ABSTRACT

Title of Document: ADVANCING ECOSYSTEM BASED FISHERIES MANAGEMENT: BIOLOGICAL REFERENCE POINTS FOR NUTRITIONAL STATUS OF STRIPED BASS (MORONE SAXATILIS).

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Nutritional condition is a valuable metric in ecosystem-based fisheries management. However, the need for lethal sampling for the most accurate indicators ethically and logistically limits sample sizes. Percent moisture has been recommended for management of striped bass *Morone saxatilis* and a management threshold has been suggested. Past researchers have used bioelectrical impedance analysis (BIA) to non-lethally estimate percent dry weight, the inverse of percent moisture. We sought to develop species-specific BIA models for striped bass in a controlled, laboratory setting and later validate those models with independent, field-collected data. BIA models were developed for five size classes and sampled across three temperatures. Results in the lab suggest BIA is an accurate and robust method for estimating percent dry weight in striped bass. However, when implemented in field surveys results are less conclusive. Possible differences between wild and hatchery-reared striped bass that effect BIA need further exploration. Additionally, the effects of salinity and stress response on BIA warrant further work.

ADVANCING ECOSYSTEM BASED FISHERIES MANAGEMENT: BIOLOGICAL REFERENCE POINTS FOR NUTRITIONAL STATUS OF STRIPED BASS (MORONE SAXATILIS).

By

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2014

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Acknowledgements

I would like to thank Reggie Harrell, John Jacobs, Kyle Hartman, Jim Uphoff, Andrew Hafs, Lonnie Gonsalves, and Mark Matsche for technical guidance. I thank John Rosendale for helping capture and maintain the larger fish used in this study. I also thank MDDNR Manning Hatchery and Horn Point Laboratory for providing fish, Cooperative Oxford Laboratory and James J. Howard Marine Sciences Laboratory for providing the necessary aquaculture facilities, and the Wye Research and Education Center for putting up with the long months of drying fish. I would like to thank Kevin Rosemary, Jimmy Ritzman, Sarah Bornhoeft, Lonnie Gonsalves, Elizabeth Friedel, Cody Shingleton, Luke Ferricher, Collette Lauzau, Kaitlyn Harrell, Eliu Seeber, Matt Rhodes, Ryan Corbett, Cameron Day, Megan O'Donnell, Alexander Noonen, Grace McIlvain, and Delan Bayce for help with data collection. Lastly I would like to thank Maryland Sea Grant, MDDNR, and NOAA for funding this project.

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Chapter 1: Introduction

The recovery of the Chesapeake Bay striped bass (*Morone saxatilis*) stock following its moratorium in the late 1980s is generally considered a management success (Richards and Rago 1999; ASMFC Striped Bass Stock Assessment Subcommittee 2011). However, in the late 1990s there were reports of emaciated striped bass and striped bass with skin lesions (Overton et al. 2003; Rhodes et al. 2004; Gauthier et al. 2008). Coinciding with the recovery of the Chesapeake Bay striped bass stock, Atlantic menhaden (*Brevoortia tyrannus*) stocks in the region declined (Richards and Rago 1999; ASMFC Atlantic Menhaden Technical Committee 2010; ASMFC Striped Bass Stock Assessment Subcommittee 2011). Several studies have found Atlantic menhaden to be the dominant species in striped bass diet's in the mid-Atlantic region (Griffin and Margraf 2003; Overton et al. 2008, 2009).

Striped bass appear to be able to limit their own prey populations (Hartman 2003; Uphoff 2003; Savoy and Crecco 2004) and prey limitation has been cited as a management issue for striped bass in lake systems (Axon and Whitehurst 1985). Uphoff (2003) estimated a major reduction in predator-prey ratios between striped bass and Atlantic menhaden that coincided with the decline in striped bass condition. This energetic interaction led to the concern that the spread of disease seen in striped bass may have resulted from prey limitation (Hartman and Brandt 1995b; Hartman and Margraf 2003; Uphoff 2003).

Similarly, the relationship between prey limitation and nutritional condition on a fishery has been seen before in the Atlantic cod (*Gadus morhua*) stocks where a

dramatic increase in natural mortality was linked to reduced condition (Lambert and Dutil 1997; Dutil and Lambert 2000; Shelton and Lilly 2000). This increased mortality came at the same time the cod's primary prey disappeared (Lilly 1994). The limited recovery in cod populations that has been observed is linked to the local recovery of prey populations (Rose and O'Driscoll 2002).

Following the appearance of fish with lesions it was found that striped bass were experiencing an epizootic of mycobacteriosis (Heckert et al. 2001; Rhodes et al. 2001, 2004; Overton et al. 2003; Kaattari et al. 2005). Two new species of mycobacteria were isolated from the Chesapeake Bay striped bass population, *M. shottsii* and *M. pseudoshottsi*i (Rhodes et al. 2001, 2005), but only *M. marinum* was demonstrated to cause the same pathology when fish were challenged experimentally (Gauthier et al. 2003). Later work by Jacobs et al. (2009b) was able to link the progression of mycobacterial disease in striped bass to the fish's diet in an experimental setting. Fish with inadequate diets had faster and more severe disease progressions than those fed an adequate ration (Jacobs et al. 2009b). Concurrent to these findings and studies, tagging effort results suggested an increase in natural mortality for striped bass in the Chesapeake Bay (Jiang et al. 2007; Sadler 2010). Epidemiological modeling done by Gauthier et al. (2008) estimated that infected fish were 69% as likely to survive as uninfected fish.

In recent years ecosystem-based fisheries management has been gaining momentum with a goal of holistically managing ecosystems instead of partitioning them into more traditional single-species management models (Pikitch et al. 2004; Link 2005). An ecosystem-based strategy, however, relies on indicators independent

of fisheries removal for management decisions (Pikitch et al. 2004; Link 2005). In light of the above findings in the Chesapeake Bay region, Maryland Sea Grant called for management efforts to make greater use of nutritional condition reference points (2009). Traditional weight and length metrics for fish condition such as Fulton's condition factor and relative weight were suggested (Maryland Sea Grant 2009); likely due to low cost and ease of measurement. However, the demonstrated relationship between these length- and weight-based indices and the nutritional condition of the fish has been inconsistent (Niimi 1972; Brown and Murphy 1991; Herbinger and Friars 1991; Plante et al. 2005; Davidson and Marshall 2010). This inconsistency is primarily due to the inverse relationship created between fat and water content in fish from their physiological reaction to starvation.

In general, as a fish progresses through starvation, glycogen and lipid consumption is coupled with tissue hydration. Protein only begins to catabolize after extreme starvation (Love 1970; Niimi 1972; Jobling 1980; Jezierska et al. 1982; Black and Love 1986). This process of tissue hydration leads to fish conserving weight (via water content versus lipid content and muscle mass) through most of the starvation process, thereby hampering the effectiveness of traditional morphometric condition indices. This relationship is why proximate analysis, the direct measurement of body composition, is still considered the standard to compare against other techniques (Brown and Murphy 1991; Jacobs et al. 2008). Despite its accuracy, the expense, processing time, and lethal sampling requirement of proximate analysis have limited its application (Walsberg 1988; Brown et al. 1993; Jacobs et al. 2008).

Although the inverse relationship between fat and water limits traditional measures of condition, it does provide another avenue to the same goal. Based on the strength of that relationship, moisture alone has been used to predict lipid content and energy density in fish (Hartman and Brandt 1995a). More recently, strong moisture-lipid relationships in striped bass have been found further limiting the need for full proximate analysis in condition management (Hartman and Margraf 2008; Jacobs et al. 2013). Jacobs et al. (2013) have also already suggested possible management thresholds for Chesapeake Bay striped bass that are based on moisture alone.

Until recently, measuring the moisture content of fish required a lethal sampling technique. A new technology called bioelectrical impedance analysis (BIA) allows for the non-lethal estimation of fish body composition (Cox and Hartman 2005). The BIA method originated in the medical field and has been used to estimate fat-free mass and hydration levels (Lukaski et al. 1985; Kushner and Schoeller 1986; Lukaski et al. 1994). It has also been correlated with health and nutritional status in human patients, from caring for hemodialysis patients (Chertow et al. 1995) to tracking HIV progression (Schwenk et al. 2000).

The ability of BIA to estimate water content could provide a method for empirically measuring fish condition non-lethally. Strong predictive models of body composition have already been developed with BIA for a number of fish species including bluefish *Pomatomus saltatrix*, brook trout *Salvelinus fontinalis*, and cobia *Rachycentron canadum* (Duncan et al. 2007; Hafs 2011; Hartman et al. 2011).

The methods for assessing fish condition with BIA are relatively simple. A small electrical current (425 µA, 50 kHz) is passed through an individual's tissue and

the resistance and reactance values are measured. Typically a Quantum II bioelectrical body composition analyzer (RJL Systems, Clinton Township, MI) is used for sampling fish (Cox and Hartman 2005; Margraf et al. 2005; Hafs and Hartman 2011; Hartman et al. 2011). Resistance and reactance, and the electrical parameters that can be derived from them, are then used to predict the proximate composition of the test subject.

The reason these electrical measures are thought to correlate to proximate composition is because fat is an electrical insulator and the lipid bilayer of cells can function as a capacitor (Lukaski 1987). Resistance is a measure of how much a substance opposes the flow of electricity through itself, therefore, the insulating properties of fat should cause resistance to increase in fish with more fat (Lukaski 1987). Reactance is the opposition of a substance to a change in voltage due to its capacitance, therefore reactance should increase in fish with healthier, fatter cells (Lukaski 1987).

After a fish is measured for BIA it is euthanized and the proximate components of interest to the user are measured using standard techniques. Models are then developed with regression procedures to predict these proximate composition values from the electrical parameters that were measured (resistance and reactance) and derived. Once these models are developed fish no longer have to be sacrificed in order to measure proximate composition as the BIA measures act as surrogates.

The equipment needed to conduct BIA on fish is also relatively inexpensive, less than $3,000 US for startup. Sampling of fish is also very economical, both in equipment and labor. The device only needs 9v batteries to operate and no other

disposable equipment is required. The time it takes to measure a fish is comparable to measuring for length-weight indices. The sampler, with practice, can complete the BIA portion of the measurements in less than a minute. This method then is considerably cheaper and faster than conducting proximate analysis in the lab.

The BIA measures in fish are, however, affected by several internal and external factors (Cox et al. 2011). Temperature, in particular, has been found to have an impact on resistance and reactance measures in both clinical and fisheries studies dictating the need for separate temperature correction models (Buono et al. 2004; Corciovă et al. 2011; Cox et al. 2011; Hartman et al. 2011; Stolarski et al. 2014). Other sources of error have mostly been accounted for as the protocol for implementing BIA has developed (Cox et al. 2011; Hafs and Hartman 2011). Erroneous readings due to alternate paths for the current other than through the tissue are controlled for by using a nonconductive sampling surface and patting the fish dry. Also, surface areas of the probes are kept constant with standardized needle gauge and penetration depth, and electrical volume of the fish can be corrected for with consistent probe placement and measuring detector length.

With a need for nutritional condition management of striped bass in the Chesapeake Bay (Maryland Sea Grant 2009), moisture is clearly a strong candidate for a future indicator. In addition, the observational body fat index (BFI) that has been in continuous use with Maryland Department of Natural Resources (MDDNR) since 1998 was validated with a moisture-based approach, providing data continuity (Jacobs et al. 2013). The development of moisture-estimation models with BIA

measures for striped bass would provide a non-lethal sampling method necessary for the ethical collection of large sample sizes without undue sacrificing of fish.

The goal of this project was to develop these moisture-estimation models with the use of BIA as well as temperature-correction models to improve the field utility. The availability of these striped bass specific BIA models could allow the MDDNR to easily take large, non-lethal, samples of striped bass condition in the Chesapeake Bay annually that are more reflective of true condition compared with more traditional means. The small size of the equipment and the ergonomics of BIA sampling would allow the MDDNR to incorporate condition sampling into their regular striped bass surveys currently being conducted. These data would then provide managers with the condition information called for by MSG that would be needed for ecosystem-based management.

Chapter 2: Development of Striped Bass Relative Condition Models with BIA and Associated Temperature Corrections.

Abstract

Nutritional condition is a valuable metric in ecosystem-based fisheries management. However, the need for lethal sampling for the most accurate indicators ethically and logistically limits sample sizes. Percent moisture has been recommended for management of striped bass *Morone saxatilis* and a management threshold has been suggested. Past researchers have used bioelectrical impedance analysis (BIA) to non-lethally estimate percent dry weight, the inverse of percent moisture. We sought to develop species-specific BIA models for striped bass in a controlled, laboratory setting. BIA models were developed for five size classes and sampled across three temperatures. Results suggest BIA is an accurate and robust method for estimating percent dry weight in striped bass with model R^2's in the range of 0.70-0.89. We recommend these models be tested with independent data collected in a field setting.

Introduction

In recent years ecosystem-based fisheries management has been gaining momentum with a goal of holistically managing ecosystems instead of partitioning them into more traditional single-species management models (Pikitch et al. 2004; Link 2005). In the Chesapeake Bay region, Maryland Sea Grant has supported efforts to transition traditional state fisheries management efforts to an ecosystem based approach. As a part of this transition, Maryland Sea Grant called for research on management efforts to make greater use of nutritional condition reference points in the striped bass (*Morone saxatilis*) fishery (2009).

This approach to transition to an expanded management philosophy in the Chesapeake Bay had its roots in the late 1990s when there were reports of emaciated striped bass and striped bass with skin lesions (Overton et al. 2003; Rhodes et al. 2004; Gauthier et al. 2008). Uphoff (2003) estimated a major reduction in predator-prey ratios between striped bass and Atlantic menhaden (*Brevoortia tyrannus*) that coincided with the decline in striped bass condition. Several studies have found Atlantic menhaden to be the dominant species in striped bass diets in the mid-Atlantic region (Griffin and Margraf 2003; Overton et al. 2008, 2009). This energetic interaction led to the concern that the spread of disease seen in striped bass may have resulted from prey limitation (Hartman and Brandt 1995b; Hartman and Margraf 2003; Uphoff 2003).

Following the appearance of fish with lesions it was found that striped bass were experiencing an epizootic of mycobacteriosis (Heckert et al. 2001; Rhodes et al. 2001, 2004; Overton et al. 2003; Kaattari et al. 2005). Later work by Jacobs et al. (2009b) in an experimental setting was able to link the progression of mycobacterial disease in striped bass to the fish's diet. Fish with inadequate diets had faster and more severe disease progressions than those fed a higher ration (Jacobs et al. 2009b).

In response to the report by Maryland Sea Grant (2009), Jacobs et al. (2013) tested several metrics for their suitability as biological reference points. Included were the traditional fisheries metrics, standard weight and Fulton's condition factor, as well as some newer methods, body fat index (BFI) and percent moisture content. While the traditional methods were suggested by Maryland Sea Grant (2009), likely due to their ease of use and low cost, Jacobs et al. (2013) found they were poorly

correlated with lipid depletion. However, BFI and percent moisture were both shown to be reliable methods for estimating lipid depletion. Moving forward, Jacobs et al. (2013) recommended percent moisture be used to assess striped bass condition and proposed a biological reference point. Their suggested management reference target was for 75% of the striped bass population to have less than the 80% moisture threshold, the level found to coincide with lipid depletion.

However, until recently, measuring the moisture content of fish required a lethal sampling technique. A new technology called bioelectrical impedance analysis (BIA) allows for the non-lethal estimation of fish body composition (Cox and Hartman 2005). The BIA method originated in the medical field and has been used to estimate fat-free mass and hydration levels (Lukaski et al. 1985; Kushner and Schoeller 1986; Lukaski et al. 1994). It has also been correlated with health and nutritional status in human patients, from caring for hemodialysis patients (Chertow et al. 1995) to tracking HIV progression (Schwenk et al. 2000). Strong predictive models (R^2>0.75) of percent dry weight, the inverse of percent moisture, have already been developed with BIA for bluefish *Pomatomus saltatrix*, brook trout *Salvelinus fontinalis*, and cobia *Rachycentron canadum* (Duncan et al. 2007; Hafs 2011; Hartman et al. 2011).

The methods for assessing fish condition with BIA are relatively simple. A small electrical current (425 µA, 50 kHz) is passed through an individual's tissue and the resistance and reactance values are measured. Typically a Quantum II bioelectrical body composition analyzer (RJL Systems, Clinton Township, MI) is used for sampling fish (Cox and Hartman 2005; Margraf et al. 2005; Hafs and

Hartman 2011; Hartman et al. 2011). Resistance and reactance, and the electrical parameters that can be derived from them, are then used to predict the percent dry weight of the test subject (which is the inverse of percent moisture).

The equipment needed to conduct BIA on fish is also relatively inexpensive, less than $3,000 US for startup. Sampling of fish is also very economical, both in equipment and labor. The device only needs 9v batteries to operate and no other disposable equipment is required. The time it takes to measure a fish is comparable to measuring for length-weight indices. With practice, the BIA portion of the measurements can be completed in less than a minute. This method then is considerably cheaper and faster than conducting proximate analysis in the lab, and it is not lethal.

The BIA measurements in fish are, however, affected by several internal and external factors (Cox et al. 2011). Temperature, in particular, has been found to have an impact on resistance and reactance measures in both clinical and fisheries studies dictating the need for separate temperature correction models (Buono et al. 2004; Corciovă et al. 2011; Cox et al. 2011; Hartman et al. 2011; Stolarski et al. 2014).

The hypothesis for this project was that BIA would be a reliable way of non-lethally estimating percent moisture in striped bass. The objectives were to: (1) develop these moisture-estimation models with the use of BIA across a range of sizes from young-of-year to mature adults and (2) develop temperature correction models to allow the use of BIA in a wide range of field conditions typically found in the Chesapeake Bay.

Methods

This study uses a hierarchical sampling structure in order to sample fish across size ranges, condition ranges, and temperatures (Figure 1). Five size classes were used: young-of-year (YOY) (~100 mm), small juveniles (~200 mm), large juveniles (~300 mm), recruits (~400 mm), and adults (550+ mm) (Table 1). These five size classes were sampled at three nutritional condition ranges based on the observational body fat index (BFI) that was used by Jacobs et al. (2013). This 0 to 3 scale is based on a visual assessment of the percent coverage of the viscera by fat (Table 2). For instance, if the sampler using standard necropsy protocol determines that 75% or more of the viscera are visually covered by fat then that fish scores a 3 on the index. If the sampler determines that fat visually covers between 25%-75% of the viscera the fish scores a 2. Fish with less than 25% coverages are scored a 1 and fish with no visible fat deposits on the viscera are scored a 0. The first condition class was comprised mainly of fish with a BFI of 3, the second class by fish with BFI's of 2, and the third and final class was fish with a BFI of 1 or 0. In order to make temperature correction models each size class, at each condition class, was sampled at three temperatures: 8°C, 18°C, and 26°C (Figure 1).

The three smallest size classes, (YOY, small juveniles, and large juveniles), were sampled at the Cooperative Oxford Laboratory (COL) Oxford, MD. Seven hundred fish were obtained from Maryland Department of Natural Resources' (MDDNR) Manning Hatchery in 2011. An additional 500 fish in 2011 and 100 fish in 2012 were received from the University of Maryland Horn Point Laboratory, Cambridge, MD. The experiment began with extra fish to ensure adequate sample sizes after possible mortality loss. The fish were transferred to COL using standard

protocols (Weirich 1997) to minimize stress where they were held and acclimated in two 3,785 L outdoor, flow-through tanks and fed size-appropriate commercial feed consisting of 2.5 mm slow-sinking pellets that contained 42% protein and 16% fat (Nicholson et al. 1992; Gatlin 1997) (Zeigler, PA). Fish were then moved into COL's eight indoor 1,135 L recirculating systems for the experimental studies. Each 1,135 L system was made up of two 568 L Polytanks® linked to the same pump and filter system. Experimental conditions were: photoperiod 12:12 fluorescent, pH 8.2, salinity 10 ppt, and temperature 21°C. Initially the experimental fish were fed a commercial striped bass grower diet to satiation daily (Melick Aquafeed, Catawissa, PA). The diet was a 4 mm sinking pellet consisting of 42% protein and 16% fat.

The BIA sampling procedure began when the fish reached an average of 100 mm, the average size of the YOY size class. At that time, 75 fish were separated into another 1,135 L system and removed from feed for the first series of experimental efforts. The remaining fish were kept on feed until they grew to the average total length of the next experimental size classes (200 mm, 300 mm), at which point 75 fish for each test group were again separated and removed from feed. All test fish in each experimental size group were sampled with the protocol described below.

Of the initial 75 fish, 25 were put in a separate 1,135 L system for the first BIA measurements. Because these fish had been fed to satiation throughout acclimation they were assumed to be in the best possible condition (a representative fish was sampled and was confirmed to have a BFI of 3, the highest condition class). These test fish were acclimated to the warmest test temperature (26°C) overnight.

They were then anesthetized in a MS-222 bath (150 mg/L) (Lemm 1993) and total length, wet-weight, and BIA measures were recorded.

After each fish was measured they were individually marked by surgically implanting passive integrated transponders (PIT) tags into the abdominal cavity and then immediately placed into a tank at the next test temperature (18°C) to recover from anesthesia and acclimate to the next temperature level. Fish were again allowed to acclimate overnight before being re-anesthetized and having impedance measures repeated 24 hours after the initial measurements. The test fish were then immediately placed into the coldest test temperature (8°C) and the process was repeated for a third time at the lowest temperature range. Once all the temperature treatments were complete all the fish were euthanized (300mg/L of MS-222), visceral lipids were visually examined to record BFI, and the fish were frozen whole at -4°C before further analysis. Each fish was later oven-dried at 80°C to constant weight and percent dry weight was calculated.

The remaining 50 fish removed from feed were sampled (25 fish per period) at roughly 6 and 12 week intervals. These intervals varied depending on fish size. Sampling intervals were determined based on the visually-estimated condition of the fish. Single fish were occasionally sacrificed in order to sample for BFI and estimate the condition of the sample group. For the second sample period (6 weeks) the fish were sampled when they appeared to be approximately a "2" on the BFI. For the last sample period fish were sampled when they appeared to be roughly half "1's" and half "0's" on the BFI.

To conduct BIA on the adult- and recruit-size classes, fish were collected off of Sandy Hook, New Jersey by NOAA and were maintained at James J. Howard Marine Science Laboratory in Sandy Hook, NJ. These fish were collected by hook and line and acclimated to indoor flow-through tanks. Recruit-size fish were fed various species of cut fish daily to satiation for approximately three months to establish feeding and build lipid reserves. To obtain the three previously mentioned condition classes 62 fish were placed in one of three treatments (~20 per treatment) for two months. The fish for the lowest condition class (BFI = 0-1) were fed once weekly; the medium condition fish (BFI = 2) were fed twice weekly to satiation; the third group representing the highest condition class (BFI = 3) were fed daily to satiation. The recruit-sized fish were then sampled for BIA values using the same temperature protocol described above.

Adult fish were sampled in two groups due to space limitations. The first group of 23 fish was sampled shortly after establishment of feeding with cut bait fish. This group was assumed to be the medium condition group (BFI = 2) due to the moderate diet they were fed and their recent feeding history as wild fish. The second group of 49 fish was split into two treatments to save time. One treatment was fed to satiation daily for approximately three months to build lipid reserves. The second treatment group was fasted for three months. These treatments were assumed to be our highest (BFI = 3) and lowest (BFI = 0-1) condition classes, respectively, based on their copious diet or lack thereof. The adult fish, when at the appropriate condition level, were then sampled with the same temperature protocol as above.

BIA Data Collection

Resistance and reactance was measured on all fish with subdermal, needle electrodes using a Quantum II bioelectrical body composition analyzer (RKL Systems, Clinton Township, MI) (Figure 2). All needles were 28 gauge, however, spacing and depth specifications for the signal and detector electrodes varied by size class to minimize harm to the fish by over-penetration (Table 1). For example, fish in the YOY size class, 50-150 mm in length, were sampled with needles set 5 mm apart and set to penetrate a maximum of 1 mm. Fish were placed on a non-conductive board with the head toward the left and the belly oriented toward the sampler. Fish were then blotted dry before measurement. These steps ensured consistent handling of the fish and minimized the possibility of alternative paths for the electrical current.

The BIA measurements were taken at two locations on the fish based on the recommendations of Hafs and Hartman (2011). The first measurement was taken immediately below the dorsal midline (DML) of the fish by placing the first electrode roughly 2-3 scale rows below the DML half way between the head and the insertion of the first dorsal fin (Figure 3). The second electrode was placed 2-3 scale rows below the DML directly anterior to the caudle peduncle. The second location was from the dorsal to ventral (DTV) area of the fish ahead of the first dorsal fin (Figure 4). For this measurement the first anterior electrode near the head was left in place and the posteriorly located second electrode was removed and relocated to an anterior spot roughly halfway between the pectoral and pelvis fins directly below the insertion of the first dorsal fin. For consistency, the detector needle of both electrodes was placed anteriorly for the first measurement and when the second electrode is moved

to the belly it is posteriorly rotated such that the detector needle is then closest to the tail. Following the direction of Hafs and Hartman (2011) we also measured the detector length as the distance between detector needles of the electrodes at each measurement location in order to calculate additional electrical parameters.

BIA Model Development

The BIA models were developed for each size class individually using the 26°C data to predict percent dry weight. Including the resistance and reactance values, there are eleven possible electrical parameters at each measurement location that potentially can be used by the models (Table 3). The nine parameters not directly measured can be calculated from the resistance, reactance, and detector length. The calculations for all the possible parameters used in the BIA models can be seen in Table 3.

Models were developed by ordinary least squares regression using the *ols* function from the *rms* package (Harrell 2012) in the program "R" (R Team 2012). Mallows' *Cp* (Mallows 1973) was then calculated for every possible model using the function *leaps* from the *leaps* package (Lumley and Miller 2009) in "R" (R Team 2012). Mallow's *Cp* values were then used to select a subset of models for validation. The subset consists of the best model (lowest *Cp* value) at each possible model size from one to fifteen variables. These fifteen BIA models were then validated using the function *validate* from the *rms* package (Harrell 2012) in "R" (R Team 2012). The validate function uses bootstrapping methods developed by Efron (1983) to randomly separate the data into training- and test-data sets. The training-data sets are then used for model development and the test-data sets are used to validate the models. The

validate function was set to run 1,000 permutations for each model and R-square and root mean square error (RSME) values were calculated on the basis of how well the test-data sets fit the models. The best model was selected based on Akaike's Information Theoretical Criterion corrected for sample size (Akaike 1973).

Temperature Correction Models

Due to mortality between temperature samplings, data was sorted to only include fish that had three complete BIA measurements, one at each of the experimental temperature treatments. Ordinary least squares regression was used to establish relationships between both resistance and temperature, and reactance and temperature at both the DML and DTV sample locations. The slopes of these relationships were used in the Temperature Correction Equation as the variable K to correct the resistance and reactance values at 18° C and 8° C to 26° C. The BIA models (Table 6) were used to predict percent dry weight and residuals were calculated for each of the three temperature categories before and after applying temperature corrections. Linear regressions were then calculated in order to check for relationships between water temperature and BIA model residuals before and after temperature correction to account for temperature variation.

$$B_1 = (T_1 - T_0) * K + B_0$$

Temperature Correction Equation

B_0 = Initial BIA measurement, DMLr, DMLx, DTVr, DTVx.
B_1 = BIA measurement corrected to T_0
T_0 = Temperature measurement is being corrected to. 18°C in this case.
T_1 = The water temperature of the sample
K = Correction constant found in Table 7

Results

A total of 347 fish were sampled for BIA model development. The largest range in condition, 17.41% measured as percent dry weight, was seen in the YOY samples (Table 4). The smallest range, 9.41%, was seen in the recruit samples (Table 4). The average percent dry weight of all samples was 28.08%. The total lengths sampled in the study ranged from 110 mm to 937 mm. After removing fish with less than three records from the data, 342 fish were used to develop temperature correction models for a total of 1,026 records.

The top performing BIA models were for the small juveniles and the adults with R^2s of 0.883 and 0.872 respectively and RMSE's of 1.530 and 1.349 respectively (Table 5). The poorest performing models were for the YOY and recruits with R^2s of 0.718 and 0.708 respectively and RMSE's of 1.930 and 1.522 respectively (Table 5). It should be noted that there is a gap in the TL and percent dry weight coverage of the data between 350 mm and 600 mm (Figure 5), and this gap is a likely cause for the lower predictive power of the recruit BIA model. Figure 6 shows predicted percent dry weights over measured percent dry weights for each BIA model. Plotted model estimates closely follow the theoretical 1 to 1 line between predicted and observed. Parameters for all of the models can be found in Table 6.

The Temperature Correction Equation constant *K* is equivalent to the slope of the linear regression between BIA measurements and water temperature. The linear regression slopes used in the Temperature Correction Equation can be found in Table 7. Before correcting for temperature, BIA model residuals had a significant

relationship with temperature at an alpha=0.05 (p-value<0.0001) (Figure 7). After applying the Temperature Correction Equation there was no significant relationship between model residuals and water temperature (p-value=0.48) (Figure 7).

The BIA models were found to be fairly insensitive to most variable changes (Figure 8). When changing inputs by ten percent the only variables found to change the final estimate by close to ten percent were the calculated DTV phase angle and the measured DTV reactance (from which DTV phase angle is calculated) for the adult model. Changing these variables by ten percent changed the final adult model estimate by roughly 9.4% and 9.9% respectively. Additionally, changing the sample temperature by 10% yielded on average a 1.9% change in final estimate. The adult model was the most sensitive to temperature change with a 3.1% - 3.3% change in the final estimate.

Discussion

The models developed in this study perform roughly on par ($R^2 > 0.80$) with those previously developed in other studies (Cox and Hartman 2005; Duncan et al. 2007; Hafs and Hartman 2011; Hartman et al. 2011). We believe the difference there might be in performance between the models in this study and those mentioned above is primarily driven by the narrow condition range in the data for this study. To achieve R^2's much above 0.80, the recommended range in percent dry weight samples is about 28% or higher (Kyle Hartman, West Virginia University, Division of Forestry and Natural Resources, 2013, personal communication). With none of our sample groups having more than 20% dry weight range in the samples, our models

are more likely to have problems associated with extrapolation than if a wider range existed in the data. Despite this three of the models did have R^2's above 0.80.

In particular, it should be noted that the highest range in condition for this study was seen in the YOY size class. One would not expect this result due to the variation in energy allocation by such small fish. Young-of-year fish typically put more energy into growth prior to their first fall before allocating more energy to fat reserves (Hurst and Conover 2003). This result suggests that the YOY size class was erroneously measured to have a higher condition range, the larger size classes had an under representation of condition range due to the feeding and fasting protocol, or both.

The equipment and facility used for drying and weighing the fish in this study could have led to the first explanation. Because the air intake for the drying oven was located on the exterior wall, outdoor fluctuations in humidity made recorded fluctuations in the measured dry weight of fish, creating inconsistency when fish were declared "dry". While repeated measures might have only varied plus or minus 0.01g, prolonged high humidity during this period could have led to an over-estimation of dry weight compared to a fish declared "dry" during a prolonged period of low humidity. Additionally, an enclosed analytical balance was not available that could contain the disposable aluminum trays that fish were dried in, allowing more error due to air movement within the room and the lower sensitivity of the balance measuring only to the nearest hundredth gram. Thus, due to these two factors, it is possible the YOY had proportionally more measurement error when sampling dry weights than the larger fish. This variation could explain why the YOY model,

despite having the widest condition range available for model development, did not perform as well as models for larger fish.

Another potential source of error could be skin temperature changes during BIA measurements on live fish. Fluctuations in skin temperature have been shown to impact BIA measures in a clinical setting (Gudivaka et al. 1996) and could change the course taken by the electrical current through the fish. The YOY in particular, due to their small size and volume, could have changed in temperature during sampling while being handled out of the water. This result would be especially evident when sampling at 8°C where the air temperature in the room exceeded the temperature of the water and thus fish by several degrees. This affect could have acted to raise the skin temperature of the fish rapidly over the course of sampling and influenced the readings – however that is only speculation.

In addition, the stress of handling and implanting PIT tags might have had more impact on smaller fish than expected, thereby creating proportionally more bruising or greater ionic change of the fish's internal environment. Either of which might impact the physiological variables that influence the electrical properties of the fish's tissue and therefore the measured resistance and reactance values. While 24 hour acclimation periods between temperature treatments were chosen to limit the possibility of condition changing between samples, this period might have been insufficient for all of the fish in this study to return to homeostasis. This protocol could have introduced a stress bias into the BIA models. A recent study found BIA measures were correlated with plasma osmolarity and salinity, however, the interaction between BIA and condition was not considered (Miller 2014). Although

the extent to which stress impacts BIA is still unclear, it appears to be a variable worth further investigation.

These potential sources of error might explain the lower performance of the YOY model, but they were more than likely a much smaller impact on the recruit model. While we consider the low condition range in the recruit data to be the likely cause, there is also the potential for user or procedural error in this group. This group contained the only data sampled by a less experienced BIA user without the supervision of one of the authors. Despite the robust nature of the BIA models as evidenced by the sensitivity analysis (Figure 8), user experience has been found to be a source of error when using this technology (Cox et al. 2011).

Recently, concern has been expressed that the primary predictive power of BIA is related to using detector-length controlled variables or using length and weight as covariates when estimating absolute values of condition (dry mass) (Klefoth et al. 2013). In support of this concern, Klefoth et al. (2013) referenced weak relationships between BIA and relative values of condition (% dry weight). However, in both of the studies referenced, BIA measures were taken at a single location along the dorsal side of the lateral line as originally performed by Cox and Hartman (2005). Their protocol failed to take into account the effect of measurement location as reported by Hafs and Hartman (2011). Our findings, using the suggested needle sampling locations by Hafs and Hartman (2011), support the utility of a dorsal to ventral sample (DTV). The DTV sample data were selected much more frequently for striped bass than DML data (Table 6). Considering fish primarily store lipids in visceral mesenteries (Love 1970; Sheridan 1988), the predictive power of this sample location physiologically

makes sense, as the viscera are supposedly crossed by the current from the BIA device. If the earlier studies had incorporated the new measurement location or tested for optimal sample locations on their species of interest the amount of variation explained by BIA may have likely improved.

The BIA technique shows great promise with striped bass. In order to verify that the strong performance seen in the lab carries over, a field validation study is highly recommended. If successfully validated in the field, BIA incorporation with regular field sampling would allow MDDNR and other states with striped bass to monitor population condition without undue removal of individuals, greatly increasing potential sample size. These large, spatially-diverse data sets would be a great asset to management and research alike, opening new opportunities to understanding the effects of nutrition at the population, community, and watershed levels. Incorporating BIA in mark-recapture studies would allow us to better understand condition seasonally and over the lifetime of individuals, potentially allowing managers to estimate future recruitment more accurately. Moving forward, BIA shows great potential as a commonplace tool in holistic fisheries management.

Chapter 3: Validating Striped Bass BIA Models in Field Conditions.

Abstract

The use of bioelectical impedance analysis (BIA) in fisheries, while novel, has shown promise in recent years. BIA has successfully been used by past researchers in laboratory settings to estimate relative and absolute measures of proximate components in a variety of fish species. These lab-derived models have rarely been tested against independent data collected in the field. We sought to validate recently developed BIA models for striped bass *Morone saxatilis* for use in regular surveys by the Maryland Department of Natural Resources (MDDNR). Data was collected during regular MDDNR surveys or with similar protocols. We found BIA consistently overestimated percent dry weight for four of the five models. Field samples also frequently fell outside the range of data used for model development. Results suggest additional data is needed for model development to better represent the conditions typically encountered by MDDNR in the Chesapeake Bay. Furthermore, additional research is needed to determine if the use of hatchery-reared fish for BIA model development is appropriate when the end goal is to sample wild fish.

Introduction

In recent years ecosystem-based fisheries management has been gaining momentum with a goal of holistically managing ecosystems instead of partitioning them into more traditional single-species management models (Pikitch et al. 2004;

Link 2005). An ecosystem-based strategy, however, relies on indicators independent of fisheries removal for management decisions (Pikitch et al. 2004; Link 2005). One of the indicators being suggested in the Chesapeake Bay region is fish condition (Maryland Sea Grant 2009). A new technology called bioelectrical impedance analysis (BIA) allows the non-lethal estimation of fish body composition (Cox and Hartman 2005). The BIA method originated in the medical field and has been used to estimate fat-free mass and hydration levels (Lukaski et al. 1985; Kushner and Schoeller 1986; Lukaski et al. 1994). It has also been correlated with health and nutritional status in human patients, from caring for hemodialysis patients (Chertow et al. 1995) to tracking HIV progression (Schwenk et al. 2000). Strong predictive models of body composition have already been developed with BIA for bluefish *Pomatomus saltatrix*, brook trout *Salvelinus fontinalis*, and cobia *Rachycentron canadum* (Duncan et al. 2007; Hafs 2011; Hartman et al. 2011).

The methods for assessing fish condition with BIA are relatively simple. A small electrical current (425 µA, 50 kHz) is passed through an individual's tissue and the resistance and reactance values are measured. Typically a Quantum II bioelectrical body composition analyzer (RJL Systems, Clinton Township, MI) is used for sampling fish (Cox and Hartman 2005; Margraf et al. 2005; Hafs and Hartman 2011; Hartman et al. 2011). Resistance and reactance, and the electrical parameters that can be derived from them, are then used to predict the proximate composition of the test subject.

The reason these electrical measures are thought to correlate to proximate composition is because fat is an electrical insulator and the lipid bilayer of cells can

function as a capacitor (Lukaski 1987). Resistance is a measure of how much a substance opposes the flow of electricity, therefore, the insulating properties of fat should cause resistance to increase in fish with more fat (Lukaski 1987). Reactance is the opposition of a substance to a change in voltage due to its capacitance, therefore reactance should increase in fish with healthier, fatter cells (Lukaski 1987). After a fish is measured for BIA it is sacrificed and the proximate components of interest to the user are measured. Models are then developed with regression procedures to predict these proximate composition values from the electrical parameters that were measured (resistance and reactance) and derived.

Once these models are developed, fish no longer have to be sacrificed in order to measure proximate composition. The BIA measures in fish are, however, affected by several internal and external factors (Cox et al. 2011). Temperature, in particular, has been found to have an impact on resistance and reactance measures in both clinical and fisheries studies (Buono et al. 2004; Corciovă et al. 2011; Cox et al. 2011; Hartman et al. 2011; Stolarski et al. 2014).

Early studies that developed BIA models in fisheries either failed to incorporate sample temperatures or did not take into consideration the effect of temperature on their measurements (Cox and Hartman 2005; Pothoven et al. 2008). More recent works that have incorporated temperature have generally developed their BIA models in the laboratory and controlled temperature (Duncan et al. 2007; Hafs and Hartman 2011; Hartman et al. 2011; Klefoth et al. 2013). Relatively few have developed temperature corrections to allow the use of BIA across a wider range of conditions (Margraf et al. 2005; Stolarski et al. 2014). We are unaware at the time of

this writing of any peer-reviewed studies other than Hafs (2011) where BIA models with temperature corrections have been validated with data independent of model development and under field conditions.

Haus (2014) developed BIA models and temperature correction models for striped bass in the Chesapeake Bay in response to Maryland Sea Grant's call for condition indices to be used in striped bass management. Based on the above considerations, the objective of this study is to validate the previous BIA models for striped bass in the field under conditions consistent with current Maryland Department of Natural Resources (MDDNR) sampling.

Methods

Fish Collection

Approximately 20 samples were collected in each of the five size classes used by Haus (2014) for BIA model development (Table 1). The fish for this study were primarily collected during existing field surveys conducted by MDDNR. Sources and associated means of collection varied, however, due to the range in sizes collected. The YOY fish in the 50-150 mm range were collected by beach seining at various locations in the Choptank River basin, similar to the methods used by MDDNR for the juvenile index (Durell and Weedon 2011). Fish in the 150-550 mm size ranges were captured by hook and line during regular MDDNR surveys and sampled as they were being collected. Due to the irregularity of fish in the 550 mm and up size range in Maryland's portion of the Chesapeake Bay, these fish were collected from commercial pound nets. All fish were euthanized by Maryland DNR upon capture and BIA was performed immediately as fish were made available.

BIA Data Collection

Resistance and reactance was measured on all fish with subdermal, needle electrodes using a Quantum II bioelectrical body composition analyzer (RKL Systems, Clinton Township, MI) (Figure 2). All needles were 28 gauge, however, spacing and depth specifications for the signal and detector electrodes varied by size class to minimize harm to the fish by over-penetration (Table 1). For example, fish in the YOY size class, 50-150 mm in length, were sampled with needles set 5 mm apart and set to penetrate a maximum of 1 mm. Fish were placed on a non-conductive board with the head toward the left and the belly oriented toward the sampler. Fish were then blotted dry before measurement. These steps ensured consistent handling of the fish and minimized the possibility of alternative paths for the electrical current.

The BIA measurements were taken at two locations on the fish based on the recommendations of Hafs and Hartman (2011). The first measurement was taken along the dorsal midline (DML) of the fish by placing the first electrode roughly 2-3 scale rows below the DML half way between the head and the insertion of the first dorsal fin (Figure 3). The second electrode was placed 2-3 scale rows below the DML directly anterior to the caudle peduncle. The second location was from the dorsal to ventral (DTV) area of the fish ahead of the first dorsal fin (Figure 4). For this measurement the first anterior electrode near the head was left in place and the posteriorly located second electrode was removed and relocated to an anterior spot roughly halfway between the pectoral and pelvis fins directly below the insertion of the first dorsal fin. For consistency the detector needle of both electrodes was placed anteriorly for the first measurement and when the second electrode is moved to the belly it is posteriorly rotated such that the detector needle is then closest to the tail.

Following the direction of Hafs and Hartman (2011) we also measured the distance between detector needles of the electrodes at each measurement location. Water temperature was recorded for each fish to later correct the BIA measurements to 26°C. After completing BIA measurements, total length (TL) was recorded in the field and the fish were put on ice. Due to samples being taken from small craft, wet weights were measured upon return to shore. Each fish was returned to the lab and oven dried at 80°C to constant weight and percent dry weight was calculated.

Data Analyses

The BIA measurements were corrected to 26°C using the Temperature Correction Equation and K-values (Table 7) from Haus (2014). Corrected resistance

$$B_1 = (T_1 - T_0) * K + B_0$$

Temperature Correction Equation

B_0 = Initial BIA measurement, DMLr, DMLx, DTVr, DTVx.
B_1 = BIA measurement corrected to T_0
T_0 = Temperature measurement is being corrected to. 18°C in this case.
T_1 = The water temperature of the sample
K = Correction constant found in Table 7

and reactance values were entered into the BIA models (Table 6) from Haus (2014). Calculations for all the possible parameters used in the BIA models can be seen in Table 3. To validate the BIA models developed by Haus (2014) predicted percent dry weights were compared to measured dry weights of the harvested fish. The R^2 and root mean square error (RMSE) estimates were calculated and used to assess the ability of the models to predict individual fish condition. Mean predicted and observed dry weights were calculated and a paired t-test was performed to assess the

ability of the model to predict mean population condition. Residuals were plotted to check for obvious patterns and field data were plotted alongside lab data obtained from Haus (2014) to compare data ranges and patterns.

Results

For this validation study 108 fish were sampled and euthanized (Table 8). Sample sizes by size class ranged from 17-24. Total length (TL) ranged from 54 mm to 773 mm and wet weight (WW) ranged from 1.56 g to 3982.90 g. Water temperature ranged from 18.3C to 30.6C. Thirty-four percent of the samples were collected from water higher than 26°C. The percent dry weight range for individual size classes was as small as 2.54% for large juveniles and as large as 10.32% for adults.

On an individual estimation basis the models performed poorly. The R^2s were negative for all of the models (Table 9). The RMSE ranged from 4.24 for the large juveniles model to 11.44 for the recruit model. The models for the 4 smallest size classes overestimated every fish (Figure 9). Although the adult model residuals were centered around 0, there was a strong relationship between the residuals and TL (Figure 10).

When estimating mean percent dry weight the predictions for the 4 smallest size classes were significantly different from the observed means (p-values< 0.01) (Table 10). The adult model's mean prediction of 25.9% was not significantly different from the observed mean of 25.0% (p-value$= 0.43$) (Table 10).

Recruit-sized fish and a portion of the smaller adult-sized fish collected in the field were outside the range of TL and WW sampled by Haus (2014) for model development (Figure 11). Fish from approximately 450 mm to approximately 600 mm are outside the data ranges collected by Haus (2014) for the recruit and adult models. Additionally, all of the YOY collected in the field were smaller than the fish used by Haus (2014) for model development (Figure 12).

Discussion

Despite the poor performance of the BIA models in this field study there is still potential they will have utility in the future. Hafs (2011) also found consistent overestimation of condition in wild fish using lab models developed with hatchery fish. The models in this study developed by Haus (2014) used hatchery reared fish for the three smallest size classes, while the recruit and adult models used samples collected from the wild. Excluding the recruit model, which will be discussed below, the results in this study follow the same pattern with models developed on hatchery fish overestimating wild fish condition.

Hafs (2011) referenced morphological and physiological stress response differences between wild and hatchery-reared fish as a likely reason for this bias. More evidence has been presented that stress response impacts BIA by recent work finding correlation between BIA measures and plasma osmolality (Miller 2014). These findings suggest the shortcomings of the BIA models used in this study are related to the data used to develop them.

The likely reason for the poor performance of the recruit model is the gap in TL and percent dry weight among the samples used to develop the model mentioned

by Haus (2014). The narrow TL range used in model development required the model to extrapolate for the larger fish sampled in the field. Similarly, with only a 9.4% range in percent dry weight used to develop the recruit model, several samples in this study had percent dry weights outside that range, requiring further extrapolation. These issues could likely be resolved with a minimal number of additional lab samples. The low condition range used for the recruit model development is likely due to the different feeding protocol used by Haus (2014) for this size class. Incorporating the fasting protocol used on the other size classes would most likely better represent the lower percent dry weight range seen in this study's field samples. Also, the fish collected in this study appear to better represent the upper half of the proposed recruit size class (450-550 mm). If possible, the field collection sites and protocols used in this study should be used for sample collection as it more closely represents the samples of the intended end user (MDDNR).

While the data gap affecting the recruit size-class partially extends in to the adult size class, this extrapolation does not fully explain the strong relationship seen between adult model residuals and total length. This fact is especially concerning when you take into account that the adult model was the only BIA model to incorporate TL as a variable, presumably accounting for variance associated with TL. This raises the question whether factors related to the data or the model development process are causing this residual pattern.

The YOY model also performed poorly; at least partially due to extrapolation. All of the fish sampled in this study for the YOY size class were smaller than those used to develop the model in the lab, despite being within the defined range of 50 mm

to 150 mm. Additionally, most of the YOY samples were taken at a higher water temperature than what was used in the lab. Fourteen of the YOY samples were taken above 30.0°C, 4°C higher than the warmest temperature used for model development, 26°C. Further refinement of the feeding protocol used to obtain the desired condition range is needed for YOY fish. The current protocol used by Haus (2014) likely allowed for more growth during the experimental period than intended, skewing the data toward the larger end of the size class. Also, the YOY were not the only fish with samples taken in water over 26°C. We propose an additional temperature sample be taken above 30°C to prevent temperature biased extrapolation.

Beyond sampling in waters warmer than anticipated by Haus (2014), there are additional sources of temperature error in the field that should be kept in mind. The water temperatures for all of the fish in the juvenile size class through adult size class were measured at the water's surface (upper 0.6 m.). The fish collected by hook and line (juveniles, large juveniles, recruits) were caught in late morning as water surface temperatures begin to change compared to the deeper water where the fish are traditionally caught. This difference could have led to measurement error by not correctly measuring the true temperature of the fish's environment or the body temperature of the fish. This error did not impact the adult size fish because they were collected from shallow pound nets before dawn when the temperature was more likely constant throughout the sampling period. The sampling temperature also could not be accurately identified for each individual due to the hook and line collection method used.

As fish were made available by the MDDNR, the fish had been on the deck various amounts of time and an individual water temperature could not be recorded accurately. Furthermore, this variable amount of time exposed to the sun likely caused skin temperature changes, which has been shown in clinical settings to impact BIA measurements (Gudivaka et al. 1996). Hafs (2011) also suggested this as a source of error using BIA in the field. We recommend that care be taken to minimize possible skin temperature shifts when using BIA in the field. Limiting the time each fish spends after capture until being sampled should be prioritized. If delay occurs in measuring the fish, we recommend using a live well to hold the fish and frequently exchanging water in order to maintain a similar temperature as the environment. Additionally, incorporate overhead shade when taking BIA measurements when possible.

An issue at present with incorporating BIA for non-lethal sampling within the current MDDNR surveys is anesthetizing fish. The standard of MS-222 cannot be used on these surveys due to the high likelihood of fish being recaptured by fisherman before the required 21 day withdrawal. Approval of a 0-day anesthetic by the FDA would eliminate this consideration.

Another challenge with implementing this technology in the field is the durability of the needle electrodes. While the electrode units were sufficiently durable, the 28 gauge needles were unstable when set to 5 mm of penetration. The added length of fine metal combined with the thicker skin and scales of the fish measured with that needle configuration led to frequent bending of the needles. Needles has to be realigned by hand, incorporating an additional source of error from

the changing widths between the needles and increasing the amount of time spent completing the measurements. Also, this frequent bending could lead to premature breakage. Heavier gauge needles would prevent this, however changes in needle gauge has been shown to effect BIA measurements (Cox et al. 2011). Slightly heavier gauge needles should be tested to see if there is still a significant source of error from the change.

Additionally, practice collecting BIA data should be conducted in the lab before taking the technology into the field as a new user. Changes in user applied pressure on the electrodes can impact resistance and reactance measures (Cox et al. 2011), and are compounded by vessel movement and rough water conditions.

Despite the present poor performance, these BIA models could still hold potential. The noted gap in the development data could be fixed with a relatively small sample of fish. With warmer waters seen during summer sampling than the warmest experimental temperature used by Haus (2014), additional lab samples above 30°C should prevent most of the temperature extrapolation errors seen in this study. At present we recommend further work be done to better understand what differences between wild and hatchery-reared striped bass affect BIA samples. Also, while more work is needed to improve these BIA models for field applications, incorporating BIA in MDDNR surveys could provide the requisite samples to create wild-fish models that correct for overestimation similar to Hafs (2011). As such, it is still too early to speak to the true potential of these models.

Conclusions

Although this study had poor results when the laboratory developed model was applied to a field situation, the value of this study is that it shows the inherent problems that one encounters when transferring a lab protocol to a field application. We quickly learned what not to do when trying to use this model because of the inherent nature of variability that cannot be controlled for within a field setting. The application of BIA in the field still has tremendous potential. Knowing some of the pitfalls outlined in this effort and taking the steps necessary to mitigate these problems, including higher temperature model development, should improve the utility of this technique considerably to the point where the accuracy of the tool to assess overall fish health by non-lethal sampling is worthwhile.

Appendix

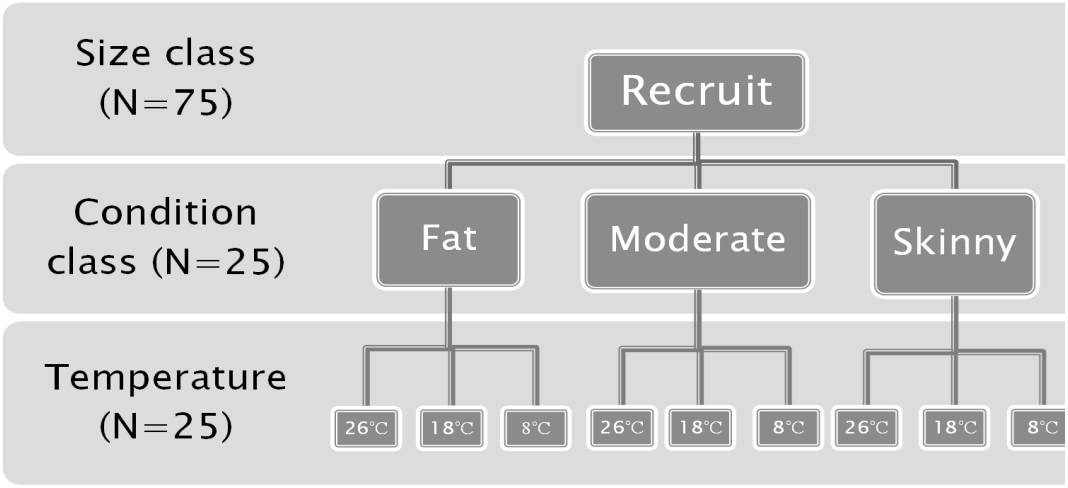

Figure 1: Hierarchical study design for striped bass BIA model development. Each experimental size class had its own sample group of 75 fish fed to satiation daily until the fish averaged the mean total length of that size class. These fish were then assumed fat and 25 fish were separated for the fat condition class sample group. The remaining fish were fasted to establish the moderate and skinny condition classes. Each of these condition classes were then sampled for BIA at three different water temperatures to develop Temperature Correction Equation.

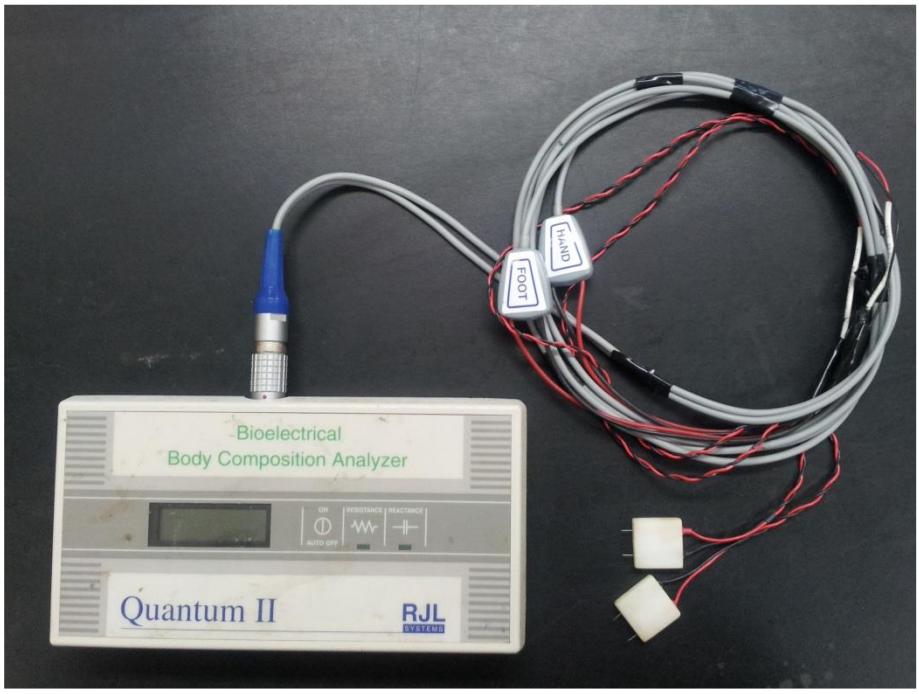

Figure 2: Quantum II bioelectrical body composition analyzer (RKL Systems, Clinton Township, MI)

Figure 3: Dorsal midline (DML) measurement location demonstrated on a palmetto bass. Note that the detector needle of each electrode (the red wire) is kept anterior of the signal needle.

Figure 4: Dorsal to ventral (DTV) measurement location demonstrated on palmetto bass. Note that the electrode anterior of the first dorsal fin near the dorsal midline was left in place from the previous measurement location (DML **Figure 3**). Also note that the detector needle (red wire) of the electrode on the ventral side of the fish is distally placed from the head while the electrode left in the dorsal side of the fish still has the detector needle (red wire) placed proximal to the head

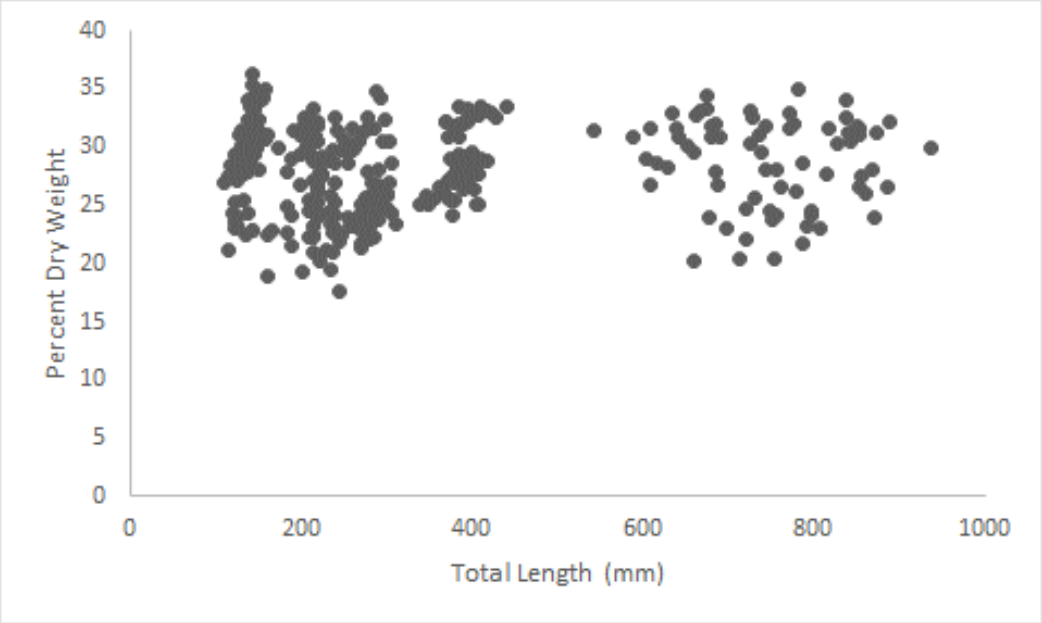

Figure 5: Percent dry weight plotted over total length for all of the striped bass used for BIA model development. Note the apparent gap in the data between 300mm and 600mm, particularly the narrower percent dry weight range from 300mm to 450mm and the lack of samples between 450mm and 550mm. This gap is the expected reason for the recruit model's poorer performance and is likely due to the different feeding regime used for that size class.

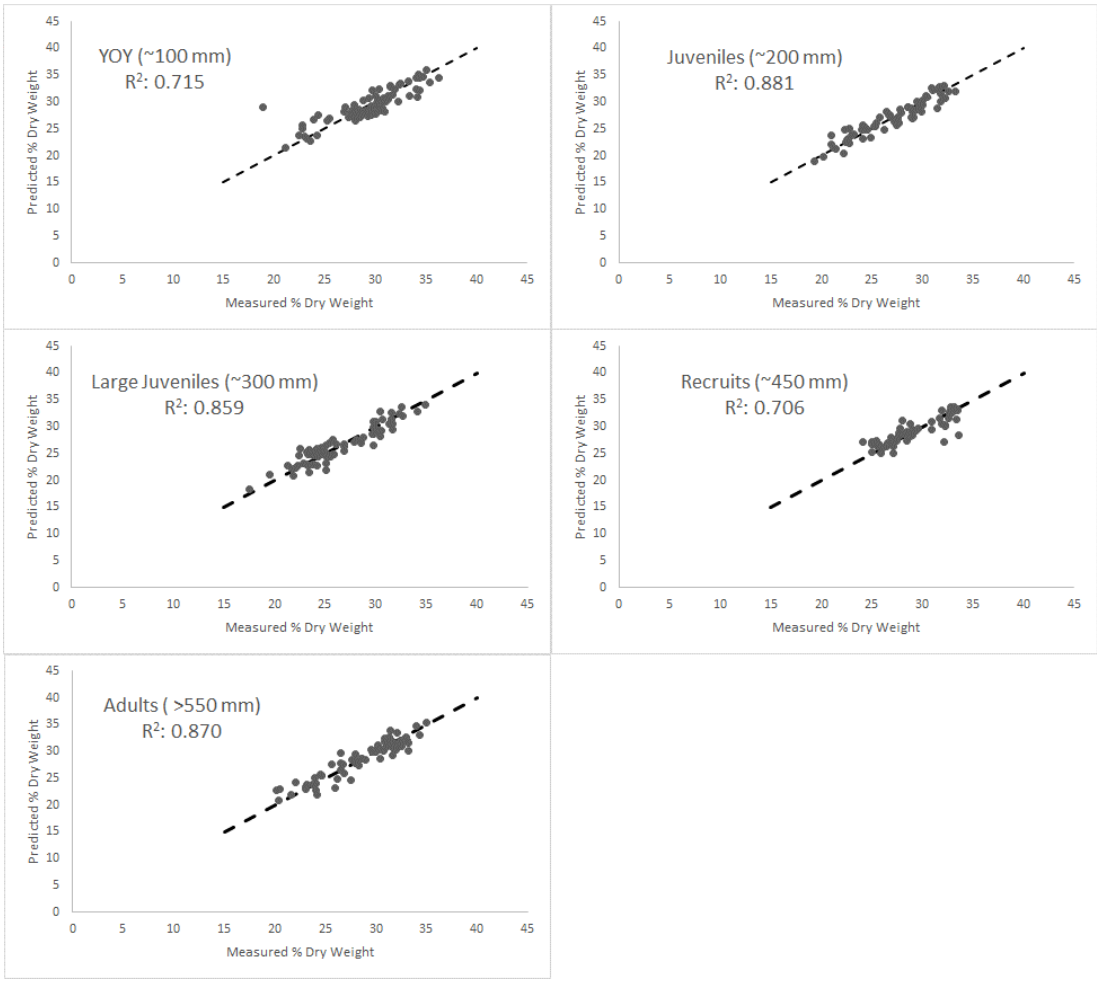

Figure 6: Predicted vs Observed percent dry weight plots for each of the five striped bass BIA models developed for the size classes listed in the figure titles. Note that the plotted points fall roughly along a 1 to 1 line between predicted and observed (represented by the black, dashed line). Model R^2s are included in each plot.

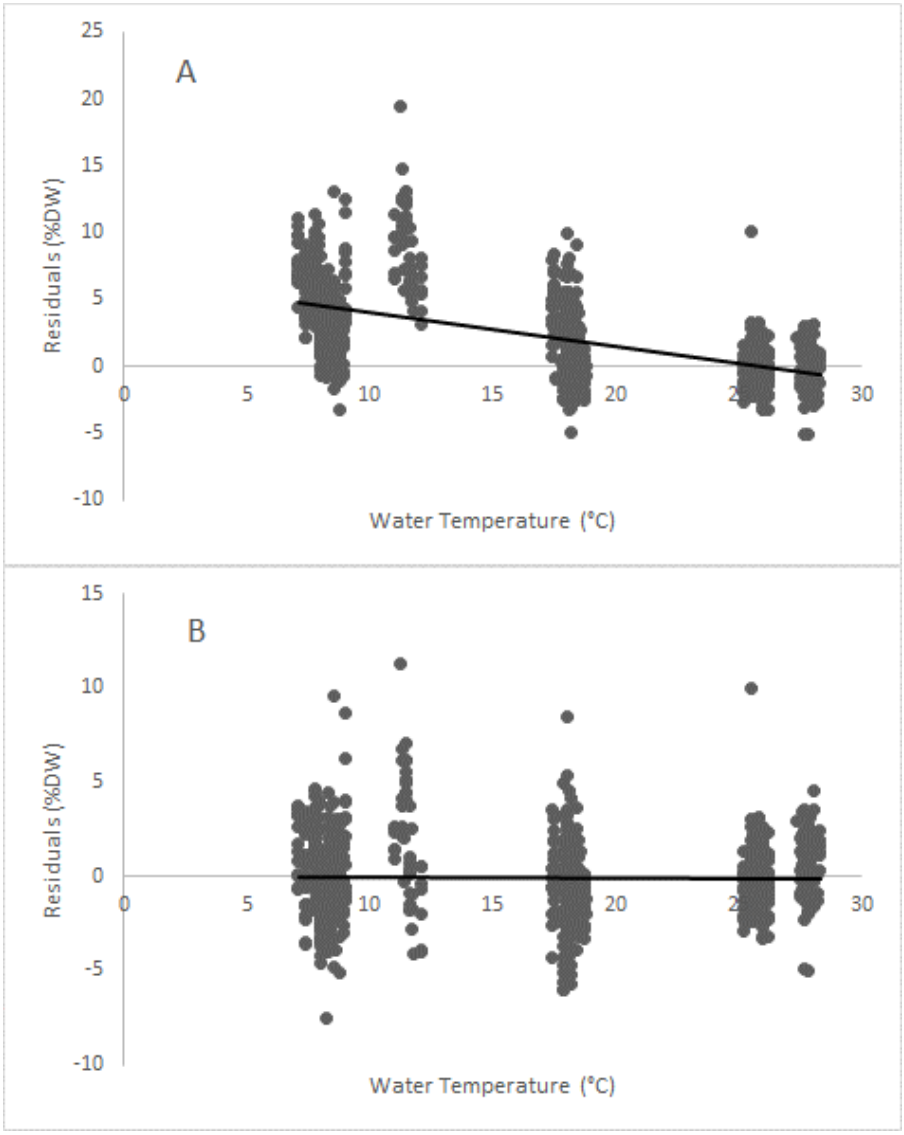

Figure 7: Striped bass BIA model residuals plotted against water temperature (°C) before (A) and after (B) applying temperature corrections. Before correcting BIA measures for temperature, model residuals had a significant relationship with water temperature (p-value: <0.0001). After applying temperature corrections with the Temperature Correction Equation, residuals no longer have a significant relationship with water temperature (p-value: .48)

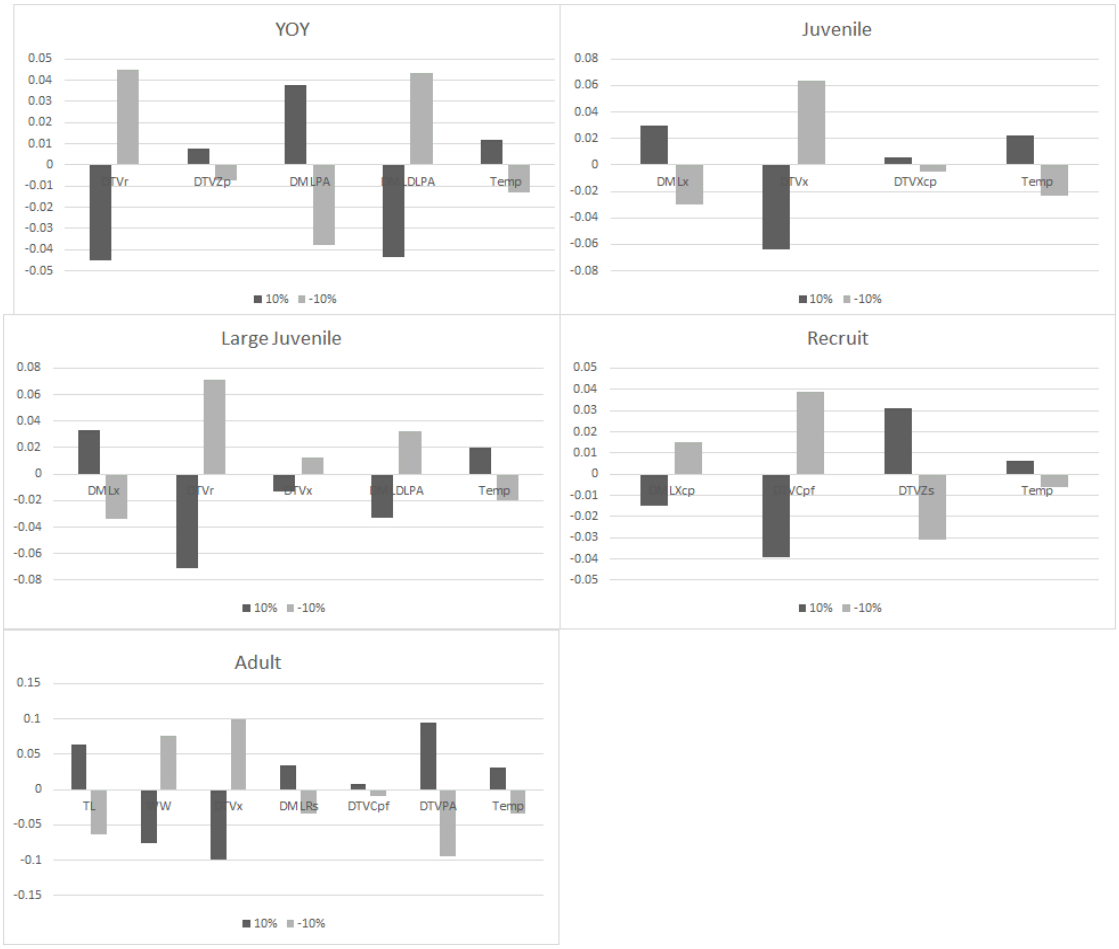

Figure 8: Results of striped bass BIA model sensitivity analysis. Model variables were changed plus or minus 10% and the resulting change in the model estimate was recorded. Additionally, sample temperature was also changed plus or minus 10% and estimate changes recorded. If the model estimate changed by more than 10% when a variable was modified, that variable was deemed sensitive. If the change in model estimate was less than 10% that variable is deemed insensitive. This was done to test how robust the striped bass BIA models were against potential measurement error. The DTVx and DTVPA variables in the adult models were the only variables in the study that changed more than 9%, however both changed less than 10% and were deemed insensitive. None of the BIA models were sensitive to changes in sample temperature. These results suggest the models are robust and not likely to be heavily impacted by sampling error.

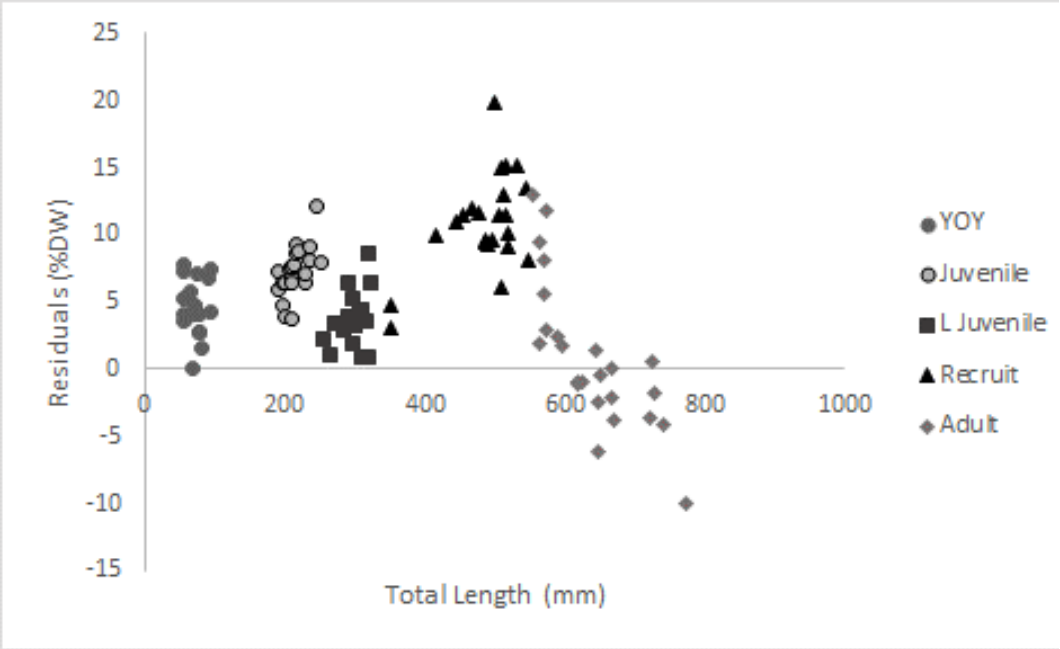

Figure 9: Field validation: striped bass BIA model residuals plotted over total length. Four of the five BIA models consistently over-estimated the percent dry weight of field samples. It should also be noted the negative trend between residuals and total length for the adult model. The over-estimation seen in this study is consistent with findings from Hafs (2011). The suspected cause lies in possible differences between wild fish and the hatchery-reared fish used for model development. If physiological stress responses or morphology differ, the ionic composition of the tissue could vary, leading to sampling bias.

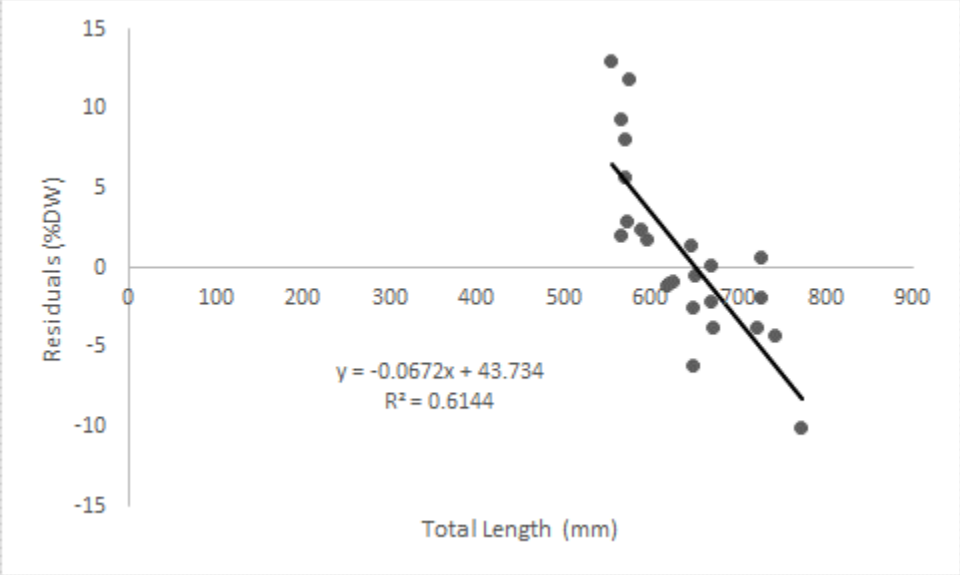

Figure 10: Striped bass BIA adult model residuals when estimating field samples plotted over total length. The strong relationship between residuals and length is unexpected considering the inclusion of total length in the adult BIA model. This raises questions as to what background factors could be the cause of this result. Whether they are related to differences between lab and field samples, or to the model development protocol is not certain.

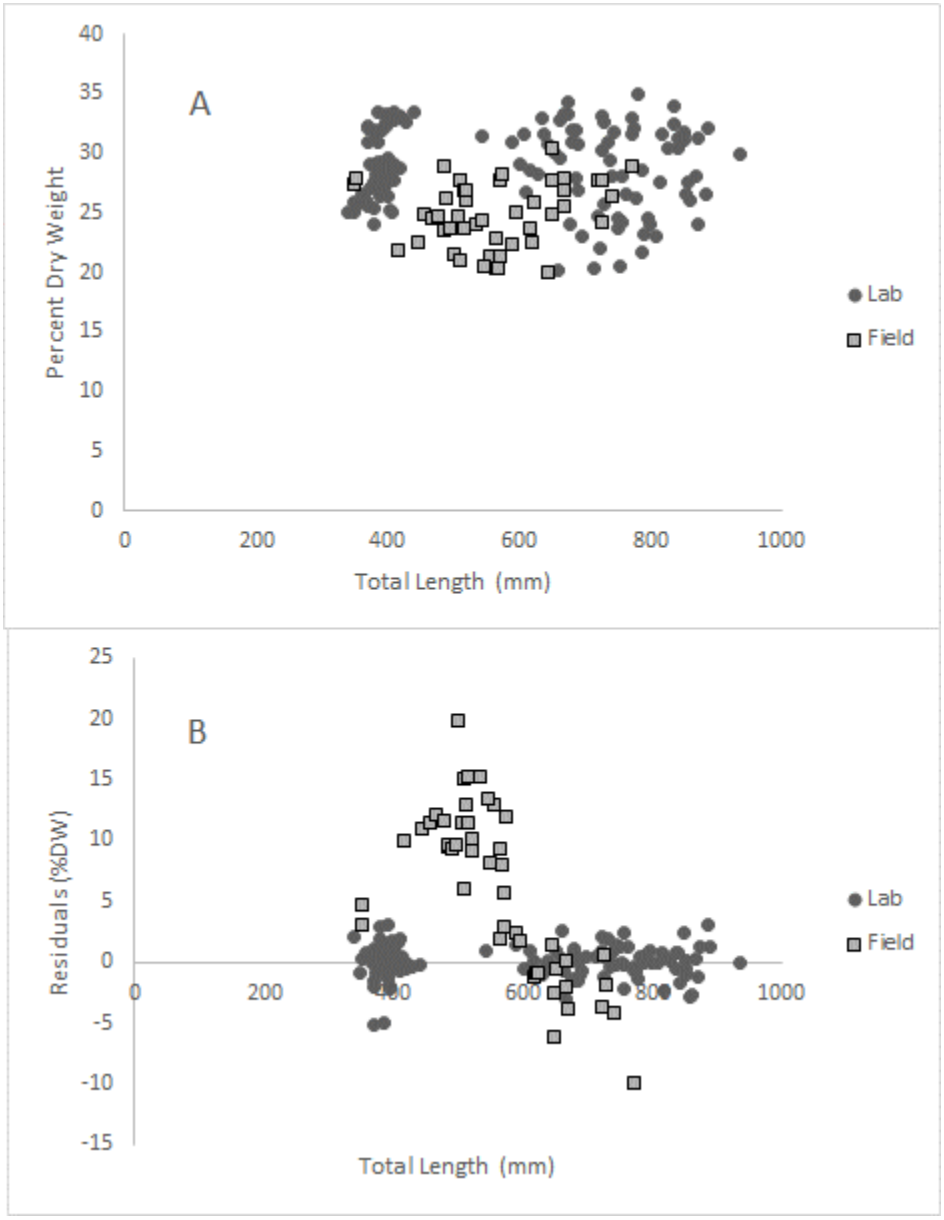

Figure 11: Striped bass percent dry weight plotted over total length using recruit and adult size class data from both the lab (Haus 2014) and the field (A). The gap in data coverage mentioned by Haus (2014) was over a size and condition range frequently encountered in the field. This result led to model extrapolation that is at least partially responsible for residual pattern seen when applying the recruit and adult models to field data (B).

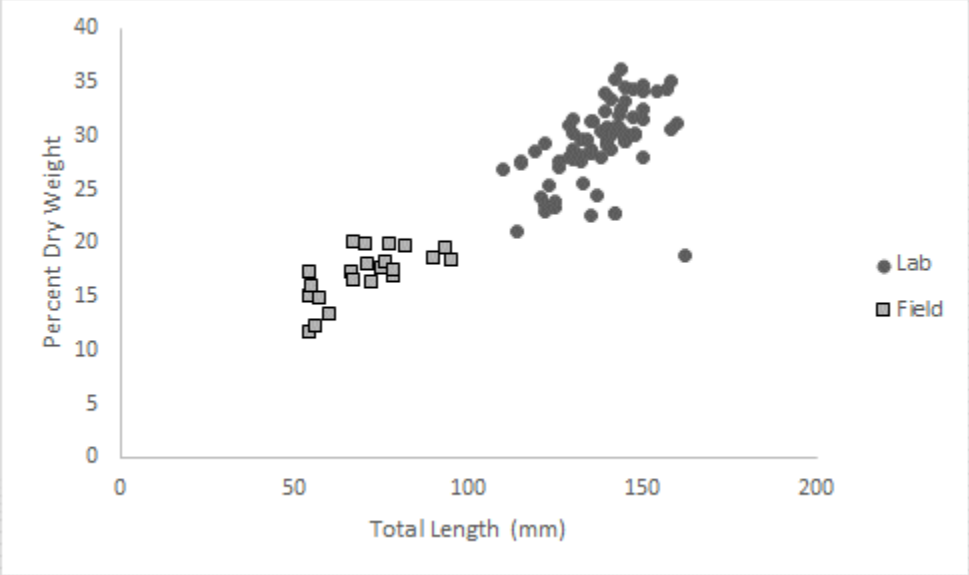

Figure 12: Striped bass percent dry weight vs total length plots for both the lab-collected model development data (Haus 2014) and the field-collected data in this study for the young-of-year (YOY) size class. Note that the YOY collected in the field were both smaller and in poorer relative condition than the samples used for model development. This result led to model extrapolation that likely contributes to the poor performance of the YOY BIA model in this study.

Table 1: The five experimental size classes used for striped bass in this study and their range in total length. Also listed are the needle configurations used in the BIA probe for each size class, with the first measurement being the needle penetration depth and the second being the distance between the signal and detector needles on each probe.

Size Class	*Total Length (mm)*	*Needle configuration*
Young-of-year (YOY)	50-150	1mm x 5mm
Small juvenile	150-250	3mm x 10mm
Large juvenile	250-350	3mm x 10mm
Recruit	350-450	3mm x 10mm
Adult	550-850	5mm x 10mm

Table 2: The 0 through 3 relative body fat index (BFI) used to determine relative condition classes fat, moderate, and skinny of striped bass in the BIA model development study. Listed are the visual percent coverage ranges used to determine index score. To determine percent coverage the abdominal cavity of the fish is opened with standard necropsy procedures. The percentage of the viscera that are visually covered by fat is then estimated. This estimate is then converted to the BFI score according to the table below.

BFI score	*Percent coverage*
0	0%
1	0-25%
2	25-75%
3	75-100%

Table 3: Provided are the parameters used in striped bass BIA model development and the equations used to calculate them.

Parameter	*Symbol*	*Units*	*Calculations*
Resistance	r	ohms	measured by Quantum II
Reactance	x	ohms	measured by Quantum II
Resistance in series	Rs	ohms	DL^2/r
Reactance in series	Xc	ohms	DL^2/x
Resistance in parallel	Rp	ohms	$DL^2/(r+(x^2/r))$
Reactance in parallel	Xcp	ohms	$DL^2/(x+(r^2/x))$
Capacitance	Cpf	picoFarads	$DL^2/((1/(2\times\pi\times50000\times r)\times(1\times10^{12}))$
Impedance in series	Zs	ohms	$DL^2/(r^2+x^2)^{0.5}$
Impedance in parallel	Zp	ohms	$DL^2/(r\times x/(r^2+x^2)^{0.5})$
Phase angle	PA	degrees	$\text{atan}(x/r)\times180/\pi$
Standardized phase angle	DLPA	degrees	$DL*(\text{atan}(x/r)\times180/\pi)$

DL= Detector length measured as the distance in millimeters between detector electrodes (red wire). When taking these measurements follow the curvature of the fish.

Table 4: Sample size, total length average and range, and percent dry weight average and range of the data used to develop each of the striped bass BIA models in this study. Of particular note is the relatively low percent dry weight range in the recruit size-class data. This was suspected as a likely cause for the lower performance of the recruit BIA model (R^2=0.7 vs R^2>0.80).

Model	*N*	*TL range (mm)*	*%DW range*	*Average TL (mm)*	*Average %DW*
YOY	74	52	17.41	137.7	29.29
Juvenile	69	80	13.95	213.2	26.92
Large Juvenile	74	75	17.27	273.7	26.46
Recruit	57	101	9.41	389.7	28.97
Adult	73	393	14.80	747.7	28.74

Table 5: Number of explanatory variables selected by each striped bass BIA model. Each BIA model also included an intercept. Also reported are the R^2's and root mean square errors calculated for the models using *validate* function in *rms* package (Harrell 2012) in program "R" (R Team 2012)

Model	*# variables*	*R^2*	*RMSE*
YOY	5	0.715	1.930
Small Juveniles	5	0.881	1.237
Large Juveniles	5	0.859	1.384
Recruits	4	0.706	1.384
Adult	7	0.870	1.349

Table 6: Coefficients for the best striped bass BIA model for each size class. Of particular interest is that the adult model was the only BIA model to incorporate total length. Also take note that the majority of the selected variables are from the dorsal to ventral (DTV) sample location. This fact intuitively makes sense physiologically; as the current is theoretically passing through the viscera at that sample location and fish are known to store the majority of their lipids in visceral mesenteries (Love 1970; Sheridan 1988). Calculations for the parameters listed are listed in Table 3.

	Model				
Parameter	YOY	Juvenile	Large Juvenile	Recruit	Adult
Intercept	16.431156	24.44	4.4195128	22.19	35.31
TL					-0.02399
WW		0.0767			0.005335
DMLx			-0.1106239		
DTVr	0.028371		0.0920166		
DTVx			0.0664780		0.4916
DMLRs					-0.0143
DMLXcp				0.1573	
DTVRs		-2.116			
DTVXcp		2.979			
DTVCpf		5.815e-23		3.943e-23	-2.49e-24
DTVZs				-0.2745	
DTVZp	-0.267424				
DMLPA	-0.989643				
DTVPA					-1.051
DMLDLPa	0.019150		0.0060499		

Table 7: Provided are the K values for Temperature Correction Equation used to correct striped bass BIA measurements to 26°C. These values were derived as the slope of the relationship between water temperature (°C) and the BIA measurements listed in the column headings.

Model	*DMLr*	*DMLx*	*DTVr*	*DTVx*
YOY	-13.42	-3.68	-4.28	-2.42
Juveniles	-8.56	-1.65	-4.24	-0.79
Large Juveniles	-8.63	-1.68	-4.45	-1.16
Recruits	-2.27	0.04	-1.36	-0.40
Adults	-2.86	-0.41	-1.88	-0.31

Table 8: The sample size and the total length (TL), wet weight (WW), and percent dry weight (%DW) range and mean for each of the size classes used in striped bass BIA model validation. Data was collected during Maryland Department of Natural Resources regular field surveys or with similar protocols.

Model	*N*	*TL range (mm)*	*WW range (g)*	*%DW range*	*Average TL (mm)*	*Average WW (g)*	*Average %DW*
YOY	22	41	6.36	8.26	70.3	3.82	17.09
Juvenile	22	60	114.66	4.87	214.4	91.23	22.92
Large Juvenile	17	68	147.79	2.54	296.9	257.38	26.52
Recruit	23	198	1048.10	8.38	484.8	990.18	24.84
Adult	24	218	2668.45	10.32	637.8	2412.84	25.00

Table 9: The R^2 and root mean square error (RMSE) estimates for the striped bass BIA model predictions with independent, field-collected data. The reported R^2's have not been adjusted to move the decimal point right (i.e. 0.80 to 80.0). The estimates of less than -1 are due to severe overestimation by the BIA model, statistically suggesting that a horizontal line through the data would explain more variance than the selected model. The RMSE estimates are double or more than the associated lab models.

Model	*R^2*	*RMSE*
YOY	-3.45	4.95
Juvenile	-44.52	7.48
Large Juvenile	-37.17	4.24
Recruit	-23.41	11.44
Adult	-2.38	5.47

Table 10: Comparing mean estimates of predicted and observed percent dry weight (%DW) of striped bass BIA models. Also reported are p-values for a paired, two-tail t-test. Predicted mean percent dry weight was significantly different from observed for four of the five models, all of which greatly overestimated. While the adult model's predicted mean was not significantly different from observed, the strong relationship between model residuals and total length (Figure 10) brings into question the validity of the model.

Model	*Observed mean %DW*	*Predicted mean %DW*	*P-value*
YOY	17.09	21.63	$5.81e^{-10}$
Juvenile	22.92	30.18	$2.04e^{-14}$
Large Juvenile	26.52	30.23	$2.03e^{-6}$
Recruit	24.84	35.71	$1.21e^{-12}$
Adult	25.00	25.90	0.43

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