Bioelectrical impedance analysis as a predictor of fish health and energy content in juvenile rainbow trout (*Oncorhynchus mykiss*)

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Thesis submitted to the Faculty of Science of the University of Ontario Institute of Technology
In Partial fulfillment of the requirements for the degree of

Master of Science

in

Applied Biosciences

December 14th, 2016
Oshawa, Ontario

Key words: bioelectrical impedance analysis, rainbow trout, *Oncorhynchus mykiss*, body composition

Abstract

Assessing the health of a fish is an essential part to aquaculture and fish research. This is traditionally done by measuring body content using bomb calorimetry or proximate composition analysis; however, both of these methods necessitate sacrificing the fish in order to obtain accurate data. This has led to research into novel non-lethal methods of analyzing fish tissue so as to determine the body composition and health condition of fish without sacrificing the animal. One such method is Bioelectrical Impedance Analysis (BIA). BIA is a quick, non-lethal procedure that can estimate the body content of fish by passing an electric current between two electrodes through the tissue of the fish. The focus of this study is to develop predictive equations non-lethally determine the water content, dry mass and energy content of juvenile rainbow trout (*Oncorhynchus mykiss*) and to determine whether the electrode position has an effect on the predictive capability of these equations. Three different sizes of juvenile rainbow trout were obtained: small (150mm length), medium (230mm length) and large (300mm length) and fasted over a period of two weeks. A third of each size group was sampled at the beginning of the fasting period and once every week after. Bioimpedance was measured along the dorsal, lateral and ventral axis of each fish. Tissue from each fish was obtained during sampling and analyzed afterward for energy content using bomb calorimetry. The results of this BIA study demonstrated a strong correlation between the BIA readings and total water content (r^2=0.9170), dry mass (r^2=0.9064) and energy content (r^2=0.9149). The best predictive equations were developed from the dorsal BIA readings. These results indicate that BIA may be used as an accurate tool to non-lethally determine the energy content and health of rainbow trout in future studies.

Acknowledgements

I would first like to thank my supervisor, Dr. Douglas Holdway, for taking me on as one of his students. I will always appreciate his constant support and encouragement in my research throughout my project helped me get through my degree in one piece.

Thank-you as well to Dr. Sean Forrester for being part of my supervisory committee and examining my thesis. His expertise and knowledge provided unique perspectives to my research.

Thank you to John Guchardi for his assistance and training in use of the bomb calorimeter and in providing me with some of the literature to start my research.

I would also like to give a big thank-you to all my lab mates, Jordan Anderson, Lindsay Beyger, Zacharias Pandelides and Erin Ussery. Their friendship and humor made my time here a memorable experience.

I promised I would thank my parents in here, so thank-you to both of them. Their endless support throughout my career as a student, even when I had no idea what I was doing with my education, means a lot.

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List of Abbreviations

ANOVA	Analysis of Variance
BIA	Bioelectrical Impedance Analysis
BIVA	Bioelectrical Impedance Vector Analysis
TOBEC	Total Body Electrical Conductivity
NIR	Near Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
CT	Computerized Tomography
EMG	Electromyography
TBW	Total Body Water
DW	Dry Weight
TBA	Total Body Ash
TBP	Total Body Protein
TBF	Total Body Fat
R	Resistance
X	Reactance
Z	Impedance
PA	Phase Angle
SPA	Standardized Phase Angle

Introduction

Monitoring the energy content of fish is an integral part to aquaculture and fish research (Pothoven *et al.*, 2008). Energy consumed by an organism is either expended (i.e. for growth or metabolism) or is stored within tissue (as fat). The amount of energy present within the tissue can be a measure of an organisms physiological status (Pothoven *et al.*, 2008). Fish which obtain large amounts of food are able to acquire larger energy deposits and are said to be healthier because of it. Thus, determining the body composition of a fish is an important part of aquaculture and fish research as it allows us to determine how healthy a fish is (Nacci, 2013).

The current standards that determine the energy content of fish are proximate composition analysis and bomb calorimetry. Unfortunately, both methods are lethal and necessitate sacrificing the organism under in question to obtain data. This is not always possible or ideal when working with endangered species of fish or in situations where obtaining large enough samples are not possible. Yet despite this disadvantage, proximate analysis and bomb calorimetry are still the standard as there is currently no standard non-lethal method which can estimate the health and energy content of a fish.

For these reasons, research into non-lethal estimates of fish condition and body composition has led to the development of a wide variety of non-lethal methods for analyzing fish. Many of these methods are still being developed but have the potential to have an impact on aquaculture and/or fish research. Non-lethal methods which have been investigated for use on fish are: condition analysis, electromyogram telemetry, total body electric conductivity (TOBEC), nuclear magnetic resonance (NMR), computerized tomography (CT), fat meter devices, near infrared spectroscopy and bioelectrical

impedance analysis (BIA) (Duncan *et al.*, 2007; Folkestad *et al*., 2008; Veliyulin *et al*., 2005).

The only non-lethal method which has been used to any extent in fish research is condition analysis; however, condition analysis suffers from an inability to yield accurate information about the composition about a fish and is only suitable at providing a rough estimate of fish health (Rasmussen *et al*., 2012). If a study requires more information about the composition or health of a fish than condition analysis can provide, the study must resort to lethal methodology (bomb calorimetry or proximate composition analysis). Existing non-lethal methods cannot provide the same information as these lethal methods. There thus exists a need to fill this gap in knowledge by developing a non-lethal method that can help researchers understand the body composition and health of fish better than condition analysis, and with similar accuracy as lethal methods.

One particular method which has potential to fill this gap is bioelectrical impedance analysis (BIA). BIA makes use of the different conductive properties of organic tissue to differentiate between healthy and unhealthy tissue, which in turn can be used to determine the body composition and health condition of the organism in question. BIA essentially works by passing an electric current between two electrodes placed at either end of an organism and measuring the electrical resistance experienced by that current as it passes through the tissue. Water within tissue and cells will conduct the current and impart a certain amount of resistance, while other tissue components (such as lipids) act as insulators and will not conduct the electrical current (Dorhofer, 2005). This means that fish with different levels of water and lipids within their tissue will impart different resistance values to a current which passes through them. These differences in

resistance values can then be used in turn to predict the health condition and body composition of the fish.

Such resistance measurements have allowed researchers to develop species specific BIA models which can predict the body composition of live whole fish (Cox & Hartman, 2005). These models are developed using regression analysis which correlate the resistance values measured using the BIA instrument to actual measured values obtained using bomb calorimetry or proximate composition analysis to develop predictive equations. These predictive equations are used to non-lethally determine the total body water (TBW), dry weight (DW), total body ash (TBA), total body protein (TBP) and energy content (in calories) which are all important metrics when assessing the health of fish (Cox & Hartman, 2005; Pothoven *et al.*, 2008).

BIA was first developed as a means to analyze fish by Bosworth and Wolters (2001) in their study of channel catfish (*Ictalurus punctatus*) fillets. Their work proved that BIA not only worked in theory, but was able to differentiate between high energy content fish tissue and low energy content fish tissue. Predictive models and equations were first developed by Cox and Hartman (2005) four years later in their study of live brook trout (*Salvelinus fontinalis*). Since then, BIA has been tested on several other fish species and has been incorporated into field studies involving bluefin tuna (*Thuunus maccoyii*) (Willis & Hobday, 2008). BIA has also been used to study real energy depletion during migration of Chinook salmon (*Oncorhynchus tsawytscha*) (Ryan *et al.*, 2009).

The use of BIA in humans has seen clinical use since the 1980's (Kristina *et al.*, 2012). As such, the application of BIA in humans is more developed than in fish. One

useful application of bioimpedance in humans that has not made its way to animal subjects is bioelectrical impedance vector analysis (BIVA). BIVA is the plotted resistance and reactance measurements (obtained from the BIA instrument) normalized against the height/length of the organism. BIVA has shown promise in its applicability to diagnose some diseases in people by showing how different disease states yield different vectors from healthy individuals (Antonio *et al*., 2002). This is an interesting avenue to explore in fish as it has previously not been done in non-human organisms.

Research has shifted into studying how abiotic factors in the aquatic environment can impact BIA readings (such as salinity, pH, water temperature etc.) (Miller, 2014) and is still ongoing. Results thus far have also not examined the effect of gender on BIA readings, and whether or not resistance measures can be used to determine the sex of fish. BIA predictive equations are also non-existent for many ubiquitous species such as rainbow trout (*Oncorhynchus mykiss*), which limits the use of current predictive models. To advance the use of BIA in fish, more models for more species are needed if BIA is to be fully developed for widespread use in fish research. Although BIA is still a relatively new method, it holds great promise to be a beneficial tool for assessing body composition and health of fish.

Literature Review

Determining the health and growth of fish is an important part of aquaculture and fish research. One way to do this is to determine the body composition of fish (Cox & Hartman, 2005). Determining the body composition can reveal the amount of fat, water and dry mass within an organism. Fish with larger energy reserves are considered healthier than fish with low energy content in their tissue due to their ability to overwinter, evade predators etc. (Biro *et al.*, 2004). Determining the energy content of fish tissue is therefore a good indicator of fish health and traditionally this has required lethal techniques (i.e. proximate composition analysis) to be employed to obtain reliable data (Duncan *et al.*, 2007). The actual energy content of a fish is closely correlated with the ratio of dry mass to wet mass of the fish (Schreckenbach *et al*., 2001). This is due to the strong inverse relations between moisture and lipid content in fish tissue (Klefoth *et al.*, 2013). Higher water content of fish tissue coincides with lower fat reserves in the same tissue while lower water content of fish tissue coincides with higher fat reserves (Pothoven *et al.*, 2008). The relative dry mass of a fish stays relatively constant and so by determining the ratio of dry to wet mass or just the amount of water itself within fish tissue can give insights to the total fat/energy content of fish tissue.

Lethal Methods

Traditional methods of energy assessment are lethal/destructive in that they sacrifice the fish in order to analyze their tissue for energy content. The two standard lethal methods for determining energy content of fish are proximate composition analysis and bomb calorimetry (Crossin & Hinch, 2005).

Bomb Calorimetry

Bomb calorimetry is an analytical method which involves combusting a sample in precisely controlled conditions and measuring the amount of energy released during that combustion reaction (Crossin & Hinch, 2005). Tissue samples of fish containing high levels of energy will have more energy-containing macromolecules (lipids, proteins, carbohydrates) which will result in more calories of energy being released during combustion. Fish with low energy stores within their tissue will possess fewer energy-containing macromolecules which will result in less calories of energy being released during combustion (Cox & Hartman, 2005; Crossin & Hinch, 2005; Doyle *et al.*, 2007).

In terms of determining the total energy content of a tissue sample, bomb calorimetry is a reliable and accurate method of procuring this information (Crossin & Hinch, 2005). A completed sample combustion in a bomb will vaporize the sample within the bomb such that the entire sample is consumed in the process, leaving only a small amount of ash. The bomb is charged with excess amounts of oxygen so that the only limiting reagent in the combustion reaction is the sample itself. This ensures that the reaction goes to completion which makes bomb calorimetry reliable since the tissue will yield the caloric value of the tissue in its entirety.

Bomb calorimetry suffers from several drawbacks, however, it is a destructive method which requires large amounts of tissue to perform (Crossin & Hinch, 2005), bomb calorimetry is also time consuming and labour intensive, which can prohibit its' use in some experiments.

Proximate Composition Analysis

Proximate composition analysis is considered the 'gold standard' in determining the energy content of fish. Proximate composition uses an array of chemical assay techniques to determine the composition of tissue from a fish and based on the assay will determine the total amount of lipids, proteins or carbohydrates present. The amount of each of these components (in grams) is then multiplied by the corresponding heat of combustion to obtain the caloric value of each component present in the tissue (Crossin & Hinch, 2005).

Since lipids, proteins and carbohydrates are all energy containing molecules, proximate composition analysis will determine not only the total amount of energy available, but the amount of energy yielded from each fraction of macromolecule (i.e. how much energy is contributed from lipids, how much from proteins, etc.) (Doyle *et al.*, 2007).

Despite providing such great detail about the energy content of fish, proximate composition has some drawbacks that make the application of this technique non-viable in certain experiments. Proximate composition is also costly (both in lab materials and tissue required) (Crossin & Hinch, 2005) and additionally, large numbers of fish must be sampled to ensure accurate results.

Non-Lethal Methods

Most current non-lethal techniques suffer from some drawbacks that have thus far prevented them from being widely adopted in aquaculture or fish research. Many of these methods are prohibitively expensive and/or are not ideal for lab or field studies (Cox & Hartman, 2005). Existing non-lethal methods to assess fish condition include: total body

electrical conductivity (TOBEC), near infrared spectroscopy (NIR), computerized tomography (CT), nuclear magnetic resonance (NMR), electromyogram telemetry, condition analysis/condition factor, fat meter (FM) devices and bioelectrical impedance analysis (BIA).

Condition Analysis

Condition analysis such as Fulton's Condition Factor, makes use of fish length and weight and correlates them to the health of the fish. In condition factor, fish are assigned a health rating (i.e. excellent, fair, poor etc.) based on the weight and length.

Condition factor has been widely used to obtain rough estimates of fish health but condition factor cannot be used to accurately determine energy content of fish (Cox & Hartman, 2005; Elaine *et al.*, 2012). Models that predict body composition have been attempted in previous studies but show low correlation between actual and predicted values for total body fat (r^2=0.318) and total body water (r^2=0.496) (Elaine *et al.*, 2012).

Electromyogram Telemetry

Electromyogram telemetry is an invasive procedure which requires the placement of an EMG-tag inside the fish, then using this tag to monitor the fish for a period of time after it is implanted. This procedure is time consuming and places a large amount of stress on the fish which makes it only feasible for small populations of fish (Ryan *et al.*, 2009). The procedure to implant the EMG-tag requires complete anaesthesia to complete the surgery successfully in addition to a recovery period and an injection of antibiotics for three days post-surgery to minimize risk of infection (Carbonara *et al.*, 2015). Once implanted, the electrode readings can be read wirelessly to give information about muscle activity.

Electromyogram telemetry has been used to study swimming behavior of fish to great effect to show how fish swim in certain conditions and how active fish are (Alexandre *et al.*, 2013; Carbonara *et al.*, 2015). Electromyogram telemetry provides valuable detail about the physiology of swimming fish in particular the heart rate, opercular rate and muscle activity (Cooke, Thorstad, & Hinch, 2004). Although this can provide a generalized statement about the energetics of the fish (i.e. fish with more energy reserves may be more active) but cannot be used to determine the actual amount of fat, dry mass or wet mass (Alexandre *et al.*, 2013; Cooke *et al.*, 2004).

Total Body Electric Conductivity

TOBEC works on the premise that different tissue types possess different conductive properties and that the conductive properties of body fat and fat free mass can be measured in an electromagnetic field. In theory, TOBEC provides accurate measurements to determine body composition but it is expensive and uses bulky equipment not ideal for field studies (Duncan *et al.*, 2007). The capacity of TOBEC to non-lethally assess the body composition of fish has been studied but failed to produce predictive models with meaningful correlations, only obtaining an r^2 value of 0.37 (Hancz, Milisits, & Horn, 2003). Despite sounding promising in theory, in application TOBEC fails at providing accurate detail about the body composition of fish.

Nuclear Magnetic Resonance

NMR provides incredible detail about the body composition of fish, however, the cost of this technology along with the size of the instrument and the complexity involved in both operating and interpreting results has prohibited its general use in fisheries research (Veliyulin *et al.*, 2005).

Recent technological advances have improved NMR instruments to produce portable NMR devices which produce highly accurate predictive models for body fat (r^2=0.92) in Atlantic salmon (*Salmo salar*) (Veliyulin *et al*., 2005).

Computerized Tomography

Computerized tomography (CT) is a non-destructive imaging technique that can be used to estimate body composition in fish species (Folkestad *et al.*, 2008). Previous studies have examined CT's use as a non-lethal assessor of body composition and produced models with high predictive capabilities for body fat percentage (r^2=0.95) (Folkestad *et al.*, 2008) and dry mass (r^2=0.92) (Rye, 1991). However, the extensive cost of this technology prohibits its use in the fisheries industry (Folkestad *et al*., 2008; He *et al.*, 2013; Rye, 1991).

Fat Meter

Fat meter (FM) devices make use of microwaves and their interaction with water molecules (Crossin & Hinch, 2005). The FM is essentially a short ranged microwave transmitter that sends out a specific frequency of microwaves into the tissue of fish. Microwaves experience a loss of energy as they pass through water (that lost energy is transferred to the water molecules). This loss in energy is measured by a sensor on the FM (Klefoth *et al.*, 2013). FM devices are an attractive alternative to lethal body composition measurements because they do not require puncturing fish with electrodes in addition to the fact that measurements can be taken quickly with minimal setup.

FM devices have been tested on fish with varying degrees of success. One study attempted to produce FM models for lipid content of three species of fish: smallmouth bass (*Micropterus dolomieu*), walleye (*Sander vitreus*) and channel catfish (*Ictalurus*

punctatus) but could only produce r^2 values of 0.02, 0.17 and 0.83 for each species respectively when comparing predicted lipid content to actual lipid content (Mesa & Rose, 2015). The authors of this study concluded that FM as a non-lethal method of determining lipid content of fish appeared to be unreliable (Mesa & Rose, 2015).

Some studies have been able to produce very good correlations between FM measurements and energy content (r^2=0.927) (Crossin & Hinch, 2005). This model was produced for pacific salmon from the average measurements across two measurements and the authors point out that the FM device has limited accuracy on fish with low lipid levels (in particular spawning salmon) which, due to the inverse relationship between water and lipids, results in high levels of water which could impair instrument accuracy (Crossin & Hinch, 2005).

Since FMs depend on the microwaves actively penetrating fish tissue enough to be read by the instrument, the readings from FM devices on larger fish tend to be inaccurate (Pothoven *et al.,* 2008). In addition to the size of the fish, the thickness of the skin of fish can heavily impact the readings obtained by the device (Mesa & Rose, 2015).

Near Infrared Spectroscopy

Near infrared spectroscopy (NIR) works in a similar manner to FM devices. The device passes NIR radiation through a fish which interacts with water within the fish tissue. This will then reflect back to a detector on the instrument which measures the loss in energy (Folkestad *et al.,* 2008). This loss in energy is dependent on the amount of water present in the fish tissue.

NIR and its application as a non-lethal assessor of body composition is relatively new and few studies have examined its application in fisheries research (Folkestad et al., 2008). Initial predictive models are promising however, and models predicting body fat percentage show strong predictive capabilities (r^2=0.94) (Folkestad *et al.*, 2008).

Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) makes use of the conductive properties of water in an organism's tissue. Extracellular and intracellular water work to conduct a current of electricity between two electrodes placed on an organism (Kushner *et al*, 1992; Pothoven *et al.*, 2008; Rasmussen *et al*, 2012). As this current passes through the tissue it experiences electrical resistance from non-conductive elements within tissue. This resistance produces a loss in voltage in the current and this drop in voltage is measured by the instrument which can then determine the resistance experienced by the current as it passed through the fish (Dorhofer, 2005; Pothoven *et al.*, 2008). In theory, fish with more water in their tissue will yield different resistance values to fish with low water content. This measured difference in moisture content should also make it possible to measure differences in energy content of fish (Cox & Hartman, 2005).

The resistance readings obtained from the instrument can then be correlated to various body composition parameters (i.e. fat, water, dry mass etc.) using equations that model the path of an electric current as it passes through a three-dimensional object (Dorhofer, 2005). These equations can be plotted against values obtained using traditional/lethal methods using linear regression analysis to determine the correlation between the resistance measures and the lethal proximate measures (Cox & Hartman, 2005; Margraf *et al.*, 2005; Pothoven *et al.*, 2008; Rasmussen *et al.*, 2012).

As a procedure, BIA is simple to employ and relatively harmless to the fish. A tetrapolar bioimpedance analyzer is used with electrodes paired into two sets of electrodes (one signal and one detector electrode) which are placed at either end of the fish. The analyzer sends a current from each signal electrode which then travels to the detector electrode. The time required to measure the resistance of fish tissue is only dependant on how fast the electric current can travel through fish, which makes taking BIA measurements extremely fast.

BIA was first developed for use in humans in the 1970's and since then, the technology has been refined for clinical use (Pothoven *et al.*, 2008). The use of BIA on non-human organisms did not begin until much later, with the technology being applied to sheep, cattle and swine in the 1990's (Bosworth & Wolters, 2001), but not applied to fish until 2001 (Bosworth & Wolters, 2001). So despite the technology being over a half-century old, the application to fish is relatively new. Since then, accurate models that predict fish body composition have been developed (first in 2005 (Cox & Hartman, 2005)) capable of predicting dry weight (DW), total body water (TBW), total body fat (TBF), total body ash (TBA), total body protein (TBP) and total energy (see Table 1 for a summary of literature BIA models).Despite the novelty of this technology, the potential benefits of its application to fisheries research is very promising.

Table 1: Existing models developed for various fish species using bioelectrical impedance analysis from the literature. For each paper published, the species of fish that the model was developed from is given along with the body composition parameter the models describe. The models published are: TBW=total body water, TBF=total body fat, DW=dry weight, TBP=total body protein, TBA=total body ash and the total energy content. The number listed next to each model is the r^2 value for that model.

Author	**Fish Species Used**	**BIA Models Developed**
(Krimmer & Rasmussen, 2008)	Brook trout (*Salvelinus fontinalis*)	TBW = 0.719 TBF = 0.718
(Bosworth & Wolters, 2001)	Channel Catfish (*Ictalurus punctatus*)	TBW = 0.43 TBF = 0.63
(Cox & Hartman, 2005)	Brook trout (*Salvelinus fontinalis*)	TBW = 0.9746 DW = 0.9726 TBF = 0.9563 TBP = 0.9727 TBA = 0.973
(Duncan *et al.*, 2007)	Cobia (*Rachycentron canadum*)	TBW = 0.989 DW = 0.929 TBP = 0.958 TBA = 0.859
(Elaine Maclean & Beth, 2012)	Atlantic salmon (*Salmo salar*)	TBW = 0.993 TBF = 0.755 TBP = 0.986
(Hafs & Hartman, 2014)	Brook trout (*Salvelinus fontinalis*)	DW = 0.86
(Klefoth *et al.*, 2013)	Eel (*Anguilla anguilla*) Carp (*Cyprinus carpio*)	Eel DW = 0.028 Carp DW = 0.261
(Margraf *et al.*, 2005)	Chinook salmon (*Oncorhynchus tshytscha*)	TBW = 0.80 TBF = 0.88 TBP = 0.87 TBA = 0.66 Total Energy = 0.84
(Pothoven *et al.*, 2008)	Yellow perch (*Perca flavescenes*) Walleye (*Sander vitreus*) Lake whitefish (*Coregonus clupeaformis*)	DW = 0.99 TBF = 0.89 Total Energy = 0.99
(Stolarski *et al.*, 2014)	Dolly varden (*Salvelinus malma*)	TBW = 0.73 TBF = 0.77

Bioelectrical Impedance Vector Analysis

Bioelectrical impedance vector analysis (BIVA) is a subset of BIA in that it forgoes the use of predictive equations and instead uses a plot of normalized resistance and reactance values (Kristina *et al*, 2012). In the literature, BIVA has only been applied to human beings thus far, and its applicability to fish or other non-human models is unknown; however, it has interesting clinical application that could be adapted for use in fish research. In BIVA, each organism has its own vector, which is based on the resistance values measured with the BIA instrument which in turn are influenced by the composition of the tissue. BIVA of people show this as people in different hydration and nutritional states will have significantly different vectors from healthy individuals (Antonio *et al*, 2002; Kristina *et al*., 2012). Due to the fact that BIVA plots are two-dimensional representations of resistance measures, any changes in tissue hydration status (and by effect energy content) might be better represented by BIVA than by phase angle alone.

Vector placement can also change over time as a response to any changes within the tissue of an organism which means BIVA can be used in temporal analysis to track and log changes with the condition of an organism (Walker-Kroker *et al*, 2011). It is this aspect of BIVA that is a promising tool for use in fish research as it would allow researchers to observe and track changes in fish health and condition in a non-lethal manner.

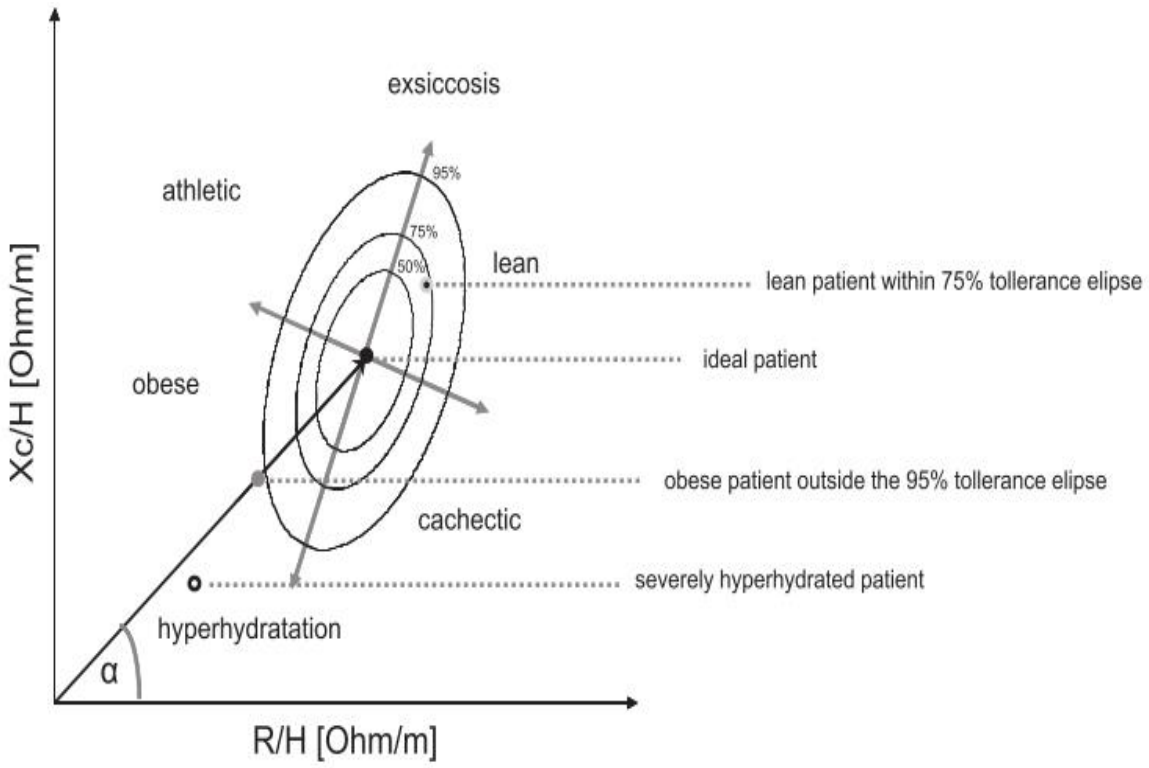

Figure 1: A visual representation of BIVA. This is labels show where different vector positions would be to represent different body composition and nutritional status (i.e. obese individuals will have a different vector from athletic or dehydrated individuals). In this graph, resistance (R) and reactance (Xc) are both normalized by the height (H) of the person measured and their vector is plotted accordingly. (Kristina *et al*., 2012)

Rainbow Trout

Habitat

The native habitat for rainbow trout lies on the Pacific drainages of the West coast of North America ranging from as far north as Alaska, to as far south as Mexico (FAO, 2015). Beginning in 1874, rainbow trout have been introduced to all continents on Earth (except Antarctica) for sport fishing and aquaculture purposes (FAO, 2015).

Fish production as a source of food in aquaculture has increased dramatically over the past century, and is becoming more relevant in recent decades as raw capture yields has stagnated (FAO, 2016; Luna & Villarroel, 2013). This is true for most fish being farmed for food, and in particular rainbow trout (see Figure 1). The production of rainbow trout has grown significantly since the 1950's. There has been a marked increase in production worldwide but rainbow trout production has seen particular success in Europe and Chile (FAO, 2015). As the fish has been introduced to other countries for aquaculture it has become an integral part of these nations' economies meaning the endemic nature of rainbow trout makes it an economically relevant species.

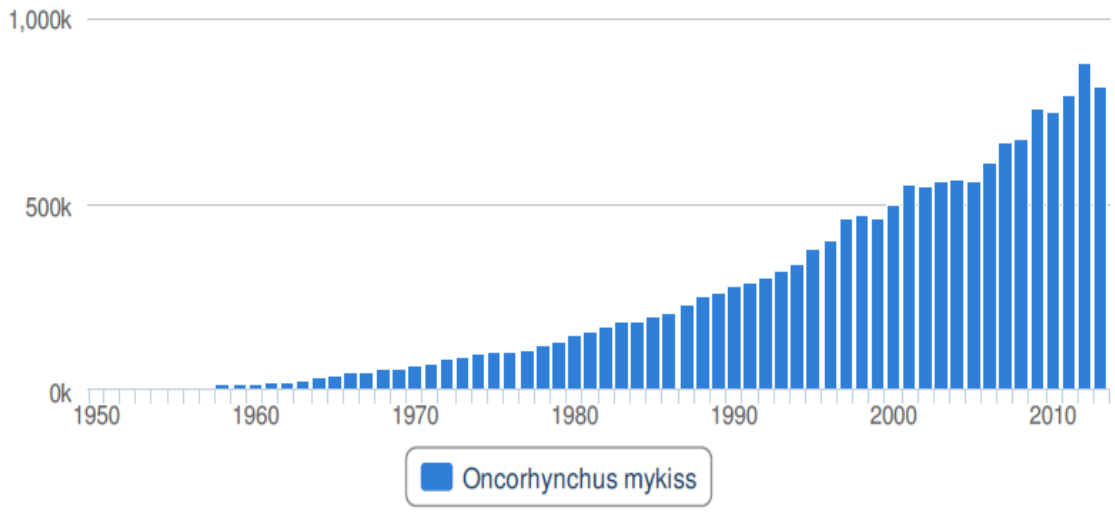

Figure 2: Annual world-wide production of rainbow trout (*Oncorhynchus mykiss*) in tonnes from 1950-2015. (FAO, 2015)

Species Classification

Rainbow trout are an anadromous and iteroparous species of fish, meaning they migrate upriver to spawn, and are able to reproduce multiple times over the course of their life (Quinn & Myers, 2004). Rainbow trout have two variants in morphology which can roughly be classified as their saltwater and freshwater forms, which are referred to as steelhead trout and rainbow trout, respectively (Quinn & Myers, 2004). Despite having different names, steelhead and rainbow trout are the same species. Rainbow trout are the more easily recognized morph of the two, as they possess the distinct red band colouring characteristic of the species. Steelhead lose this colouring as they swim out to the ocean, adopting a more silvery appearance.

Rationale

Rainbow trout make an ideal study species as the wide ranging geographical distribution of the species means studies using rainbow trout are globally relevant. Their use in aquaculture makes them an economically important species of interest (FAO, 2015). This study aims to assist in assessing the growth and condition of rainbow trout non-lethally and is relevant both to aquaculture and to future research applications. Moreover, BIA predictive models do not exist for rainbow trout and so this study will produce models which will add to the number of species of fish which BIA can be applied to.

Research Objectives

Overall Objective

The overall objective of this research is to develop bioelectrical impedance analysis (BIA) for use in predicting the energy content of juvenile rainbow trout (*Oncorhynchus mykiss*).

Specific Objectives

1. To develop species specific predictive equations for rainbow trout for water mass, dry mass and energy content by correlating conductor volume equations for transformed bioelectrical impedance data to actual values.
2. To determine if the position of electrodes during BIA can be used to improve the predictive equations.
3. To evaluate the use of bioelectrical impedance vector analysis (BIVA) in juvenile rainbow trout and determine if healthy and unhealthy fish have significantly different vectors.

Research Hypotheses

Starvation Study

The null hypothesis was there was no correlation between BIA readings and observed values. Alternate hypotheses were that correlations were caused by fish size or length instead of BIA readings.

Growth Study

The null hypothesis was that growth had no impact on BIA and BIVA. Alternative hypotheses were that growing fish had higher BIA readings which impacted BIA/BIVA models.

Materials & Methods

Conductor Volume Equations for BIA Analysis

R = measured resistance (Ω)

X = measured reactance (Ω)

L = detector length (cm)

Resistance in Series (Rs)

$$R_s = \frac{L^2}{R}$$

Resistance in Parallel (Rp)

$$R_p = \frac{L^2}{R + \left(\frac{X^2}{R}\right)}$$

Reactance in Series (Xs)

$$X_s = \frac{L^2}{X}$$

Reactance in Parallel (Xp)

$$X_p = \frac{L^2}{X + \left(\frac{R^2}{X}\right)}$$

Impedance in Series (Zs)

$$Z_s = \frac{L^2}{\sqrt{R^2 + X^2}}$$

Impedance in Parallel (Zp)

$$Z_p = L^2 \cdot \left(\frac{X \cdot R}{\sqrt{X^2 + R^2}}\right)$$

Capacitance (C)

$$C = \frac{L^2}{\left(\frac{1}{1 \times 10^{17} \cdot \pi \cdot R}\right)}$$

Phase Angle (PA)

$$PA = \tan^{-1}\left(\frac{X}{R}\right) \cdot \frac{180^{\circ}}{\pi}$$

Standardized Phase Angle (SPA)

$$SPA = L \cdot PA$$

Condition Factor (CF)

$$CF = \frac{100 \cdot weight}{(fork\ length)^3}$$

Actual Dry Weight (DW)

$$DW = \%dry\ mass \cdot weight$$

Actual Water Mass (TBW)

$$TBW = \%WM \cdot weight$$

Actual Energy Content

$$Energy\ Content = energy\ density \cdot weight$$

Obtaining Trout

Ninety juvenile rainbow trout were acquired from the local fish hatchery (Linwood Acres) and transported to the aquatic toxicology lab at UOIT. The fish were split into three different tanks based on their total length, either 150mm, 250mm or 300mm. The fish were housed in 1000L tanks filled with circulating 12°C water. The fish were acclimatized to lab conditions over a two week period. During this time the fish were fed twice daily by hand *ad libitum* and the tanks were vacuumed immediately prior to all feeding times. After acclimation, the fish were subjected to a feeding period lasting two weeks, in which they were again fed twice daily until the end of the feeding period. At the end of this feeding period, the fish were fasted for the remainder of the experiment.

Sampling Procedure

Sampling of the fish followed CCAC recommended protocols and involved euthanasia by tricainemethylsulfonate-222 (TMS-222) at a concentration of 150mg/L. Once euthanized, the fish were removed from the bucket of anesthetic with a net, excess water was allowed to drain off back into the bucket. The fish were then weighed on a scale and then moved to a measuring board. The standard length, fork length and total length of the fish were recorded. The left facing sides of the fish were then dried with paper towel and the BIA readings were taken using an RJS Quantum-II bioelectrical impedance analyzer. BIA readings were taken along the dorsal midline (DML), lateral line (LL) and the ventral midline (VML) see figure 3. The resistance and reactance obtained from the instrument were recorded. The left side of each fish was then filleted with a fillet knife and six muscle tissue samples were then removed from the anterior, medial and posterior sections of the fillet. Each muscle tissue sample was then placed

onto a weighed piece of labelled aluminum foil and weighed on a mass balance. The muscle tissue was then wrapped in the aluminum foil and placed on ice. This process was repeated on the rest of the fish during the sampling period. Once all the tissue samples were collected, they were transferred to a sealable plastic bag and then stored in a freezer at -80^{o}C for later use analysis.

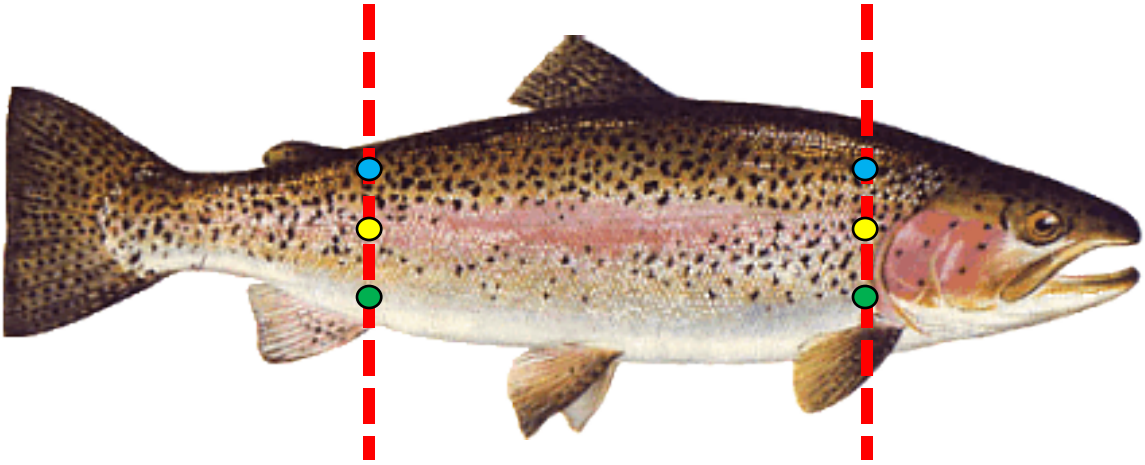

Figure 3: Position of all electrode positions. The dorsal electrode position (blue circles), lateral electrode position (yellow circles) and ventral electrode position (green circles). Morphometric markers were used to help place the electrodes consistently on the fish (red dashed lines). Electrodes were placed immediately posterior to the operculum and immediately anterior to the adipose fin.

Freeze Drying

A drum freeze dryer was used which houses samples during the drying process in 600mL – 900mL sized beakers. Tissue samples were placed into the freeze dryer and the aluminum foil was opened at one end (to allow adequate air flow from the tissue during the drying) prior to being placed into the dryer. The tissue packets were arranged inside these beakers such that air adequate sublimation of ice from the tissue in the freeze dryer. Due to size constraints of the freeze dryer, the fish tissue was dried in several batches. Once placed in the freeze dryer the samples were left to dry until the dryer showed a stable temperature and pressure reading inside the dryer (this took approximately five days). Once dried, the samples were removed and immediately placed into a desiccator and the freeze dryer was cleaned and dried for further use.

Measuring Dry Weights

Once all fish tissue was dried the tissue was then weighed again to obtain the dry weight of the fish. Since the aluminum foil was weighed previously, the dried tissue weight was obtained by subtracting the foil weight from the measured total weight obtained from the balance.

Pulverizing and Homogenizing Fish Tissue

The tissue samples from each fish were then pulverized and homogenized into a fine powder using an Ika analytical mill. The homogenized tissue was then transferred to 2mL micro centrifuge tubes using an antistatic scoop. The tissue was stored in a desiccator once the pulverizing was complete.

Making Tissue Pellets

The powdered tissue was then compressed into 0.5cm sized pellets and stored inside 2mL micro centrifuge tubes and stored in a desiccator in preparation for performing bomb calorimetry.

Bomb Calorimeter Standard operating Procedure

The bomb calorimeter consisted of the Parr 6725 semi-micro calorimeter, the Parr 6772 calorimetric thermometer/control unit (with printer) and two Parr 1109A semi-micro oxygen bomb.

Prior to use, the oxygen bomb was wetted with a small amount of water (according to calorimeter operating instructions) and any excess moisture was dried with a Kim wipe. A 10cm length of fuse wire was cut and attached to the bomb head by wrapping the fuse around the electrode hooks and creating a five turn helical coil using an Allen wrench such that the coil was left to hang between the electrode hooks. The fuse connections was then tested using a voltmeter by temporarily connecting the electrodes from the voltmeter to the bomb head and terminal nut. If inadequate resistance was measured on the voltmeter, the fuse was removed and reattached to the electrode hooks to create adequate contact between the fuse wire and the bomb.

If this failed to produce sufficient resistance on the voltmeter, the fuse was removed and the electrode hooks were thoroughly scrubbed with emery paper and cleaned again. The fuse was then reattached and the fuse connection tested again. These two troubleshooting steps were sufficient in resolving any connection issues during the time period that bomb calorimetry was performed.

The sample pan for the bomb was then placed onto the supporting loop attached to the bomb head. The pelleted sample was then weighed (weight was recorded) and placed in the sample pan. Using metal tweezers, the helical coil of the fuse wire was then manipulated so that the coil was pressing against the pellet. This ensured that heat from the fuse ignition would concentrate onto the pellet and also helped to ensure that the pellet tablet did not slip or fall off the sample pan while the bomb was being handled.

The bomb head was then placed into the bomb body and the screw cap was placed over the bomb head and then tightened to form an airtight seal. The bomb was then charged with oxygen by using a pin wrench to open the air valve on top of the gas tube one full turn and filling the bomb with 35 atmospheres of pure oxygen. The air valve was then closed with the pin wrench sealing the bomb.

The bomb was then placed inside of the bomb holder from the calorimeter. Two lead wires from the control unit of the calorimetric thermometer were attached to the bomb: one wire plugged into the terminal nut of the bomb head, the other into the bomb holder itself.

The bucket inside the calorimeter was then placed on a balance, tared and filled with water to a mass of 450 ± 0.05g. The bucket was then placed in the calorimeter jacket. The bomb holder (containing the charged bomb) was then lowered into the bucket submerging the bomb under water so that the water level in the bucket completely submerged the bomb just past the air valve inlet. The calorimeter lid was placed over the bucket submerging the thermometer and stirrer into the water of the bucket in such a way that they did not make any physical contact with the bomb or its holder. The stir belt was

then attached to the stirrer connecting the stirrer to the motor, thus completing the physical setup of the bomb calorimeter.

The calorimeter operations were then accessed on the calorimeter control unit to begin the calorimeter run. The weight of the sample was entered into the calorimeter along with the sample ID and bomb ID. The bomb run consists of a preparatory period during which time the jacket temperature and bucket temperature were equilibrated to ensure that a stable water temperature was measured by the instrument. The fuse was then ignited which combusted the sample. The heat released from this combustion reaction was released into the surrounding water and the temperature rise was recorded by the thermometer. A post period followed the ignition to determine the maximum temperature rise that resulted from the combustion of the sample. Once the bomb run was completed, the run print out was obtained and the bomb was removed from the calorimeter. The air valve was opened slowly to depressurize the bomb. Once excess air was removed from the bomb, the screw cap was removed and the bomb was opened and thoroughly cleaned. The remaining non-combusted fuse wire was removed from the electrode hooks with tweezers and the length of the fuse wire was measured with a ruler. This was done to determine the fuse correction of the combustion reaction.

Bomb Calorimetry Calibration

To calibrate the bomb, ten benzoic acid tablets were combusted to obtain the energy equivalent (W) value of the bomb. Two bombs were used for the duration of the experiment necessitating the combustion of twenty benzoic acid tablets to completely calibrate the bombs. The energy equivalent was calculated by the instrument using the following equation:

$$W = \frac{6318 \cdot m + f}{\Delta T}$$

Where *W* represents the energy equivalent of the bomb, *m* is the mass of the benzoic acid sample, 6318 is the standard heat of combustion of benzoic acid, *f* represents the fuse correction (in calories) and ΔT is the temperature change (determined by the calorimeter). The average energy equivalent for each bomb was programmed into the calorimeter for use in later sample runs.

Bomb Calorimetry Sample Runs

Once calibration was complete, the calorimeter was put into determination mode in order to calculate the heat of combustion of the sample. Duplicate samples were run for each fish for a total of 180 bombs. The formula used to determine the heat of combustion is as follows:

$$H_g = \frac{\Delta T \cdot W - f}{m}$$

Where Hg is the gross heat of combustion of the sample (in calories per gram), W is the energy equivalent of the bomb (determined during calibration), f is the fuse correction and m is the mass of the tissue sample.

Growth Study Data

Two data sets of BIA on rainbow trout were analyzed. One set was obtained from the starvation study outlined above, the other was obtained from unpublished data from 2012 examining the effect of growth on BIA results. For the purpose of this research, the results were used to construct separate BIA models and BIVA graphs to compare to the

starvation study. For a full breakdown of methods used in the study, refer to Bourdages (2011). A brief summary of this growth study is outlined below.

Rainbow trout were subjected to one of three feeding regimens, maintenance (0.4% body weight), optimal (1.9% body weight) or satiation (3.4% bodyweight) for ninety days and twelve rainbow trout from each feeding regimen were sampled in the same way for the starvation study every 30 days. Tissue samples were excised from these fish, frozen, freeze dried and analyzed via bomb calorimetry.

Constructing BIA Models

BIA models were constructed in Excel. The resistance and reactance values obtained during sampling were put through each electric volume equation: resistance in series (R_s), resistance in parallel (R_p), reactance in series (X_s), reactance in parallel (X_p), impedance in series (Z_s), impedance in parallel (Z_p) capacitance (C), Phase Angle (PA) and standardized phase angle (SPA). These values were graphed on the y-axis as the dependent variable plotted against the actual measured values obtained from the fish tissue (i.e. dry weight, wet mass, energy content) on the x-axis representing the independent variable. The values obtained from each electric volume equation was graphed as a scatter plot to determine if there was any correlation between the electric volume equation and the actual measured values. This produced nine models total and was repeated for the resistance and reactance values obtained from the other electrode positions taken during sampling for a total of twenty-seven models for dry mass, wet mass and energy content.

Linear regression was analysis was performed on each model to determine which model produced the best r^2 value. The model which had the highest r^2 value was selected

as the best predictive model. The equation for the slope of this model ($y=mx+b$) was rearranged to solve for the independent variable (x) to create a predictive equation that solves for the fish parameter of interest (dry mass, wet mass or energy content). These values were then graphed against the actual measured values in a scatter plot to produce a final "predicted vs actual" model.

From this final predicted vs actual model linear regression analysis was done to obtain the equation of the line of best fit. This equation is the final predictive equation which converts the measured resistance values into actual predictions of a fish parameter (dry mass, wet mass, energy content).

BIVA

For the BIVA graphs, all resistance and reactance values for each fish were normalized by dividing by the detector length. The average value of for each set of normalized resistance values from each sampling group was then plotted on an R/X graph to show the average vector for each sample group.

Results

BIA Index

All fish euthanized in the growth and starvation studies were used for the development of predictive models for the BIA index. Measurements show a wide range of fish length, weight, condition and energy content (see table 2). The bioimpedance readings obtained during the study also show a wide range of resistance values. These values were used to develop the BIA models which make up the BIA index.

Table 2: Summary of the range of variation of the fish for the starvation study.

Variable		**Mean (± SE)**	**Range**
Total Weight (g)		268 ± 17.9	65.8 - 628
Total Length (cm)		26.7 ± 0.5	18.5 - 35.0
Condition Factor		1.34 ± 0.02	0.44 - 1.97
Resistance (Ω)	**Dorsal**	460 ± 6.5	314 - 692
	Lateral	415 ± 11.0	292- 1280
	Ventral	487 ± 9.5	359- 899
Reactance (Ω)	**Dorsal**	65.9 ± 3.8	1.4 - 135
	Lateral	43.8 ± 2.5	3.0 - 99.2
	Ventral	53.9 ± 3.1	4.9 - 116

Bomb Calorimetry: Starvation Study

The bomb calorimetry data from the starvation study is summarized in table 3. Over the course of the study, there was a decrease in the energy density of the tissue; however, the differences were not significant. The data was tested for normality using a Chi-Square test and found the data to be normal. A one-way analysis of variance (ANOVA) comparing the energy density of tissue from fish in the small size class across sampling days shows no significant change in the energy density of the tissue ($p=0.37$). An ANOVA was also done for the medium size class of fish ($p=0.23$) and the large size class fish ($p=0.49$).

Table 3: Bomb calorimetry results from the starvation study. Results shown are in calories per gram of tissue ± standard error. Results are grouped according to size and days fasted. For each cell, n=10.

	Day 1	**Day 7**	**Day 14**
Small	5980 ± 69.8	5960 ± 82.4	5840 ± 76.2
Medium	6020 ± 85.3	6070 ± 45.9	5920 ± 42.2
Large	6140 ± 75.9	6050 ± 61.7	6030 ± 62.8

BIA Models: Starvation Study

Each bioelectric volume equation was plotted against the body parameter of interest (dry mass (DM), total body water (TBW) or energy content). This was done for the resistance values obtained from each electrode position (dorsal, lateral and ventral) for a total of seventy-two models (twenty-four models for each body parameter) (see table 4). Most models displayed some level of correlation between although a few bioelectrical equations failed to produce any relationship between the bioimpedance readings and the actual parameter. For each body parameter, the model with the highest r^2 value was selected as the best predictor model, and all showed strong correlations for DW (r^2=0.9064), TBW (r^2=0.9170) and energy content (r^2=0.9149). A summary of the predictive equations for TBW, DW and energy content is given in table 6. For all three models, the resistance in parallel equation using dorsal measurements provided the best regression.

Linear regression analysis was performed on the BIA models and the correlation of each of these models was found to be significant ($p<0.0001$ for all). The linear equation derived from these models were all rearranged to solve for the predicted body parameter and all BIA values were then put through this equation to produce predicted values for DM, TBW and energy content. These predictive values were then plotted against the actual values to produce predictive models for DM, TBW and energy content (see figure 4-6).

Table 4: A summary of the r^2 values for each model produced during data analysis. The models listed in the second column are the electric volume equations used to estimate the body parameter of interest. For each body parameter, the body parameters are listed as: resistance (R) in series, resistance in parallel, reactance (X) in series, reactance in parallel, impedance (Z) in series, impedance in parallel, capacitance (C) and phase angle (PA) using the dorsal resistance measurements (R_D), lateral measurements (R_L) and the ventral measurements (R_V). Bolded r^2 values represent the highest value for each body parameter.

Body Parameter	Model	R^2 Value
Dry Mass	L^2 / R_{Ds}	0.8942
	L^2 / R_{Dp}	**0.9064**
	L^2 / X_{Ds}	0.0312
	L^2 / X_{Dp}	0.0393
	L^2 / Z_{Ds}	0.9009
	$L^2 \cdot Z_{Dp}$	No correlation
	L^2 / C_D	0.1715
	L^2 / PA_D	0.1715
	L^2 / R_{Ls}	0.8609
	L^2 / R_{Lp}	0.8645
	L^2 / X_{Ls}	0.2234
	L^2 / X_{Lp}	0.1710
	L^2 / Z_{Ls}	0.8628
	$L^2 \cdot Z_{Lp}$	0.0052
	L^2 / C_L	0.2057
	L^2 / PA_L	0.1813
	L^2 / R_{Vs}	0.8684
	L^2 / R_{Vp}	0.8767
	L^2 / X_{Vs}	0.1163
	L^2 / X_{Vp}	0.1596
	L^2 / Z_{Vs}	0.8728
	$L^2 \cdot Z_{Vp}$	0.0096
	L^2 / C_V	0.2968
	L^2 / PA_V	0.0482
	L^2 / R_{Ds}	0.9037
	L^2 / R_{Dp}	**0.9170**
	L^2 / X_{Ds}	0.0355
	L^2 / X_{Dp}	0.0396
	L^2 / Z_{Ds}	0.9110
	$L^2 \cdot Z_{Dp}$	No correlation
	L^2 / C_D	0.1831

Total Body Water	L^2 / PA_D	0.1813
	L^2 / R_{Ls}	0.8702
	L^2 / R_{Lp}	0.8739
	L^2 / X_{Ls}	0.2011
	L^2 / X_{Lp}	0.1727
	L^2 / Z_{Ls}	0.8722
	$L^2 \cdot Z_{Lp}$	0.0535
	L^2 / C_L	0.2210
	L^2 / PA_L	0.1856
	L^2 / R_{Vs}	0.8798
	L^2 / R_{Vp}	0.8870
	L^2 / X_{Vs}	0.1177
	L^2 / X_{Vp}	0.1701
	L^2 / Z_{Vs}	0.8836
	$L^2 \cdot Z_{Vp}$	0.0086
	L^2 / C_V	0.3196
	L^2 / PA_V	0.0465
Total Energy Content	L^2 / R_{Ds}	0.9008
	L^2 / R_{Dp}	**0.9149**
	L^2 / X_{Ds}	0.0338
	L^2 / X_{Dp}	0.0399
	L^2 / Z_{Ds}	0.9085
	$L^2 \cdot Z_{Dp}$	No correlation
	L^2 / C_D	0.1853
	L^2 / PA_D	0.1759
	L^2 / R_{Ls}	0.8630
	L^2 / R_{Lp}	0.8670
	L^2 / X_{Ls}	0.2086
	L^2 / X_{Lp}	0.1645
	L^2 / Z_{Ls}	0.8651
	$L^2 \cdot Z_{Lp}$	0.0487
	L^2 / C_L	0.2160
	L^2 / PA_L	0.1875
	L^2 / R_{Vs}	0.8736
	L^2 / R_{Vp}	0.8816
	L^2 / X_{Vs}	0.1218
	L^2 / X_{Vp}	0.1671
	L^2 / Z_{Vs}	0.8778
	$L^2 \cdot Z_{Vp}$	0.0089
	L^2 / C_V	0.3141
	L^2 / PA_V	0.0465

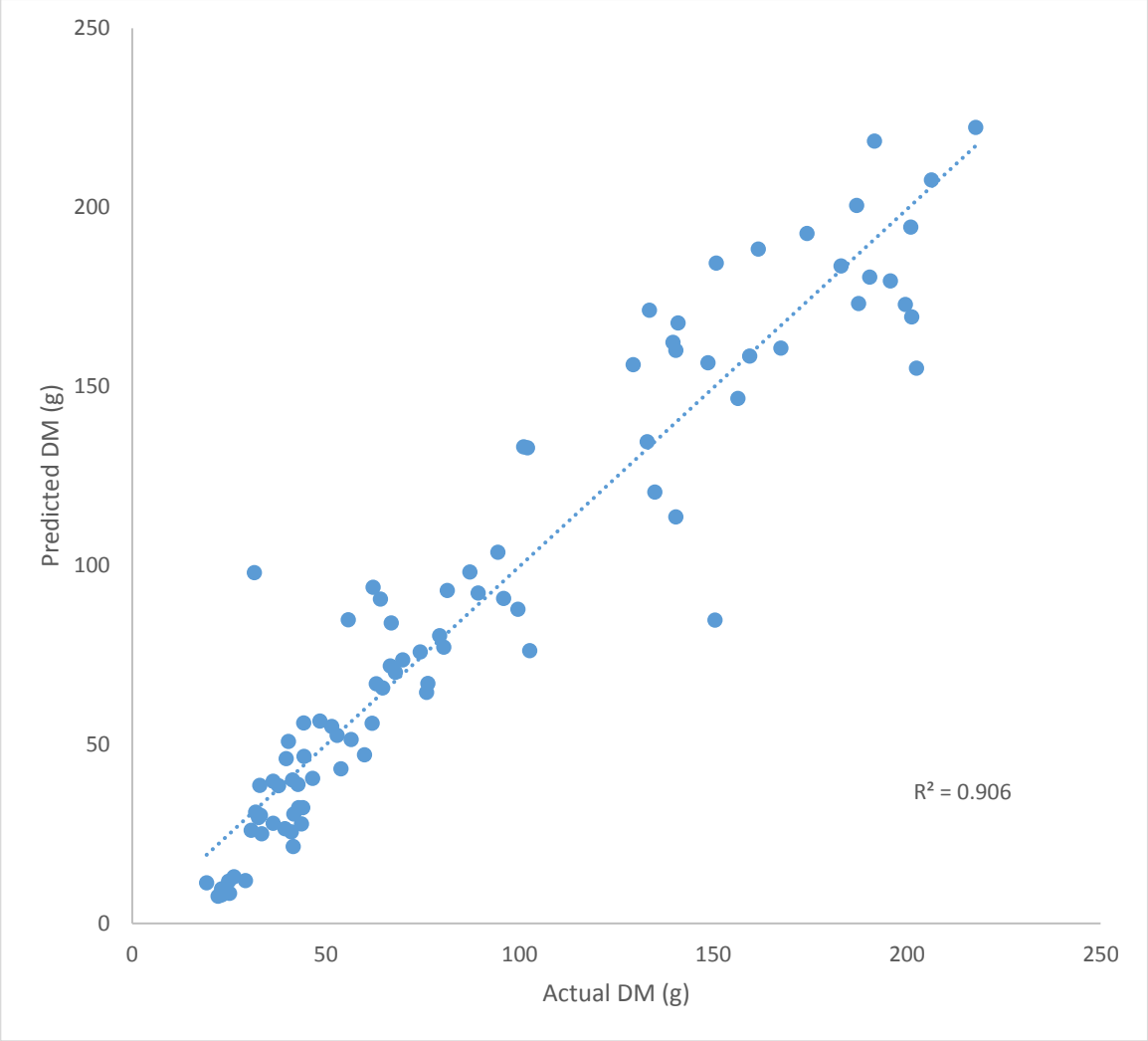

Figure 4: Model comparing predicted values of total dry mass of rainbow trout (*Oncorhynchus mykiss*) from the starvation study to actual dry mass. Predicted values are based on bioelectrical impedance measurements. Actual measurements are based on the mass of dried fish tissue samples.

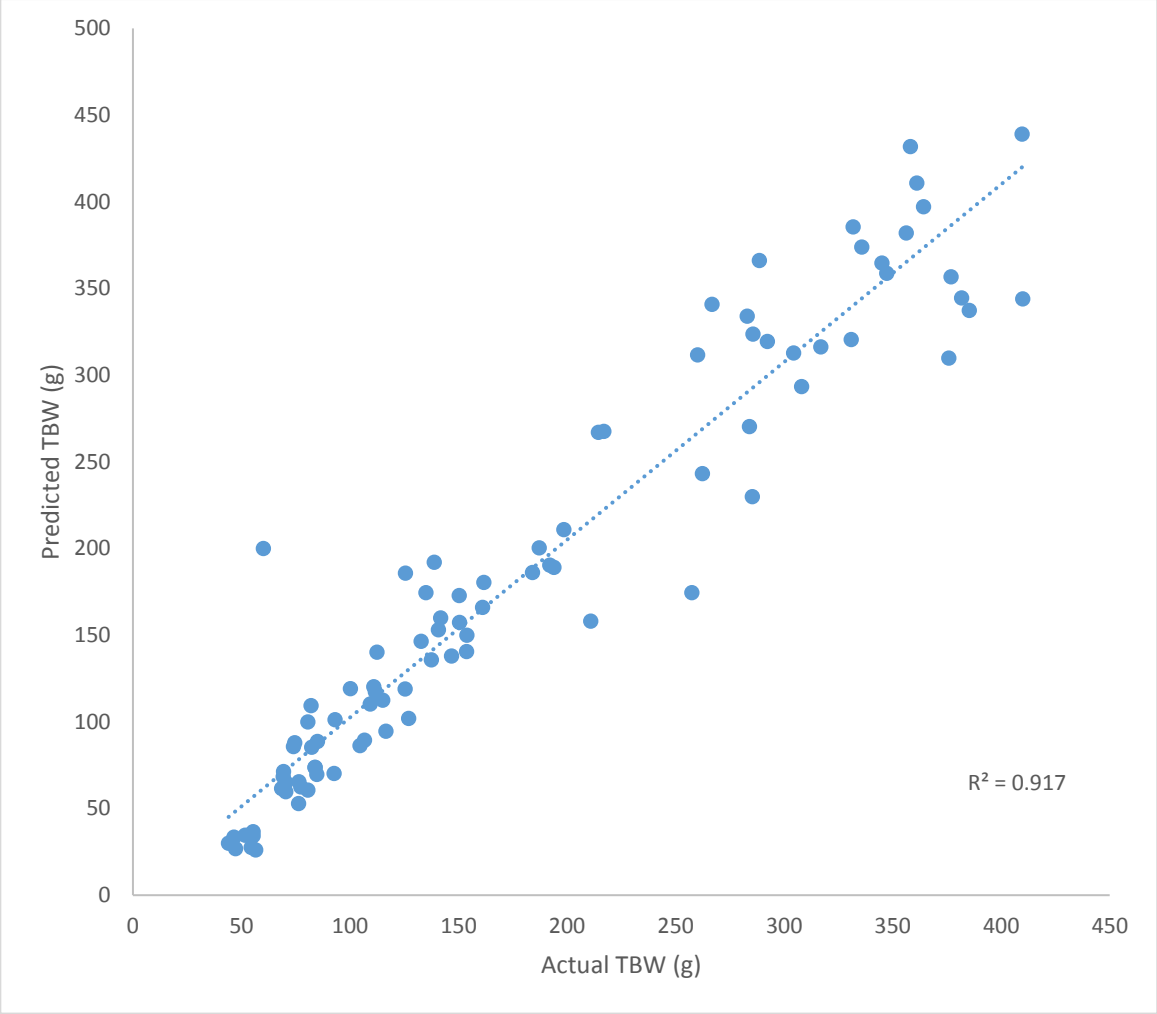

Figure 5: Model comparing predicted values of total water weight of rainbow trout (*Oncorhynchus mykiss*) from the starvation study to actual water weight. Predicted values are based on bioelectrical impedance measurements. Actual values were obtained from the difference between wet and dried tissue samples of the fish.

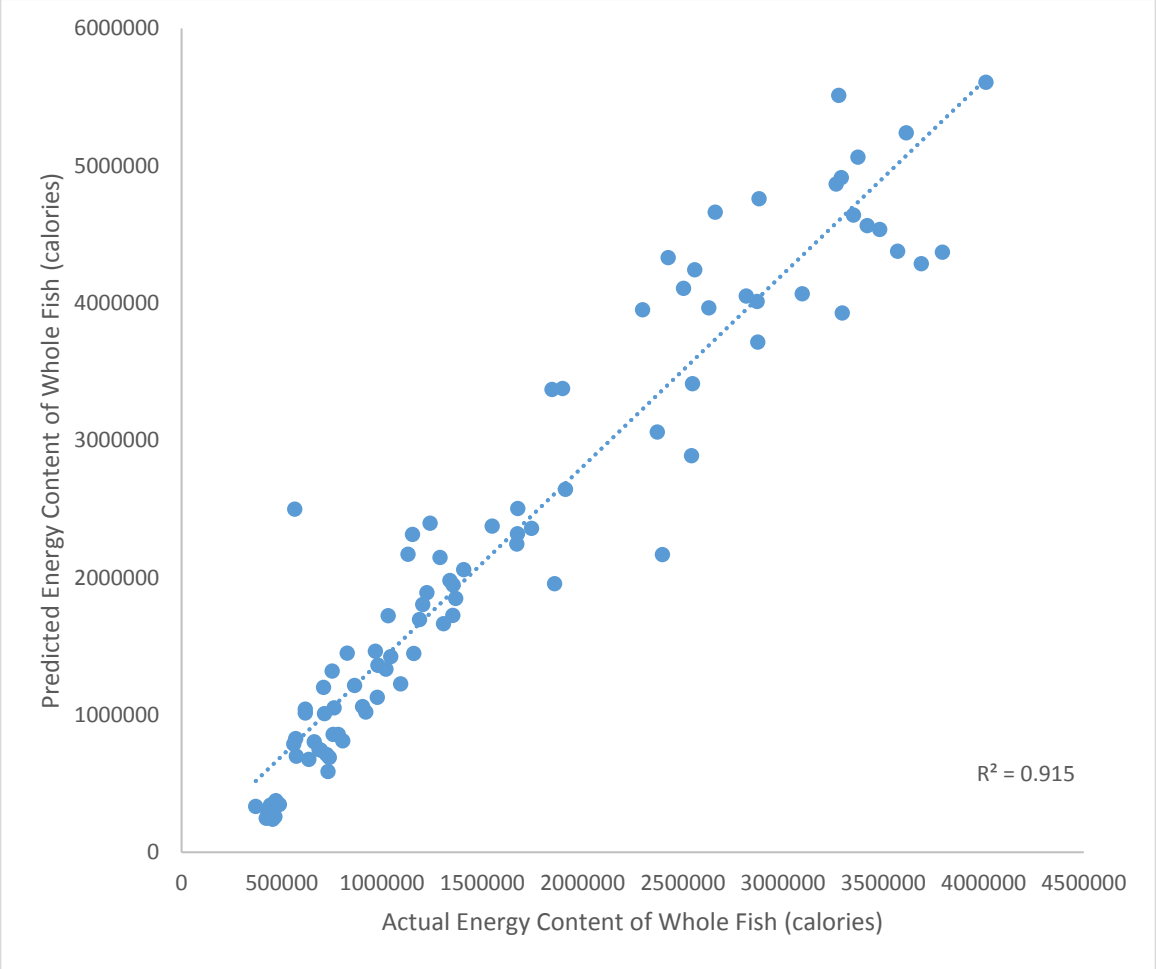

Figure 6: Model comparing predicted energy content (in calories) vs actual energy content of rainbow trout (*Oncorhynchus mykiss*) from the starvation study. Predicted values are based on bioelectrical impedance measurements. Actual values were obtained through combustion of dried fish tissue via bomb calorimetry.

Table 5: A summary of the predictive equations obtained from the starvation study of juvenile rainbow trout (*Oncorhynchus mykiss*). To obtain the predicted body parameter, substitute L with the length between electrodes (in cm) and R and X with their respective measured resistance (in Ω).

Body Parameter	**Predictive Equation**
Dry Weight (DW)	$DW = \frac{\left(\frac{L^2}{R + \left(\frac{X^2}{R}\right)} - 0.072\right)}{0.0025}$
Total Body Water (TBW)	$TBW = \frac{\left(\frac{L^2}{R + \left(\frac{X^2}{R}\right)} - 0.057\right)}{0.0013}$
Total Energy Content	$Energy\ Content = \frac{\left(\frac{L^2}{R + \left(\frac{X^2}{R}\right)} - 0.067\right)}{1 \times 10^{-7}}$

BIA Models: Growth Study

For the data set from the growth study, BIA measurements were only taken along the lateral line of the fish. Models were derived using the bioelectric volume equations as above. Models were constructed for TBW, DW and energy content. These models produced regression coefficients of r^2=0.8858, r^2=0.8304 and r^2=0.8108 for TBW, DW and energy content respectively (see figures 6-8). Linear regression analysis on these models shows that the regression coefficient is significant ($p<0.05$). These models are less powerful than predictors than those produced from the starvation study. For all three models, the resistance in parallel equation using dorsal measurements provided the best regression.

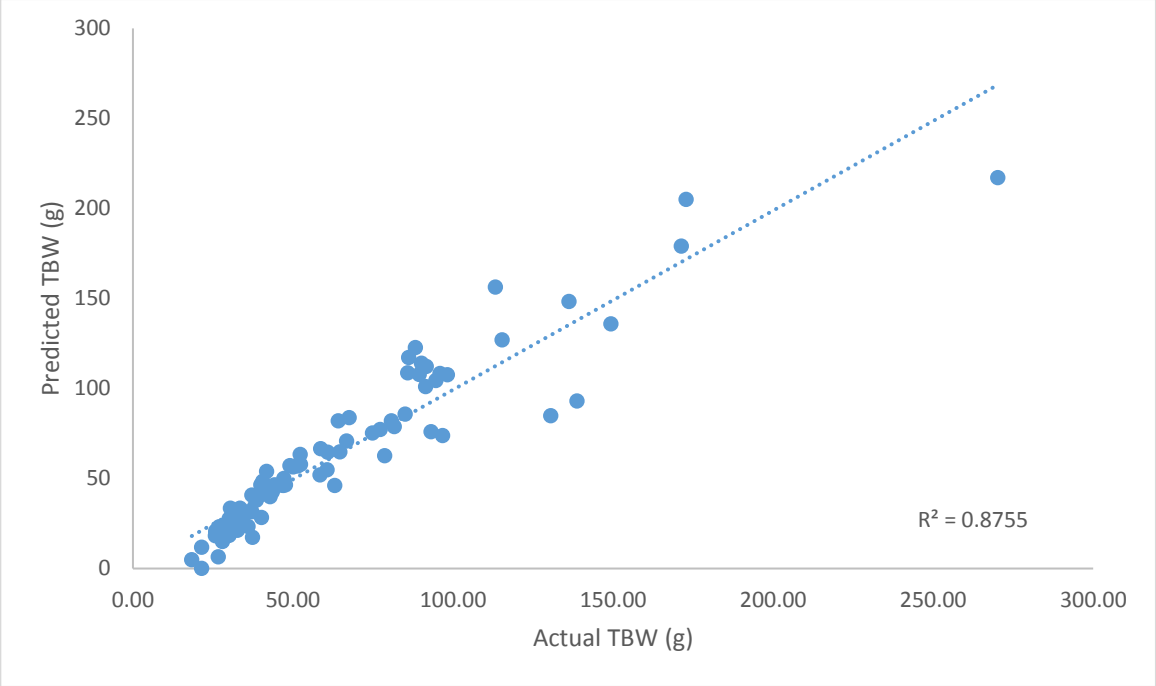

Figure 7: Bioimpedance value of juvenile rainbow trout (*Oncorhynchus mykiss*) from the growth study plotted against actual water weight values. Predicted values are based on bioimpedance measurements, actual values are based on weights obtained during sampling.

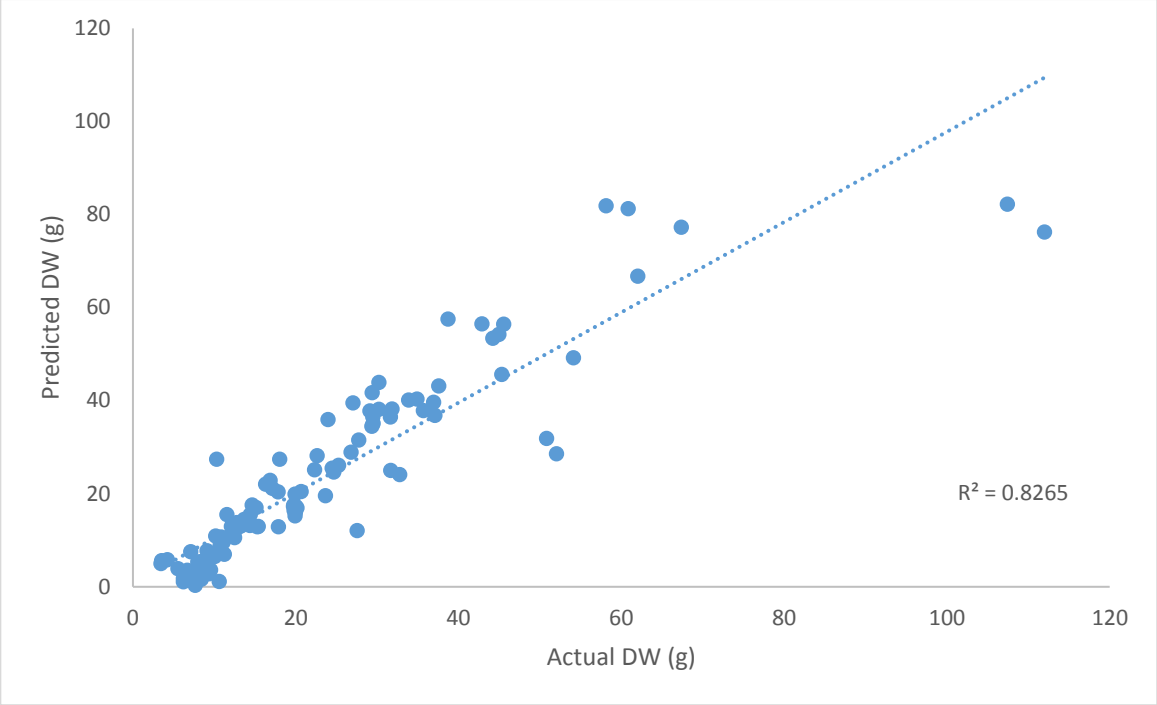

Figure 8: Bioimpedance values from of juvenile rainbow trout (*Oncorhynchus mykiss*) from the growth study plotted against actual dry weight values. Predicted values are based on bioimpedance measurements, actual values are based on weights obtained during sampling.

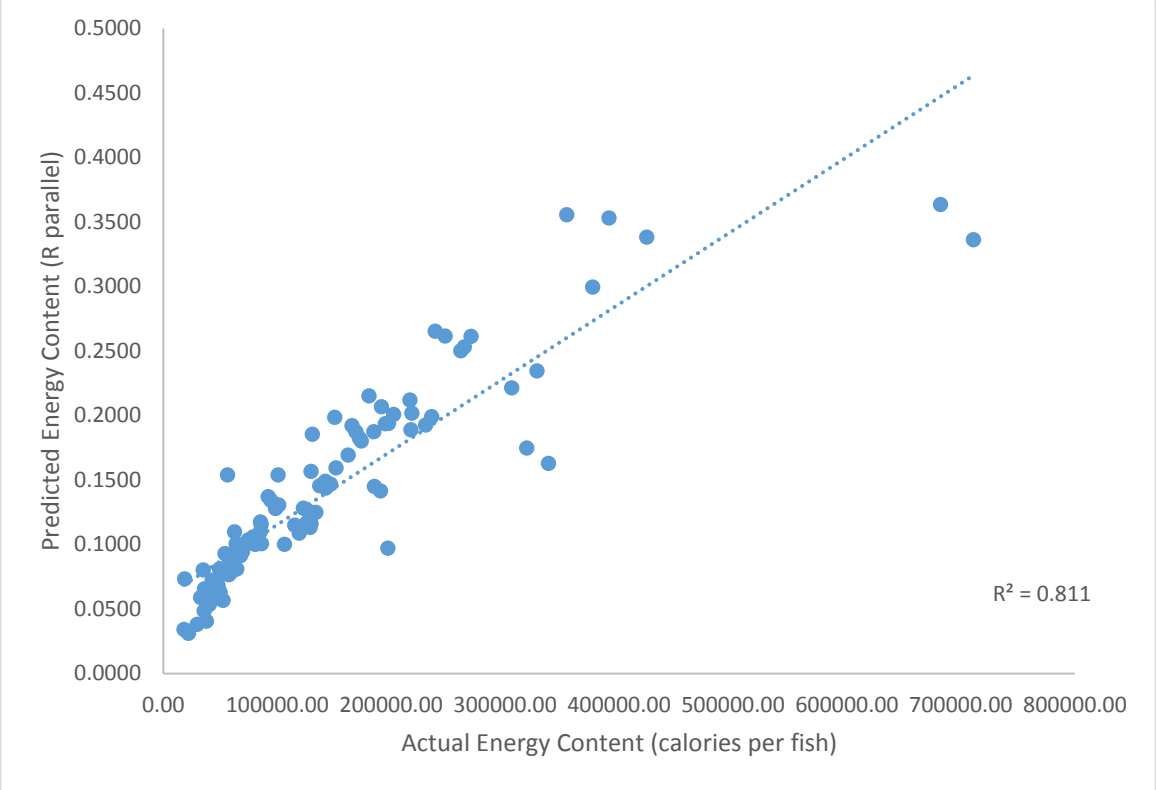

Figure 9: Bioimpedance values from of juvenile rainbow trout (*Oncorhynchus mykiss*) from the growth study plotted against actual energy content value of the whole fish (in calories). Predicted values are based on bioimpedance measurements, actual values are based on weights obtained via bomb calorimetry.

Phase Angle

Phase angle is a potential measure of the health condition of fish and is measured using the resistance values obtained during BIA, and using the formula ($PA = \tan^{-1}\left(\frac{X}{R}\right) \cdot \frac{180^{\circ}}{\pi}$) where X and R represent the measured reactance and resistance respectively.

Sex based differences in bioimpedance readings were examined. Phase angle measurements were compared to see if there was a significant difference between male and female fish using dorsal, lateral and ventral measurements. A t-test comparing male to female fish for each electrode position showed no significant differences for any electrode position (see figure 9).

A t-test comparing the average phase angle of male and female fish for each sampling day was done and found no significant difference between the male and female fish at any point ($p>0.05$). For all future phase angle tests, male and female fish were combined. After determining there was no significant difference between male and female fish for each sampling day, the differences in phase angle measurements between sampling groups was examined (see figure 10). A one-way ANOVA comparing the average phase angles of fish from sampling days one, seven and fourteen do not show a significant difference in dorsal measurements ($p=0.444$), lateral measurements ($p=0.499$) or ventral measurements ($p=0.964$). This suggests that the phase angle did not change significantly over the course of the study.

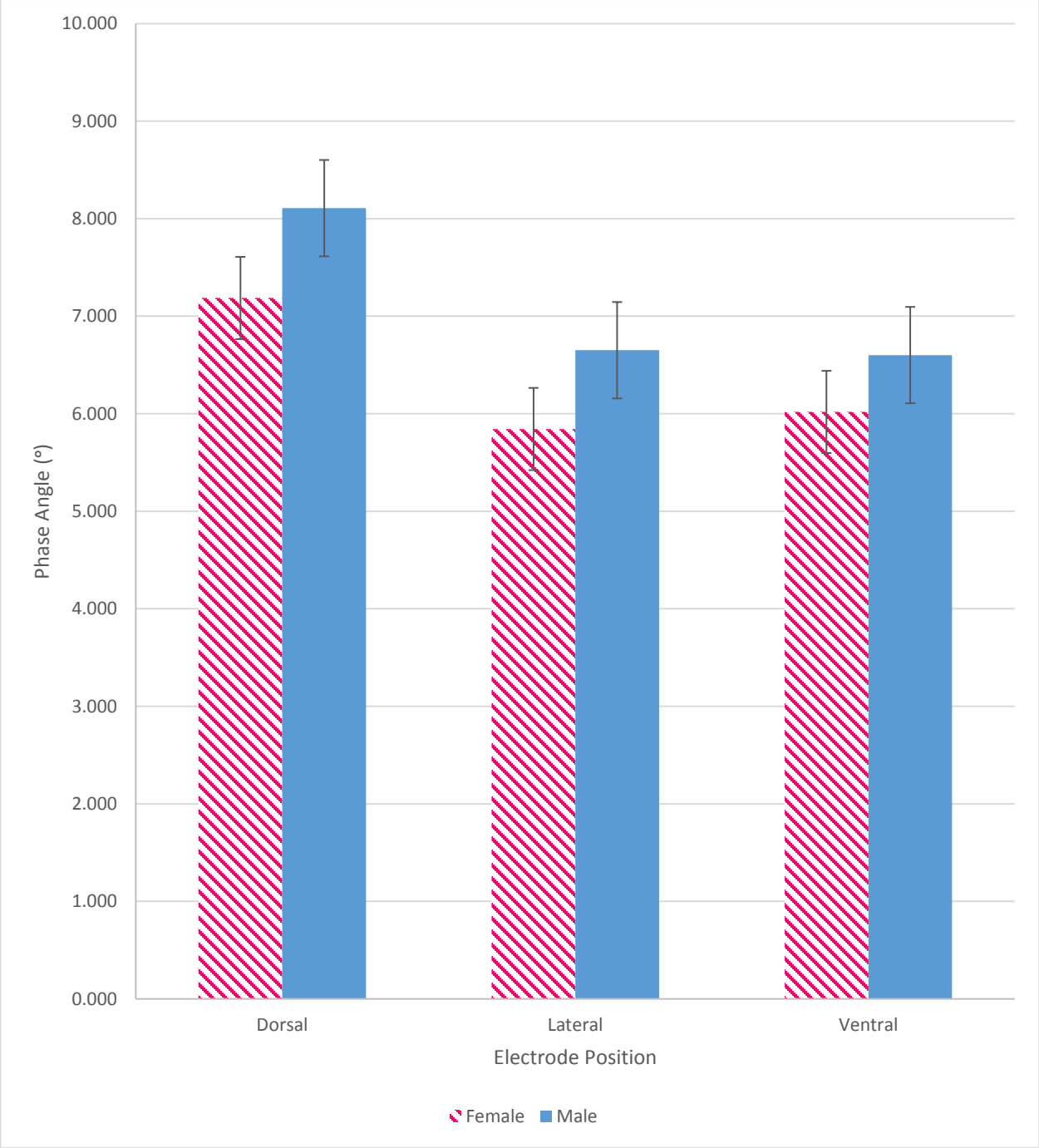

Figure 10: Comparison of the average phase angle of male and female fish for dorsal, lateral and ventral measurements. Error shown is standard error. No significant difference between male and female fish for dorsal, lateral or ventral phase angles.

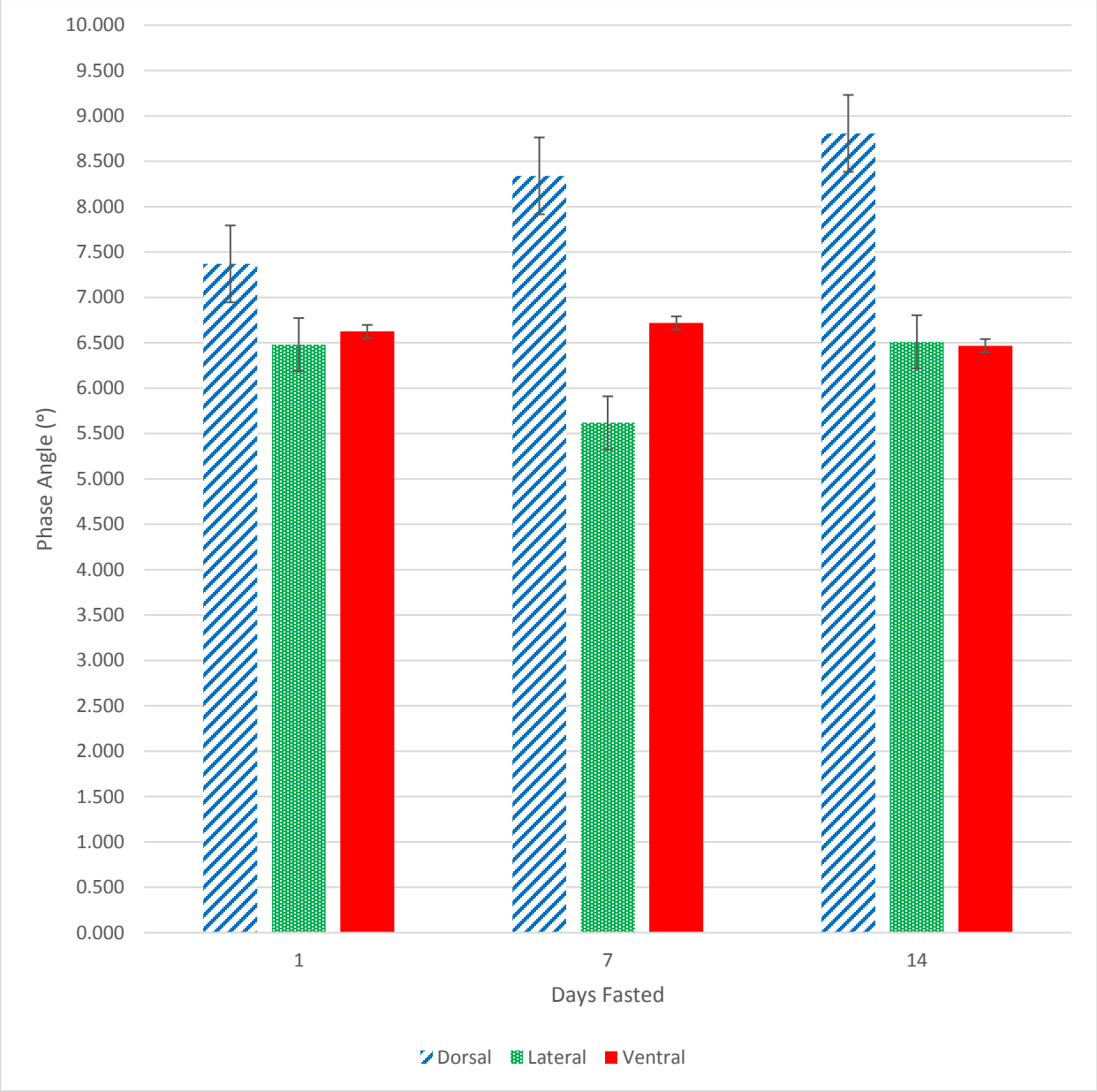

Figure 11: Average phase angle for dorsal, lateral and ventral electrode position for each sampling day. Error shown is standard error.

BIVA: Starvation Study

BIVA of the fish was done to determine if there was a change in in the average vector for each size class of fish during each sampling day. BIVA was performed for each electrode position to see if there was more/less change observed in one electrode position over another at each sampling day (see figures 11-13). The vectors shown are normalized by the distance (in centimetres) between electrodes.

For each BIVA graph, the average vector for each sampling day is shown (labelled as one, seven or fourteen days into the fast) for each size class of fish. To determine if the average vector position changed significantly during the course of the starvation period a Hotellings T^2 test was performed to test for difference between sampling days. For figure 11 (dorsal electrode position) significant differences were observed between days one and fourteen for the small ($p<0.05$) and large sized fish ($p<0.005$) but not in medium sized fish ($p>0.05$). The average vectors between small and medium size groups and medium and large size groups were significant ($p<0.05$ for all). For figure 12 (lateral electrode position) average vectors were not significant between sampling days within size groups ($p>0.05$) but average vectors were significantly different between small and medium sized groups and medium and large size groups ($p<0.05$). The same significant differences were observed in figure 13 (ventral electrode position) in addition to there being significant differences between day one and day seven vectors for small fish only ($p<0.05$).

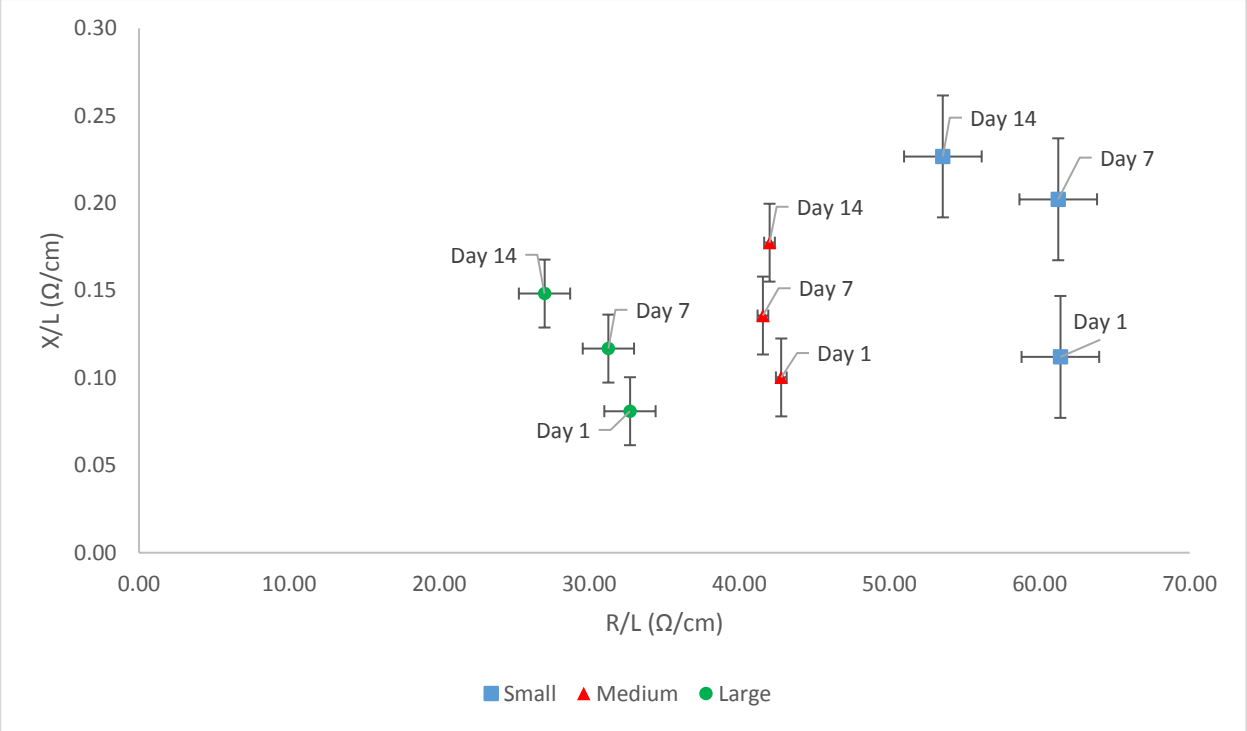

Figure 12: Vector analysis of bioelectrical impedance readings made from the dorsal electrode position. The average vector for each size class of fish (small, medium and large) is shown and labelled with the number of days the fish were fasted (1, 7 or 14). Average vectors were significantly different ($p<0.05$) between small, medium and large fish. Average vectors were also significantly different between day one sampled fish and day fourteen sampled fish for small and large fish. Error shown is standard error.

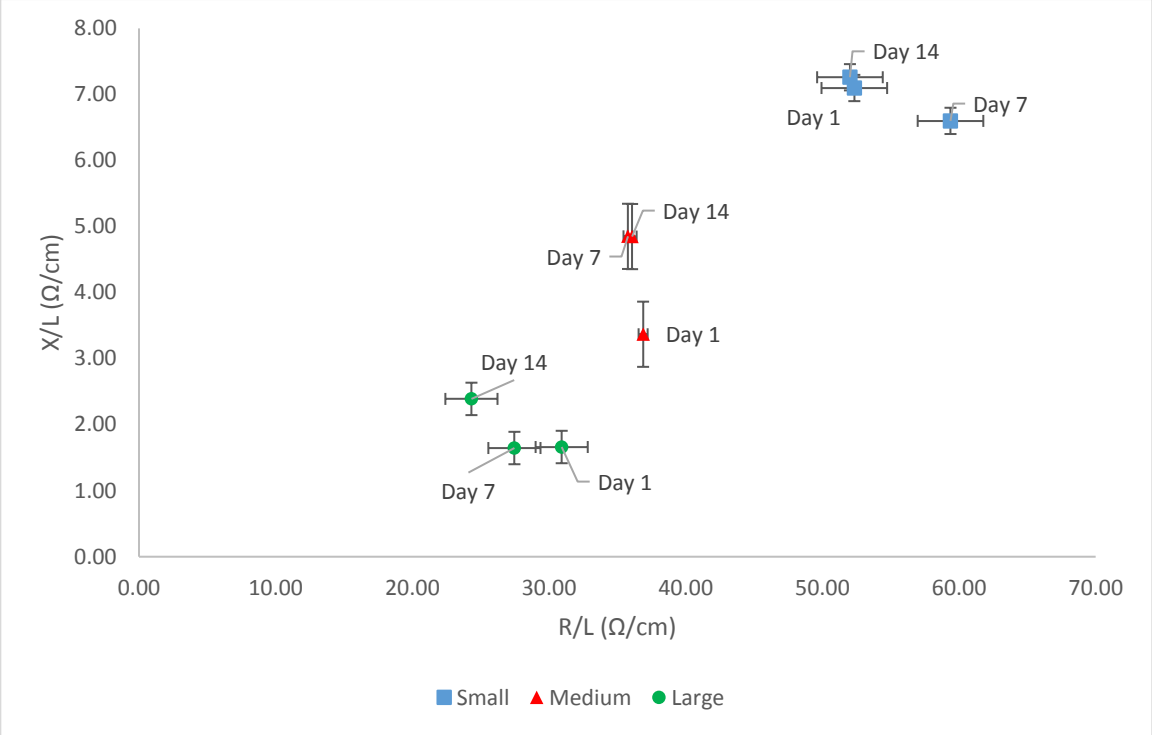

Figure 13: Vector analysis of bioelectrical impedance readings made from the lateral electrode position. The average vector for each size class of fish (small, medium and large) is shown and labelled with the number of days the fish were fasted (1, 7 or 14). Average vectors were significantly different ($p<0.05$) between small, medium and large fish. Error shown is standard error.

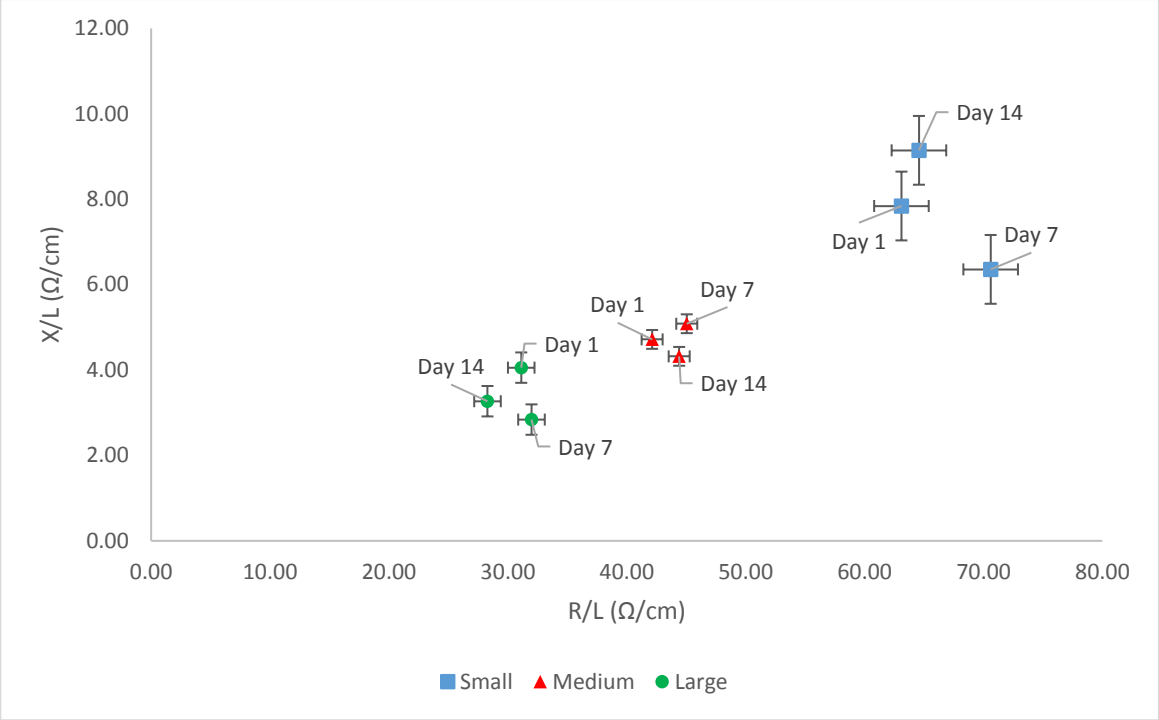

Figure 14: Vector analysis of bioelectrical impedance readings made from the ventral electrode position. The average vector for each size class of fish (small, medium and large) is shown and labelled with the number of days the fish were fasted (1, 7 or 14). Average vectors were significantly different ($p<0.05$) between small, medium and large fish. Error shown is standard error.

BIVA: Growth Study

BIVA from the growth study of rainbow trout was performed to determine if the average vector of the fish would change during the growth period (see figure 14). A Hotellings T^2 test was used to test for differences in the average vector between sampling days. For the satiation diet, there was a significant difference between the fish sampled on day 30 and day 60 ($p<0.05$) though interestingly not between day 30 and 90. For the optimal diet fed fish, there was a significant difference between the day 30 and 90 fish. No significance was found between the vectors of the maintenance fed fish. This is expected as the fish fed on the maintenance diet should have experienced no growth. The vector change for the optimal and satiation fed fish are expected as the fish with increasing energy stores should have a lower resistance value which would correspond to a change in vector position.

The BIVA for the growth study show that the vector position did change as the fish grew. It is most likely that this change is due to the change in the size of the fish rather than the diet they were fed. As seen in the BIVA from the starvation study, larger fish have separate vector positions from smaller fish, the same is likely happening in the BIVA from the growth study as the fish grew in size from the feeding regimen.

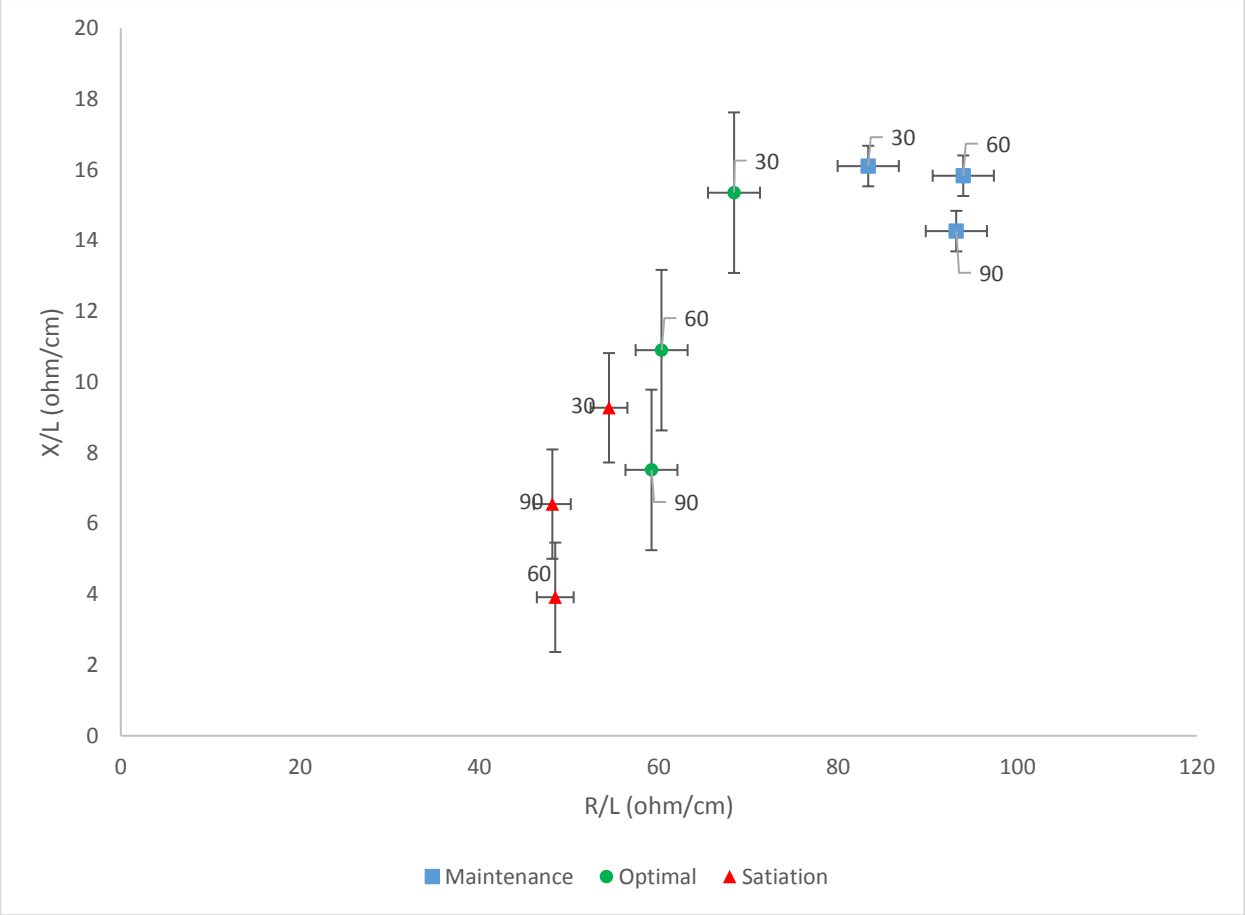

Figure 15: Vector analysis of bioelectrical impedance analysis from the growth study of juvenile rainbow trout (*Oncorhynchus mykiss*). Shown are the average vectors of fish fed on the maintenance (0.4% body weight), optimal (1.6% body weight) and satiation (3.4% body weight) diets sampled at 30, 60 and 90 days into their feeding regimen. 30 and 60 day vectors for the satiation diet are significantly different ($p<0.05$) and for the 30 and 90 day vectors from the optimal diet ($p<0.05$). Error shown is standard error.

Discussion

Understanding the energy content of the tissue within fish is an integral part to understanding the health and well-being of the organism (Pothoven *et al*., 2008). Understanding the health condition of fish is currently only possible with accuracy using bomb calorimetry or proximate composition analysis (Duncan *et al*., 2007) both of which are lethal procedures; however, it is sometimes necessary to obtain a complete understanding of the health and well-being of a fish. BIA represents one possible way in which it is possible to obtain similar information as bomb calorimetry or proximate composition analysis but in a non-lethal manner. BIA is a promising method for studying the body composition of fish tissue as it is a quick, non-lethal procedure that is applicable to both field studies and laboratory use. The development of predictive models for BIA will enable researchers in future studies to quickly estimate the body composition of fish.

The BIA models from the study yielded several interesting results. Since multiple electrode positions were examined in this study the effect of electrode position on the models could be examined. The models derived from the dorsal measurements showed a stronger correlation than models derived from the lateral or ventral measurements. The best predictive equations for total body water, dry mass and energy content had strong correlations of: r^2=0.9170, r^2=0.9064 and r^2=0.9149 respectively. The best predictive equations were derived from the resistance measured from the dorsal position.

The electrode position did have an impact on the regression coefficient of the predictive equations. The dorsal electrode position produced the strongest possible predictive equations. The majority of BIA models in the literature are produced by placing the electrodes of the BIA instrument at or slightly above the lateral line (Cox &

Hartman, 2005; Krimmer & Rasmussen, 2008; Pothoven *et al.*, 2008; Stolarski *et al.*, 2014). Few studies have examined the impact of electrode position on the predictive power of BIA models. Bosworth and Wolters (2001) examined dorsal and ventral electrode positions on channel catfish fillets and found dorsal measurements to be superior. Duncan (2007) also performed BIA using dorsal electrode positions and produced models all with r^2 values above 0.90. The BIA models produced from this starvation study on rainbow trout contribute to the evidence that the dorsal electrode position provides better predictive power than lateral positions and future research should consider using dorsal measurements as well.

The best models produced from the starvation and growth studies were constructed using the resistance in parallel bioelectrical volume equation. Previous studies tend to find that resistance in series or parallel are the best descriptive equations (Cox & Hartman, 2005; Krimmer & Rasmussen, 2008; Willis & Hobday, 2008).

Comparing the predictive capability of the models developed in this study to those developed in the literature is difficult as the authors of many of these papers do not supply the equation used to correlate the predicted values to actual values. In the majority of papers, only the regression coefficient is supplied (Bosworth & Wolters, 2001; Duncan *et al*., 2007; Elaine *et al*., 2012; Hafs & Hartman, 2014; Pothoven *et al*., 2008). This makes comparing the predictive equations developed in this study to other species difficult. In addition to this, many of these predictive equations acknowledge using a multiple regression combining transformed resistance values with other variables (weight, length etc.) to improve their regression coefficients, but do not state how significant each variable in the multiple regression is.

A summary of the regression coefficients for all BIA models developed for fish species shows that the predictive equations developed from this study are comparable to those in the literature (Table 1). Of the fish BIA models in the literature, the only other species that shares the same genus as rainbow trout are from a study on Chinook salmon (*Oncorhynchus tshyscha*) (Margraf *et al*., 2005). The models from this study produced correlations for total water content (r^2=0.80) and total energy content (r^2=0.84) and used a lateral electrode position (Margraf *et al*., 2005). These are lower correlations than what was found in rainbow trout during the starvation study, but comparable to those produced from the growth study.

There are some sources of error in the starvation study. There was no significant difference in caloric value of the fish tissue across sampling days which was the fasting period was designed to accomplish. The fasting period of two weeks was based off a similar study in brook trout of the same size (Cox & Hartman, 2005) which did produce significant changes in energy content. The predictive equations developed from the starvation study may not be accurate for fish with lower caloric content. If this study was repeated, a longer fasting period should be used. Other sources of error, which could account for the prediction error of the BIA models would come from the fact that only samples of muscle tissue were analyzed via bomb calorimetry, rather than performing calorimetric analysis of the whole fish. A more accurate model could be made from using energy values obtained from the whole fish carcass.

Ambient room temperature and fish temperature were not accounted and corrected for which could also have interfered with the BIA measurements. Temperature has been shown to effect BIA readings to some degree (Miller, 2014). The water temperature was

kept constant for the duration of the fasting period which would help mitigate this error, although it is still possible that temperature changes between sampling days could still have interfered with BIA readings.

There are also some limitations in the use of the predictive equations developed in this study. As mentioned above, since fish with very low energy levels were not obtained during the starvations study, these may not be as accurate on fish in such a condition. Juvenile rainbow trout were also used during this study, not adult fish which could mean the predictive models may not be accurate for adult fish. This could be avoided in research studies by using juvenile rainbow trout wherever possible.

Phase angle measurements are an interesting concept in that they could potentially replace condition factor as a tool for determining the health of fish. Since there is an inverse correlation between water content and fat content (Ryan *et al.*, 2009) and since fish with more fat are said to be healthier, fish which possess a larger phase angle (as a result of increased water content contributing to increased resistance) could potentially be said to be less healthy than fish with smaller phase angles (high fat and low water imparts lower resistance levels). Existing non-lethal measures of fish health condition rely on condition analysis (Fulton's conditions factor). Condition factor does not measure the lipid levels of fish but only makes assumptions of fish health based on fish weight and length (i.e. bigger fish are healthier). In theory this makes BIA/phase angle measurements a better measure of fish health since it measures the contents of the fish itself rather than making assumptions based on weight or length.

During the fasting period of this study, a drop in the lipid levels of fish theoretically should have corresponded with larger phase angles. The expected result

would have seen an increase in phase angles across the fasting period. The average phase angle of sampled fish was analyzed for each electrode position. An increase in the average phase angle measured from the dorsal position was observed but it was not statistically significant. No significant change in average phase angle was observed for either of the other two electrode positions (lateral and ventral). This lack of observed phase angle change could be due to too short of a fasting period. A longer fasting period may have produced a greater change in phase angle measurements by more greatly depleting the energy stores of the fish.

The use of phase angle as a means of differentiating between male and female fish was examined, and it was found that in juvenile rainbow trout there was no significant difference between male and female fish. Interestingly, electrode position did not appear to make any meaningful difference in the phase angle measurements in the same way it did for the predictive equations. Dorsal, lateral and ventral phase angle measurements all failed to show significant differences between male and female fish. This could be due to the fact that only juvenile rainbow trout were used, and as such, the sex characteristics of the fish were not fully developed. It is possible that adult rainbow trout might show differences based on sex, but more research is needed to determine if this is true or not. Phase angle measurements have also been based upon the health of the organism being examined (Dorhofer, 2005), so future studies will need to account for this when examining phase angles in fish.

Another potential measure of fish health is bioelectrical impedance vector analysis (BIVA). BIVA is an appealing aspect of BIA as it does not require any predictive

equations to use. Simply plotting the normalized resistance and reactance values obtained from the instrument is sufficient.

In human BIVA, the resistance and reactance values are normalized by dividing those values by the height of the individual. In fish, resistance and reactance values can be normalized similarly using the length of the individual fish. When making the BIVA plots, normalization was attempted with total length, standard length and detector electrode length. The distribution of vectors did not change when normalized with different length variables. Since the detector length will always be measured when conducting BIA, it makes sense to normalize the vectors with the detector length since the value will already be measured, eliminating the need take another measurement of the fish, which speeds up the sampling process.

BIVA was performed for all three electrode positions, and for all three graphs the only consistent observation observed was the difference in vector position based on size (length) of the fish. Each size class of fish yielded significantly different vectors from each other. This is likely a result of the larger fish having higher energy stores in their tissues than the smaller trout. Due to the inverse relationship between water and lipid content in tissue (Dorhofer, 2005), fish with higher energy stores in their tissue will have less water to impart resistance on an electric current which would cause the vector distribution observed from the starvation study. Within size groups, it was expected that as the fish were fasted, their corresponding vector would also change. The expected change would be an increase in vector position along the reactance axis (due to the depletion of lipids in tissue) but this was only partly observed on the BIVA for the dorsal measurements. A significant difference in average vector was observed between the day

one and day fourteen fasted fish for the small and large sized fish but not the medium sized fish. Unfortunately, this does not correspond to a significant change in energy content in the tissue as measured by bomb calorimetry. It may be that any physiological change was not due to the depletion of energy stores measured within the analyzed tissue samples but from somewhere else inside the fish.

The BIVA results from the growth study show some interesting results. Over the course of the growth period, fish fed the most (i.e. the fish that grew the most) showed the largest vector change from initial sampling. Fish fed on a satiation diet showed a significant vector change from the maintenance fed fish. The vector change between sampling periods of the fish fed the satiation diet showed a vector change from a high resistance/reactance value to a lower resistance/reactance value, likely due to the increased lipid content in the fish. These fish showed similar vector positions to the large size class of rainbow trout from the starvation study. The maintenance fed fish did not change a significant amount between sampling periods, this is expected as fish fed on a maintenance diet should not have experienced any significant change in lipid/water content. Fish fed on the optimal diet showed a vector change in between maintenance and satiation fed fish. If the fish are accumulating lipid levels within their tissue, then these results make sense as fat cells are less metabolically active (Dorhofer, 2005) which would result in a lower reactance vector than a fish with less fat in its tissue. The utility of BIVA to show large scale changes in tissue composition in fish is limited by the scope of this study. More research is needed to ascertain the full capabilities of BIVA on fish models.

In humans BIVA can also be used as a diagnosis tool because people suffering from different health conditions will have different phase angles (Walker-Kroker *et al.*,

2011). This can only be applied to conditions which effect the amount of fluid in the body's tissues (since water conducts the current in BIA). Individuals suffering from exsiccosis, anorexia or obesity all effect the electric current experienced (Kristina *et al*., 2012). Ideally this concept could be applied to fish, although the data required to conduct such analysis is not available. The results of the BIVA graphs from both the starvation study and the growth study show that larger, growing fish have an average vector position characterized by low resistance and reactance. This could indicate that normal, healthy fish should possess vectors similar to the ones found in this study, in which case future studies using BIVA could use these results as a way of checking the health of the fish; however, more research is needed to corroborate these findings.

BIA has several interesting avenues for future research. Incorporating the use of BIA into surveys of wild fish populations could be used to track any changes in energy content from one time point to the next (Willis & Hobday, 2008). Annual fish surveys are conducted on multiple species of fish around the world and although the use of condition analysis can be used to track changes in some physiological condition, it can only provide limited information about the fish. BIA could be added on to some of these surveys to provide additional information about fish populations.

The non-lethal aspect of BIA enables temporal analysis to be conducted on fish. Since the fish is not destroyed upon analysis when using BIA, the changes in energy content of that fish's tissue could be tracked over time. Future studies could be focused around this concept and instead of using large samples of fish from a population they could instead opt to follow changes within a single organism over a period of time. This

concept could be further expanded upon in toxicological studies to track the real time changes in fish tissue when exposed to a compound.

BIVA is another interesting concept for future research, as it is a largely unexplored concept of BIA in organisms other than humans. A possible study based on BIVA could involve comparing the change in impedance vectors over time of fish exposed to a compound versus the change in impedance vectors of unexposed fish. The vector analysis from the growth study shows that as fish grow and their condition improves, their vector also changes. This could be explored further to determine how and if contaminants in the water effect the growth or health of a fish. It would be interesting to see if fish exposed to different compounds produced distinct vectors that are distinct from each other and/or the vectors of healthy fish.

Conclusions

The development of non-lethal methods of assessing fish condition is an ongoing avenue of research. BIA represents a very promising use of technology which can rapidly and easily estimate the body composition and health of fish species with the aid of predictive models. The results from this study on juvenile rainbow trout contribute to the growing body of literature that supports the use of BIA as a non-lethal method of analyzing fish. BIA was shown to accurately predict the water mass (r^2=0.9170), dry mass (r^2=0.9064) and energy content (r^2=0.9149) of juvenile rainbow trout. These predictive models can be used in future BIA studies on juvenile rainbow trout to non-lethally study these fish. The electrode placement on the fish improved the regression coefficients for all models, with the dorsal position giving the best possible predictive power. Future studies on other fish should also examine electrode position to determine if a similar effect is observed in other species. The BIVA performed on the rainbow trout in this study was able to show that different sized fish yielded different vector positions. It could potentially be a useful indicator of fish health condition; however, more research is required to confirm if this is possible.

The development of non-lethal methods of analyzing fish is ongoing, but the results from this study, along with the available studies in the literature show BIA to be a promising prospect going forward.

Bibliography

Alexandre, C., Quintella, B., Silva, A., Mateus, C., Romao, F., Branco, P., Ferreira, M., Almeida, P. (2013). Use of electromyogram telemetry to assess the behaviour of the Iberian barbel (*Luciobarbus bocagei* Steindachner, 1864) in a pool-type fishway. *Ecological Engineering*, *51*, 191–202.

Antonio, P., Luana, P., & Dumler, F. (2002). Impedance Vector Distribution by Sex, Race, Body Mass Index, and Age in the United States: Standard Reference Intervals as Bivariate Z scores. *Nutrition*, *18*, 153–167.

Biro, P., Morton, A., Post, J., & Parkinson, E. (2004). Over-winter lipid depletion and mortality of age-0 rainbow trou (*Oncorhyncus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences*, *61*(8), 1513–1520.

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

Bourdages, C. G. (2011). Use of bioelectrical impedance analysis (BIA) to predict water and energy content of juvenile Rainbow trout (*Oncorhynchus mykiss*), MSc Thesis, University of Ontario Institute of Technology, 141.

Carbonara, P., Scolamacchia, M., Spedicato, M., Zupa, W., McKinley, R., & Lembo, G. (2015). Muscle activity as a key indicator of welfare in farmed European sea bass (*Dicentrarchus labrax* L. 1758). *Aquaculture Research*, *46*, 2133–2146.

Cooke, S. J., Thorstad, E. B., & Hinch, S. G. (2004). Activity and energetics of free-swimming ® sh : insights from electromyogram telemetry.

Cox, K., & Hartman, K. (2005). Nonlethal Estimation of Proximate Composition in Fish. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*, 269–275.

Crossin, G. T., & Hinch, S. G. (2005). A Nonlethal, Rapid Method for Assessing the Somatic Energy Content of Migrating Adult Pacific Salmon. *Transactions of the American Fisheries Society*, *134*(October), 184–191.

Dorhofer, R. (2005). The BIA Compendium. (M. Pirlich, Ed.). Germany: Data-Input The B.I.A. Company.

Doyle, T., Houghton, J., McDevitt, R., Davenport, J., & Hays, G. (2007). The energy density of jellyfish: Estimates from bomb-calorimetry and proximate-composition. *Journal of Experimental Marine Biology and Ecology*, *343*, 239–252.

Duncan, M., Craig, S. R., Lunger, A., Kuhn, D. D., Salze, G., & Mclean, E. (2007). Bioimpedance Assessment of Body Composition in Cobia (*Rachycentron canadum*). *Aquaculture*, *271*, 432–438.

Elaine Maclean, C., & Beth, S. (2012). Evaluation of Bioelectrical Impedance Analysis and Fulton's Condition Factor as Nonlethal Techniques for Estimating Short-Term Responses in Postsmolt Atlantic Salmon (*Salmo salar*) to Food Availability. *Fishery Bulletin*, *110*(2), 257–270.

FAO. (2015). Cultured Aquatic Species Information Programme. Retrieved from http://www.fao.org/fishery/culturedspecies/Oncorhynchus_mykiss/en

FAO. (2016). *The State of World Fisheries and Aquaculture*.

Folkestad, A., Wold, J. P., Rorvik, K. A., Tschudi, J., Haugholt, K. H., Kolstad, K., & Morkore, T. (2008). Rapid and non-invasive measurements of fat and pigment concentrations in live and slaughtered Atlantic salmon (*Salmo salar* L.). *Aquaculture*, *280*(1–4), 129–135.

Hafs, A., & Hartman, K. (2014). Developing Bioelectrical Impedance Analysis Methods for Age-0 Brook Trout. *Fisheries Management and Ecology*, *21*, 366–373.

Hancz, C., Milisits, G., & Horn, P. (2003). In vivo measurement of total body lipid content of common carp (*Cyprinus carpio* L.) by electrical conductivity. *Archiv Fur Tierzucht*, *46*(4), 397–402.

He, H.-J., Di, W., & Da-Wen, S. (2013). Nondestructive Spectroscopic and Imaging Techniques for Quality Evaluation and Assessment of Fish and Fish Products. *Critical Reviews in Food Science and Nutrition*, *55*(6), 864–886.

Klefoth, T., Skov, C., Aarestrup, K., & Arlinghaus, R. (2013). Reliability of Non-Lethal Assessment Methods of Body Composition an Energetic Status Exemplified by Applications to Eel (*Anguila anguilla*) and Carp (*Cyprinus carpio*). *Fisheries Research*, *146*, 18–26.

Krimmer, A., & Rasmussen, D. J. (2008). *Behavioural and Physiological Response of Overwintering Brook Trout (Salvelinus fontinalis) to Instream Flow Manipulations from the Canadian Rocky Mountains*. *Biological Sciences*. University of Lethbridge.

Kristina, N., Nicole, S., Matthias, P., & Anja, B.-W. (2012). Bioelectrical phase angle and impedance vector analysis - Clinical relevance and applicability of impedance parameters. *Clinical Nutrition*, *31*, 854–861.

Kushner, R. F., Schoeller, D. a, & Carla R Fjeld, and L. D. (1992). Is the impedance index (ht2 / R) predicting total body water? *Am J Clin Nutrion*, *56*, 835–839.

Luna, J., & Villarroel, D. M. (2013). *Effect of short-term fasting prior to slaughter on welfare and meat quality of rainbow trout (Oncorhynchus mykiss)*. University of Madrid.

Margraf, J., Hartman, K., Cox, K., & Fairbanks, U. of A. (2005). *Non-Lethal Estimation of Energy Content of Yukon River Chinook Salmon*. (J. Margraf, Ed.), *Arctic-Yukon-Kuskokwim Sustainable Salmon Initiative*. AK, United States: Alaka Cooperative Fish and Wildlife Research Unit Institute of Arctic Biology.

Mesa, M., & Rose, B. (2015). An assessment of morphometric indices, blood chemistry variables and an energy meter as indicators of the whole body lipid content in *Micropterus dolomieu*, *Sander vitreus* and *Ictalurus punctatus*. *Journal of Fish Biology*, *86*, 755–764.

Miller, S. (2014). *Effects of Salinity and Temperature on Bioelectrical Impedance*

Analysis of Gulf Killifish, Fundulus Grandis. *Agricultural and Mechanical College*. Louisiana State University.

Nacci, D. (2013). Electronic Assessments of Small Fish Health. (U. S. E. P. A. O. of R. and D. A. E. Division, Ed.). United States: EPA.

Pothoven, S., Ludsin, S., Hook, T., Fanslow, D., Mason, D., Collingsworth, P., & Tassell, J. (2008). Reliability of Bioelectrical Impedance Analysis for Estimating Whole-Fish Energy Density and Percent Lipids. *Transactions of the American Fisheries Society*, *137*, 1519–1529.

Quinn, T. P., & Myers, K. W. (2004). Anadromy and the marine migrations of Pacific salmon and trout: Rounsefell revisited. *Reviews in Fish Biology and Fisheries*, *14*(4), 421–442.

Rasmussen, D. J., Krimmer, A., Paul, A. J., & Hontela, A. (2012). Empirical Relationships Between Body Tissue Composition and Bioelectrical Impedance of Brook Trout Salvelinus fontinalis from a Rocky Mountain Stream. *Journal of Fish Biology*, *80*, 2317–2327.

Ryan, M., Christopher, P., Amy, P., & Christopher, A. (2009). *Energy use, migration time, and spawning success of adult spring-summer Chinook slamon returning to spawning areas in the South Fork Salmon River in Central Idaho* (Vol. 4). University of Idaho, Fish Ecology Research Lab.

Rye, M. (1991). Prediction of carcass composition in Atlantic salmon by computerized tomography. *Aquaculture*, *99*(1–2), 35–48.

Schreckenbach, K., Knosche, R., & Ebert, K. (2001). Nutrient and Energy Content of Freshwater Fishes. *Journal of Applied Ichthyology*, *17*, 142–144.

Stolarski, J. T., Margraf, F. J., Carlson, J. G., & Sutton, T. M. (2014). Lipid and Moisture Content Modeling of Ampidromous Dolly Varden Using Bioelectrical Impedance Analysis. *North American Journal of Fisheries Management*, *34*(3), 471–481.

Veliyulin, E., Van Der Zwaag, C., Burk, W., & Erikson, U. (2005). In vivo determination of fat content in Atlantic salmon (Salmo salar) with a mobile NMR spectrometer. *Journal of the Science of Food and Agriculture*, *85*(8), 1299–1304.

Walker-Kroker, A., Kroker, A., Mattiucci-Guehlke, M., & Glaab, T. (2011). A Practical Guide to Bioelectrical Impedance Analysis Using the Example of Chronic Obstructive Pulmonary Disease. *Nutrition Journal*, *10*(35), 1–8.

Willis, J., & Hobday, A. (2008). Application of Bioelectrical Impedance Analysis as a Method for Estimating Composition and Metabollic Condition of Southern Bluefin Tuna (Thunnus maccoyii) During Conventional Tagging. *Fisheries Research*, *93*, 64–71.