biology of Fishes **40**:109-124.

Brown, M. L. and B. R. Murphy. 1991. Relationship of relative weight (Wr) to proximate composition of juvenile striped bass and hybrid striped bass. Transactions of the American Fisheries Society **120**:509-518.

Burrell GA, Siebert FM. 1916. Gases found in coal mines. Miner's Circular 14.BureauMines, US Department of Interior, Washington, DC.

Caldarone, E. M., S. A. MacLean, and B. Sharack. 2012. Evaluation of bioelectrical impedance analysis and Fulton's condition factor as nonlethal techniques for estimating short-term responses in postsmolt Atlantic salmon (Salmo solar) to food availability. Fishery Bulletin **110**:257-270.

Ciereszko, R. E., K. Dabrowski, A. Ciereszko, J. Ebeling, and J. S. Ottobre. 1997. Effects of temperature and photoperiod on reproduction of female yellow perch *Perca flavescens*: Plasma concentrations of steroid hormones, spontaneous and induced ovulation, and quality of eggs. Journal of the World Aquaculture Society **28**:344-356.

Cone, R. S. 1989. The need to reconsider the use of condition indexes in fishery science. Transactions of the American Fisheries Society **118**:510-514.

Copeland, T., B. R. Murphy, and J. J. Ney. 2008. Interpretation of relative weight in three populations of wild bluegills: A cautionary tale. North American Journal of Fisheries Management **28**:368-377.

Cox, M. K. and K. J. Hartman. 2005. Nonlethal estimation of proximate composition in fish. Canadian Journal of Fisheries and Aquatic Sciences **62**:269-275.

Cox, M. K. and R. Heintz. 2009. Electrical phase angle as a new method to measure fish condition. Fishery Bulletin **107**:477-487.

Cox, M. K., R. Heintz, and K. Hartman. 2011. Measurements of resistance and reactance in fish with the use of bioelectrical impedance analysis: sources of error. Fishery Bulletin **109**:34-47.

Crockett, E.L. and R. L. Londraville. 2005. Temperature. pp. 231-269. In: The Physiology of Fishes 3rd Edition. Ed. by D.H. Evans abd J.B. Crc Press, Boca Raton, FL.

Devries, A. L. and Wohlschl.De. 1969. Freezing resistance in some antarctic fishes. Science **163**:1073-&.

Diana, J. S. 1995. Biology and Ecology of Fishes. Cooper Publishing Group. Michigan

Dibble, K. L. and L. A. Meyerson. 2012. Tidal flushing restores the physiological condition of fish residing in degraded salt marshes. Plos One **7**.

dos Anjos, N. A., T. Schulze, W. Brack, A. L. Val, K. Schirmer, and S. Scholz. 2011. Identification and evaluation of cyp1a transcript expression in fish as molecular biomarker for petroleum contamination in tropical fresh water ecosystems. Aquatic toxicology **103**:46-52.

Duncan, M., S. R. Craig, A. N. Lunger, D. D. Kuhn, G. Salze, and E. McLean. 2007. Bioimpedance assessment of body composition in cobia *Rachycentron canadum* (L. 1766). Aquaculture **271**:432-438.

Elliott, J. M. 1976. The energetics of feeding, metabolism, and growth of brown trout (*Salmo trutta L*.) in relation to body weight, water temperature and ration size. Journal of Animal Ecology **45**: 923-948

Evans, M. S., G. E. Noguchi, and C. P. Rice. 1991. The biomagnification of polychlorinated-biphenyls, toxaphene, and ddt compounds in a lake-michigan offshore food web. Archives of Environmental Contamination and Toxicology **20**:87-93.

Febry, R. and P. Lutz. 1987. Energy partitioning in fish - the activity-related cost of osmoregulation in a euryhaline cichlid. Journal of Experimental Biology **128**:63-85.

Federal Water Pollution Control Act (Clean Water Act) (33 U.S.C. 1251 - 1376; Chapter 758; P.L. 845, June 30, 1948; 62 Stat. 1155).

Folch, J., M. Lees, G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues, Journal of Biological Chemistry **226**:497-509.

Fox, B. K., L. G. Riley, T. Hirano and E. G. Grau. 2006. Effects of fasting on growth hormone, growth hormone receptor, and insulin-like growth factor-I axis in seawater-acclimated tilapia. General and Comparative Endocrinology **147**: 340-347.

Fritz, E.S. and E.T.Garside. 1974. Salinity preferences of *Fundulus heteroclitus* and *F. diaphanus* (Pices: Cyprinodontidae): their role in geographic distribution. Canadian Journal of Zoology **52**:997-1003.

Gagnon, M. M., D. Bussieres, J. J. Dodson, and P. V. Hodson. 1995. White sucker (Catostomus commersoni) growth and sexual maturation in pulp mill-contaminated and reference rivers. Environmental Toxicology and Chemistry **14**:317-327.

Germian, P. and G. Pinte. 1990. NP-237 In the marine-environment determination in animal and plant-species in the english-channel - biological indicators and trophic relationships. Journal of Radioanalytical and Nuclear Chemistry-Articles **138**:49-61.

Gilliers, C., O. Le Pape, Y. Desaunay, J. Morin, D. Guerault, and R. Amara. 2006. Are growth and density quantitative indicators of essential fish habitat quality? An application to the common sole Solea solea nursery grounds. Estuarine Coastal and Shelf Science **69**:96-106.

Gray, James R. 2004. Conductivity Analyzers and Their Application. In Down, R.D; Lehr, J.H. Environmental Instrumentation and Analysis Handbook. Wiley. pp. 491–510.

Green, A. J. 2001. Mass/length residuals: Measures of body condition or generators of spurious results? Ecology (Washington D C) **82**:1473-1483.

Grier, J. W. 1982. Ban of ddt and subsequent recovery of reproduction in bald eagles. Science **218**:1232-1235.

Gunasekera, R.M.; K.F. Shim; T.J. Lam. 1995. Effect of dietary-protein level on puberty, oocyte growth and egg chemical-composition in the tilapia, *Oreochromis niloticus*. Aquaculture **134**:169-183.

Hafs, A. W. and K. J. Hartman. 2011. Influence of electrode type and location upon bioelectrical impedance analysis measurements of brook trout. Transactions of the American Fisheries Society **140**:1290-1297.

Hanson, K. C., K. G. Ostrand, A. L. Gannam, and S. L. Ostrand. 2010. Comparison and validation of nonlethal techniques for estimating condition in juvenile salmonids. Transactions of the American Fisheries Society **139**:1733-1741.

Hartman, K. J., B. A. Phelan, and J. E. Rosendale. 2011. Temperature effects on bioelectrical impedance analysis (bia) used to estimate dry weight as a condition proxy in coastal bluefish. Marine and Coastal Fisheries **3**:307-316.

Hayashi, M. 2004. Temperature-Electrical Conductivity Relation of Water for Environmental Monitoring and Geophysical Data Inversion. Environmental Monitoring & Assessment **96**:119-128.

Hazel, J. R. and C. L. Prosser. 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiological Reviews **54**:620-677.

Herbinger, C. M. and G. W. Friars. 1991. Correlation between condition factor and total lipid content in Atlantic salmon, Salmo salar L., parr. Aquaculture & Fisheries Management **22**:527-529.

Henderson, B. A., N. Collins, G. E. Morgan, and A. Vaillancourt. 2003. Sexual size dimorphism of walleye (*Stizostedion vitreum vitreum*). Canadian Journal of Fisheries and Aquatic Sciences **60**:1345-1352.

Hendry, A. P., O. K. Berg, and T. P. Quinn. 1999. Condition dependence and adaptation-by-time: breeding date, life history, and energy allocation within a population of salmon. Oikos **85**:499-514.

Hicken, C. E., T. L. Linbo, D. H. Baldwin, M. L. Willis, M. S. Myers, L. Holland, M. Larsen, M. S. Stekoll, S. D. Rice, T. K. Collier, N. L. Scholz, and J. P. Incardona. 2011. Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. Proceedings of the National Academy of Sciences of the United States of America **108**:7086-7090.

Iwama, G. K., P. T. Thomas, R. H. B. Forsyth, and M. M. Vijayan. 1998. Heat shock protein expression in fish. Reviews in Fish Biology and Fisheries **8**:35-56.

Izquierdo, M.S.; H. Fernandez-Palacios; A.G.J. Tacon. 2001 Effect of broodstock nutrition on reproductive performance of fish. Aquaculture **197**:25-42.

Jackson, J. R., A. J. VanDeValk, T. E. Brooking, O. A. vanKeeken, and L. G. Rudstam. 2002. Growth and feeding dynamics of lake sturgeon, *Acipenser fulvescens*, in Oneida Lake, New York: results from the first five years of a restoration program. Journal of Applied Ichthyology **18**:439-443.

Jakob, E. M., S. D. Marshall, and G. W. Uetz. 1996. Estimating fitness: A comparison of body condition indices. Oikos **77**:61-67.

Kammerer, B. D., J. J. Cech, and D. Kultz. 2010. Rapid changes in plasma cortisol, osmolality, and respiration in response to salinity stress in tilapia (*Oreochromis mossambicus*). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology **157**:260-265.

Karnaky, K.J., L. B. Kinter, W. B. Kinter, C. E. Stirling. 1976. Teleost chloride cell, autoradiographic localization of gill na, k-atpase in killifish fundulus-heteroclitus adapted to low and high salinity environments. Journal of Cell Biology **70**:157-177.

Kerambrun, E., F. Henry, L. Courcot, F. Gevaert, and R. Amara. 2012. Biological responses of caged juvenile sea bass (*Dicentrarchus labrax*) and turbot (*Scophtalmus maximus*) in a polluted harbour. Ecological Indicators **19**:161-171.

Kidder, G. W., C. W. Petersen, and R. L. Preston. 2006. Energetics of osmoregulation: II. Water flux and osmoregulatory work in the euryhaline fish, Fundulus heteroclitus. Journal of Experimental Zoology Part a-Comparative Experimental Biology **305A**:318-327.

Kyle, U. G., I. Bosaeus, A. D. De Lorenzo, P. Deurenberg, M. Elia, J. M. Gómez, B. L. Heitmann, L. Kent-Smith, J.-C. Melchior, M. Pirlich, H. Scharfetter, A. M. W. J. Schols, and C. Pichard. 2004. Bioelectrical impedance analysis—part I: review of principles and methods. Clinical Nutrition **23**:1226-1243.

Lambert, Y. and J. D. Dutil. 2000. Energetic consequences of reproduction in Atlantic cod (Gadus morhua) in relation to spawning level of somatic energy reserves. Canadian Journal of Fisheries and Aquatic Sciences **57**:815-825.

Lankford, T. E. and T. E. Targett. 1994. Suitability of estuarine nursery zones for juvenile weakfish (*Cynoscion regalis*) - effects of temperature and salinity on feeding, growth and survival. Marine Biology **119**:611-620

Larose, C., R. Canuel, M. Lucotte, and R. T. Di Giulio. 2008. Toxicological effects of methylmercury on walleye (Sander vitreus) and perch (*Perca flavescens*) from lakes of the boreal forest. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **147**:139-149.

Lecren, E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca-fluviatilis*). Journal of Animal Ecology **20**:201-219.

Lee, David S., C.R. Gilbert, R.E. Jenkins, D. E. Mcallister, J. R. Stauffer, Jr. 1990. Atlas of the North American Freshwater Fishes. North Carolina State Museum of Natural History, North Carolina.

Light, T. S. 1984. Temperature dependence and measurement of resistivity of pure water. Analytical Chemistry **56**:1138-1142.

Lleonart, J., J. Salat, and G. J. Torres. 2000. Removing allometric effects of body size in morphological analysis. Journal of Theoretical Biology **205**:85-93.

Lowe, T. E., J. M. Ryder, J. F. Carragher, and R. M. G. Wells. 1993. Flesh quality in snapper, pagrus-auratus, affected by capture stress. Journal of Food Science **58**:770-&.

Lukaski, H. C., P. E. Johnson, W. W. Bolonchuk, and G. I. Lykken. 1985. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. American Journal of Clinical Nutrition **41**:810-817

Lukaski, H. C., W. W. Bolonchuk, C. B. Hall, and W. A. Siders. 1986. Validation of tetrapolar bioelectrical impedance method to assess human body composition. Journal of Applied Physiology **60**:1327-1332.

Maetz, J., M. Bornancin. 1975 Biochemical and biophysical aspects of salt excretion by chloride cells in teleosts. *Fortschritte Der Zoologie* **23:**322-362.

Martinez, M. L., C. Landry, R. Boehm, S. Manning, A. O. Cheek, and B. B. Rees. 2006. Effects of long-term hypoxia on enzymes of carbohydrate metabolism in the Gulf killifish, *Fundulus grandis*. Journal of Experimental Biology **209**:3851-3861.

Mazor, R. D., T. B. Reynoldson, D. M. Rosenberg, and V. H. Resh. 2006. Effects of biotic assemblage, classification, and assessment method on bioassessment performance. Canadian Journal of Fisheries and Aquatic Sciences **63**:394-411.

McNutt, M. K., S. Chu, J. Lubchenco, T. Hunter, G. Dreyfus, S. A. Murawski, and D. M. Kennedy. 2012. Applications of science and engineering to quantify and control the Deepwater Horizon oil spill. Proceedings of the National Academy of Sciences of the United States of America **109**:20222-20228.

Metz, J. R., E. H. van den Burg, S. E. W. Bonga, and G. Flik. 2003. Regulation of branchial Na+/K+-ATPase in common carp Cyptinus carpio L. acclimated to different temperatures. Journal of Experimental Biology **206**:2273-2280.

Myers, M. S., C. M. Stehr, O. P. Olson, L. L. Johnson, B. B. McCain, S. L. Chan, and U. Varanasi. 1994. Relationships between toxicopathic hepatic-lesions and exposure to chemical contaminants in english sole (*Pleuronectes vetulus*), starry flounder (*Platichthys stellatus*), and white croaker (*Genyonemus lineatus*) from selected marine sites on the pacific coast, usa. Environmental Health Perspectives **102**:200-215.

Morita, S. H., K. Morita, and A. Nishimura. 2012. Sex-biased dispersal and growth in sablefish (*Anoplopoma fimbria*) in the northeastern Pacific Ocean. Environmental Biology of Fishes **94**:505-511.

National Research Council 1987. Biological markers in environmental health research. Committee on Biological Markers of the National Research Council. Environmental Health Perspectives **74**:3-9.

National Oceanographic and Atmospheric Administration. Fisheries of the United States, Comercial Landings 2012. http://www.st.nmfs.noaa.gov/commercial-fisheries/fus/fus11/index

Nicieza, A. G. and N. B. Metcalfe. 1997. Growth compensation in juvenile Atlantic salmon: Responses to depressed temperature and food availability. Ecology **78**:2385-2400.

Niemeijer, D. 2002. Developing indicators for environmental policy: data-driven and theory-driven approaches examined by example. Environmental Science & Policy **5**:91.

Patterson, J., C. Bodinier, and C. Green. 2012. Effects of low salinity media on growth, condition, and gill ion transporter expression in juvenile Gulf killifish, Fundulus grandis. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology **161**:415-421.

Peakall, D. 1992. Animal biomarkers as pollution indicators. Chapman and Hall, New York.

Peig, J. and A. J. Green. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos **118**:1883-1891.

Pethig, R. 1979. Di Electric And Electronic Properties Of Biological Materials. John Wiley and Son. Chichester, New York

Piazza, B. and M La Peyre. 2010. Using Gambusia affinis growth and condition to assess estuarine habitat quality: a comparison of indices. Marine Ecology Progress Series **412**:231-245.

Picha, M. E., J.T. Silverstein and R. J. Borski. 2006. Discordant regulation of hepatic IGF-I mRNA and circulating IGF-I during compensatory growth in teleost, the hybrid striped bass (*Morone chrysops x Morone saxatilis)* General and Comparative Endocrinology **147**:196-205

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, R.M. Hughes. 1989. Rapid assessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. EPA: Washington, D.C.

Poole, W. R. and J. D. Reynolds. 1996. Growth rate and age at migration of *Anguilla anguilla*. Journal of Fish Biology **48**:633-642.

Pothoven, S. A., S. A. Ludsin, T. O. Hook, D. L. Fanslow, D. M. Mason, P. D. Collingsworth, and J. J. Van Tassell. 2008. Reliability of bioelectrical impedance analysis for estimating whole-fish energy density and percent lipids. Transactions of the American Fisheries Society **137**:1519-1529.

Quinn, T. P. and C. J. Foote. 1994. The effects of body-size and sexual dimorphism on the reproductive-behavior of sockeye-salmon, *Oncorhynchus nerka*. Animal Behaviour **48**:751-761.

Rasmussen, J. B., A. N. Krimmer, A. J. Paul, and A. Hontela. 2012. Empirical relationships between body tissue composition and bioelectrical impedance of brook trout *Salvelinus fontinalis* from a Rocky Mountain Stream. Journal of Fish Biology **80**:2317-2327.

Ratcliff.Da. 1970. Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some british birds. Journal of Applied Ecology **7**:67-&.

Reist, J. D. 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Canadian Journal of Zoology **63**:1429-1439.

Rezac, P. 2008. Potential applications of electrical impedance techniques in female mammalian reproduction. Theriogenology **70**:1-14.

Ribeiro, F., P. K. Crain, and P. B. Moyle. 2004. Variation in condition factor and growth in young-of-year fishes in floodplain and riverine habitats of the Cosumnes River, California. Hydrobiologia **527**:77-84.

Robertson, J. C. and J. R. Hazel. 1999. Influence of temperature and membrane lipid composition on the osmotic water permeability of teleost gills. Physiological and Biochemical Zoology **72**:623-632.

Sardet, C., M. Pisam, J. Maetz. 1979. The surface epithelium of teleostean fish gills. Cellular and junctional adaptations of the chloride cell in relation to salt adaptation. The Journal Of Cell Biology **80**:96-117.

Schiemer, F. 2000. Fish as indicators for the assessment of the ecological integrity of large rivers. Hydrobiologia **422**:271-278.

Schulte-Hostedde, A. I., B. Zinner, J. S. Millar, and G. J. Hickling. 2005. Restitution of mass-size residuals: Validating body condition indices. Ecology **86**:155-163.

Searcy, S. P., D. B. Eggleston, J. A. Hare. 2007. Is growth a reliable indicator of habitat quality and essential fish habitat for a juvenile estuarine fish? Canadian Journal of Fisheries and Aquatic Sciences, **64**: 681–691.

Segal, K. R., S. Burastero, A. Chun, P. Coronel, R. N. J. Pierson, and J. Wang. 1991. Estimation of extracellular and total body water by multiple-frequency bioelectrical-impedance measurement. Journal Name: American Journal of Clinical Nutrition; (United States); Journal Volume: 54:1:Medium: X; Size: Pages: 26-29.

Shanholtzer, B. A. and S. A. Patterson. 2003. Use of bioelectrical impedance in hydration status assessment: reliability of a new tool in psychophysiology research. International Journal of Psychophysiology **49**:217-226.

Shime, N., H. Ashida, E. Chihara, K. Kageyama, Y. Katoh, M. Yamagishi, and Y. Tanaka. 2002. Bioelectrical impedance analysis for assessment of severity of illness in pediatric patients after heart surgery. Critical Care Medicine **30**:518-520.

Simpson, D. G. and G. Gunter. 1956. Notes on habitats, systematic characters and life histories of Texas salt water cyprinodonts. Tulane Stud. Zool. 4(4):115-134.

Singh, R. B., R. Beegom, S. S. Rastogi, G. L. Zheng, and S. M. Zhou. 1998. Association of low plasma concentrations of antioxidant vitamins, magnesium and zinc with high body fat per cent measured by bioelectrical impedance analysis in Indian men. Magnesium Research **11**:3-10.

Smith, A., J. Alam, P. V. Escriba, and W. T. Morgan. 1993. Regulation of heme oxygenase and metallothionein gene expression by the heme analogs, cobalt-, and tin-protoporphyrin. The Journal Of Biological Chemistry **268**:7365-7371.

Sogard, S. M. and M. L. Spencer. 2004. Energy allocation in juvenile sablefish: effects of temperature ration and body size. Journal of Fish Biology **64**:726-738.

Souza-Bastos, L. R. and C. A. Freire. 2011. Osmoregulation of the resident estuarine fish Atherinella brasiliensis was still affected by an oil spill (Vicuña tanker, Paranaguá Bay, Brazil), 7months after the accident. Science of the Total Environment **409**:1229-1234.

Spies, R. B., J. J. Stegeman, D. E. Hinton, B. Woodin, R. Smolowitz, M. Okihiro, and D. Shea. 1996. Biomarkers of hydrocarbon exposure and sublethal effects in embiotocid fishes from a natural petroleum seep in the Santa Barbara Channel. Aquatic toxicology **34**:195-219.

Stebbing, A. R. D., S. M. T. Turk, A. Wheeler, and K. R. Clarke. 2002. Immigration of southern fish species to south-west England linked to warming of the North Atlantic (1960–2001). Journal of the Marine Biological Association of the United Kingdom **82**:177-180.

Stevenson, R. D. and W. A. Woods. 2006. Condition indices for conservation: new uses for evolving tools. Integrative and Comparative Biology **46**: 1169-1190.

Tabb, Durbin C., Raymond B. Manning. 1961. A checklist of flora and fauna of norhtern Flordia Bay and adjacent brackish waters of the Flordia mainland collected during the period July 1957 through September 1960 Bulletin of Marine Science Gulf and Caribbean **11**:552-649.

Thomasset, A. 1963. Bio-electric properties of tissues. Estimation by measurement of impedance of extracellular ionic strength and intracellular ionic strength in the clinic. Lyon medical **209**:1325-1350.

Van Gestel, C. A. M. and T. C. Van Brummelen. 1996. Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. Ecotoxicology **5**:217-225.

Wallaert, C. and P. J. Babin. 1994. Thermal adaptation affects the fatty-acid composition of plasma phospholipids in trout. Lipids **29**:373-376.

Wiemeyer, S. N., T. G. Lamont, C. M. Bunck, C. R. Sindelar, F. J. Gramlich, J. D. Fraser, and M. A. Byrd. 1984. Organochlorine pesticide, polychlorobiphenyl, and mercury residues in bald eagle eggs - 1969-79 - and their relationships to shell thinning and reproduction. Archives of Environmental Contamination and Toxicology **13**:529-549.

Wierner. J. G. & Hannesan. W. R. 1982. Growth and condition of bluerrills in Wisconsin lakes. Effects of population density and lake pH. Transactions of the American Fisheries Society **111**:761-767.

Willis, J. and A. J. Hobday. 2008. Application of bioelectrical impedance analysis as a method for estimating composition and metabolic condition of southern bluefin

tuna (*Thunnus maccoyii*) during conventional tagging. Fisheries Research **93**:64-71.

Wodtke, E. 1978. Lipid adaptation in liver mitochondrial-membranes of carp acclimated to different environmental temperatures - phospholipid composition, fatty-acid pattern, and cholesterol content. Biochimica Et Biophysica Acta **529**:280-291.

Young, K. A. 2005. Life-history variation and allometry for sexual size dimorphism in Pacific salmon and trout. Proceedings of the Royal Society B-Biological Sciences **272**:167-172.

Zadunaisky, J. A., S. Cardona, L. Au, D. M. Roberts, E. Fisher, B. Lowenstein, E. J. Cragoe, and K. R. Spring. 1995. Chloride transport activation by plasma osmolarity during rapid adaptation to high salinity of *Fundulus heteroclitus*. Journal of Membrane Biology **143**:207-217.

Zapfe, G.A., C.F Rakocinski. 2008. Coherent growth and diet patterns in juvenile spot (*Leiostomus xanthurus Lacepéde*) reflect effects of hydrology on access to shoreline habitat. Fisheries Research **91**:107-111.

APPENDICES

APPENDIX A. N, mean, and standard error by day for all metrics for Study 1, fed twice daily ad libitum for 28 days. Lipid content was only collected on day 0 and day 28.

	Day 0			Day 7			Day 14			Day 21			Day 28		
Metric	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**
L	33	96.76	1.85	18	95.22	2.30	20	95.25	2.42	15	92.33	2.20	19	93.05	1.49
W	33	11.93	0.73	18	11.96	0.89	20	13.34	1.02	15	11.82	0.82	19	11.79	0.64
Kn	33	0.99	0.01	18	1.06	0.01	20	1.16	0.01	15	1.15	0.02	19	1.13	0.02
Organ Lipid %	15	0.30	0.03										19	3.50	0.29
Body Lipid %	15	6.63	0.35										19	27.25	1.98
Plasma Osmolarity	30	367.10	2.20	18	349.11	1.65	20	356.65	3.02	15	360.07	2.54	15	350.07	2.97
R	33	341.80	6.59	18	360.65	9.08	20	352.04	7.44	15	338.95	4.56	19	326.05	6.09
Xc	33	81.71	1.50	18	88.95	2.96	20	91.59	2.38	15	125.86	2.84	19	118.78	3.71
PA	33	76.52	0.16	18	76.17	0.22	20	75.42	0.23	15	69.65	0.27	19	70.03	0.40
R_{para}	33	361.43	6.80	18	382.69	9.84	20	375.98	7.93	15	385.83	5.74	19	369.70	7.48
Xc_{para}	33	1519.05	39.78	18	1558.41	40.83	20	1451.53	37.90	15	1041.52	16.00	19	1021.45	23.32
PA_{para}	33	13.48	0.16	18	13.83	0.22	20	14.58	0.23	15	20.35	0.27	19	19.97	0.40
R_{length}	33	26.88	1.40	18	24.40	1.56	20	24.93	1.55	15	22.34	1.10	19	23.81	1.04
Xc_{length}	33	6.51	0.39	18	5.97	0.37	20	6.54	0.47	15	8.32	0.45	19	8.64	0.41
PA_{length}	33	700.57	23.41	18	668.14	38.67	20	627.47	26.58	15	421.32	17.44	19	438.33	15.53
R_{probe}	33	0.0121	0.0007	18	0.0122	0.0010	20	0.0147	0.0010	15	0.0127	0.0008	19	0.0135	0.0009
Xc_{probe}	33	0.0029	0.0002	18	0.0030	0.0002	20	0.0039	0.0003	15	0.0047	0.0003	19	0.0049	0.0003
PA_{probe}	33	0.32	0.01	18	0.34	0.03	20	0.37	0.02	15	0.24	0.01	19	0.25	0.02

APPENDIX B. N, mean, and standard error by day for all metrics for Study 2, fasted for 28 days. Lipid content was only collected on day 0 and 28.

	Day 0			Day 7			Day 14			Day 21			Day 28		
Metric	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**
L	35	95.03	1.81	35	92.06	1.68	33	86.94	1.61	33	91.42	1.59	26	92.50	1.80
W	35	11.29	0.68	35	9.97	0.62	33	7.59	0.51	33	8.76	0.46	26	8.87	0.60
Kn	35	0.99	0.01	35	0.96	0.01	33	0.88	0.01	33	0.88	0.02	26	0.85	0.02
Organ Lipid %	16	0.31	0.02										26	0.19	0.01
Body Lipid %	16	6.71	0.32										26	3.99	0.23
Plasma Osmolarity	31	366.61	2.28	33	336.09	2.38	31	338.35	2.07	33	336.94	1.93	22	326.64	4.02
R	35	354.26	8.31	35	364.03	6.20	33	387.66	7.37	33	348.13	7.94	26	341.59	8.95
Xc	35	84.32	1.89	35	81.52	2.06	33	75.49	1.37	33	89.44	2.97	26	89.50	3.47
PA	35	76.57	0.15	35	77.34	0.27	33	78.91	0.22	33	75.67	0.31	26	75.39	0.37
R_{para}	35	708.52	16.62	35	728.07	12.41	33	775.32	14.74	33	696.25	15.89	26	683.18	17.90
Xc_{para}	35	104.47	2.37	35	100.09	2.95	33	90.39	1.83	33	112.74	3.99	26	113.30	4.73
PA_{para}	35	81.58	0.11	35	82.14	0.21	33	83.30	0.15	33	80.85	0.23	26	80.63	0.29
R_{length}	35	13.35	0.70	35	12.09	0.63	33	10.10	0.54	33	12.44	0.60	26	13.09	0.76
Xc_{length}	35	89.69	4.14	35	88.05	4.15	33	85.81	3.72	33	81.43	6.78	26	83.58	7.66
PA_{length}	35	112.15	4.24	35	104.46	3.86	33	91.83	3.61	33	104.36	3.66	26	107.06	4.06
R_{probe}	35	0.0062	0.0003	35	0.0062	0.0003	33	0.0052	0.0003	33	0.0060	0.0003	26	0.0059	0.0004
Xc_{probe}	35	0.0414	0.0021	35	0.0453	0.0023	33	0.0442	0.0022	33	0.0393	0.0035	26	0.0378	0.0038
PA_{probe}	35	0.05	0.00	35	0.05	0.00	33	0.05	0.00	33	0.05	0.00	26	0.05	0.00

APPENDIX C. N, mean, and standard error for Study 3, wild-caught temperature (2, 12, 24^{0}C) and salinity (2,12, 24 ppt) exposure.

	Temperature									Salinity (ppt)								
	2 ^{0}C			12 ^{0}C			24 ^{0}C			2			12			24		
Metrics	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**
W	12	9.11	0.80	12	9.15	1.10	12	9.10	0.69	12	8.06	0.64	12	9.56	1.00	12	9.74	0.87
Kn	12	1.19	0.02	12	1.12	0.02	12	1.14	0.02	12	1.15	0.02	12	1.14	0.02	12	1.16	0.03
Organ Lipid %	12	41.62	4.40	12	43.90	5.58	12	56.39	3.10	12	44.02	4.24	12	46.67	6.02	12	51.23	3.87
Body Lipid %	12	3.81	0.48	12	4.32	0.68	12	5.39	0.41	12	3.93	0.56	12	4.48	0.59	12	5.11	0.51
Muscle Na^+	12	14230.86	1512.25	12	12532.89	1086.60	12	12360.63	652.71	12	10135.38	695.24	12	13030.69	518.68	12	15958.31	1339.50
R	12	443.01	21.84	12	418.24	16.13	12	362.91	7.90	12	452.18	19.36	12	404.95	16.13	12	367.03	11.81
Xc	12	115.03	10.93	12	121.52	8.29	12	110.57	4.93	12	141.16	4.44	12	109.46	4.54	12	96.50	9.04
PA	12	75.81	0.76	12	73.99	0.75	12	73.11	0.51	12	72.55	0.40	12	74.87	0.23	12	75.49	1.05
R_{para}	12	473.76	25.73	12	454.31	18.76	12	396.98	9.62	12	496.52	19.85	12	434.62	17.25	12	393.90	14.80
Xc_{para}	12	1882.37	56.40	12	1606.73	71.35	12	1314.79	35.95	12	1598.20	91.13	12	1611.83	70.76	12	1593.87	104.33
PA_{para}	12	14.19	0.76	12	16.01	0.75	12	16.89	0.51	12	17.45	0.40	12	15.13	0.23	12	14.51	1.05
R_{length}	12	15.73	1.58	12	16.52	1.55	12	18.72	1.33	12	13.87	1.02	12	17.76	1.66	12	19.35	1.36
Xc_{length}	12	3.84	0.28	12	4.76	0.50	12	5.64	0.38	12	4.37	0.34	12	4.83	0.48	12	5.05	0.51
PA_{length}	12	521.70	47.56	12	461.58	34.15	12	439.00	27.28	12	388.56	21.92	12	494.09	32.91	12	539.63	43.00
R_{probe}	12	0.0059	0.0006	12	0.0064	0.0007	12	0.0071	0.0007	12	0.0053	0.0004	12	0.0069	0.0008	12	0.0072	0.0005
Xc_{probe}	12	0.0014	0.0001	12	0.0018	0.0002	12	0.0021	0.0002	12	0.0017	0.0001	12	0.0019	0.0002	12	0.0019	0.0002
PA_{probe}	12	0.20	0.02	12	0.18	0.02	12	0.17	0.01	12	0.15	0.01	12	0.19	0.02	12	0.20	0.02

APPENDIX D. N, mean, and standard error for Study 4, lab-conditioned temperature (2, 12, 24^{0}C) and salinity (2, 12, 24 ppt) exposure.

	Temperature									Salinity								
	2 ^{0}C			12 ^{0}C			24 ^{0}C			2			12			24		
Metrics	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**	**N**	**Mean**	**Stderr**
L	12	80.92	1.03	12	81.17	0.68	12	81.75	0.86	12	81.92	1.05	12	80.92	0.73	12	81.00	0.78
W	12	7.30	0.28	12	7.34	0.25	12	7.48	0.30	12	7.67	0.29	12	7.32	0.25	12	7.13	0.27
Kn	12	1.09	0.03	12	1.09	0.02	12	1.08	0.03	12	1.10	0.02	12	1.10	0.04	12	1.06	0.01
Organ Lipid %	12	6.41	0.76	12	5.18	0.56	12	6.48	0.54	12	73.63	8.73	12	61.98	3.47	12	77.29	15.93
Body Lipid %	12	68.18	6.55	12	72.34	15.86	12	72.38	7.62	12	6.59	0.83	12	6.02	0.34	12	5.46	0.66
Plasma Osmolarity	9	337.89	15.82	10	317.70	10.51	11	318.64	15.43	9	285.00	17.51	10	337.30	4.72	11	344.09	10.71
Plasma Na^+	2	66595.00	7105.00	11	63196.36	2655.98	8	60497.50	5133.65	8	57578.75	4620.75	6	63406.67	4712.61	7	67322.86	2183.74
Muscle Na^+	12	15205.35	840.78	12	13485.35	813.54	12	12856.91	875.96	12	12390.34	1170.98	12	14493.01	680.39	12	14664.28	528.33
R	12	472.63	14.12	12	435.93	14.54	12	381.41	14.45	12	458.80	13.44	12	410.36	19.59	12	420.81	17.88
Xc	12	105.03	5.66	12	98.11	5.61	12	91.34	8.23	12	115.82	6.86	12	89.70	4.84	12	88.96	5.16
PA	12	77.51	0.48	12	77.33	0.50	12	76.79	0.65	12	75.89	0.62	12	77.65	0.42	12	78.09	0.35
R_{para}	12	945.25	28.25	12	871.86	29.08	12	762.82	28.89	12	917.59	26.89	12	820.72	39.19	12	841.62	35.77
Xc_{para}	12	128.77	7.75	12	120.62	7.81	12	113.87	11.54	12	145.73	9.83	12	109.57	6.30	12	107.97	6.71
PA_{para}	12	82.27	0.36	12	82.13	0.38	12	81.70	0.51	12	81.02	0.48	12	82.38	0.31	12	82.71	0.26
R_{length}	12	7.00	0.25	12	7.67	0.31	12	8.91	0.39	12	7.42	0.35	12	8.20	0.45	12	7.95	0.35
Xc_{length}	12	53.07	3.43	12	56.58	2.80	12	64.56	5.65	12	48.74	3.67	12	62.57	4.64	12	62.90	3.32
PA_{length}	12	79.72	1.96	12	80.31	1.46	12	81.95	1.90	12	82.98	2.10	12	79.55	1.39	12	79.44	1.66
R_{probe} *100	12	0.00	0.00	12	0.00	0.00	12	0.00	0.00	12	0.00	0.00	12	0.00	0.00	12	0.00	0.00
Xc_{probe} *100	12	0.03	0.00	12	0.03	0.00	12	0.03	0.00	12	0.02	0.00	12	0.03	0.00	12	0.03	0.00
PA_{probe}	12	0.04	0.00	12	0.04	0.00	12	0.04	0.00	12	0.04	0.00	12	0.04	0.00	12	0.04	0.00

APPENDIX E. Table of N, mean, and standard error for Study 1 (fed) and Study 2 (fasted) sorted into small and large on the mean length. P-values are from a Tukey test and significant values are in bold.

	Fed							Fasted						
	Small			Large				Small			Large			
Metric	**N**	**Mean**	**Std Err**	**N**	**Mean**	**Std Err**	**p-Value**	**N**	**Mean**	**Std Err**	**N**	**Mean**	**Std Err**	**p-Value**
L	49	86.84	0.76	56	101.96	0.83	**<.01**	84	83.54	0.53	78	100.28	0.66	**<.01**
W	49	9.11	0.27	56	14.83	0.40	**<.01**	84	6.67	0.15	78	12.23	0.31	**<.01**
Kn	49	1.08	0.01	56	1.08	0.01	0.92	84	0.90	0.01	78	0.94	0.01	**0.01**
Organ Lipid %	16	2.58	0.38	18	1.65	0.48	0.05	16	0.25	0.02	26	0.23	0.02	0.94
Body Lipid %	16	22.48	2.99	18	14.31	2.69	0.14	16	5.00	0.49	26	5.05	0.34	0.59
Plasma Osmolarity	46	354.96	1.97	52	360.65	1.72	**0.03**	78	339.62	1.91	72	343.89	2.42	0.16
R	49	357.15	5.17	56	331.98	3.72	**<.01**	84	382.64	4.56	78	335.39	4.26	**<.01**
Xc	49	102.87	3.04	56	93.45	2.57	**0.02**	84	86.75	1.63	78	80.61	1.46	**0.01**
PA	49	73.89	0.47	56	74.27	0.41	0.55	84	77.17	0.23	78	76.49	0.19	**0.02**
Rpara	49	388.15	5.32	56	359.42	4.00	**<.01**	84	765.28	9.13	78	670.79	8.52	**<.01**
Xcpara	49	1397.43	44.32	56	1317.27	34.12	0.15	84	106.97	2.30	78	100.30	2.02	**0.03**
PApara	49	16.11	0.47	56	15.73	0.41	0.55	84	81.99	0.17	78	81.49	0.15	**0.03**
Rlength	49	19.75	0.50	56	29.36	0.72	**<.01**	84	9.31	0.19	78	15.29	0.32	**<.01**
Xclength	49	5.74	0.23	56	8.23	0.25	**<.01**	84	68.22	1.80	78	104.91	3.33	**<.01**
PAlength	49	486.15	14.22	56	687.88	20.67	**<.01**	84	85.44	1.09	78	123.88	1.69	**<.01**
Rprobe	49	0.0102	0.0004	56	0.0154	0.0005	**<.01**	84	0.0045	0.0001	78	0.0074	0.0002	**<.01**
Xcprobe	49	0.0030	0.0002	56	0.0044	0.0002	**<.01**	84	0.0331	0.0010	78	0.0512	0.0018	**<.01**
PAprobe	49	0.25	0.01	56	0.36	0.01	**<.01**	84	0.04	0.00	78	0.06	0.00	**<.01**

VITA

Shea Miller is a native of Louisiana and received his Bachelors in Biology from the University of Louisiana at Lafayette. He worked as a Research Associate in LSU's School of Renewable Natural Resources for two years before starting his graduate degree. He will receive his MS from Louisiana State University in August 2014. He plans on pursuing a career in coastal ecology and aquaculture.