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# ESTIMATING SEASONAL VARIATION IN CONDITION OF YELLOWPERCH

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ESTIMATING SEASONAL VARIATION IN CONDITION OF YELLOW PERCH

Emily Powers

Research has shown that Yellow Perch *Perca flavescens*, found throughout lakes and streams of North America, are good indicators of environmental health. Due to the correlation between fish condition and ecosystem health it is essential to reliably estimate condition to better manage our fisheries. Therefore, the study objectives were to measure the seasonal changes in adult Yellow Perch percent dry weight (PDW), and compare the reliability of bioelectrical impedance analysis (BIA) and morphometric-based estimates of condition. The seasonal fluctuations in average monthly PDW were measured over a two-year period and were sinusoidal in shape with peaks in August 2015 (25.44 %) and July 2016 (26.34 %) when water temperatures were above 22 °C. The valleys occurred in February 2015 (22.73 %) and April 2016 (22.96 %) when temperatures were below 5 °C. When the best supported BIA lab model was used to estimate condition of Yellow Perch in the lab it explained 18 % more of the variation in PDW ($R^2 = 0.60$; RMSE = 0.92) when compared to morphometric-based models ($R^2 = 0.42$; RMSE = 1.09). BIA and morphometric-based models were unable to reliably estimate condition of Yellow Perch measured in the field ($R^2 \leq 0.18$). The sources of error associated with standard fish condition estimates need to be identified and methods improved if we are to successfully manage our local and global fisheries.

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# Chapter 1: Literature Review

The ability to reliably and accurately estimate fish condition is fundamental for fisheries biologists and managers to determine the status of a population, prey availability, and prevalence of competition and predation (Moyle and Cech 2004). Condition refers to the overall physiological well-being of an organism (Pope and Kruse 2007) and may be assessed by measuring growth rate and/or fat content (Caldarone et al. 2012). Rapid growth in early life stages of fish increases the probability of survival and recruitment as vulnerability to predation and starvation decreases (Fonseca and Cabral 2007). Growth is also related to reproductive success and habitat quality (Brandt et al. 1992; Roy et al. 2004; Amara et al. 2009; Vehanen et al. 2009). In addition, reserves of energy such as lipids, are strong indications of condition (Hanson et al. 2010) as an increase in reserves improves the probability of survival for overwintering (Sullivan 1985), are used during parental care of young (Mackereth et al. 1999), and are stored for use during migration (Cooke et al. 2006).

The earliest assessment of fish condition was based on simple length-weight relationships (Le Cren 1951). The widely-accepted theory is that heavier fish of a given length are in better condition (Lambert and Dutil 1997). Condition indices, such as Fulton's condition factor (Ricker 1975), relative condition (LeCren 1951), and relative weight ($W_r$) (Wege and Anderson 1978), use length-weight relationship's that can be compared to standardized values of a fish known to be in good condition. Using a standard weight allows for fish shape to be compensated for so $W_r$ values can be compared between individual and populations of fish species (Wege and Anderson 1978; Murphy et al. 1990). $W_r$, for instance, is used by fisheries managers in deciding appropriate fish stocking and management actions by comparing an individual or population's condition to prey abundance. For example, when condition estimates are low at the height of the growing season this may indicate lower prey abundance due to competition. $W_r$ is the most commonly used condition index in fisheries science (Blackwell et al. 2000) and is calculated by dividing the weight of the individual by the length-specific standard weight predicted by a geographically broad species-specific weight-length regression. While morphometric-based condition indices are most commonly used to assess fish condition (Green 2001), they are not without problems. For instance, fish are comprised of 60-90 % water and often compensate for lack of fat by acquiring more water internally (Shearer 1994; Breck 2008; Hartman and Margraf 2008). As water and fat cannot be differentiated when measuring wet weight, $W_r$ values may introduce error and negatively influence condition estimates.

The standard method accepted by the scientific community for measuring body composition (AOAC 1990), known as proximate analysis, is used to determine how much of the fish is fat, protein, carbohydrates, ash, minerals, and water. To determine the concentration of such components, the fish and/or sample tissue from the fish must first be homogenized. Depending on what component is being estimated, a mixture of chemicals is added to the sample to separate cellular components so they can be quantified. For example, when using the Bligh and Dyer method to measure fat content, methyl alcohol, chloroform, and water are added to the sample, mixed, and after layers have clarified into an aqueous layer and a chloroform layer, the chloroform and trace amounts of water are removed through evaporation and the remaining flask contents can be weighed (Aitken et al. 2001). When the specific energy content of tissues or whole organisms needs to be quantified, the AOAC (1990) favors the use of bomb calorimetry. Bomb calorimetry measures the heat of combustion when a sample is combusted in a sealed chamber surrounded by water (Crossin and Hinch 2005). The heat absorbed by the water then represents the sample's total energy. While proximate analysis and bomb calorimetry are effective, they are not always suitable for one or more of the following reasons: expense, use of noxious chemicals, lengthy procedures, requirement of a laboratory setting, or lethality to the organism (Cox and Hartman 2005; Crossin and Hinch 2005; Duncan et al. 2007; Pothoven et al. 2008). These limitations have increased the interest in developing non-lethal, cost-effective, efficient, portable, and accurate methods for assessing fish condition.

Bioelectrical impedance analysis (BIA) has been used since the 1970's for the assessment of human body condition (Pethig 1979) and first became popular in the 1980's to estimate body fat of specific ethnic groups (Dehghan and Merchant 2008). As techniques improved, BIA became useful within the medical community to assess serious health conditions, including the human immunodeficiency virus (Corcoran et al. 2000; Schwenk et al. 2000; Eisenmann et al. 2004), obesity (Lohman et al. 2000), coeliac disease (Ratsch and Catassi 2001), malnutrition (Barbosa-Silva et al. 2003; Pirlich et al. 2004), and muscular dystrophy (Mok et al. 2006). BIA also became a popular method used to assess body composition of other mammals, including Wild Turkey *Meleagris gallopavo* (Grimes et al. 1990), Grey Seal *Halichoerus grypus* (Gales et al. 1994), Black Bear *Ursus americanus* and Brown Bear *Ursus arctos* (Hilderbrand 1998), North American Moose *Alces alces* (Hundertmark 2002), and Striped Skunk *Mephitis spoligale* (Hwang et al. 2005).

BIA first appeared in fisheries literature when it was successfully used to estimate the amount of fat mass in Channel Catfish *Ictalurus punctatus* (Bosworth and Wolters 2001). Techniques rapidly evolved and BIA research transitioned from mass-based estimates to percent-based estimates of proximate content (Pothoven et al. 2008). To date BIA models have been successfully developed to predict proximate composition estimates for mass (Bosworth and Wolters 2001; Cox and Hartman 2005; Duncan et al. 2007; Pothoven et al. 2008) and percent dry weight (Hafs 2011; Hafs and Hartman 2014). Recent studies have shown percent dry weight (PDW) is correlated to proximate composition (Margraf and Hartman 2008) and energy density (Hartman and Brandt 1995). Therefore, model equations capable of predicting PDW can be used to estimate other body components, such as fat and protein, and offer nonlethal estimates of energy density (Hartman and Brandt 1995).

BIA generates a small alternating current between two pairs of electrodes placed on or penetrating beneath the skin of the fish (Caldarone et al. 2012). The low voltages and high frequencies allow current to pass through extracellular fluids but not cell membranes (Duncan 2009), allowing for measurement of resistance and reactance. Resistance measures how well electricity can pass through a substance and reactance measures the ability of a substance to hold a charge (Lukaski 1987). Values from resistance and reactance are correlated to measures in proximate composition. Water within a body is inversely related to body fat (Craig 1977; Schreckenback et al. 2001) so resistance is largely influenced by the concentration of water found within the individual (Schoeller 2000). Higher resistance values signify higher amounts of lipids or other nonconductive materials (Lukaski et al. 1985; Jackson et al. 1988; Kyle et al. 2004), indicating healthier individuals. Cell membranes are surrounded by lipid bilayers, which have protein channels that allow materials to pass in and out of the cell, maintaining an ionic gradient between the inside of the cell and the environment outside of the cell. When an electric current is applied using low voltage and high frequencies the current passes through the extracellular fluids but not the cell membranes. The current thereby charges the cell, which acts as a capacitor, and relays reactance for that tissue. A higher reactance indicates healthier individuals (Lukaski 1987). BIA therefore, has the potential to estimate cellular components without sacrificing the fish.

Past studies have concluded BIA provides good predictions of total dry mass, total energy, and total lipids (Cox and Hartman 2005; Pothoven et al. 2008). BIA measurements explained 95 % of the variation in dry body mass, total lipid content, and total water content of Brook Trout *Salvelinus fontinalis* (Cox and Hartman 2005). BIA lab models developed using ventral and lateral BIA measurements explained 32 % more variation in proximate composition compared to length-weight models for Dolly Varden *Salvelinus malma* (Stolarski et al. 2014). Models developed using BIA measurements to predict percent dry mass explained 12 % more variation in percent dry mass over $W_r$ for Brook Trout (Hafs and Hartman 2014). While BIA models have been successful at estimating proximate content in salmonids, with coefficients of determination ranging between 0.72 and 0.86 (Hafs and Hartman 2011; 2014), there are conflicting results when using BIA on other fish species. For example, coefficients of determination were 0.18, 0.31, and 0.53 for BIA models used to estimate the percent lipid content of Yellow Perch *Perca flavescens*, Walleye *Sander vitreus*, and Lake Whitefish *Coregonus clupeaformis* (Pothoven et al. 2008). The large variation in successful proximate composition estimates from previous studies may be the result of species-specific characteristics and/or differences in methodology. For example, whereas salmonids have thin cycloid scales, Yellow Perch and Walleye have large thick ctenoid scales. The ability of an electric current to pass through different scale types has yet to be investigated but could potentially affect resistance and reactance. Additionally, variations in methodology may have influenced proximate composition estimates as different electrode types were used, sample sizes varied, and the temperature effects on BIA measurements were not always controlled.

Research has shown that factors such as electrode location, electrode type, procedural consistency, user experience, and temperature can affect BIA measurements (Cox et al. 2011). Electrodes placed on the dorsal (muscle tissue) or ventral region (internal organs) of the fish will capture different values of resistance and reactance. Additionally, electrodes with smaller surface area generate more resistance (Cox et al. 2011). Temperature has a significant influence on conductivity and viscosity of materials. For example, when water temperatures are adjusted by 1 °C, conductivity may be affected up to 5 % (Hayashi 2004). Since temperature affects the conductivity of materials (Gray 2004) when an electrical current is applied, resistance and reactance values will widely vary for fish found in environments where large seasonal fluctuations in water temperature occur. Furthermore, because temperature affects cell membrane viscosity by affecting its rigidity (Hazel 1995; Farkas et al. 2001), some fish will compensate by altering the composition of membrane lipids (Hazel and Prosser 1974; Wodtke 1978; Wallaert and Babin 1994). After 10 days of cold acclimation in juvenile Rainbow Trout the fatty acid concentrations in plasma phospholipids become significantly higher (Wallaert and Babin 1994), leading to higher resistance and reactance values. At the same time, unsaturated fats increased allowing for more fluid-transport of ions (Wallaert and Babin 1994).

Previous BIA models used to predict proximate composition in fish have used a variety of methods when considering temperature. Many BIA models were developed for fish held at a constant temperature (Bosworth and Wolters 2001; Duncan et al. 2007;

Hafs and Hartman 2014). While researchers have also measured fish over a range of temperatures (Cox and Hartman 2005; Hafs 2011; Hartman et al. 2011; Hafs and Hartman 2015), only a few researchers have applied temperature corrections to their models (Cox et al. 2011; Stolarski et al. 2014; Hafs and Hartman 2015). Despite the negative relationship between impedance and temperature (Slanger and Marchello 1994), only several models using temperature correction equations have been published (Cox et al. 2011; Stolarski et al. 2014; Hafs and Hartman 2015).

Temperature correction techniques can be used in model development to eliminate error attributable to fluctuations in temperature for model predictions. By developing temperature-corrected equations for BIA electrical parameters, variations in resistance and reactance can be accounted for thereby improving percent dry weight predictions. Several temperature correction methods have been utilized in fisheries research. Cox (2015) assessed the effects of temperature on BIA measurements by comparing body composition and condition estimates before and after freezing Albacore Tuna *Thunnus alalunga*. Stolarski et al. (2014) used temperature correction equations to assess Dolly Varden resistance and reactance changes across a temperature range of 3-19 °C. Hafs and Hartman (2015) established relationships between BIA measures and temperature for age-0 and adult Brook Trout and determined temperature correction equations developed over a wide range of temperatures improved BIA model predictions of percent dry weight.

The successful use of BIA as a management tool in fisheries, however, relies on the standardization of techniques. To understand how measures of energy content change seasonally and geographically it is imperative to continue advancement and refinement of technologies offering cost-effective, efficient, and nonlethal methods for measuring fish condition (Hanson et al. 2010). This information is especially important for conservation and management of rare, threatened, and endangered species who are particularly sensitive to increasing physiological and environmental changes (Hanson et al. 2010).

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## Chapter 2: Introduction

Yellow Perch *Perca flavescens* are found throughout lakes and streams of North America, and are an important species economically, recreationally, and ecologically. Yellow Perch have a wide distribution extending as far north as British Columbia (Scott and Crossman 1973; Collette and Banarescu 1977) and as far south as Florida (Clugston et al. 1978). Research has shown that Yellow Perch are good indicators of environmental health (Adams 2005). For example, Yellow Perch exposed to high concentrations of metals had lower condition estimates (Farag et al. 1995; Audet and Couture 2003; Giguère et al. 2005) and impaired growth (Sherwood et al. 2000, Eastwood and Couture 2002). In the last several decades' Yellow Perch have experienced large fluctuations in population size due to unstable recruitment patterns and exploitation brought on by recreational and commercial fish harvests (Bronte et al. 1993; Marsden and Robillard 2004). As a highly valued sustainable resource to humans, fur-bearing mammals, birds, and other fish species, reliable estimates of fish condition are needed to ensure the continued recovery and success of Yellow Perch populations.

Condition indices are an important tool for studying population dynamics and are useful in many contexts found throughout conservation and environmental biology. Throughout the year, variation in fish condition results from changes in temperature, nutrition, reproductive state, photoperiod, competition for food, and spawning behavior (Seddon and Prosser 1997). For Yellow Perch fat increases during the summer when water temperatures are warm and when food is most abundant. As temperatures decrease in the fall, aquatic vegetation secedes, lakes turnover, and Yellow Perch use energy accumulated throughout summer to migrate from shallow to deeper water and begin producing eggs and milt. For temperate regions winter is a critical time for Yellow Perch as light levels and low temperatures reduce food availability and intake (Eckman 2004). Yellow Perch, however, are resilient to such conditions having evolved adaptions to reduce energy depletion by feeding when food is available and retaining lower metabolic rates during winter (Eckman 2004). Nevertheless, when energy stores are insufficient at the onset of winter and fish experience a long cold winter with large amounts of snow-covered ice, fish may become stressed and more susceptible to disease, have reduced fecundity, and experience starvation (Oliver et al. 1979; Henderson et al. 1988; Fullerton et al. 2000; Sogard and Olla 2000). Energy reserves carried over from winter are used to spawn in the spring, when energy stores are lowest. Replenishing lost fat from spawning and overwintering activities largely depends on food supply during the growing season and lake productivity (Eckman 2004).

With climate change projecting rising temperatures, especially for arctic and temperate regions, changes in fish condition will be more frequent as fish populations struggle to adapt to longer growing seasons, lower dissolved oxygen concentrations, and earlier and longer periods of lake stratification (Ficke et al. 2007). Understanding and having the ability to predict changes in fish condition as a function of seasonal fluctuations of water temperature will support fisheries managers' efforts in developing effective fisheries management plans and enforcing restrictions that protect fish species that are rare, threatened, exploited, or in poor health.

Relative weight ($W_r$) (Wege and Anderson 1978) is the most commonly used condition index in fisheries science (Blackwell et al. 2000). $W_r$ is calculated by dividing the weight of the individual by the length-specific standard weight predicted by a geographically broad species-specific weight-length regression. When $W_r$ values for an individual or group are below 100 there may be inherent problems in food or feeding conditions (Murphy and Willis 1996). A mean $W_r$ of 100 across size-groups for a given fish species reflects optimum ecological and physiological conditions for a population (Murphy and Willis 1996). However, use of mean $W_r$ for an entire population can hide important trends in fish condition such as slow growth rates for individual length-classes (Murphy et al. 1991).

Methods used to determine compositional differences, such as bomb calorimetry and proximate analysis, are more accurate in assessing fish condition than morphometrics due to their ability to quantify lipid and water concentrations. However, due to high cost, the restriction of use to a laboratory setting, labor-intensive methods, and lethality to fish they are far less practical to fishery biologists interested in measuring condition of large quantities of fish in the field. Bioelectrical impedance analysis (BIA), like proximate composition analysis and bomb calorimetry, has the potential to estimate various components of an organism. However, BIA requires far less training, is less expensive, and can be used in the field where fish are obtained. Additionally, once models have been developed BIA is nonlethal and minimally invasive. Because BIA has the potential for assessing individuals under conditions of fluctuating temperatures, seasonal variation in fish condition can be evaluated. Previous studies have shown percent dry weight (PDW) is correlated to proximate composition (Hartman and Margraf 2008) and energy density (Hartman and Brandt 1995), and therefore can be used to estimate fish condition. Thus, the study objectives were to measure seasonal changes in adult Yellow Perch PDW, and compare the reliability of BIA and morphometric-based estimates of condition.

# Chapter 3: Methods

## Study Site

Lake Bemidji, located in northern Minnesota, is a 2,362 $m^2$ mesotrophic lake, with a maximum depth of 23.2 m (MNDNR 2014). The Mississippi River flows into Lake Bemidji on the west bank from Lake Irving and exits on the eastern side. Lake Bemidji is a reliable Yellow Perch fishery and as of 2012, 12 % of the Yellow Perch captured exceeded 22.86 cm in length (MNDNR 2014).

## Fish Sampling

A total of 612 Yellow Perch were collected by means of angling from Lake Bemidji between December 2014 and October 2016. A minimum length of 175 mm was required for Yellow Perch obtained to ensure each electrode type could be utilized. Of the total fish collected 150 were used during BIA model development, 270 were used during temperature correction procedures, and 202 were used over the two-year field validation portion of the study.

## Bioelectrical Impedance Analysis

To measure resistance and reactance (Ohms) of tissues found in individual Yellow Perch, a Quantum IV body composition analyzer (RJL Systems, Clinton Township, MI) was used to supply a small electronic current (400 µA – 50 KHz) through the body of the fish using external and/or subdermal needle electrodes. Each electrode type (Figure 1) had 2 pairs of electrodes, with one acting as the signal and the other acting as the detector (Cox and Hartman 2005). The first electrode type was comprised of mounted subdermal 22-gauge needle electrodes (SD), developed by the researcher, with signal and detecting electrodes spaced 10 mm apart. These were pressed through the ctenoid scales of the fish and into the muscle tissue to a depth of 5 mm. The second electrode type was a flat bottom compression electrode measuring 14 mm in diameter (CE14) fastened directly to the Quantum IV analyzer by means of threaded screws. The two pairs of electrodes are spaced 50 mm apart when measured from the electrode center and electrodes are not adjustable. The total distance measured between the two signal electrodes (outside electrodes) was 117 mm. The last electrode type was a flat bottom compression electrode measuring 9 mm (CE9) in diameter and the spacing between pairs of electrodes could be adjusted depending on fish length.

Resistance and reactance were measured for dorsal total length (DTL) and ventral total length (VTL) locations on each fish (Figure 2). For the DTL location, the first pair of electrodes were placed so the signal electrode was midway between the lateral line and the leading edge of the anterior dorsal fin. The second pair of electrodes was placed so the signal electrode was midway between the lateral line and the trailing edge of the posterior dorsal fin. For the VTL location, the first pair of electrodes was placed so the signal electrode was aligned and 1 cm above the leading edge of the pelvic fin to avoid the pelvic girdle and the pectoral fin was moved slightly to accommodate this placement. The second pair of electrodes was placed so the signal electrode was aligned midway between the lateral line and the trailing edge of the anal fin. The distance between the two detecting electrodes was measured each time BIA measurements were taken when using CE9 and SD electrodes. Since the signal and detector distance, as well as the distance between pairs of electrodes was fixed for CE14 electrodes only the first pair of electrodes were placed in accordance with the descriptions above for the DTL and VTL locations. The second pair of electrodes were placed slightly to the left or right of the trailing edge of the anal fin depending on the length of the fish. To reduce bias due to temperature changes from handling Yellow Perch, the order of both the electrode type and location (DTL or VTL) were changed for each fish.

**Lab Model Development**

The 150 Yellow Perch used for model development were captured by means of angling on Lake Bemidji and transported to Bemidji State University. Yellow Perch were held in re-circulating tanks measuring 61 cm x 61 cm x 183 cm. Since the optimum temperatures of adult Yellow Perch during the growing season is from 19-24 °C (Scott and Crossman 1973), and since Yellow Perch are minimally stressed when handled at 20 °C (Eissa and Wang 2013) the water temperature of each tank where Yellow Perch were held was monitored and maintained at a temperature of 19 °C. To assure condition was different among fish measured throughout the 10-week study period, food rations were withheld for the duration of the study. Measurements were conducted once a week for a total of 10 weeks. A total of 50 fish were measured using each of the three electrode types. Each week 18 fish were randomly selected from the tanks to measure, with exception to weeks 8-10 when there were not enough fish remaining to measure. Of the 18 fish measured each week, 6 were randomly assigned to be measured using CE14, CE9, and SD electrode types.

Each week individual fish were randomly obtained from the tanks using a dip net and sacrificed through an overdose of clove oil. Yellow Perch were patted dry using a paper towel and placed on a nonconductive surface facing left. The internal temperature of the fish was measured by inserting an electronic meat thermometer into the fish mouth and gently pushing the probe into the abdominal cavity. Dorsal and ventral BIA measurements were conducted immediately after taking the internal temperature. In addition, total length (TL; mm) and wet weight (WW; g) were measured and the sex of the fish was determined. Following BIA measurements each fish was oven dried at 80 °C until a constant dry mass was obtained. Percent dry weight (PDW) was calculated by dividing dry weight (g) by wet weight (g) and multiplying by 100. Yellow Perch relative weight was calculated by dividing the fish's wet weight (WW) by the standard weight ($W_s$), as seen below:

$$W_s = 10\text{^} (-5.386 + 3.230 * \log10 (TL)) \qquad \text{(Willis et al. 1991)}$$

$$W_r = (WW / W_s) * 100$$

**Temperature Corrections**

Three different temperature correction methods were used to assess the effect of temperature on resistance and reactance. Each correction was developed using 90 Yellow Perch captured by means of angling on Lake Bemidji. Again, fish were transported to Bemidji State University where they were held in 61 cm x 61 cm x 183 cm recirculating tanks. Yellow Perch were allowed two days for acclimation before any changes to the water temperature took place. Each tank's initial water temperature was held at 19 °C.

For the first temperature correction (Controlled Environment Room or CER) a total of 90 Yellow Perch were evenly divided into three recirculating tanks so each tank held 30 fish. The water temperature of each tank was warmed slowly over the course of five days to 25 °C. Individual fish were then sacrificed using an overdose of clove oil, whereupon they were patted dry using a paper towel and placed on a nonconductive surface facing left. The internal temperature of the fish was measured followed by BIA measurements taken at DTL and VTL locations. After the first measurement was completed at 25 °C the fish was brought into a controlled environment room set to 0 °C.

Resistance and reactance were then measured each time the internal temperature of the fish decreased by 2 °C, concluding when the internal fish temperature was 5 °C. It took approximately 90 minutes to measure each fish using BIA. Thirty fish were measured using each of the electrode types.

For the second temperature correction (Repeated Measures or RM) a total of 90 Yellow Perch were evenly divided into three recirculating tanks so each tank held 30 fish. The water temperature of each tank was warmed slowly over the course of five days to 25 °C. A barrier was placed inside the tank so all Yellow Perch were on the same side before BIA measurements began. Individual fish were then anesthetized using a 50 mg/L ratio of clove oil to water, following methods described by Kennedy et al. (2007). Fish were monitored while immersed in the anaesthetization mixture and were removed once the operculum had slowed significantly. Yellow Perch were patted dry with a paper towel and placed on a nonconductive surface facing left. Internal temperature was measured first followed by BIA measurements, total length (mm), and wet weight (g). Following the first BIA measurements a numbered Floy tag (Floy Manufacturing Inc., Seattle, WA) was inserted into the fish near the anterior portion of the first dorsal fin. Floy tags were used to ensure each individual fish was measured using the same electrode type and the distance between pairs of electrodes remained constant for subsequent BIA measurements. When measurements had been completed for all 90 fish, the temperature was decreased and the BIA measurements were performed again at 15 and 5 °C.

For the third temperature correction (Batch) fish were split into 5 groups of 18 fish. Each group was designated a tank with a water temperature of 5, 10, 15, 20, or 25 °C. The water temperature inside each tank was adjusted from 19 °C over five days until each of the designated temperatures had been reached. Fish were allowed one day to acclimate to the new water temperature. After the acclimation period, individual fish were selected randomly and anaesthetized using clove oil. Each fish was patted dry with a paper towel and placed on a nonconductive surface facing left. The internal temperature was measured first, followed by BIA measurements.

**Field Validation**

Ten Yellow Perch were collected from Lake Bemidji during the second week of every month by means of angling, with exception to April 2015 and November 2015/2016. Ice conditions on Lake Bemidji were unsafe during the second week of April 2015 and no fish were caught via angling during November2015/2016 months. Fish obtained while angling were kept in the water to maintain their internal body temperature before being measured. Individual fish were sacrificed using an overdose of clove oil. Internal temperature was measured first, followed by six BIA measurements, including dorsal and ventral measurements for each electrode type. Both electrode type and location of electrode was rotated for individual fish to reduce bias attributed to changes in fish temperature and internal physiology.

After the last BIA measurement was taken, internal temperature was recorded again, followed by total length (mm). Yellow Perch were placed in individual sealed Ziploc bags to reduce moisture loss and brought back to Bemidji State University. Wet weight (g) was measured, the sex of the fish was determined, and then each fish was oven dried at 80 °C until a constant dry mass was obtained. Percent dry weight was calculated by dividing dry weight (g) by wet weight (g) and multiplying by 100.

**Data Analysis**

Following the methods outlined by Cox and Hartman (2005) and Cox et al. (2011) electrical parameters were calculated from measurements of resistance and reactance for each electrode type and location. The following electrical parameters and morphometric-based measurements used in regression analysis are outlined in Table 1: resistance (r), reactance (x), resistance in series ($R_s$), reactance in series ($X_c$), phase angle (PA), sex, total length (mm), wet weight (g), and relative weight ($W_r$).

Prior to determining the best supported lab model for each electrode type the function leaps was used to calculate Mallow's $C_p$ (Mallows 1973) in program R (R Development Core Team 2013). Mallow's $C_p$ takes the set of variables and produces a candidate model for each possible model size. Morphometric-based candidate models were also selected using this method and included only TL, WW, $W_r$, and sex as possible predictor variables.

All candidate models were then cross-validated, whereby training data sets were used to develop the models using 75 % of the original data and test data was used to validate the model. R-square and root mean square error (RMSE) were then calculated based on how well the test data fit the models. The cross-validation function was set so 1,000 permutations were run to develop each model. The best supported model for predicting percent dry weight was selected for each electrode type based on the largest $R^2$ value and the lowest RMSE value. This same process was conducted to select the best supported morphometric-based model.

Generalized linear mixed-effects models within R (R Development Core Team 2013) were used to establish relationships between BIA impedance measures (resistance and reactance) and temperature for temperature corrections where fish were measured numerous times as the internal temperature of the fish changed (CER and RM). Simple linear regression analysis was used to assess the relationship between BIA measurements and temperature when there was only a single measurement performed on each fish (Batch). The slopes from the models were used to correct all field BIA measurements to a standardized lab temperature of 19 °C using:

$$b * (19 – ((T_0 + T) / 2) + FM$$

b is the slope (see Figure 6)

$T_0$ is the internal temperature of fish at first BIA measurement

T is the internal temperature of fish at last BIA measurement

FM is the field BIA measurement undergoing correction

Seasonal fluctuations in fish condition, represented by PDW, were tracked using observed PDW values from harvested validation Yellow Perch. Temperature corrected resistance and reactance were entered into the best supported BIA lab model developed for each electrode type to provide estimates of PDW. BIA models were then validated by comparing predicted and observed values from Yellow Perch harvested in the field. $R^2$ and RMSE were calculated and used to determine the reliability of BIA under field conditions. The best supported morphometric-based model to provide estimates of PDW was also validated by comparing observed and predicted PDW values from fish harvested in the field. To determine whether BIA or morphometric models were more reliable in estimating PDW under field conditions, $R^2$ and RMSE were compared.

# Chapter 4: Results

A total of 202 Yellow Perch were measured during field validations beginning in December 2014 and ending in October 2016. Fish TL ranged 172-300 mm and the range in WW was 49.10-377.20 g. Average internal temperature of Yellow Perch ranged from 0.10 to 25.20 °C. ANOVA results revealed a significant change in body condition as observed by changes in PDW ($p = 0.03$). The seasonal fluctuations in average monthly PDW were sinusoidal in shape with peaks in August 2015 (25.44 %) and July 2016 (26.34 %) when water temperatures were above 22 °C. The valleys occurring in February 2015 (22.73 %) and April 2016 (22.96 %) when temperatures were below 5 °C (Table 2). The months with the largest range in PDW were June 2015 (5.69 %) and August 2016 (3.65 %).

A total of 150 Yellow Perch ranging 175-321 mm TL and 41.59-418.20 g WW were used to develop BIA lab models. During model development, Yellow Perch PDW declined, on average 2.12 % per week with the maximum PDW being 27.59 % and the minimum being 20.25 % (Figure 3). Model validation established that the best supported BIA lab models explained more variation in PDW than the best morphometric-based model. There was a significant relationship between PDW and morphometric variables ($W_r$ and WW) for Yellow Perch in the lab, yet a large portion of the variation in PDW was still unexplained ($p < 0.01$, $R^2 = 0.42$, RMSE = 1.08; Figure 4). The CE14 lab model produced $R^2$ of 0.60 and RMSE of 0.92 (Figure 5). The CE9 lab model produced $R^2$ of 0.52 and RMSE of 0.93. The SD lab model produced $R^2$ of 0.44 and RMSE of 1.09. The best supported BIA lab model for each electrode type was:

Equation 1 (CE14) $\quad PDW = 22.37 + (DX * -0.12) + (Vp * 0.50)$

Equation 2 (CE9) PDW = 2.06 + (TL * 0.024) + (DX * -0.026) + ($W_r$ * 0.20)

Equation 3 (SD) PDW = 2.66 + ($W_r$ * 0.21) + (D_Xc * 0.035)

PDW is predicted percent dry weight

DX is dorsal reactance (ohms)

Vp is ventral phase angle (degrees)

TL is total length (mm)

$W_r$ is relative weight

D_Xc is dorsal reactance in series (ohms)

When developing temperature corrections, Yellow Perch had a range in TL of 165-284 mm and a WW range of 47.80-296.30 g. Linear mixed effects (CER and RM) and regression analysis (Batch) indicated that resistance and reactance decreased as temperature increased. This inverse relationship found during regression analysis was consistent across all electrode types and temperature correction methods (Figure 6). Slopes were significantly different among the three temperature corrections as indicated by the lack of overlap when comparing confidence intervals (Figure 6).

Lab models used to predict PDW under field conditions for Yellow Perch were unsuccessful as indicated by low $R^2$ values and large RMSE values during validations. Predictions using the best supported CE14 electrode and CER, Batch, and RM temperature corrections were $R^2$ 0.01 (RMSE = 2.78), $R^2$ 0.08 (RMSE = 1.59) and $R^2$ <0.01 (RMSE = 2.07; Figure 7), respectively. Predictions using the best supported CE9 electrode and CER, Batch, and RM temperature corrections were $R^2$ was <0.01 (RMSE = 1.83), $R^2$ <0.01 (RMSE = 1.99), $R^2$ 0.01 (RMSE = 2.06), respectively. Predictions using the best supported SD electrode and CER, Batch, and RM temperature corrections were $R^2$ <0.01 (RMSE = 1.66), $R^2$ <0.01 (RMSE = 1.82), and $R^2$<0.01 (RMSE = 1.77) respectively. Regardless of electrode type or temperature correction used when attempting to predict PDW, BIA models were unable to explain much of the variation in condition observed in validation Yellow Perch. For example, the best supported BIA model used to predict average monthly PDW of Yellow Perch in the field produced an $R^2$ value of 0.14 and RMSE of 0.97 (Figure 8). Additionally, the best supported morphometric-based model developed using lab Yellow Perch and including $W_r$ and WW as predictor variables, resulted in an $R^2$ of 0.03 and RMSE of 1.57 when used to predict PDW in the field.

Due to the poor performance of lab models in predicting PDW of validation Yellow Perch, field data from 2015 fish were used to develop morphometric-based and BIA models that could be used to predict PDW of 2016 fish. The first set of models developed excluded temperature corrections and instead included the average internal temperature of the fish as a potential predictor. Mallow's $C_p$ and cross validation were used to produce the best supported morphometric-based and BIA field models for each electrode type based on highest $R^2$ and the lowest RMSE:

Equation 7 (CE14) $PDW = 22.49 + (Temp * 0.07) + (VX * 0.018)$

Equation 8 (CE9) $PDW = 22.88 + (Temp * 0.048) + (Vp * 0.056)$

Equation 9 (SD) $PDW = 20.15 + (Temp * 0.11) + (DR * 0.0070)$

Equation 10 (Morph) $PDW = 25.07290 + (TL * -0.005)$

PDW is the predicted percent dry weight

Temp is the average internal temperature of fish (°C)

VX is the ventral reactance (ohms)

Vp is the ventral phase angle (degrees)

DR is the dorsal resistance (ohms)

TL is the total length of the fish (mm)

Each model was used to predict the PDW of individual Yellow Perch captured during the second year of field validations. CE14, CE9, and SD field models, excluding temperature corrections, produced $R^2$ values of 0.19 (RMSE = 1.32; Figure 9), 0.18 (RMSE = 1.30) and 0.08 (RMSE = 1.39), respectively. The best field models developed without temperature corrections and using 2015 data to predict 2016 PDW of Yellow Perch explained approximately 18% more of the variation in individual PDW over lab BIA models. Morphometric-based field models were unable to explain much of the seasonal fluctuations in PDW for Yellow Perch ($R^2$ = 0.09; RMSE =1.49). The second set of BIA models were developed using temperature corrections developed from the lab. Field data from 2015 was first corrected to 19 °C using each of the temperature corrections. Field models were then developed using the same methods described during lab model development. Equations can be found in Table 3. The best field models developed using temperature-corrections explained 11-17 % more of variation in PDW over lab BIA models but were unable to explain any more of the variation in PDW when compared to field models utilizing mean temperature as a predictor variable.

## Chapter 5: Discussion

Yellow Perch measured from Lake Bemidji throughout the two-year study period showed significant changes in body condition as observed by changes in PDW. PDW peaked in July-August when water temperatures were above 22 °C, coinciding with Pearse's (1925) findings for adult female Yellow Perch whose percent fat was highest in July. The peaks, unsurprisingly occurring during the summer when more food and light are available for foraging. As temperatures declined in September-October PDW also declined despite the onset of egg and sperm production in mature Yellow Perch. When temperatures fall below 12 °C in the fall Yellow Perch begin producing egg and sperm in preparation for spring spawning (Hokanson 1977).

From November-January PDW slowly declined, contrary to Eckman (2004), who found the largest loss of fat in Yellow Perch to occur during this period. Based on the mean monthly PDW from this study, the largest loss in PDW was from January-February in 2015 (1.04 % PDW) and from February-March in 2016 (0.92 % PDW). This coincides with Pearse (1925), who found that percent fat in adult female Yellow Perch was lowest in February when water was cold and food was less abundant. Differences in lake productivity and may offer some insight into the conflicting findings between results from this study and Eckman's. The warm-monomictic Lake Constance found in the European Alps, previously eutrophic in status, was becoming oligotrophic during the study (Eckman 2004), likely causing a reduction to food abundance available to Yellow Perch. This coupled with Ruffe *Gymnocephalus cernuus* competition for food resources is likely to have influenced the earlier fat loss seen in Yellow Perch. Pearse (1925), on the other hand studied Yellow Perch from Lake Mendota (Wisconsin), which, much like Lake Bemidji is mesotrophic suggesting a more stable food supply. Therefore, due to similarities between Lake Mendota and Lake Bemidji, the greatest loss in PDW was observed in February for adult Yellow Perch.

The lowest PDW observed for Yellow Perch from this study occurred between February-April when temperatures were below 5 °C. During this time, Yellow Perch are most likely in maintenance mode, whereby fish feed when food is available yet retain lower metabolic rates (Eckman 2004) to preserve energy stores for spawning. Spawning typically occurs between April-June when water temperatures reach 7-13 °C (Harrington 1947, Wells 1968, Scott and Crossman 1973) and coincides with the lowest PDW as revealed by results from this study. Once spawning is complete Yellow Perch strive to replenish lost energy reserves from overwintering and reproductive activities. As light availability increases in May-June, water temperatures warm, aquatic vegetation grows, and phytoplankton communities increase rapidly. The increase in algal biomass occurs just as zooplankton, benthic invertebrates, and age-0 Yellow Perch begin hatching. Warming water, greater light availability, and an increase in food resources found during late spring and early summer are concurrent with a sharp increase in PDW observed in adult Yellow Perch.

Fisheries managers typically conduct field work in temperate regions during the summer season when water temperatures are warm, males and females have spawned, and PDW is highest. As seasonal fluctuations in fish condition are strongly influenced by water temperature, food abundance, light availability needed for foraging, and reproduction, condition estimates at this time may not fully represent the status of a fishery. Therefore, to effectively manage fish populations, condition estimates are needed for seasons that are suitable and optimal for growth but also those maintenance periods where energy reserves are depleted such as during reproduction and spawning.

In an effort to determine the most reliable method for assessing fish condition as it relates to seasonal fluctuations in PDW, BIA and morphometric-based model predictions of PDW were compared. While BIA has shown potential in measuring compositional differences in fish, there is still uncertainty as to its reliability in estimating condition. For example, Stolarski (2013) reported coefficients of determination for moisture and lipid predictions using BIA models to be 0.73 and 0.77 for Dolly Varden, respectively. Likewise, the predictive abilities of other BIA models developed for Brook Trout and Bluefish *Pomatomus saltatrix* had $R^2$ values ranging from 0.72 to 0.86 (Hafs and Hartman 2011; Hartman et al. 2011; Rasmussen et al. 2012). These results conflict with findings from Pothoven (2008) who found coefficients of determination for energy density and percent lipids using BIA models to be 0.18 and 0.17 for Yellow Perch, respectively. BIA models from this study produced coefficients of determination for percent dry weight ranging 0.44-0.60 when used on Yellow Perch in the lab. However, when these same models were applied in a field setting $R^2$ values were reduced to 0.08 or less. Similarly, the best-supported morphometric-based model produced an $R^2$ value of 0.42 when used in the lab and $R^2$ was reduced to 0.03 in the field.

The large disparity in the predictive abilities of BIA in estimating PDW may be a result of methodological differences. Research has shown factors such as electrode location, electrode type, procedural consistency, user experience, and temperature can affect BIA measurements (Cox et al. 2011). However, the direct measurements used to develop morphometric-based models (TL, WW, and PDW) are unlikely to incorporate the amount of error that would be needed to cause such large reductions in estimates from the lab to the field as seen in this study. Since fish are comprised of 60-90 % water and compensate for lack of fat by acquiring more water internally (Shearer 1994; Breck 2008;

Hartman and Margraf 2008), the lack of reliability in condition estimates when using morphometrics is not surprising as water and fat cannot be differentiated. Therefore, in addition to methodological differences between studies, other factors such as internal physiology and species-specific behaviors may be influencing the reliability of model predictions.

This study utilized previous research (Hafs and Hartman 2011) to design and manufacture subdermal needles that were known to produce reliable composition estimates. However, due to the variation in scale thickness between Brook Trout (cycloid) and Yellow Perch (ctenoid), subdermal needles in this study were manufactured to penetrate 5 mm instead of 3 mm and used 22 gauge needles instead of 28. Despite these adjustments, subdermal BIA model prediction in PDW were the lowest seen out of any previously published research. The lack of reliability in condition estimates, therefore, may be less a function of instrumentation and more a result of differences in fish physiology. Teleost fish scales (cycloid and ctenoid) vary in thickness and size but are comprised mainly of collagen, which acts as an insulator. Materials acting as insulators are less conductive than muscle due to the reduction in water concentration. Resistance to an electric current, therefore, is greater when more insulative material is present. The larger and thicker ctenoid scales found on Yellow Perch, consequently, may have produced higher resistance values due to interference at the surface of the fish.

Physiological changes in fish are well documented as they relate to fluctuations in temperature. How internal physiological changes affects the form and function of cellular membranes during warm and cold periods, however, is complex and relies on a more comprehensive understanding of fish biology. Because fish are poikilotherms, maintaining an internal body temperature equivalent to the surrounding water, a physiological response to combat cell membrane rigidity when conditions are cold is to alter the composition of membrane lipids (Hazel and Prosser 1974; Wodtke 1978; Wallaert and Babin 1994). When cell membranes are more rigid, resistance is higher and minerals are less able to be transported to the cell. By altering membrane lipids, fish can maintain ionic regulation when environmental conditions are not favorable for doing so. This response may cause variability in resistance and reactance values at high and low temperatures as the structural form of various membrane lipids are being altered. It could be argued, therefore, that model predictability would improve by using temperature corrections as variability to BIA measurements as a function of temperature would be reduced. However, results from this study revealed that there was not a significant difference in the model ability to estimate Yellow Perch condition regardless of the temperature correction method used. This finding conflicts with previous research that found composition estimates improved when temperature corrections were used during validation studies of BIA models (Hartman et al 2011; Stolarski et al. 2013). The mean difference between the start and end internal temperatures for Yellow Perch being measured in the field was 0.88 °C. This would suggest that the effect of temperature on BIA measurements is unlikely to have caused increased error that would be associated with decreased model reliability. However, during the first four months (12-2014 through 03-2015) of BIA measurements, as the researcher was becoming acquainted with BIA, 14 of the 40 Yellow Perch had variations in temperature that exceeded 2 °C. As a result, PDW estimates during this time were potentially less reliable as error was likely introduced as temperature-deviations, resulting from more handling time, increased.

The most significant difference between lab and field measurements for Yellow Perch was the quantity of BIA measurements and the amount of handling time required to complete measurements. Research has revealed that cortisol levels have been linked to changes in temperature and in repeated handling of Yellow Perch (Eissa and Wang 2013). High cortisol levels, caused by stress, result in loss of active tissue mass. When tissues are damaged or die, conductivity is reduced and resistance increases. After handling Yellow Perch at 14 and 26 °C, research revealed a one to fivefold increase in cortisol levels, with greater increases found at lower temperatures (Eissa and Wang 2013). Yellow Perch handled at 20 °C showed the lowest increase in cortisol, suggesting minimal stress (Eissa and Wang 2013). During the development of lab models Yellow Perch were held in water at 19 °C and each fish was measured using a single electrode taking approximately 30 seconds to complete. During field validations, Yellow Perch were captured across a range of temperatures by angling and then measured using all three electrode types for a total of six BIA measurements taking several minutes to complete. The influence of temperature on stress level and physiological responses coupled with an increase in handling time and amount of BIA measurements conducted for each fish could explain why lab BIA models were less reliable when applied to Yellow Perch in the field. To effectively eliminate variation in condition estimates associated with stress and the physiological changes that manifest therein researchers should try to reduce handling times and stress wherever possible.

Nevertheless, the variability in condition estimates, whether using BIA or morphometrics to predict PDW, indicate there are far more factors affecting seasonal fluctuations in condition then what is being assessed. Due to the inability of methods to reliably measure compositional difference in Yellow Perch throughout the year, the use of growth estimates and/or proximate composition analysis are suggested for assessing condition.

## Chapter 6: Conclusion

Yellow Perch PDW, a proxy for assessing condition, fluctuates annually as a function of changes in seasonal water temperatures and reproductive timing. To better assess fish populations and the likelihood of their adaptability to a changing climate, it is essential to continue to pursue research avenues focused on improving estimates of fish condition. To accomplish this cost-effective, non-invasive, and reliable fish condition methods are needed to assess fish populations residing and/or migrating between lotic and lentic systems. While this research suggests BIA has the potential to measure Yellow Perch condition when fish are held in the lab, methods breakdown when applied in a field setting. Despite this finding, morphometric-based estimates of fish condition, currently the most widely used method for assessing fish condition, were also unable to explain a large portion of the variation in PDW. Rather than relying on unreliable methods, the focus of fisheries research should be on identifying the sources of error found within current fish condition estimates and finding solutions so we can better manage the valuable and finite resources of our local and global fisheries communities.

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**Appendix A**

Table 1 – Calculated BIA electrical parameters (converted to electrical volume when $DL^2$ is included in equation) used in lab model development (from Hafs and Hartman 2011).

| Parameter | Symbol | Units | Calculations |
|---|---|---|---|
| Resistance | r | ohms | measured by Quantum IV |
| Reactance | x | ohms | measured by Quantum IV |
| Resistance in series | $R_s$ | ohms | $DL^2/r$ |
| Reactance in series | $X_c$ | ohms | $DL^2/x$ |
| Phase angle | PA | degrees | atan(x/r)*108/π |

DL = detector length

**Appendix B**

Table 2 – The range in dry weight (%), mean dry weight (%), and the mean internal temperature (°C) for the ten Yellow Perch measured during field validations.

| Date mo./yr. | Range in Dry Weight % | Mean Dry Weight % | Mean Internal Temp (°C) |
|---|---|---|---|
| 12/14 | 22.23 - 24.95 | 23.58 | 1.21 |
| 01/15 | 21.98 - 25.43 | 23.77 | 2.02 |
| 02/15 | 20.89 - 24.28 | 22.73 | 0.68 |
| 03/15 | 22.50 - 24.63 | 23.36 | 0.88 |
| 05/15 | 21.04 - 25.24 | 23.67 | 8.23 |
| 06/15 | 19.48 - 25.17 | 23.29 | 19.74 |
| 07/15 | 23.73 - 25.73 | 24.50 | 23.76 |
| 08/15 | 24.52 - 26.26 | 25.44 | 24.62 |
| 09/15 | 23.55 - 26.79 | 25.23 | 18.04 |
| 10/10 | 24.38 - 24.96 | 24.53 | 12.88 |
| 12/15 | 23.30 - 25.33 | 24.40 | 1.51 |
| 01/16 | 22.98 - 25.93 | 24.39 | 0.28 |
| 02/16 | 23.52 - 25.17 | 23.99 | 0.79 |
| 03/16 | 21.61 - 23.07 | 23.07 | 0.34 |
| 04/16 | 22.42 - 23.73 | 22.96 | 4.64 |
| 05/16 | 21.93 - 24.64 | 23.07 | 12.88 |

| | | | |
|---|---|---|---|
| 06/16 | 22.49 - 26.03 | 23.97 | 18.00 |
| 07/16 | 24.20 - 27.23 | 26.34 | 22.21 |
| 08/16 | 23.86 - 27.51 | 25.69 | 21.75 |
| 09/16 | 24.11 - 25.68 | 25.03 | 19.85 |
| 10/16 | 24.98 - 26.50 | 25.57 | 7.39 |

# Appendix C

A comparison of the best supported lab and field BIA models used to predict percent dry weight of Yellow Perch in Lake Bemidji, MN

| Model | Electrode Type | Temperature Correction | Model Equation | Monthly Average | | Individual | |
|---|---|---|---|---|---|---|---|
| | | | | $R^2$ | RMSE | $R^2$ | RMSE |
| Lab | CE14 | CER | 22.37 + (DX*-0.12) + (Vp*0.50) | 0.14 | 2.27 | 0.01 | 2.78 |
| Lab | CE14 | Batch | 22.37 + (DX*-0.12) + (Vp*0.50) | 0.14 | 0.97 | 0.08 | 1.59 |
| Lab | CE14 | RM | 22.37 + (DX*-0.12) + (Vp*0.50) | 0.04 | 1.60 | <0.01 | 2.07 |
| Field | CE14 | ------ | 20.15 + (Temp*0.11) + (DR*.007) | 0.32 | 1.04 | 0.19 | 1.32 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Field | CE14 | CER | 24.14 + (Dp*0.11) + (Vp*-0.10) | 0.17 | 1.13 | 0.03 | 1.42 |
| Field | CE14 | Batch | 22.84 + (Dp*0.11) | 0.41 | 1.09 | 0.17 | 1.35 |
| Field | CE14 | RM | 16.42 + (TL*0.023) + (VX*0.078) | 0.20 | 1.09 | 0.10 | 1.36 |
| | | | | | | | |
| Lab | CE9 | CER | 2.06 + (TL*0.023) + (DX*-0.026) + ($W_r$ *0.2) | 0.01 | 1.42 | <0.01 | 1.83 |
| Lab | CE9 | Batch | 2.06 + (TL*0.023) + (DX*-0.026) + ($W_r$ *0.2) | 0.08 | 1.64 | <0.01 | 1.99 |
| Lab | CE9 | RM | 2.06 + (TL*0.023) + (DX*-0.026) + ($W_r$ *0.2) | 0.11 | 1.72 | 0.01 | 2.06 |
| Field | CE9 | ------ | 22.88 + (Temp * 0.048) + (Vp * 0.056) | 0.30 | 1.02 | 0.18 | 1.30 |
| Field | CE9 | CER | 24.30 + (D_Rs*-0.015) | <0.01 | 1.20 | <0.01 | 1.45 |
| Field | CE9 | Batch | 23.71 + (VX*0.0049) | <0.01 | 1.21 | <0.01 | 1.44 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Field | CE9 | RM | 23.68 + (DX*0.006) | 0.24 | 1.15 | 0.11 | 1.39 |
| Lab | SD | CER | 2.66 + ($W_r$ *0.21) + ($DX_c$*0.035) | <0.01 | 1.26 | <0.01 | 1.66 |
| Lab | SD | Batch | 2.66 + ($W_r$ *0.21) + ($DX_c$*0.035) | 0.05 | 1.41 | <0.01 | 1.82 |
| Lab | SD | RM | 2.66 + ($W_r$ *0.21) + ($DX_c$*0.035) | 0.03 | 1.37 | <0.01 | 1.77 |
| Field | SD | ------ | 20.15 + (Temp*0.11) + (DR*0.007) | 0.19 | 1.12 | 0.08 | 1.39 |
| Field | SD | CER | 22.03 + (DX*0.019) | 0.48 | 1.27 | 0.06 | 1.47 |
| Field | SD | Batch | 21.58 + (DX*0.025) | 0.15 | 1.21 | <0.01 | 1.42 |
| Field | SD | RM | 21.61 + (DX*0.025) | 0.29 | 1.23 | <0.01 | 1.44 |
| Lab | Morph | ------ | 2.22 + (WW*0.010) + (Wr*0.22) | <0.01 | 1.25 | 0.03 | 1.57 |
| Field | Morph | ------ | 25.07 + (TL*-0.005) | 0.16 | 1.24 | 0.09 | 1.49 |

**Appendix D**


Figure 1. Electrode types used in this study: (A) external adjustable 9 mm spring-loaded compression electrode (CE9), (B) external fixed 14 mm spring-loaded compression electrode (CE14), (C) adjustable subdermal needle electrode (SD) manufactured by experimenter.

**Appendix E**

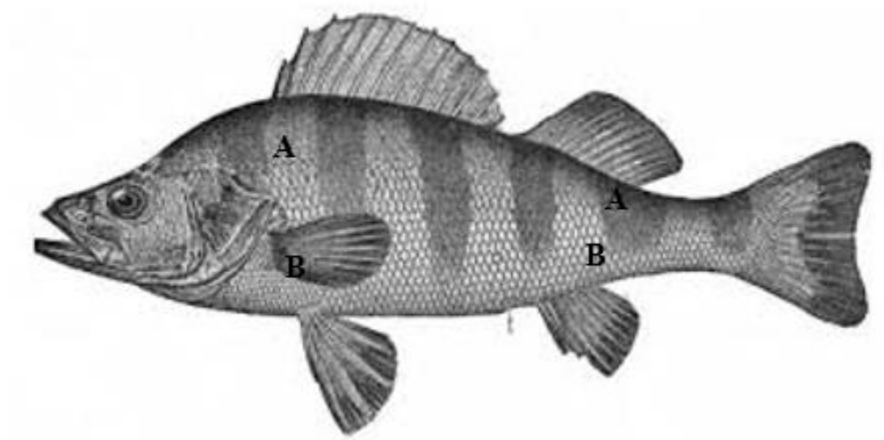



Figure 2. Electrode locations: (A) dorsal total length (DTL), (B) ventral total length (VTL).

**Appendix F**

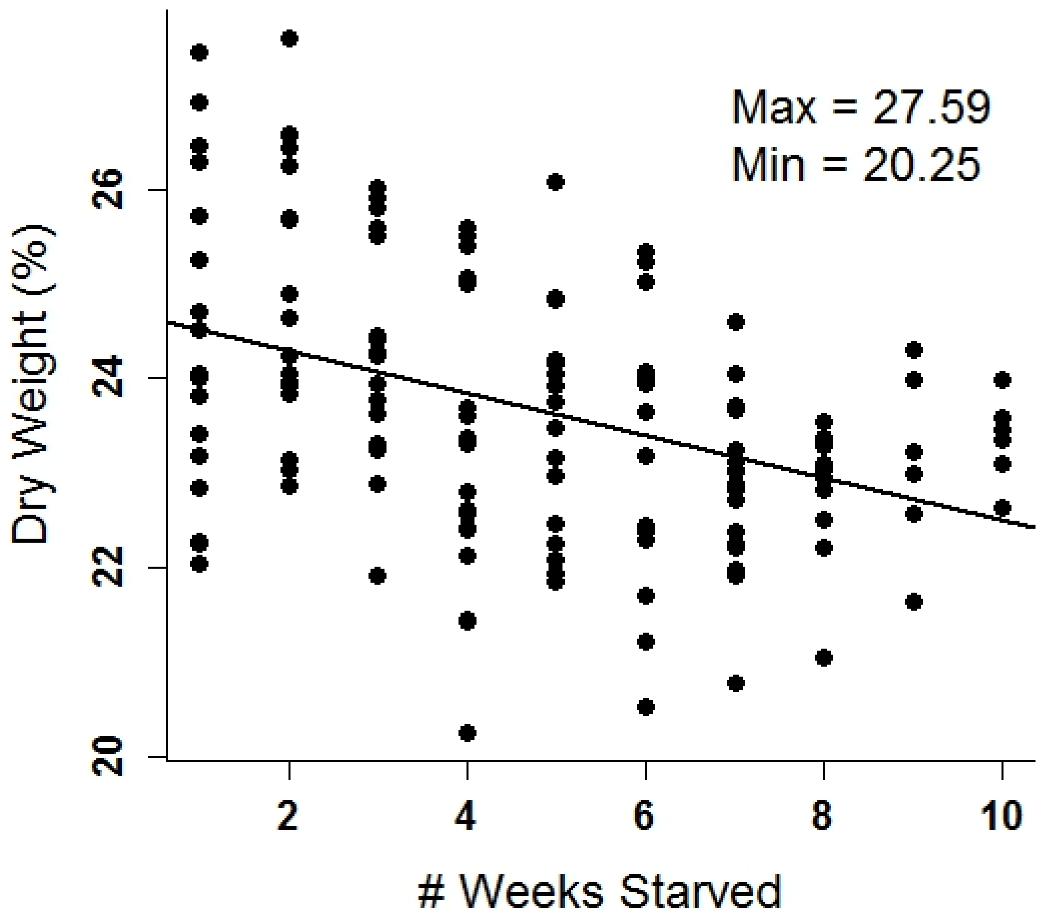



Figure 3. Change in dry weight (%) of Yellow Perch as a function of the number of weeks fasted during lab model development.

**Appendix G**

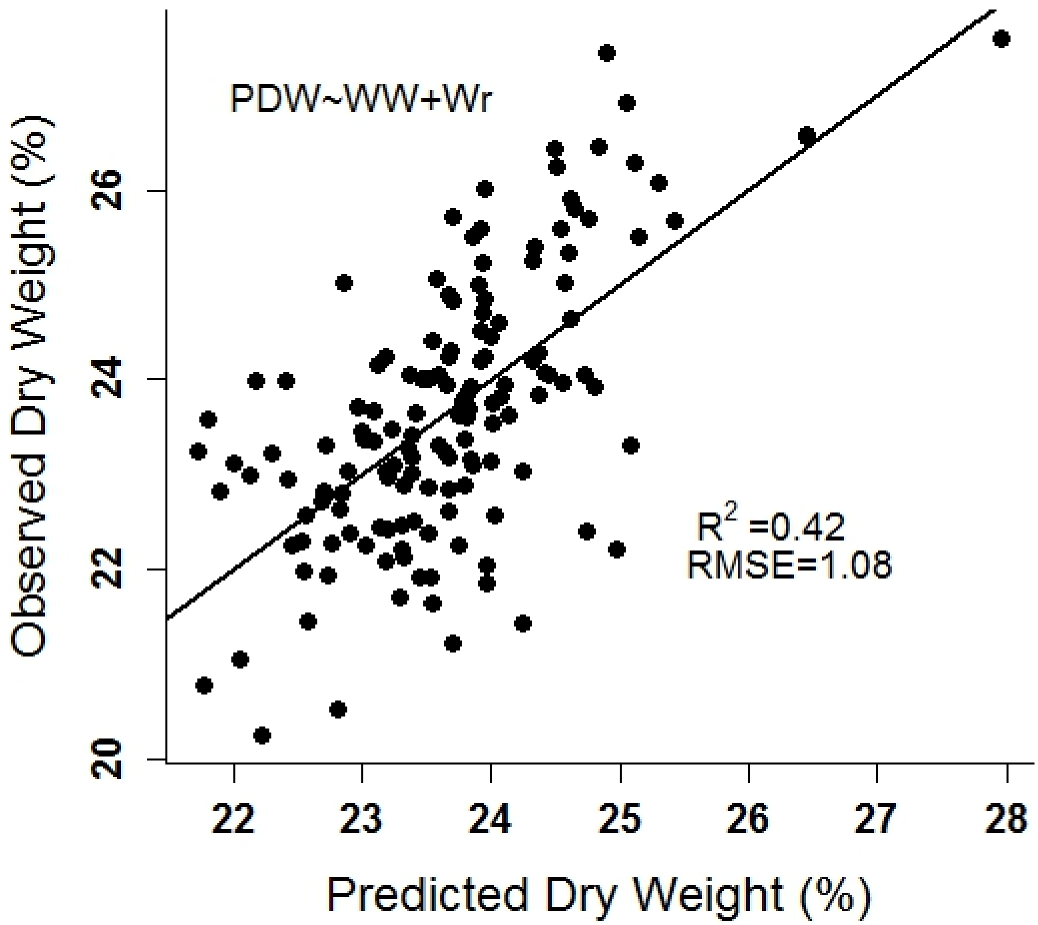



Figure 4. Using the best supported morphometric-based lab model, including wet weight (WW) and relative weight ($W_r$) as predictor variables, this figure identifies the relationship between observed and predicted dry weight (%) for Yellow Perch measured in the lab.

**Appendix H**

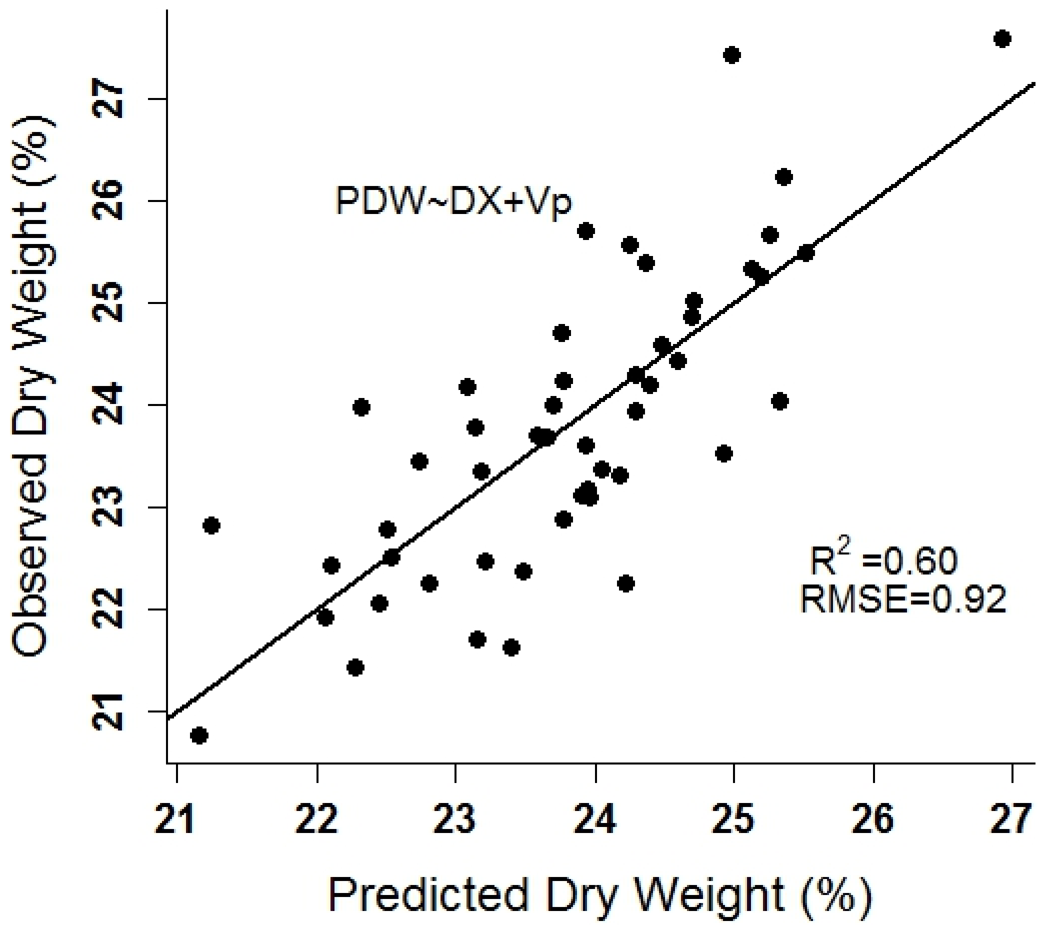



Figure 5. Using the best supported CE14 BIA lab model, including dorsal reactance (DX) and ventral phase angle (Vp), this figure identifies the relationship between observed and predicted dry weight (%) for Yellow Perch measured in the lab. See Appendices A-B for additional figures.

**Appendix I**

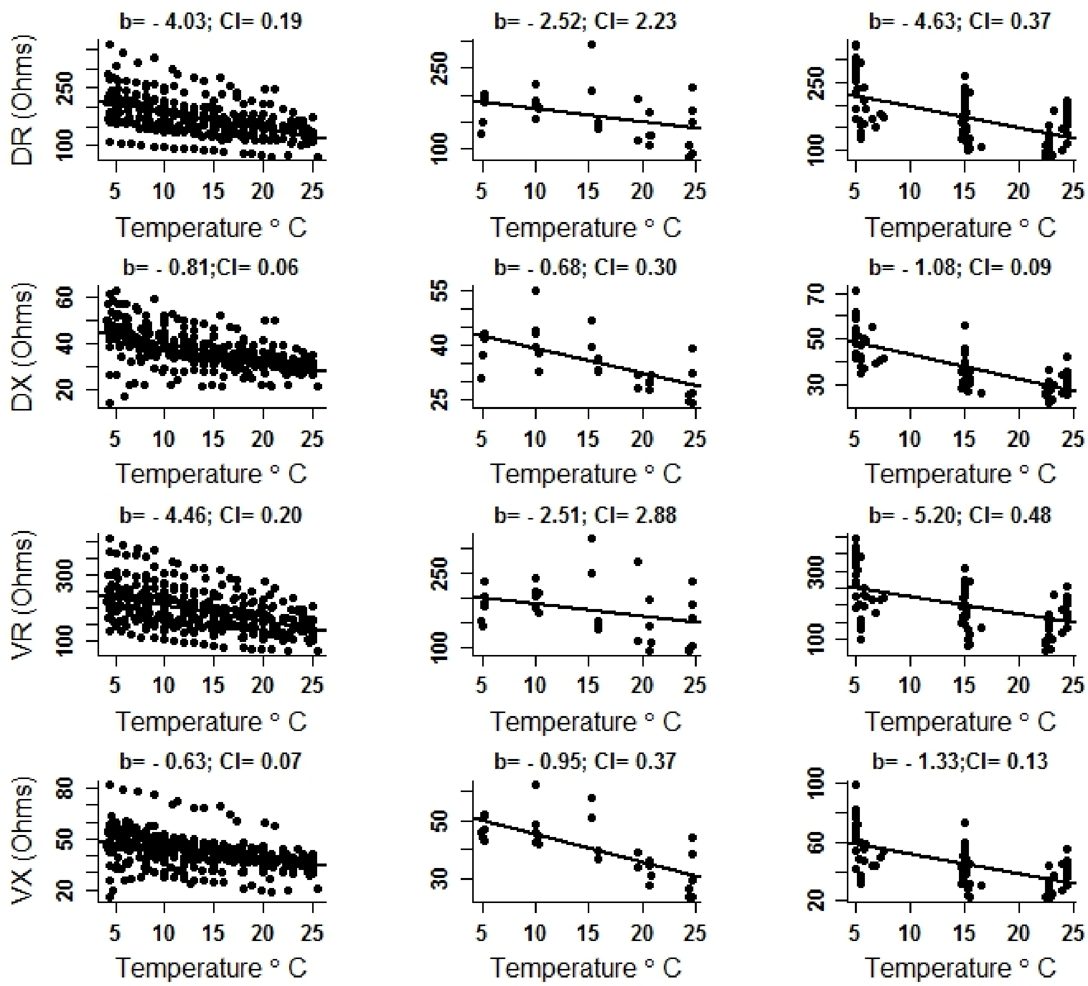



Figure 6. The change in BIA electrical parameters, using CE14 electrode, as a function of temperature (°C). The slope (b) of each line is represented at the top of each graph in addition to the 95% confidence intervals (CI) for the slope. Electrical parameters, measured in Ohms, include: dorsal resistance (DR), dorsal reactance (DX), ventral resistance (VR), ventral reactance (VX). Each column represents a different temperature correction method (CER, Batch, RM), respectively. See Appendices C-D for additional figures.

## Appendix J

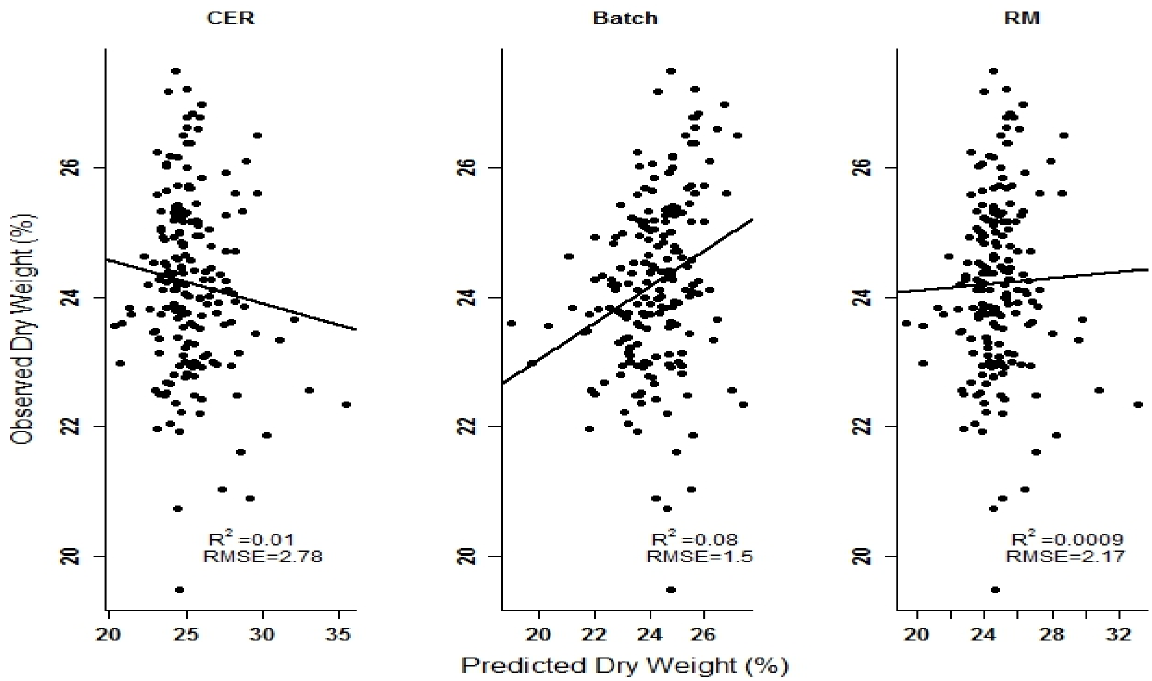



Figure 7. The relationship between observed and predicted dry weight (%) for Yellow Perch captured from Lake Bemidji, MN using the best supported and temperature-corrected (CER, Batch, RM) CE14 lab model. See Appendices E-F for additional figures.

**Appendix K**

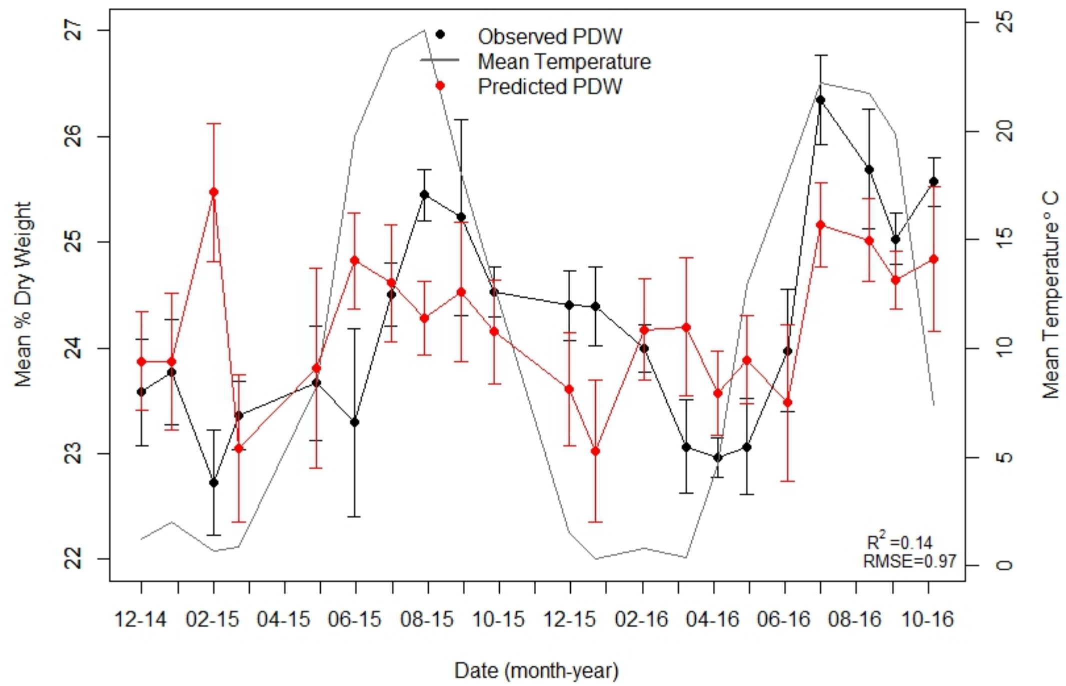



Figure 8. The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW using the best supported CE14 lab BIA model and Batch temperature corrections. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line. See Appendices G-N for additional figures.

**Appendix L**

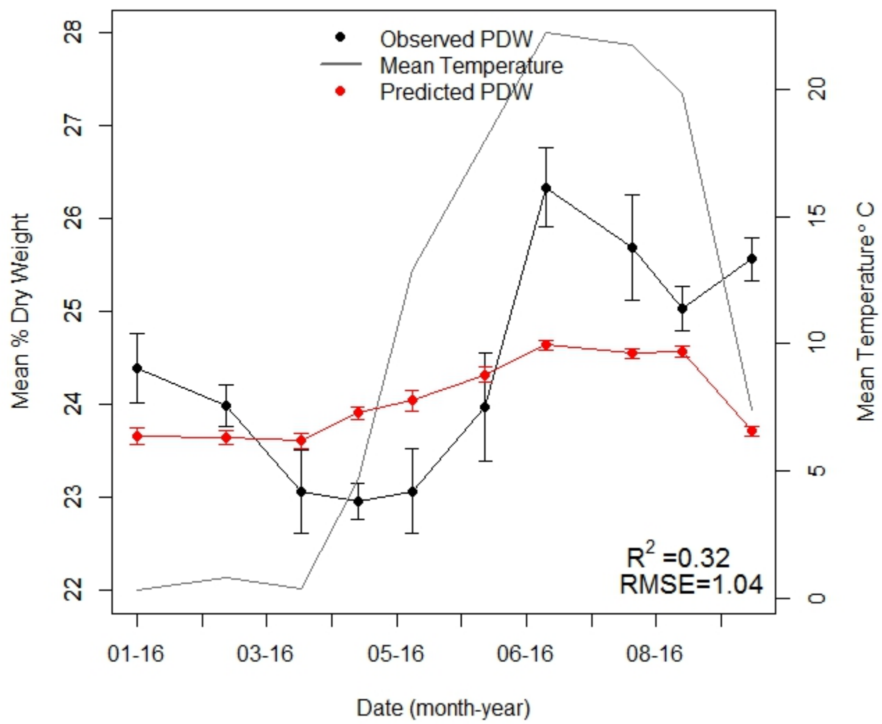



Figure 9. The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW for 2016 Yellow Perch using the best supported CE14 field BIA model developed from 2015 data. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line. See Appendices O-P for additional figures.

# Appendix M

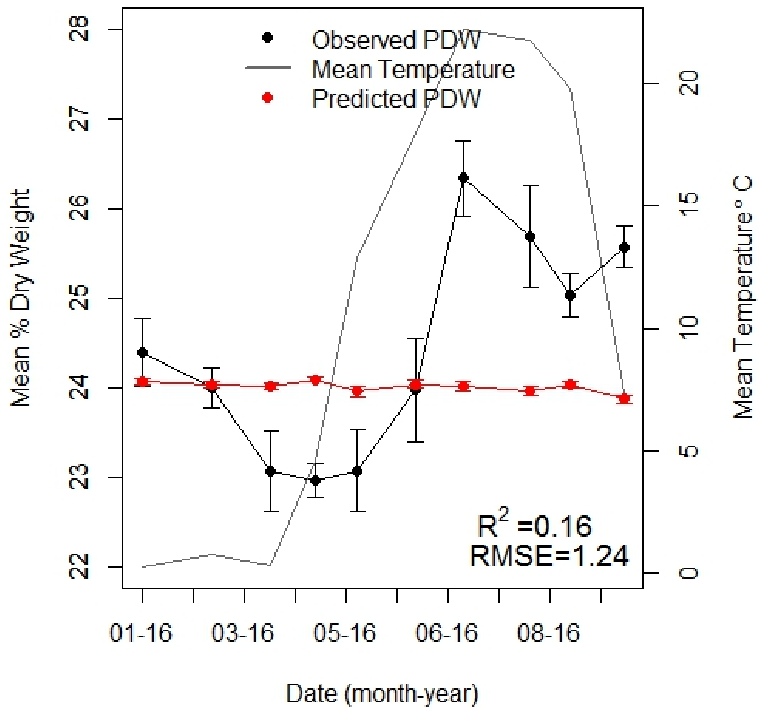
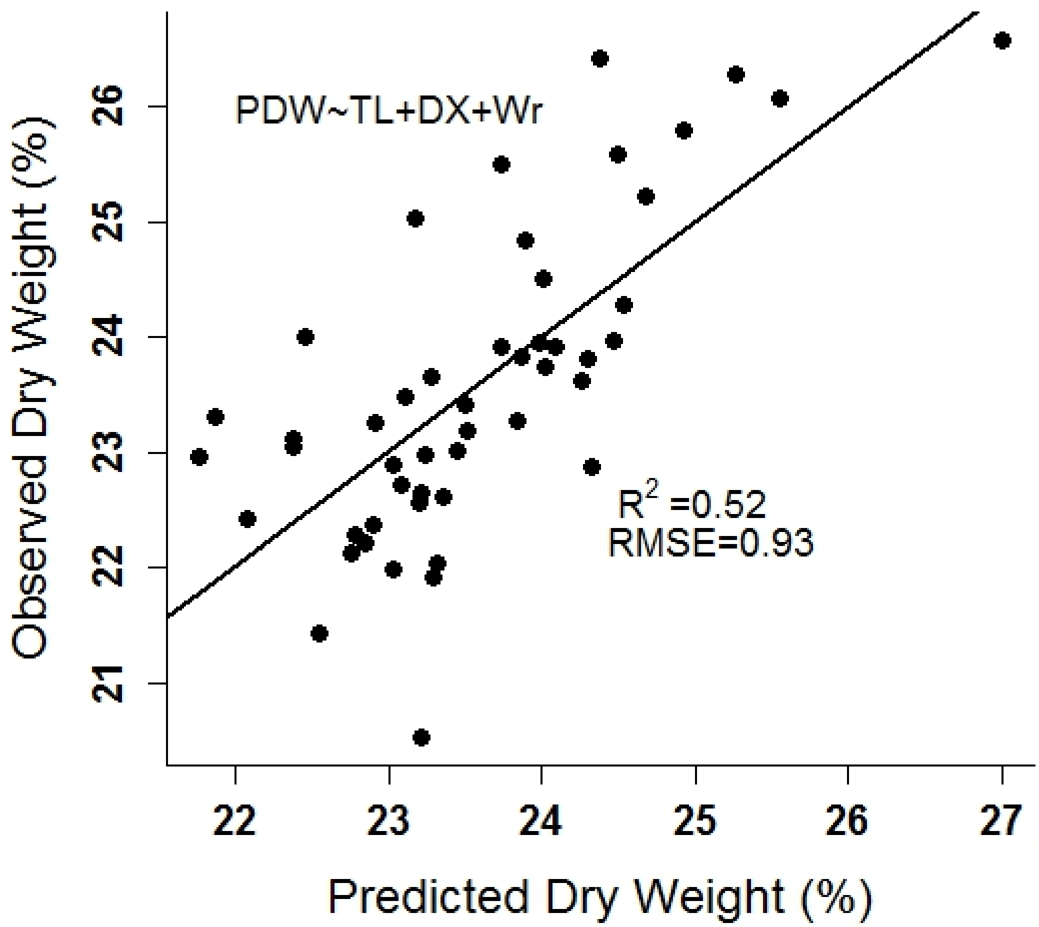



Figure 10. The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW for 2016 Yellow Perch using the best supported morphometric-based field model developed from 2015 data. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.

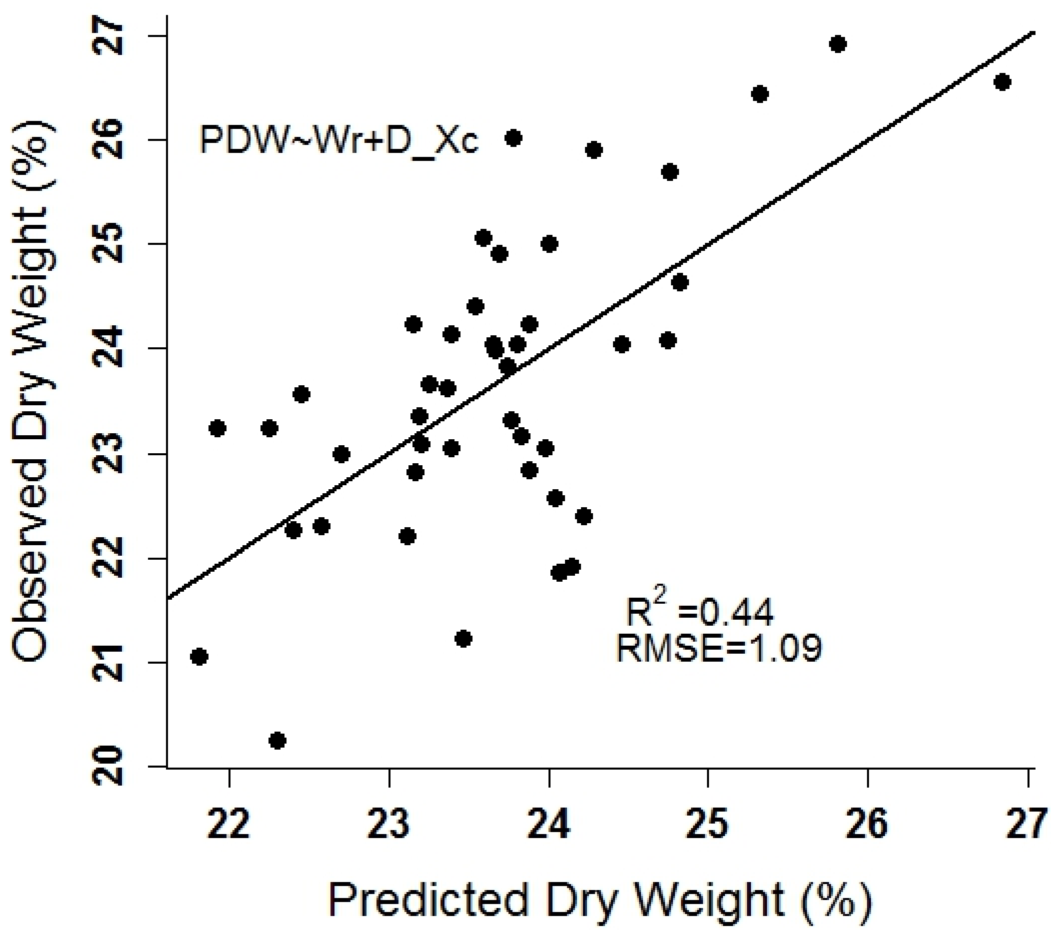
**Appendix N**

Using the best supported CE9 BIA lab model, including total length (TL), dorsal reactance (DX), and relative weight ($W_r$) as predictor variables, this figure identifies the relationship between observed and predicted dry weight (%) for Yellow Perch measured in the lab.



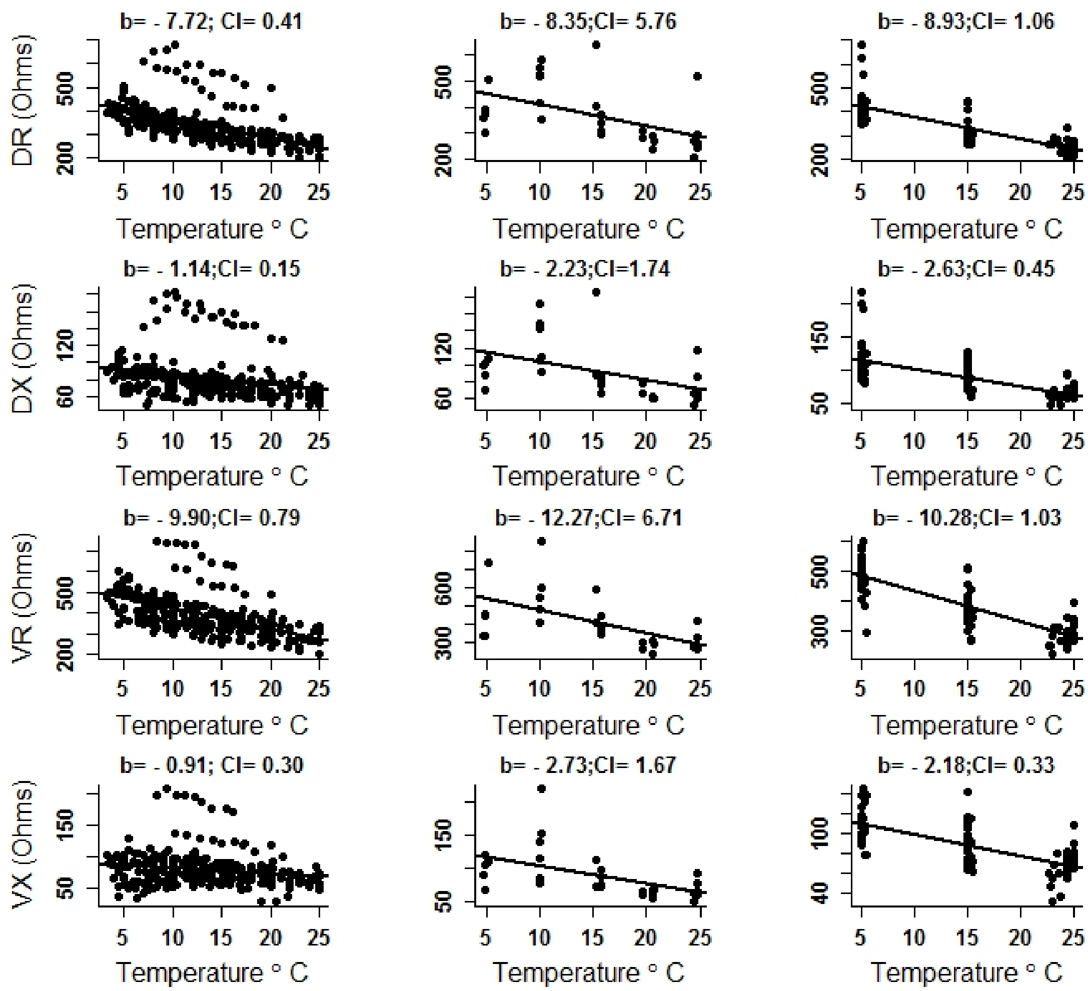
**Appendix O**

Using the best supported SD BIA lab model, including relative weight ($W_r$) and dorsal reactance in series (D_Xc) as predictor variables, this figure identifies the relationship between observed and predicted dry weight (%) for Yellow Perch measured in the lab.



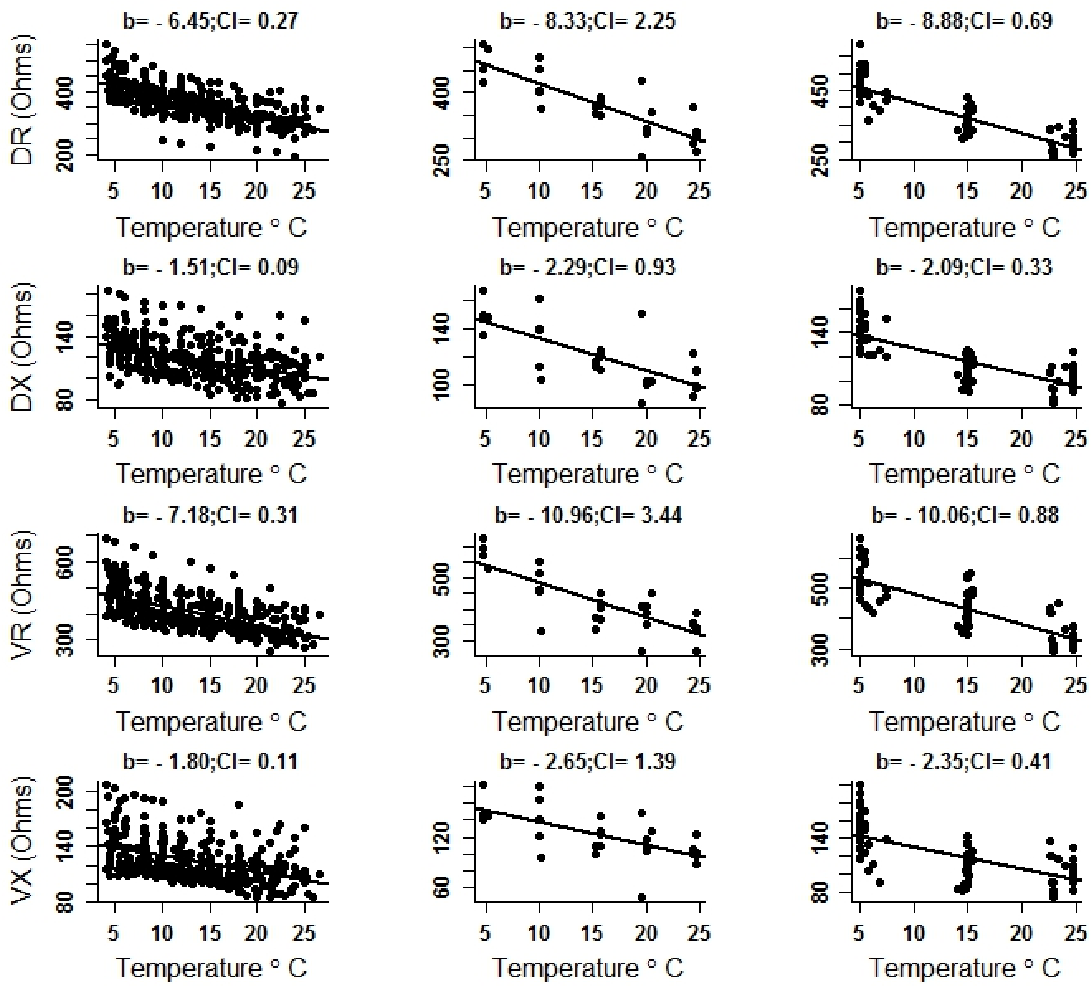
## Appendix P

The change in BIA electrical parameters, using CE9 electrode, as a function of temperature (°C). The slope (b) of each line is represented at the top of each graph in addition to the 95% confidence intervals (CI) for the slope. Electrical parameters, measured in Ohms, include: dorsal resistance (DR), dorsal reactance (DX), ventral resistance (VR), ventral reactance (VX). Each column represents a different temperature correction method (CER, Batch, RM) used, respectively.



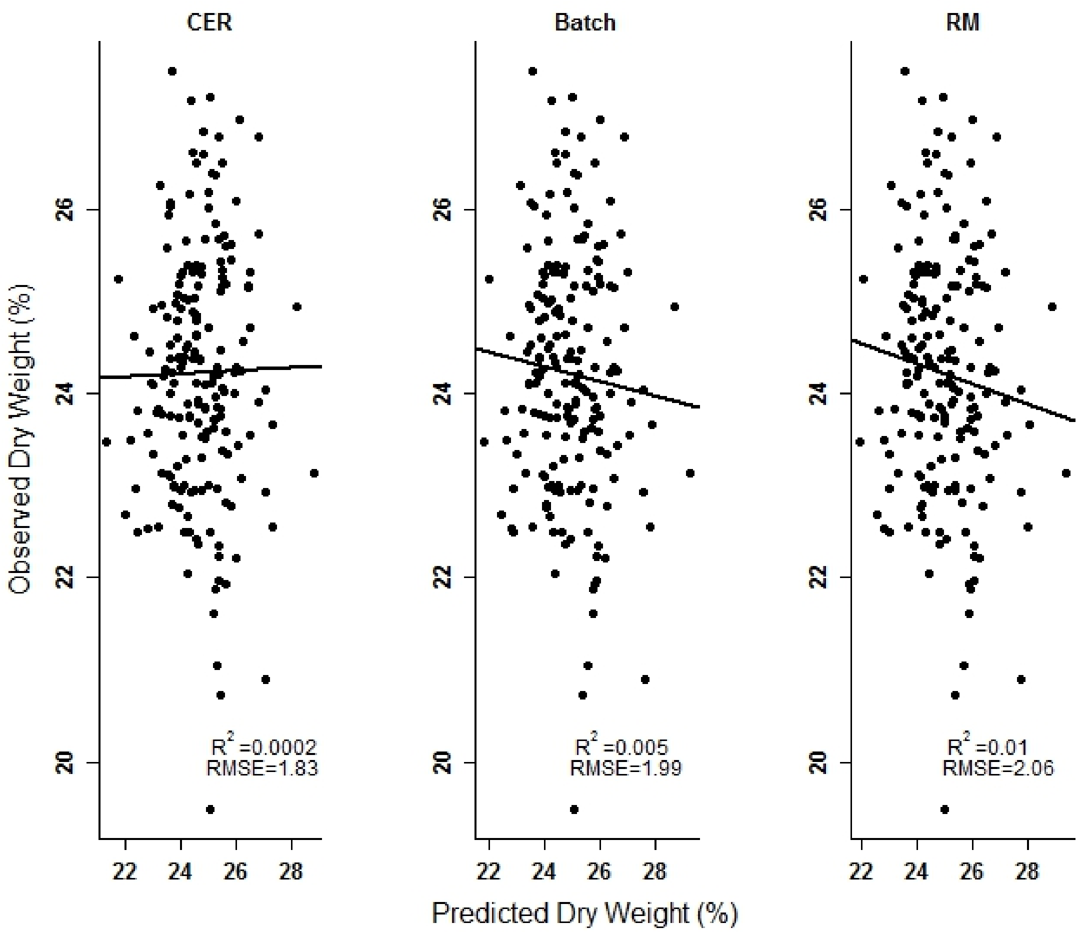
**Appendix Q**

The change in BIA electrical parameters, using SD electrode, as a function of temperature (°C). The slope (b) of each line is represented at the top of each graph in addition to the 95% confidence intervals (CI) for the slope. Electrical parameters, measured in Ohms, include: dorsal resistance (DR), dorsal reactance (DX), ventral resistance (VR), ventral reactance (VX). Each column represents a different temperature correction method (CER, Batch, RM) used, respectively.



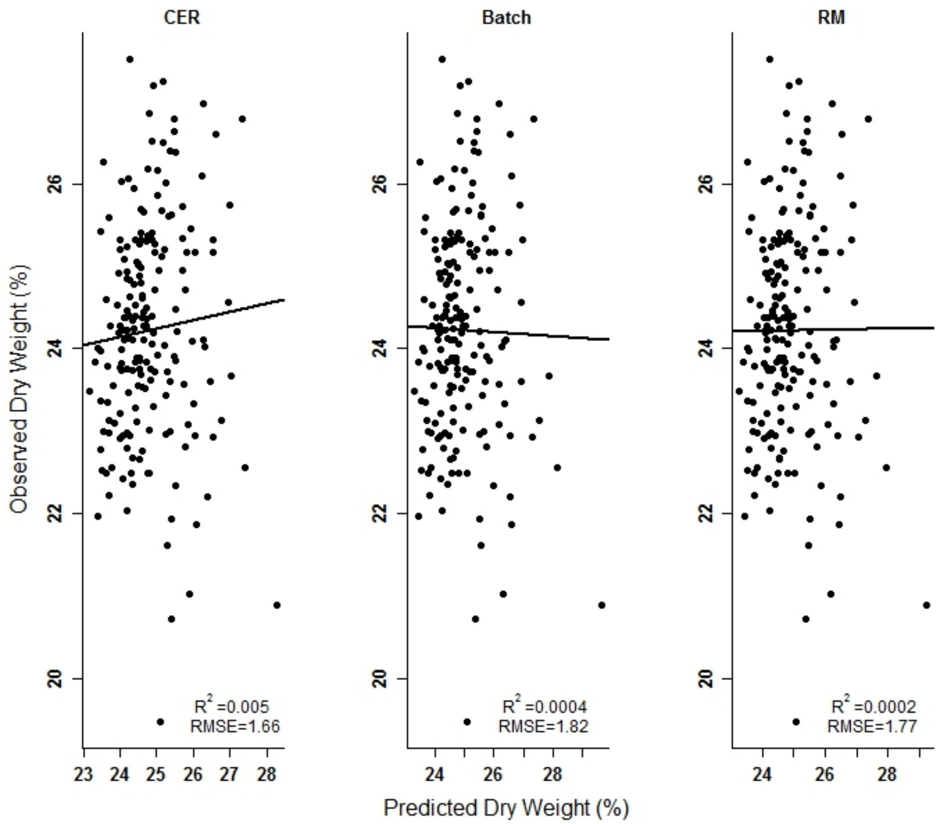
**Appendix R**

The relationship between observed and predicted dry weight (%) for Yellow Perch measured from the field using the best supported and temperature-corrected (CER, Batch, RM) CE9 lab model.



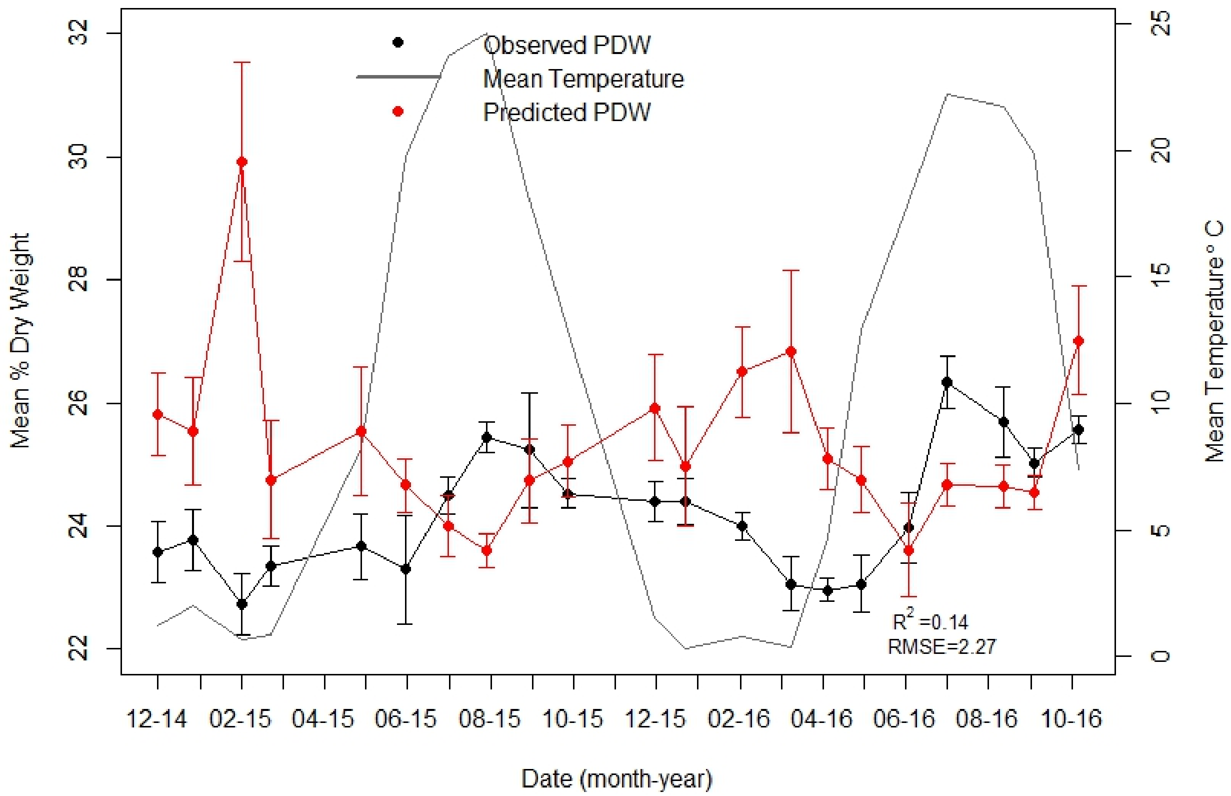
**Appendix S**

The relationship between observed and predicted dry weight (%) for Yellow Perch measured from the field using the best supported and temperature-corrected (CER, Batch, RM) SD lab model.



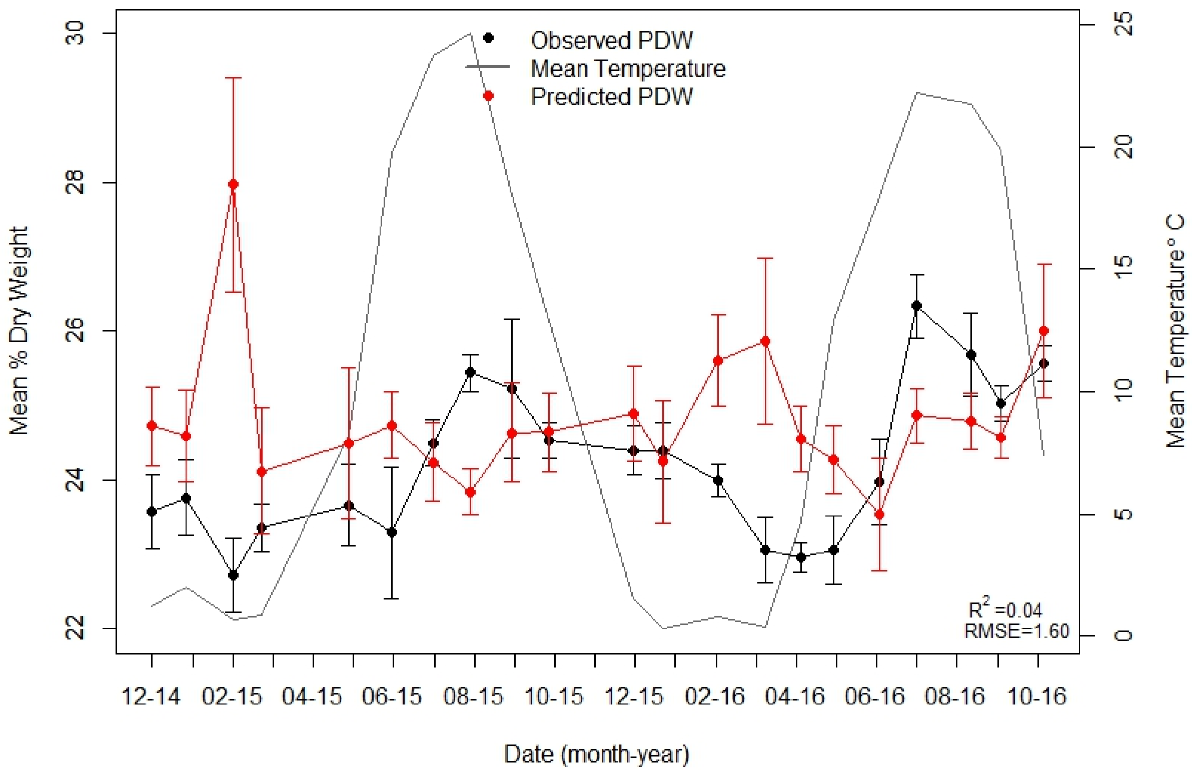
**Appendix T**

The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW using the best supported CE14 lab BIA model and CER temperature corrections. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.



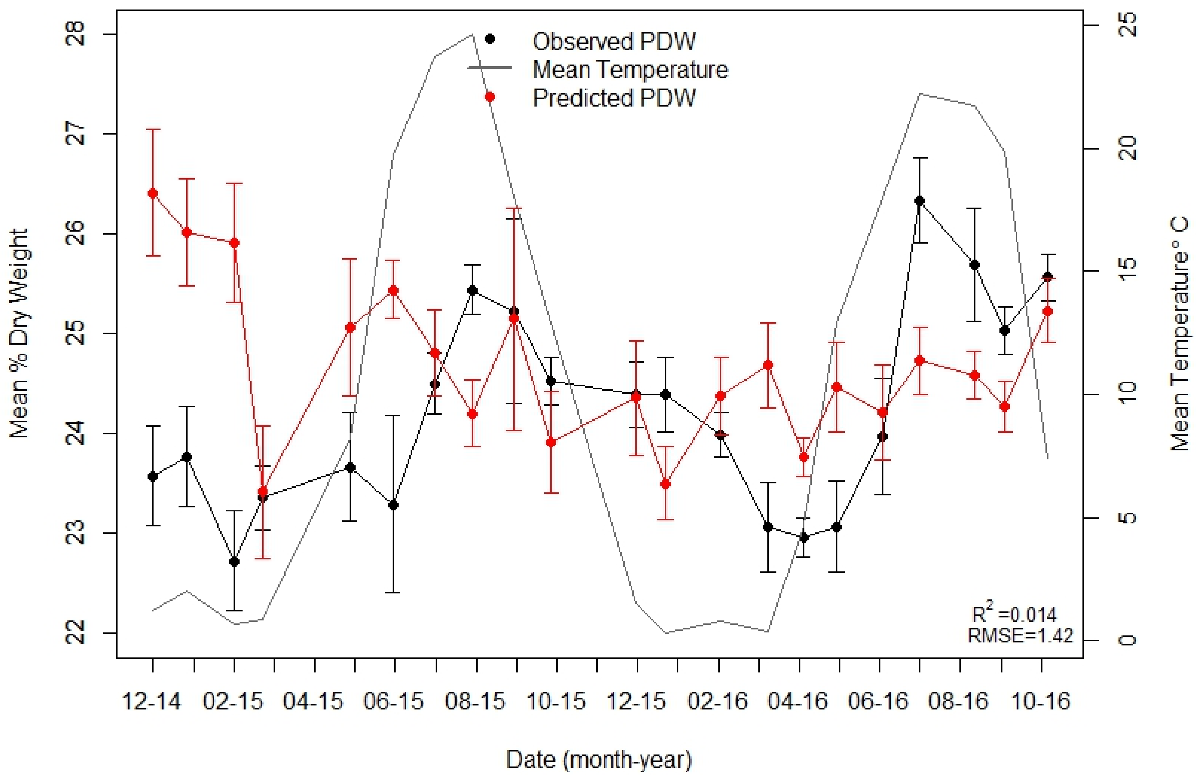
**Appendix U**

The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW using the best supported CE14 lab BIA model and RM temperature corrections. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.



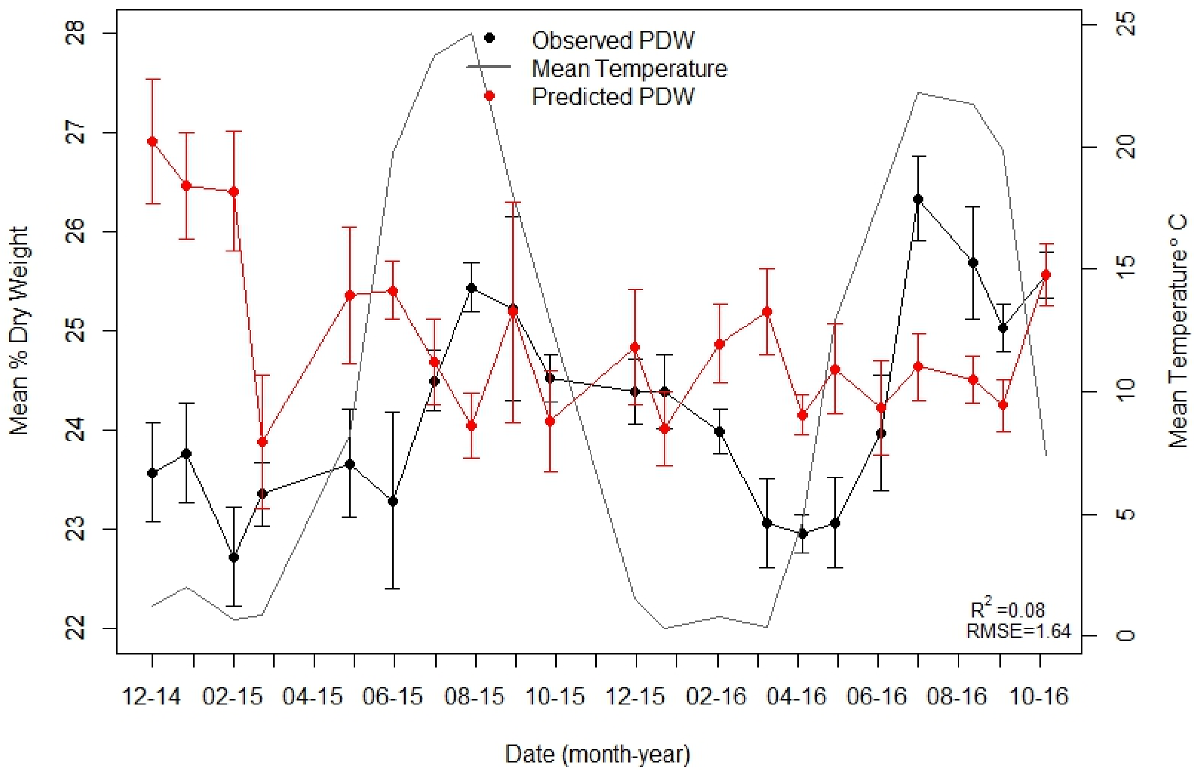
**Appendix V**

The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW using the best supported CE9 lab BIA model and CER temperature corrections. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.



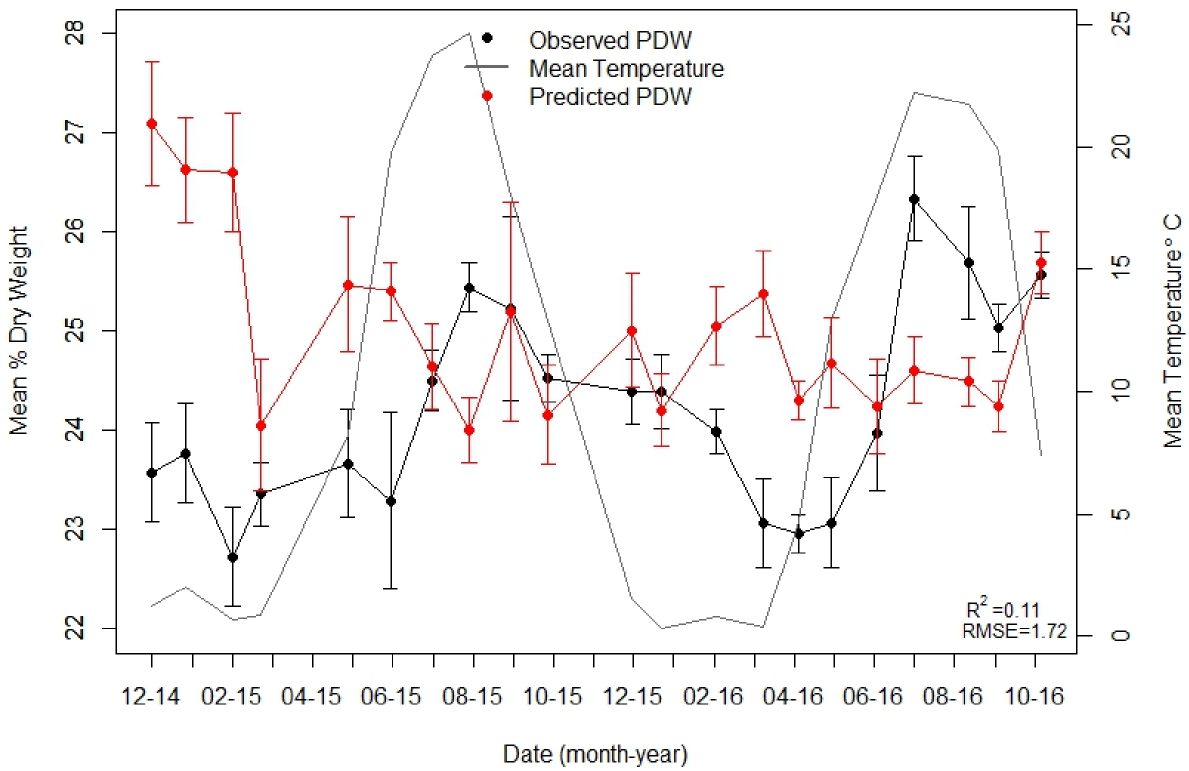
## Appendix W

The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW using the best supported CE9 lab BIA model and Batch temperature corrections. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.



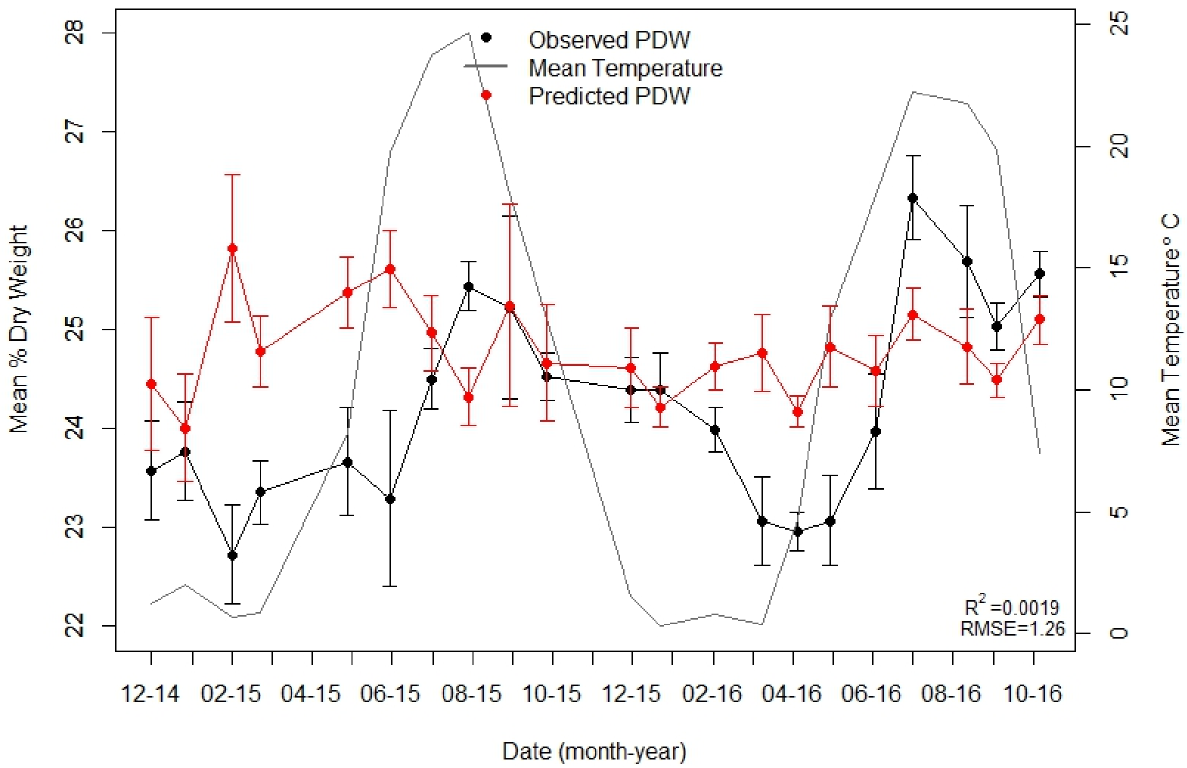
## Appendix X

The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW using the best supported CE9 lab BIA model and RM temperature corrections. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.



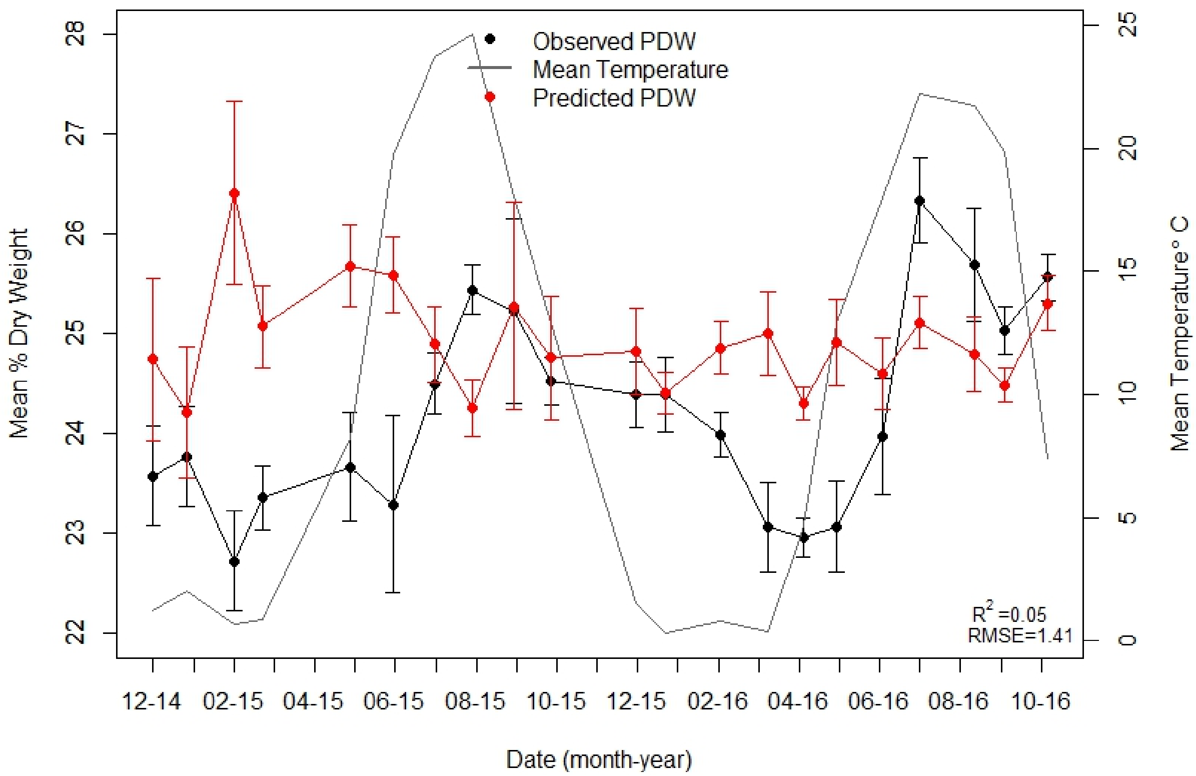
**Appendix Y**

The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW using the best supported SD lab BIA model and CER temperature corrections. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.



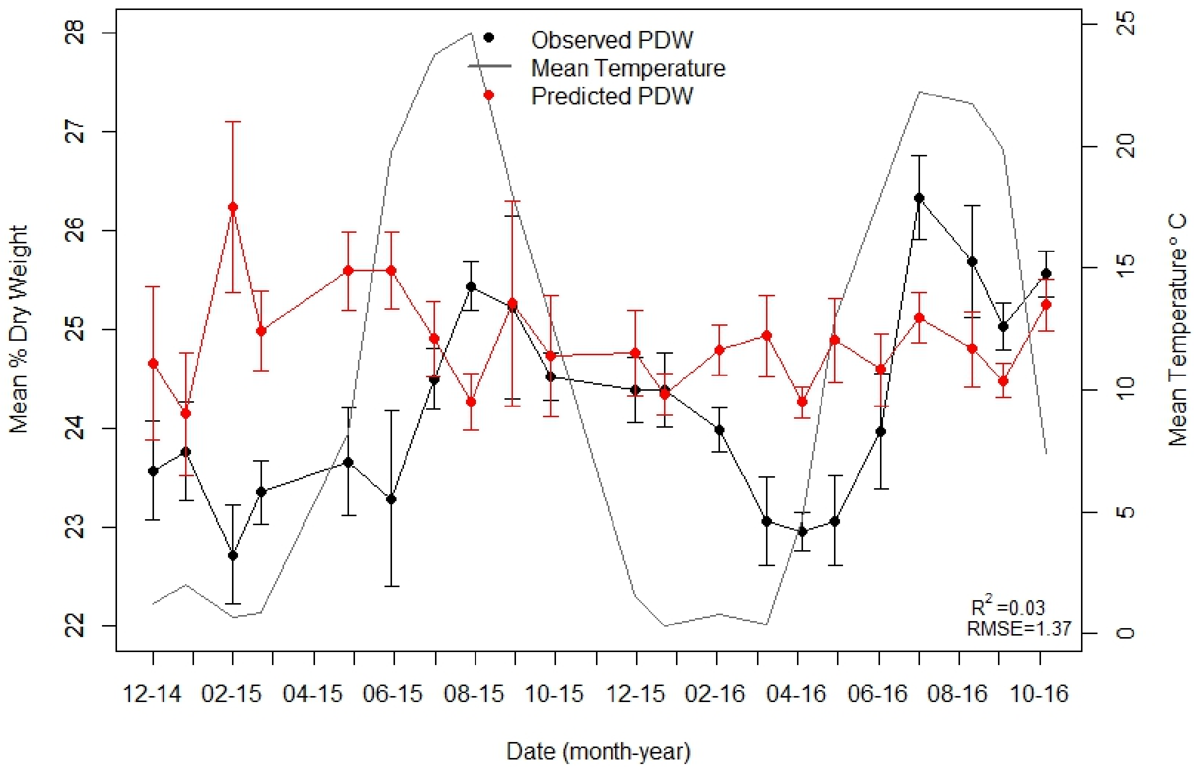
**Appendix Z**

The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW using the best supported SD lab BIA model and Batch temperature corrections. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.



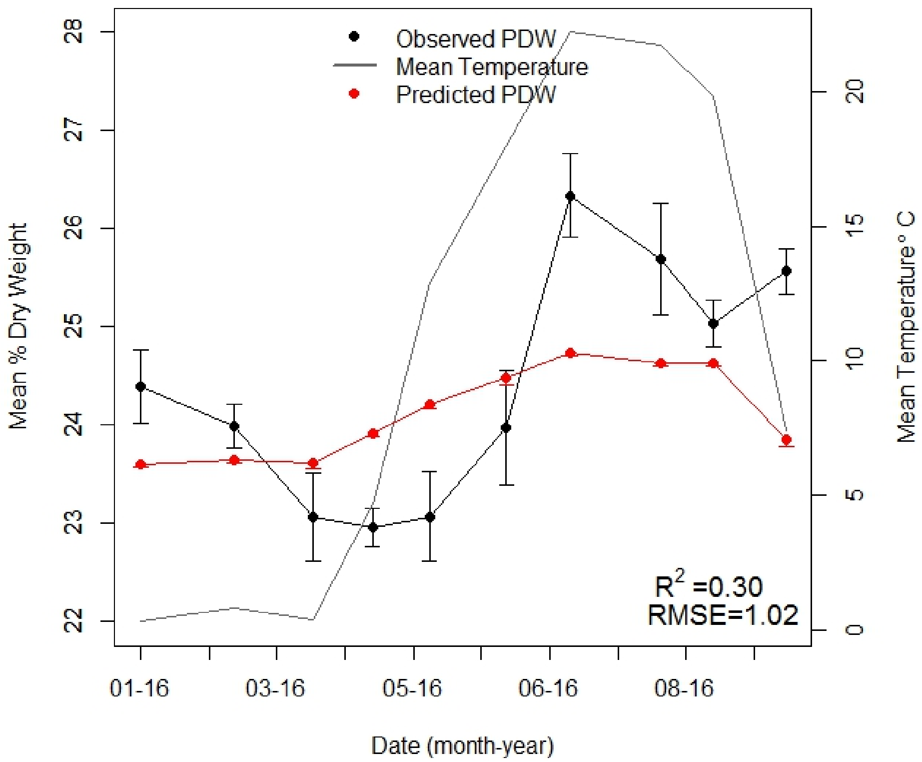
**Appendix AA**

The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW using the best supported SD lab BIA model and RM temperature corrections. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.



## Appendix BB

The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW for 2016 Yellow Perch using the best supported CE9 field BIA model developed from 2015 data. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.



# Appendix CC

The mean observed monthly percent dry weight (PDW) in comparison to the mean predicted PDW for 2016 Yellow Perch using the best supported SD field BIA model developed from 2015 data. The mean internal temperature of the Yellow Perch measured is depicted by the gray solid line.