Use of Bioelectrical Impedance Analysis (BIA) to Predict Water and Energy Content of Juvenile Rainbow Trout (*Oncorhynchus mykiss)*

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ABSTRACT

Accurate measurements of energy content and body composition are essential to effectively assess the well-being of fish. Bomb calorimetry and proximate analysis are currently the most dependable and accurate methods to estimate energy content and body composition. However, bioenergetic studies that employ the traditional methodology necessitate the killing of fish to determine physiological composition and energy content in a target tissue. The killing of the individual negates the ability for repeated measures on the same individual, and also suppresses compositional studies involving endangered or threatened species. Recent research has shown Bioelectrical Impedance Analysis (BIA), a quick, easy-to-use, non-invasive, and most importantly, non lethal technique to be an effective method for estimating the proximate composition and energy content of fish. The focus of this research is to evaluate the capability of BIA to accurately assess the bioenergetics of juvenile rainbow trout (*Oncorhynchus mykiss*), and to develop species-specific indices to predict energy content, total body water and dry mass. To do this, juvenile rainbow trout were subjected to one of three ration regimes: maintenance (0.4 % bw/day), optimum (1.9 % bw/day) and satiation (3.4 % bw/day) for 90 days. Subsamples from each treatment were taken every 30 days to be subjected to BIA testing. Tissue samples were collected from the subsampled trout for future caloric and compositional analysis via bomb calorimetry and proximate analysis. It was found that BIA demonstrated a strong predictive relationship with regard to energy content ($r^2 = 0.90$), total body water ($r^2 = 0.89$) and dry mass ($r^2 = 0.80$). BIA was also able to successfully reflect a notable statistical difference between treatments with regard to total energy content, energy density, total body water, dry mass. These results, along with much of the existing literature, indicate that BIA may be an accurate and reliable tool to estimate the bioenergetics and proximate composition of fish.

Keywords: Rainbow Trout, *Oncorhynchus mykiss*, bioelectrical impedance analysis, body composition, condition, proximate analysis.

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LIST OF ABBREVIATIONS

ATP = adenosine triphosphate

BIA = bioelectrical impedance analysis

DM = dry mass

FFM = free fat mass

GSI= gonadosomatic index

HSI= hepatosomatic index

K = condition factor

MS-222 = tricane methane sulphonate

TBA= total body ash

TBF = total body fat

TBP=total body protein

TBW = total body water

TOBEC = total body conductance

CT = computerized tomography

NMR = nuclear magnetic resonance

NIR = near-infrared spectroscopy

EMG = electromyogram

MT = microwave transmission

1.0 INTRODUCTION

The study of bioenergetics involves the examination of the losses, gains and transfers of energy within biological organisms (Jobling, 1994). All energy acquired through the ingestion of food is ultimately used in metabolic processes, deposited as new body tissue or lost as waste (Jobling, 1994). A basic truth involving the bioenergetics of fish is that the energy content that fish obtained from external sources must equal the same amount of energy required for metabolism, maintenance and activity in order for body mass to remain constant (Diana, 2004). When the energy intake of a fish is in excess of those needs, growth and energy storage will occur (Jobling, 1994). Conversely, if the expenditure of energy is not met by food intake, previously acquired lipid and protein energy stores will be metabolized and the fish will lose weight (Jobling, 1994).

The proximate composition and energy content of fish play a fundamental role with regard to growth, reproduction and overwinter survival (Hanson *et al.*, 2010; Kimberlea *et al.,* 2011). High caloric energy content in muscle is indicative of a healthy fish that has a surplus of calories that can be directed towards growth, reproduction and overwinter survival (Lee and Putnam, 1973). Conversely, tissues that are low in energy content may be indicative of an unhealthy fish that has not obtained enough calories to supplement growth, reproduction and overwinter survival (Lee and Putnam, 1973). Consequently, aquatic biologists commonly examine proximate composition and energy content to characterize the condition of fish (Cox and Hartman, 2005; Hanson *et al.,* 2010).

Proximate analysis and bomb calorimetry are the current standards that aquatic biologists rely upon to determine the proximate composition and energy content of fish (Crossin and Hinch, 2005; Hanson *et al.*, 2010). Proximate analysis determines the energy concentration of a tissue through lipid, protein, carbohydrate and water estimation, whereas; bomb calorimetry determines the caloric content of tissue by measuring the heat released during its combustion (AOAC, 1990; Crossin and Hinch, 2005). Both methods provide reliable energetic estimates in the laboratory (AOAC, 1990); however, the cost and time associated with both methods are quite prohibitive and impracticable for field work (Cox and Hartman, 2005; Crossin and Hinch, 2005). Furthermore, bioenergetic studies that employ these traditional methods necessitate the killing of the fish to determine the energy content in a target tissue (Cox and Hartman, 2005; Crossin and Hinch, 2005; Duncan *et al.*, 2007; Pothoven *et al.*, 2008; Hanson *et al.*, 2010).

The killing of the fish negates the ability for repeated measures on the same fish, and also suppresses compositional studies involving endangered or threatened species (Cox and Hartman, 2005; Crossin and Hinch, 2005; Duncan *et al.*, 2007; Pothoven *et al.*, 2008; Hanson *et al.*, 2010). Moreover, the killing of the fish is typically followed by lengthy procedures that can only be done in the laboratory, thus illustrating the impracticality of the traditional methodology in the field (AOAC, 1990). These limitations have increased interest to develop nonlethal techniques that are quicker, more portable and less expensive than the traditional methodology.

As an alternative to the traditional methodology, researchers often rely on a variety of condition indices, generally relating length to wet weight to provide an estimate of 'fatness', for their assessments (Schneider, 2000). However, under energy stress, such as migration, reproduction, or toxic stress, condition indices may produce inconsistent results (Cone, 1989). Inconsistencies with condition indices can lead to inaccurate characterizations of test subjects and inappropriate management strategies (Cone, 1989). Like the traditional methodology, the shortcomings of condition indices have also motivated researchers to develop a series of other nonlethal methods to assess proximate composition and energy content in fish.

Over the last twenty years, a series of nonlethal electronic methods have been developed to estimate proximate composition and energy content. One such technique, near-infrared spectroscopy, measures the reflectance, interactance and absorption of water, lipid and protein content at different concentrations (Downey, 1996). Although a promising technique, the equipment for this approach is quite expensive and not field appropriate (Crossin and Hinch, 2005). Similarly, computerized tomography (Rye, 1991) and nuclear magnetic resonance (Burgetz *et al.,* 1998), although used to measure proximate composition and energy status with some success, are also not well suited for field use (Crossin and Hinch, 2005).

Conversely, a technique that uses microwave transmissions (Crossin and Hinch, 2005) and non-destructive physiological examinations (Crawford *et al.,* 1977; Hruska *et al.,* 2007) can be non-lethally applied in the field. However, their

accuracy and reliability involving fish with either low or non-uniform energy levels is limited (Hruska *et al.,* 2007; Crossin and Hinch, 2005). Similarly, total body electrical conductivity (TOBEC), an approach that uses an electromagnetic field to measure the conductivity of the fish, thereby estimating its internal composition, also suffers from issues involving accuracy (Fischer, 1996). Several studies suggest that TOBEC cannot reliably estimate lipid mass, and that errors increase with increasing total body weight and total body water (Novinger and Del Rio, 1999). Furthermore, the instrumentation associated with TOBEC is rather large and not well suited for field studies (Scott *et al.,* 2001). Therefore, the potential for TOBEC to accurately estimate the internal composition of fish in the field and in the laboratory is limited (Crossin and Hinch, 2005).

Lastly, electromyogram telemetry is a nonlethal approach that can be carried out in the field and is fairly accurate; however, its individual-specific calibration procedure makes it very impracticable to employ in the field (Hruska *et al.,* 2007). The complexity, field limitations, costs, and impracticality of the aforementioned nonlethal approaches limit their potential to be considered as viable alternatives to the traditional methodology. These limitations further emphasize the need for the development of a broadly applicable, nonlethal technique that can be reliably implemented in the field on a variety of species.

Bioelectrical impedance analysis (BIA), a well established method used to evaluate the body composition, energy content and the nutritional status by the medical community (Gupta, 2009), is said to hold great promise by aquatic biologists as a means to provide researchers with a viable alternative to proximate

analysis and bomb calorimetry (Cox and Hartman 2005; Duncan *et al.,* 2007; Pothoven *et al.,* 2008). The bioelectrical impedance analyzer, a user-friendly, handheld instrument that makes BIA possible, works by measuring the resistance and reactance along the length of the fish (Cox and Hartman 2005; Duncan *et al.,* 2007; Pothoven *et al.,* 2008). Resistance is the restriction to the flow of electric current through the body, and is proportional to the amount of water present in the tissue (Gupta *et al.,* 2009); whereas, reactance is the resistive effect caused by the cell membranes and tissue interfaces (Willis and Hobday, 2008). Measurements of resistance and reactance are then used to quantify proximate body composition and energy content of the fish.

Researchers use resistance and reactance measurements to characterize whole-body electrical impedance for particular organisms (Cox and Hartman 2005; Duncan *et al.*, 2007; Pothoven *et al.*, 2008). The characterization of whole-body impedance has helped researchers to develop species-specific body composition indices (Cox and Hartman 2005; Duncan *et al.,* 2007; Pothoven *et al.*, 2008). Using regression analysis, researchers can correlate the BIA estimated proximate composition and energy content of a particular species with their actual body composition, determined through proximate analysis and bomb calorimetry, to develop predictive compositional equations. Such equations are useful to fishery and aquatic biologists as they have been shown to successfully predict total body water (TBW) total body fat (TBF), fat-free mass (FFM), total body ash (TBA), total body protein (TBP), dry mass (DM) and energy content, all of which are important when assessing fish health (Cox and Hartman 2005; Duncan *et al.,*

2007; Pothoven *et al.*, 2008). These predictive equations can provide researchers with species-specific nonlethal estimators of body composition and energy content for fish in the laboratory and in the field.

Bosworth and Wolters (2001) pioneered BIA as a means to estimate the fat and water content of channel catfish *Ictalurus punctatus*, while Cox and Hartman (2005) were able to accurately correlate BIA resistance and reactance with the energy density, fat mass, protein mass, dry mass, water mass, and ash mass of live brook trout *Salvelinus fontinalis*. Over the last few years, there have been a limited number of subsequent studies to use BIA as a means to estimate the proximate composition and energy content of a variety of species with notable success. Although successful, caution should be heeded as there are still many questions about BIA that remain unanswered.

One such question that remains unanswered is how differential growth can influence BIA readings. Understanding how differential growth can impact BIA will allow researchers and biologists to account for changes in body composition, energy content, and ultimately condition, in the laboratory and in the wild. Furthermore, there has yet to be a BIA study to use rainbow trout *Oncorhynchus mykiss* as its test species. Rainbow trout is the world's most extensively farmed fish species, and generates billions of dollars for the aquaculture industry on an annual basis. BIA could provide rainbow trout farming operations with a tool to continually assess the body composition and energy content of their product. Furthermore, understanding how ration impacts BIA predictions of body composition and energy content for rainbow trout can help to increase profit

margins by eliminating wasteful feeding regimes. The current system relies on weight measurements to assess the growth and condition of aquaculture fish, however, assessments on weight alone may produce inaccurate results. Therefore, BIA would be an enormous asset to the trout farming industry. Additionally, being able to non-lethally determine the bioenergetic impacts of contaminant exposure to fish will provide an important new tool for assessing fish ecotoxicology of chemicals. Although a relatively new method, BIA appears to hold great promise as a means to assess the body composition energy content of fish.

2.0 LITERATURE REVIEW

2.1 Bioenergetics of Fish

The study of fish bioenergetics focuses primarily on the relationship between ration and the growth rate of fish (Jobling, 1994). The performance of any activity requires the expenditure of energy, and energy production requires the ingestion of food (Jobling, 1994). The sources of ingested food energy are lipids, proteins and carbohydrates, each of which yield different amounts of energy when metabolized (Diana, 2004). Foods found in the wild come in a myriad of shapes and sizes, ranging from other fish species and crustaceans to aquatic plants and algae (Jobling, 1994). Once ingested, food energy is then liberated through digestion. Heat is created as a by-product of the digestive process, and then quickly lost to the surrounding aquatic environment (Jobling, 1994). The energy liberated through digestion is stored as adenosine triphosphate (ATP), which powers cellular processes such as the manufacture of proteins to facilitate muscle growth (Diana, 2004).

2.2 Energy Flow in Fish

All food ingested by fish has a gross energy content (Diana, 2004). However, not all of this energy is accessible or usable by fish. A portion of the food consumed is indigestible and eventually lost as fecal matter (Figure 1) (Jobling, 1994). Furthermore, not all of the digestible components of ingested food can be completely metabolized, resulting in further energy loss (Jobling, 1994). Additional energy is lost via respiration across the gills and through the excretion of urine (Figure 1) (Jobling, 1994). The remaining energy that can be

metabolized is then used for movement, growth and maintenance (Figure 1) (Moyle and Cech, 2004).

2.3 Metabolism

The metabolism of fat, protein and carbohydrates are of great importance in terms of energy allocated for growth and maintenance (Diana, 2004). Proteins are digested into amino acids, carbohydrates into simple sugars, and fats into free fatty acids and glycerol (Moyle and Cech, 2004). Once absorbed, these constituents are transported to the liver (Diana, 2004). Amino acids are removed from the blood and stored in the liver temporarily until ready for transport though the body to build new proteins (Diana, 2004). Fish continuously breakdown and synthesize proteins to be used in enzymes, cells and tissues (Moyle and Cech, 2004). Excess amino acids are broken down into carbon dioxide, water and ammonia (Lee and Putnam, 1973). Absorbed dietary carbohydrates are converted to glucose in the liver (Evans and Claiborne, 2006). Glucose is then either recombined back into glycogen and stored in the liver as an energy reserve, or used to fulfill the energetic requirements of movement and maintenance (Warren and Davis, 1967). Fish readily catabolize fat to supply the daily energy required for movement and maintenance (Tocher, 2003). Lipids are the preferred source of stored energy because they provide twice as much ATP as proteins or carbohydrates when broken down (Tocher, 2003). When current energy expenditure is not met by food intake, or during times of complete starvation, fish will lose weight (Moyle and Cech, 2004). Conversely, fat deposition will occur, often in the liver or as mesenteric fat deposits, when fish are either overfed for a

long period of time or when there is too much fat in their diet (Moyle and Cech, 2004). It is very easy for fish to accumulate fat from their diet because lipids can be synthesized from glucose, amino acids, glycerol and free fatty acids (Tocher, 2003). When energy intake exceeds the caloric metabolic requirements, an accumulation of energy stores may occur, resulting in growth (Moyle and Cech, 2004).

2.4 Growth

Growth, the basis of bioenergetics, is the ultimate integrative expression of well-being at the individual level (Cox and Hartman, 2005). As a result, growth has become a well studied indicator of fish health in aquatic habitats (Cox and Hartman, 2005; Pothoven, 2008; Moyle and Cech, 2004; Diana, 2004). Rapid growth indicates an abundance of food and favorable conditions, whereas slow growth is likely to indicate the opposite (Moyle and Cech, 2004). Furthermore, biologists use growth and growth rate as indicators of reproductive fitness and survival (Johnson and Bjornsson, 2001). Larger fish tend to produce more eggs, thus increasing their potential to successfully reproduce (Pennington and Kapuscinski, 2011). Furthermore, young fish that grow large quickly will often become less vulnerable to predation (Pennington and Kapuscinski, 2011). Therefore, the survival of offspring generally increases with increasing growth and growth rate.

Growth is also the first aspect of the energy budget to be affected when fish are stressed energetically (Cox and Hartman, 2005; Pothoven, 2008). For instance, growth is inhibited by the energetic demands associated with toxicant removal (Erickson *et al.,* 2011). When fish are exposed to a toxicant, energy is diverted away from growth and directed toward the liver to facilitate increased detoxification activity and liver growth (Erickson *et al.,* 2011). Similarly, when fish are subjected to starvation conditions involving food scarcity, growth is inhibited so that basal metabolism may continue to function (Moyle and Cech, 2004). As illustrated by the examples above, the growth of fish is strongly related to, if not dictated by, the environment in which it resides. The environmental factors that can significantly impact the growth of fish include competition for food, fish density, photoperiod, water temperature and ration (Diana, 2004; Warren and Davis, 1967; Evans and Claiborne, 2006; Moyle and Cech, 2004).

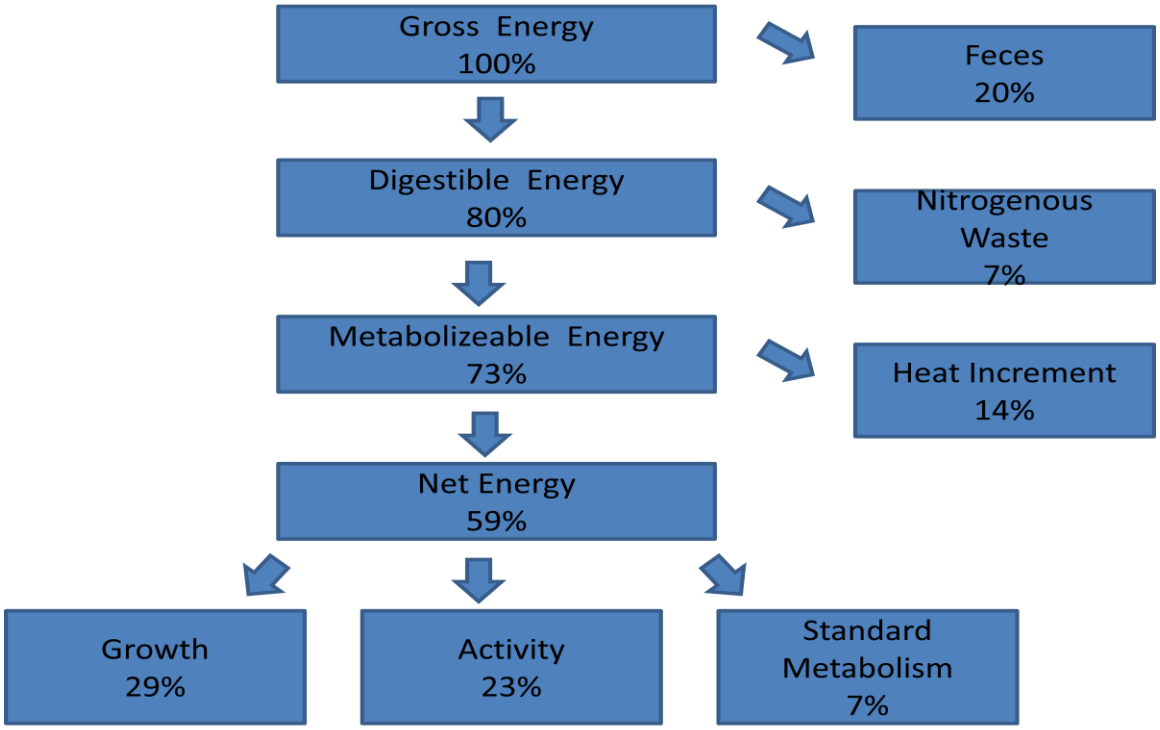

Figure 1: The energy budget for an average carnivorous fish. This figure provides an estimate of the breakdown of the each parameter in the energy budget. This figure was adapted from Diana, 2004.

2.4.1 **Environmental Factors influencing Growth**

2.4.1.1 Competition

Competition is the demand for the same environmental resources, either within or among species, in excess of immediate supply (Moyle and Cech, 2004). Competition for a limited amount of food may result in depensatory growth to occur within a population of fish (Moyle and Cech, 2004). Depensatory growth, a common phenomenon observed in standard growth studies, results in the bigger, more dominant fish acquiring most of the food (Ryer and Olla, 1996). Fish populations experiencing depensatory growth are typically composed of a few large dominant fish and many smaller submissive fish (Quinn, 2005). Furthermore, fish populations generally experience higher depensatory growth at low rations due to the limited availability of food (Ryer and Olla, 1996). The high occurrence of depensatory growth in standard growth experiments is due in part to space limitations in experimental containers (Diana, 2004). Depensatory growth is a limitation of aquaculture systems, and is commonly dealt with by putting fish that are as near in size as possible in the same tanks, or by feeding each tank its associated ration once per day (Diana, 2004). Depensatory growth is less likely to occur in the field because space limitations similar to those encountered in the laboratory do not happen in the field (Diana, 2004).

2.4.1.2 Density

Density-dependant growth is another commonly observed phenomenon in both nature and aquaculture (Diana, 2004). As density increases, the competition

for space and food intensifies; this increased competition among fish within a tank causes growth rates to decline (Kostow, 2009). Competition for food and space is low in tanks with low population densities (Quinn, 2005). Low competition will result in higher growth rates because fish are not expending as much energy competing for food and space (Quinn, 2005). This phenomenon is commonly observed in aquacultural settings because resources are restricted within the holding tanks (Diana, 2004).

The only response for fish stocked at excessive densities is to compete for food when resources are restricted (Ryer and Olla, 1996). It is often difficult to accurately determine optimum density requirements for fish due to a lack of understanding with regard to the optimum densities for different species at various sizes (North *et al.,* 2006). For example, wide discrepancies exist with regard to optimum stocking density recommendations for rainbow trout (Ellis *et al.,* 2002). Akbulut *et al.,* (2002) estimated that the optimum stocking density to rear juvenile rainbow trout is in between 4 - 5 kg/m^3; whereas, the Official Journal of the European Union suggests that the optimum stocking density of rainbow trout is closer to10 kg/m^3. North *et al.,* (2006) suggests that rainbow trout can be adversely affected at both low and high densities. High population densities lead to high competition for food which may stress the fish and result in poor growth. Low population densities result in low motivation to compete for food also resulting in poor growth. Moderate food competition is needed to facilitate growth; this can be achieved through the use of an intermediate stocking density. However, the contention in the literature concerning density puts the onus

on the researcher to determine an adequate stocking density that will simultaneously avoid adverse density-dependant growth impacts, while maintaining a statically relevant number of test organisms.

2.4.1.3 Temperature

Fish are poikilotherms, and therefore rely on the external environment to control their internal body temperature, basal metabolic rate and feed efficiency. Growth associated with feed efficiency increases with increasing water temperature (Azevedo *et al.,* 1998). Increasing water temperature increases the digestibility of dietary dry matter, nitrogen and energy derived from the diet (Azevedo *et al.,* 1998). However, standard growth experiments involving fish often employ species-specific intermediate temperatures because ration regimes are typically more efficient at intermediate temperatures (Diana, 2004). Growth associated with low rations increases continually with increasing temperature, whereas growth from high rations will reach optimum efficiency at intermediate temperatures (Diana, 2004). Growth associated with high rations tapers off and begins to decline once an intermediate temperature is exceeded (Diana, 2004). Therefore, both controlled and satiation rations would experience optimum growth at an intermediate temperature (Moyle and Cech, 2004).

2.4.1.4 Photoperiod

Photoperiod, or the length of time during a 24 hour period where fish are exposed to natural or artificial light, can significantly affect growth rate (Moyle

and Cech, 2004). Fish require a particular balance between light and dark phases, reflective of their natural habitat, to facilitate growth (Komourdjian *et al.*, 1989). Photoperiods consisting of continuous light or continuous dark will inhibit growth, resulting in weight loss (Taylor *et al.*, 2005). However, a properly regulated photoperiod can significantly improve growth rate and metabolism of fish (Komourdjian *et al.*, 1989). For instance, rainbow trout grow significantly better when subjected to extended photoperiods, consisting of 18 hours of light and 6 hours of dark each day, than when subjected to shorter photoperiods (Taylor *et al.*, 2005)

2.4.1.5 Ration

The quality and quantity of food are the most important factors influencing growth (Moyle and Cech, 2004). A complete diet, consisting of essential amino acids, fatty acids, vitamins and minerals, is required for growth (Moyle and Cech, 2004). Furthermore, diets that are high in protein content often stimulate increased growth (Moyle and Cech, 2004). For example, rainbow trout that are fed a diet consisting of approximately 400-460 g/kg protein will experience optimal growth (Cowey and Sargent, 1972).

The quantity of food available to fish can also significantly impact growth (Moyle and Cech, 2004). Fish that are starved will lose weight; therefore fish that are fed zero rations will experience negative growth (Diana, 2004). Furthermore, fish that are fed rations at levels less than those needed to fulfill basic energy requirements will also experience negative growth (Diana, 2004). Fish deprived

of full rations will lose weight, but not as quickly as fish that are fed nothing at all (Diana, 2004). To achieve zero growth, a fish will require at least some level of ration (Nourollahi-Fard & Woo, 2008; Russell, 1977; Diana, 2004). There are three levels of ration involving zero or positive growth - the maintenance ration, optimum ration and satiation ration (Figure 2) (Diana, 2004). The maintenance ration is the amount of food required to maintain the existing body weight of the fish (Figure 2) (Nourollahi-Fard & Woo, 2008; Russell, 1977; Diana, 2004). The optimum ration produces the highest gross conversion efficiency, or the most efficient growth (Figure 2) (Russell, 1977; Nepal *et al.*, 2002; Diana, 2004). The satiation ration involves feeding the fish the maximum amount of food that the fish can eat at one time (Boujard & Medale, 1994; Glencross *et al.*, 2006; Diana, 2004). The satiation ration will result in maximum growth of the fish (Figure 2) (Boujard & Medale, 1994; Glencross *et al.*, 2006; Diana, 2004). Conversion efficiencies are high at high rations, whereas, conversion efficiency is low at maintenance ration due to the majority of energy being allocated towards fulfilling basal metabolic requirements (Diana, 2004). Furthermore, the lack of food associated with the maintenance ration can cause stress that results in even poorer conversion efficiency, poor growth and ultimately poor health. In the field, individual fish growth is difficult to measure; however, the fatness of the fish is easy to measure and is an excellent indicator of condition.

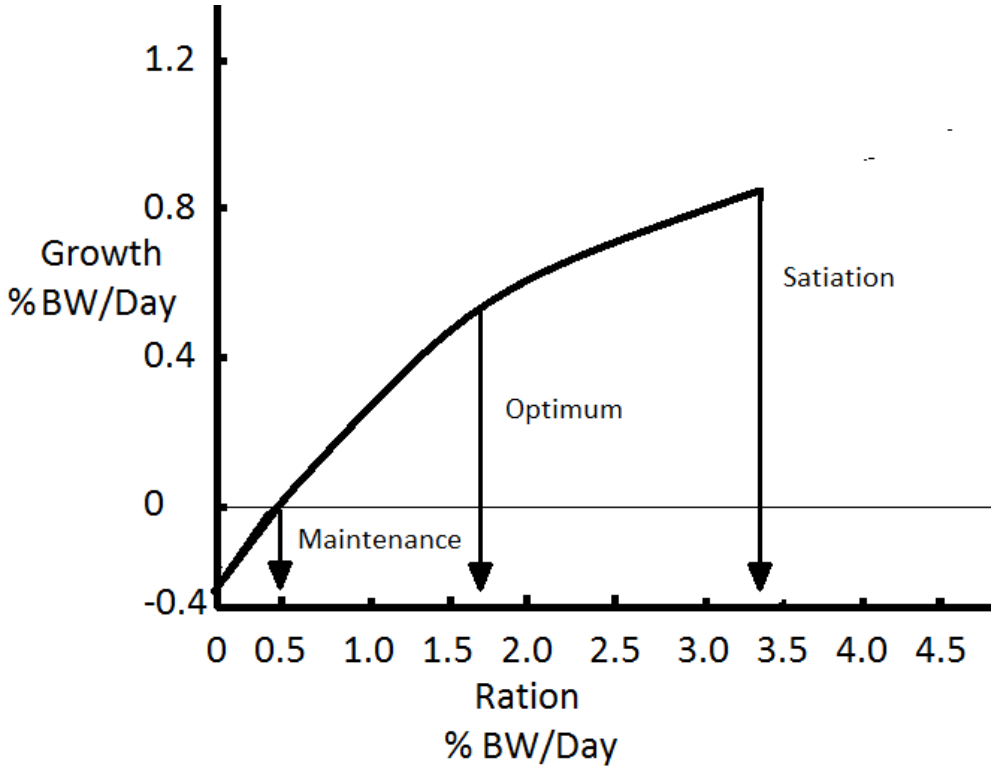

Figure 2: Relationship between growth and ration for juvenile rainbow trout. This figure was adapted from Russell, 1977.

2.5 Condition

Condition is described as the relative fatness, or energetic state of the fish (Schulte-Hostedde *et al.,* 2001). Organisms with a high amount of energy in reserve tend to be fairly fat, and are therefore considered to be in good condition (Cox and Hartman, 2005; Pothoven, 2008; Moyle and Cech, 2004; Diana, 2004; Blackwell *et al.,* 2000). Conversely, fish with a low amount of energy in reserve appear thin, and might be considered to be in poor condition (Cox and Hartman, 2005; Pothoven, 2008; Moyle and Cech, 2004; Diana, 2004; Blackwell *et al.,* 2000). Fish with low energy reserves may be considered to be in poor condition because they are unable to meet their energetic requirements, resulting in an emaciated appearance (Moyle and Cech, 2004; Diana, 2004; Blackwell *et al.,* 2000). However, fish with a high amount of energy in reserve are considered to be in good condition because they can meet their energetic requirements, and are able to allocate some of that energy toward growth, leading the fish to grow fat (Moyle and Cech, 2004; Diana, 2004; Blackwell *et al.,* 2000).

Fish that are considered to be in good condition are also considered to have a higher fitness than fish in poor condition (Moyle and Cech, 2004; Diana, 2004; Blackwell, 2000). Fat or fit fish indicate favourable environmental conditions, such as ample food availability, low food competition or low predator interaction (Moyle and Cech, 2004; Diana, 2004; Blackwell, 2000). Whereas, thin or unfit fish indicate less favourable environmental conditions, such as food scarcity, increased predation, or high food competition (Moyle and Cech, 2004; Diana, 2004; Blackwell, 2000). As a result, aquaculture and fishery biologists

believe the utility of being able to determine the condition of fish to be invaluable as it can help to provide insight into the fitness of the organism as well as the status of the community in which it resides (Cox and Hartman, 2005; Pothoven, 2008; Moyle and Cech, 2004; Diana, 2004; Blackwell *et al.*, 2000; Duncan *et al.*, 2007).

2.5.1 **Condition indices**

A widely accepted theory is that fish weight is directly proportional to fish length cubed (Anibeze, 2000; Anderson and Neumann, 1996; Simpkins and Hubert 1996; Blackwell *et al.*, 2000). This relationship between length and weight is the cornerstone of fishery research and management (Anibeze, 2000; Anderson and Neumann, 1996; Simpkins and Hubert 1996; Blackwell *et al.*, 2000). Aquatic and fisheries biologists use many different techniques that rely on this relationship, described by species-specific ratios, to determine the condition of fish (Anibeze, 2000; Anderson and Neumann, 1996; Simpkins and Hubert 1996; Blackwell *et al.*, 2000). These ratios can be used to measure the impact that biotic and abiotic factors have on the condition of fish (Schlenk 2008). The condition indices that are primarily used by the scientific community to evaluate the condition of fish include Fulton's condition factor, the gonadosomatic index and the hepatosomatic index.

2.5.1.1 Fulton's condition Factor (K)

Fisheries managers often assess fish condition using Fulton's condition factor (K) as it provides a comprehensive measure of fish fatness (Moyle and

Cech, 2004). For rainbow trout, condition factor is expressed as a percentage of body weight compared to the cubed length of the fish (Parisi *et al.*, 2003). A high condition factor is indicative of good fish health and high energy content, whereas a low condition factor is indicative of poor fish health and low energy content (Fulton, 1904). The formula for calculating K is:

$$K = (\text{Total Body Weight} \times 100) \div \text{Total Length}^3$$

One of the problems associated with Fulton's condition factor is that comparing K values across species is impossible because different fish shapes result in different ranges of values for each species of fish (Blackwell, 2000). One major limitation of condition factor is that its results can become confounded by increased energy diversion and growth of specific tissues or organs (Cone, 1989). For instance: Hoque *et al.*, 1998 found that when *Mystus nemurus* , the common Baung catfish, is exposed to hydrogen sulphide, energy is diverted away from somatic or reproductive growth and directed toward the liver to facilitate increased detoxification activity and liver growth. The growth of the liver may confound the results of certain indices such as Fulton's condition factor (Moyle and Cech, 2004). However, if the hepatosomatic index (HSI) was used either alone or in conjunction with condition factor, the effects of the contaminant would likely be detected as the hepatosomatic index is a more sensitive indicator of liver toxicity than Fulton's condition factor (K) (Hoque *et al.*, 1998).

2.5.1.2 Hepatosomatic Index

The Hepatosomatic index (HSI) is widely used as an indicator of fish wellness and increased liver activity and growth (Schlenk 2008). The hepatosomatic index compares the relationship between the weight of the liver and the total weight of the fish (Hoque *et al.*, 1998). The HSI is based on the broad assumption that large livers indicate greater liver activity and growth, and a decreased overall wellness of the fish, possibly from toxicant removal (Hoque *et al.*, 1998). The general procedure associated with determining the HSI involves humanely euthanizing and weighing the fish, and then surgically removing and weighing the liver (Schlenk 2008). The HSI can then be calculated with the formula:

HSI = (Weight of Liver ÷ Total Body Weight) x 100

2.5.1.3 Gonadosomatic Index

Gonadosomatic index (GSI) is used as a general measure of the overall condition of fish and as an indicator of fish sexual maturation and spawning readiness (Schlenk 2008). Biologists use GSI to measure the relationship between the weight of the gonads and the total weight of the fish (Schlenk 2008). GSI is based on the broad assumption that proportionally larger gonads indicate greater sexual development and overall wellness of the fish, whereas, small, underdeveloped gonads are indicative of inhibited sexual function, maturation and poor condition (Moyle and Cech, 2004; Diana, 2004; Schlenk 2008). The general procedure associated with determining the GSI involves humanely euthanizing

and weighing the fish, and then surgically removing and weighing the gonads (Schlenk 2008). The GSI can then be calculated with the formula:

GSI = (Gonad Weight ÷ Total Body Weight) x 100

2.6 Traditional Methods to Determine Body Composition and Energy Content

2.6.1 Proximate Analysis

Proximate analysis is the most reliable method for determining body composition, and is thus considered the standard method by the scientific community (AOAC, 1990). Proximate analysis uses a variety of chemical analyses to determine ash, protein, lipid and water content of specific organs and tissues, or of the entire organism (AOAC, 1990). The problem with proximate analysis is that its associated techniques are lethal, lengthy and expensive (Cox and Hartman, 2005, Duncan *et al.*, 2007; Pothoven *et al.*, 2008; Crossin and Hinch, 2005). Furthermore, its associated complex and time-consuming laboratory procedures make it difficult and costly to analyze large sample sizes (Duncan *et al.*, 2007). The lethality of proximate analysis prevents the possibility for researches to perform repeated measures on the same individual, and impedes research involving body composition analysis on rare, threatened or endangered species (Cox and Hartman, 2005, Duncan *et al.*, 2007; Pothoven *et al.*, 2008; Crossin and Hinch, 2005). The shortcomings of proximate analysis motivated researchers to develop nonlethal methods for determining body composition.

2.6.2 Bomb Calorimetry

Bomb calorimetry is widely considered the most reliable method for determining the energy content in specific organs and tissues, or in the entire

organism (AOAC, 1990). Bomb calorimetry indirectly determines the caloric content of a tissue by measuring the change in water temperature which occurs when the tissue undergoes complete combustion and energy, in the form of heat, is released (AOAC, 1990; Crossin and Hinch, 2005). High caloric content in muscle is indicative of a healthy fish that has obtained a sufficient amount of calories, so that a surplus can be directed towards growth (Crossin and Hinch, 2005). Conversely, fish with a lack of energy in their tissues are unable to allocate energy toward somatic growth, and may be unhealthy (Crossin and Hinch, 2005).

The process of bomb calorimetry is a very long and tedious one, and requires expensive instrumentation that can only be found in the laboratory (Cox and Hartman, 2005, Duncan *et al.*, 2007; Pothoven *et al.*, 2008; Crossin and Hinch, 2005). Although bomb calorimetry provides reliable energetic estimates in the laboratory (AOAC, 1990); its cost and immobility make it quite prohibitive and impracticable for field work (Cox and Hartman, 2005). Furthermore, bioenergetic studies employing this method necessitate the killing of the fish to determine its energy content (AOAC, 1990; Cox and Hartman, 2005). The killing of the individual negates the ability for repeated measures on the same individual, and also may prevent studies involving endangered or threatened species (Cox and Hartman, 2005; Pothoven *et al.*, 2008). There are presently very few practical and reliable methods that can determine the bioenergetics of fish in a rapid and nonlethal manner (Crossin and Hinch, 2005). The lack of an efficient, reliable, and inexpensive method has limited more precise measurements of energy content in most growth studies (Cox and Hartman, 2005). The

shortcomings of bomb calorimetry have motivated researchers to develop a series of nonlethal methods for determining energy content.

2.7 Nonlethal Methodology

2.7.1 Total Body Electrical Conductivity

Total body electrical conductivity (TOBEC) uses an electromagnetic field to measure the conductivity of fish, thereby estimating internal composition (Fischer, 1996). The TOBEC method is based on the principle that the electrical conductance of an organism is proportional to its lean mass (Fischer, 1996). The TOBEC method held great promise until several studies suggested that its potential to accurately estimate the internal composition of fish was limited (Crossin and Hinch, 2005). These studies demonstrated that TOBEC did not reliably estimate lipid mass, and that errors increase with increasing total body weight and total body water (Novinger and Del Rio, 1999). Furthermore, the instrumentation associated with TOBEC is rather large and not well suited for field studies (Scott *et al.*, 2001).

2.7.2 Near-infrared Spectroscopy

Near-infrared Spectroscopy (NIR) is a spectroscopic method that uses the near-infrared region of the electromagnetic spectrum to measure the absorption, interactance and reflectance of water, lipid and protein (Downey, 1996). NIR has successfully been used to predict oil and protein concentrations in soy bean, oats, corn, and tobacco, and the fat, water and protein concentration in mice, pork, beef and fish, including rainbow trout (Lee *et al.*, 1992). Although a promising

technique, the equipment associated with NIR is expensive and not field appropriate (Lee *et al.,* 1992).

2.7.3 **Computerized Tomography**

Computer Tomography (CT) has been successfully used to determine and monitor changes to body composition and energy content in whole-fish over time (Hancz *et al.,* 2003). Computer tomography takes a cross-sectional image of the fish through the use of a rotating x-ray scanner (Hancz *et al.,* 2003). CT works to determine different components of the body based on their ability to block the X-ray beam (Hancz *et al.,* 2003). Although CT can accurately determine the body composition of fish, its associated equipment is expensive and not well suited for field use (Crossin and Hinch, 2005).

2.7.4 **Nuclear Magnetic Resonance**

Nuclear magnetic Resonance (NMR) has been successfully used to assess of the composition and energy content of muscle tissue, and to monitor the impacts that feed additives have on fat and protein deposition (Beauvallet, 1992). NMR works to determine the molecular structure of tissue by measuring the quantity of radio frequency that cellular nuclei absorb when exposed to a strong magnetic field (Beauvallet, 1992). Although NMR is proven to accurately determine body composition and energy content, its instrumentation is quite expensive and delicate, and therefore not well suited for the rigours associated with field studies (Sacchi *et al.,* 1993).

2.7.5 **Microwave Transmission**

Microwave transmission is another recently developed nonlethal and non-invasive method for estimating the body composition and energy content of fish (Whiterod, 2009). MT meter works by emitting low-powered microwaves to measure body composition (Crossin and Hinch, 2005). The MT meter is highly portable, making it well suited for field studies (Crossin and Hinch, 2005). The problem with microwave transmission is that the meter saturates when tissue energy densities are very low or when water levels are exceedingly high, as is the case with spawning Pacific salmon (Whiterod, 2009; Crossin and Hinch, 2005). The saturation of the microwave transmission meter causes a deterioration of accuracy and inconsistent results (Whiterod, 2009). Although this technique is ideal for field work due to its portability, accuracy may decline when testing fish with low lipid levels (Crossin and Hinch, 2005)

2.7.6 **Ultrasound**

Ultrasound, a technique commonly used by the medical community to determine the boundaries between different tissues, has been successfully used to measure the lipid content of fish (Probert and Shannon, 2000). Ultrasound works by measuring the velocity and attenuation of sound as it echoes through the body (Shannon *et al.,* 2004). Different tissue types conduct sound differently, and thus will produce different echoes (Probert and Shannon, 2000). Ultrasound waves pass quickly and easily through water and lean tissues, however, fattier tissue cause the velocity of the echo to significantly increase and the attenuation to

decrease (Shannon *et al.,* 2004). Although this technique has shown promise as a means to determine body composition, its resolution drastically decreases at depths greater than a few millimetres (Probert and Shannon, 2000). High powered ultrasound devices can penetrate further to assess subcutaneous fat deposits, but may pose a health risk to fish (Probert and Shannon, 2000).

2.7.7 **Electromyogram radio telemetry**

Electromyogram (EMG) radio telemetry is a recently developed nonlethal and non-invasive method for estimating the energetic cost of activity of fish (Hruska *et al.,* 2007). This technique measures the change of bioelectrical voltage of fish while swimming (Hinch and Rand, 1998). This technique uses EMG tags that emit pulses of averaged EMG signals over a time interval (Hinch and Rand, 1998). EMG radio telemetry makes it possible to quantify the metabolic cost of activity by calibrating the averaged EMG signals with swimming speed, oxygen consumption and tail-beat frequency (Hinch and Rand, 1998). The problem with this technique is that each individual tag must be calibrated to each individual fish (Hruska *et al.,* 2007). The calibration procedure associated with this technique makes research on large populations of fish very impractical and expensive (Hruska *et al.,* 2007).

2.7.8 **Non-destructive Physiological Examinations:**

Another nonlethal method for estimating the body composition and energy content of fish is by means of non-destructive physiological examinations (Hruska *et al.,* 2007). This technique involves excising a small piece of muscle tissue, and then performing bomb calorimetry and proximate analyses on that sample to

determine the energy content and body composition of the fish (Crawford *et al.*, 1977). The problem associated with this technique is that the amount of tissue that can be removed from a fish before it starts to severely impact the health of the fish is limited, and it is an invasive procedure that increases the risk of infection (Crossin and Hinch, 2005).

2.8 Bioelectrical Impedance Analysis

Bioelectrical impedance analysis is the study of the relationship between biological material and electrical impedance (Kyle *et al.*, 2004). This relationship was known to exist since the late 1960's (Hoffer *et al.,* 1969), however, research into using BIA as a means to measure the well-being of humans was not published until 1985 (Lukaski *et al.,* 1985). Over the last 25 years, BIA has become a very popular method to estimate body composition in people (Dehghan and Merchant, 2008). As of 2008, more than 1600 published articles have been reported to use BIA as a tool to measure body composition since 1990 (Dehghan and Merchant, 2008). Today, the medical community commonly uses BIA as a rapid, reliable, nonlethal technique, to estimate and monitor changes in body composition and to assess nutritional status (Gupta *et al.,* 2009). Over the years, however, BIA has been used to assess patients suffering from Duchenne Muscular Dystrophy (Mok *et al.,* 2006), HIV (Eisenmann *et al.,* 2004), obesity (Lohman *et al.*, 2000), Coeliac disease (Ratsch and Catassi, 2001), AIDS wasting (Corcoran *et al.,* 2000) and malnutrition (Pirlich *et al.*, 2004).

According to Ohm's law, resistance is proportional to the voltage of an applied current as it passes through a substance (resistance = voltage divided by

the current); whereas, reactance is directly proportional to the opposition of a current by a capacitor (Kyle *et al.,* 2004). Researchers use BIA to measure the resistance and reactance of their test subjects by recording the voltage drop of applied current once it has traveled through the body (Gupta *et al.,* 2009). In this case, resistance is the restriction to the flow of electric current through the body, and is directly proportional to the amount of lipid present in the tissue, and indirectly proportional to the amount of water present in the tissue (Gupta *et al.,* 2009). Reactance is the capacitive effect caused by the cell membranes and tissue interfaces, and is directly proportional the health of cells in the body (Willis and Hobday, 2008). Water and muscle are much better conductors of electricity than bone and fat, therefore, an increase in water and lean muscle mass and a decrease in bone and fat mass will lessen the overall resistive force of the body (Duncan *et al.,* 2007).

Cell membranes are made up of a phospholipid bilayer, which consists of one layer of non-conductive lipid material situated between two layers of conductive protein molecules (Figure 3). The selective permeability of the cell membrane functions as an ionic gradient by using its protein channels to only allow certain materials to pass into and out of the cell (Figure 3) (Liedtke, 1997). The electrical signal from BIA passes into the cell via the protein channels, causing the cell to act as a capacitor (Figure 3) (Kyle *et al.,* 2004). The capacitance of the cell causes a drop in the applied current, which is then detected and recorded by the analyzer as the reactance of the tissue (Duncan *et al.,* 2007).

The signal that the BIA analyzer emits is of a low voltage and high frequency (800 μA, AC and 50 kHz) (Duncan *et al.,* 2007). The electrical properties of the signal allow the current to pass through extracellular fluids but not through cell membranes (Figure 3) (Kyle *et al.,* 2004). The resistance of the tissue is directly proportional to the voltage of the current as it passes through its extracellular fluid (Figure 3) (Kyle *et al.,* 2004). Since fat cells have no cell membrane, reactance is not impacted by the quantity of body fat in the tissue (Liedtke, 1997). Only cell membranes offer capacitance, therefore, reactance can be used as a measure of the cell membrane capacitance and body cell mass (Liedtke, 1997). Body water and extracellular body fat offer resistance to an applied electrical current, therefore, resistance can be used to measure total body water, total body ash, fat-free mass, total body protein, dry mass, total body fat and energy content (Liedtke, 1997).

Researchers can transform resistance and reactance measurements from a series orientation to a parallel orientation to better explain the electrical properties of a biological organism (Cox and Hartman, 2005; Pothoven *et al.*, 2008; Duncan *et al.,* 2007). These transformed measurements can then be used to develop BIA equations, such as the Conductor-Volume model (L^2/R_p) to determine proximate body composition and energy content (Cox and Hartman, 2005; Pothoven *et al.,* 2008; Duncan *et al.,* 2007). Researchers can then develop species-specific models for both proximate composition and energy content by relating the BIA electrical equations with the actual composition values determined through bomb calorimetry and proximate analysis (Cox and Hartman, 2005; Pothoven *et al.,*

2008; Duncan *et al.*, 2007). The utility of BIA is not limited to research studying human physiology, but can also be applied to plant, animal, avian and fish studies as well.

As BIA became established in the medical literature, it also started to be applied in studies involving a variety of plant, animal, bird and fish species. The livestock industry has applied BIA to assess the body composition and condition of a variety of farmed species including cows (Marchello *et al.*, 1999, Marchello and Slanger, 1994), sheep (Berg *et al.*, 1996), goats (Kohli *et al.*, 1998), and pigs (Marchello *et al.*, 1999). BIA has also been successfully used to assess body composition and well-being in wildlife studies involving species of bear *Ursus americanus and Ursu arctos* (Hilderbrand, 1998), skunk *Mephitis spoligale* (Hwang *et al.*, 2005), seal *Halichoerus grypus* (Gales *et al.*, 1994), moose *Alces alces* (Hundertmark, 2002) and turkey *Meleagris gallopavo* (Grimes *et al.*, 1990).

BIA first appeared in the fisheries literature when Bosworth and Wolters (2001) determined fat content of channel catfish *Ictalurus punctatus* fillets; and when Cox and Hartman (2005) were able to correlate BIA readings of resistance and reactance with energy density, fat mass, protein mass, dry mass, total body water mass, and ash mass of live brook trout *Salvelinus fontinalis* with notable success. A limited number of subsequent studies used BIA to estimate the proximate composition and energy content of a variety of fish species including chinook salmon *Oncorhynchus tshawytscha*, chum salmon *Oncorhynchus keta* (Margraf *et al.*, 2006), cobia *Rachycentron candadum* (Duncan *et al.*, 2007), yellow perch *Perca flavescens*, walleye *Sander vitreus*, lake whitefish *Coregonus*

clupeaformis (Pothoven *et al.*, 2008), southern bluefin tuna *Thunnus maccoyii* (Willis and Hobday, 2008), sea bass *Dicentrarchus labrax* (Vidachek *et al.*, 2008), red hake *Urophycis chuss*, Acadian redfish *Sebastes fesiatus*, haddock *Melanogrammus aeglefinus*, Atlantic cod *Gadus morhua*, pollock *Pollachius virens*, winter flounder *Pseudopleuronectes americanaus*, American plaice *Hippoglossoides platessoides*, yellowtail flounder *Limanada ferruginea*, Atlantic herring *Cluppea harrengus*, tilefish *Lopholatitus chameleonticeps* and black sea bass *Centropristis striata* (Fitzhugh *et al.*, 2010). All of these studies were remarkably successful using BIA as a means to determine body composition and energy content.

The success of BIA studies involving fish is attributed to the suitability of most fish species to BIA testing (Bosworth and Wolters, 2001; Cox and Hartman, 2005). Fish typically have cylindrically-shaped bodies that lack complication (Cox and Hartman, 2005). A lack of structural complications, such as appendages, enables BIA to accurately characterize the electrical impedance of most fish (Pothoven *et al.*, 2008). Although the success of BIA is making it increasingly popular to use in fishery and aquatic research, caution is required as there are still unanswered questions. One such question is how differential growth influences BIA. Understanding the effect that growth has on BIA will enable researchers and biologists to better assess changes in body composition and energy content, and ultimately condition, in both the laboratory and in the wild.

BIA appears to be a promising technique for assessing the body composition and energy content of fish. The electrodes associated with BIA leave

only slight bruising on the epidermis of the fish, of which is quick to heal (Cox and Hartman, 2005). Furthermore, the voltage and frequency of the applied current are well below the pain threshold, so fish do not feel any pain during the test (Cox and Hartman, 2005). Moreover, BIA is nonlethal and takes approximately 30 seconds to perform, and therefore can work in situations where traditional methods are considered inadequate (Cox and Hartman, 2005; Pothoven *et al.,* 2008; Duncan *et al.,* 2007). Although the use of BIA in fishery science is still quite young, it's quickly becoming increasingly popular in aquacultural and ecotoxicological studies as a tool to assess the bioenergetics of fish.

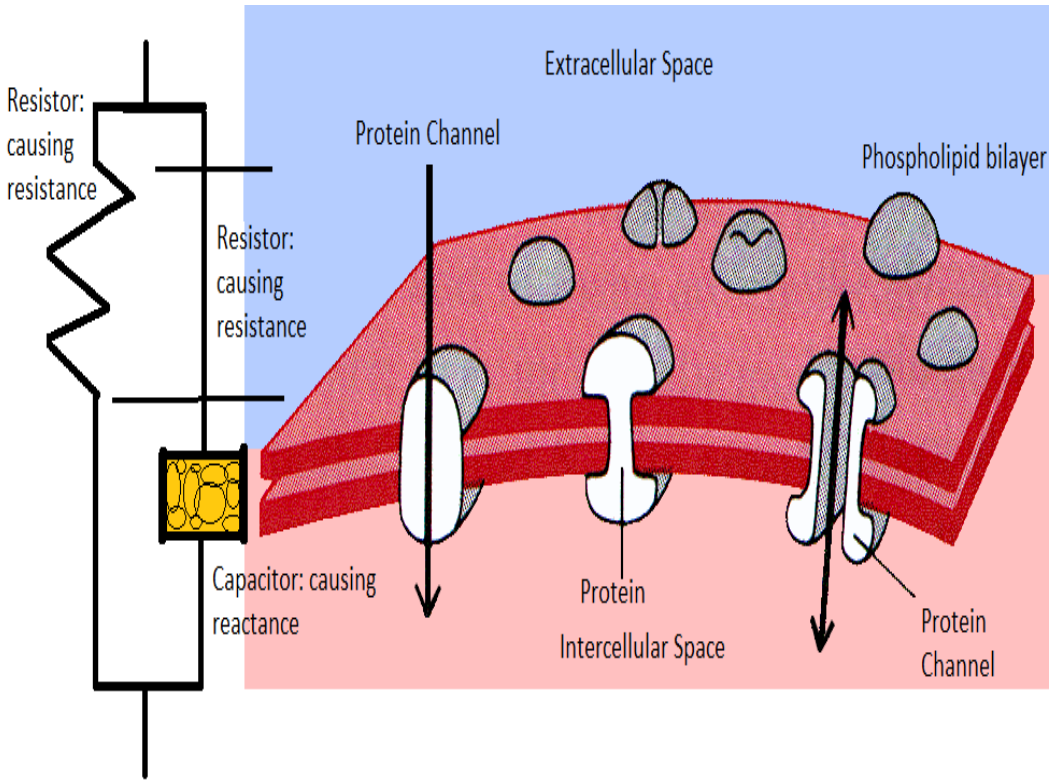

Figure 3: The Plasma Membrane of a Cell and its Electrical Equivalent. (Adapted from Liedtke, 1997)

2.9 Rainbow trout (*Oncorhynchus mykiss*)

2.9.1 Classification

Oncorhynchus mykiss, formerly *Salmo gairdneri*, is a species of salmonid native to North America and Asia (Quinn, 2004). Their native range in North America extends from the northwest part of Mexico to the Kuskokwim River in Alaska (Ross, 2001). Their Asian range is limited to the Kamchatka Peninsula in Northern Russia (Quinn, 2004). There are two genetically distinct ecotypical forms of *Oncorhynchus mykiss* (Moyle and Cech, 2004). Rainbow trout are the freshwater form, and steelhead trout are the anadromous form (Figure 4) (Moyle and Cech, 2004). Rainbow trout reside within freshwater for their entire lives, whereas; steelhead leave their freshwater spawning grounds, after about a year as smolts, only to return to spawn after a few years at sea (Quinn, 2004). Rainbow trout and steelhead are iteroparous species of fish, able to spawn several times over the course of their lives (Quinn, 2004).

2.9.2 Physical Characteristics

The body of a rainbow trout is cylindrical in shape, almost like a torpedo (Figure 4) (MNR, 2011). Its fine scales are silver in colour along the sides and bluish green along the back (Figure 4) (Quinn, 2004). Rainbow trout have a spattering of black spots along the back and sides, with a horizontal pinkish band along the lateral line that can vary in intensity (Figure 4) (MNR, 2011). Rainbow trout mature between four to six years, and can live up to a maximum of 11 years (Carlander, 1969).

2.9.3 **Food**

Rainbow trout and steelhead trout are opportunistic feeders that will eat a wide variety of food items (MNR, 2011) In lakes and streams, invertebrates such as leeches, plankton, snails, insects and crayfish, as well as small fish and fish eggs serve as sources of food for rainbow trout (Needham, 1969). Steelhead trout feed upon the considerable amount of small fish and shrimp in the ocean (Needham, 1969). The abundance of small fish and shrimp in the ocean are considerable and provides food for rapid growth (Quinn, 2004).

2.9.4 **Growth**

Rainbow trout grow at highly variable rates, relying heavily on the habitat in which they reside. Steelhead trout encounter more food in the ocean than freshwater rainbow trout, and thus grow at a faster rate (Quinn, 2004). Moreover, rainbow trout and steelhead grow indeterminately and allometrically (Anderson and Neumann, 1996). Rainbow trout never cease to stop growing throughout their entire lives and, as they lengthen and increase in mass, the relationship between length and weight is not linear (Anderson and Neumann, 1996).

2.9.5 **Validity as a Test Species**

Rainbow trout is an ideal test species as it can be obtained all year from commercial suppliers (EPA, 2002), can be easily reared in the lab, are not easily stressed out when handled, and can be found in the local environment (Figure 5) (EPA, 2002) Furthermore, rainbow trout and its associated subspecies are

approved by the U.S. Environment Protection Agency and Environment Canada as a coldwater indicator species for acute and chronic freshwater aquatic toxicity testing (USEPA, 2009; Environment Canada, 2007). This designation has greatly influenced the use of rainbow trout as the test organism in this research. Furthermore, subsequent growth inhibition experiments following the completion of this study intend to apply BIA techniques, calibrated by the research, to determine the impact that toxicant exposure has on the bioenergetics of rainbow trout.

Figure 4: Rainbow trout *Oncorhynchus mykiss* in its freshwater (top) and anadromous form (bottom). Rainbow trout are the freshwater form, and steelhead trout are the anadromous form. (Modified from MNR, 2010).

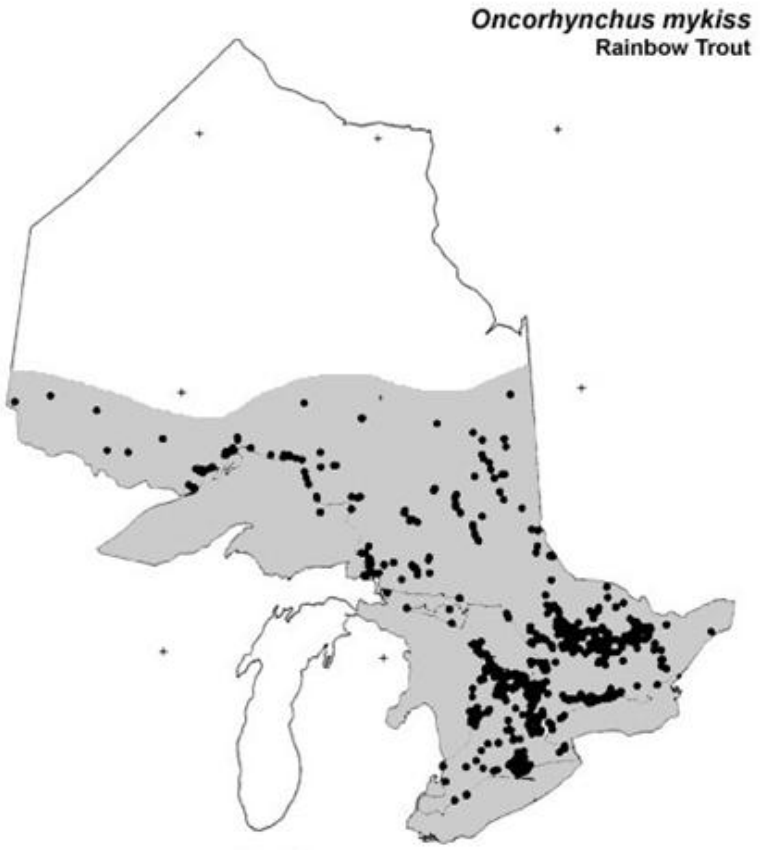

Figure 5: Distribution of rainbow trout in southern and central Ontario. (Adapted from Mandrak and Crossman, 1992).

3.0 RESEARCH OBJECTIVES:

3.1 Overall Objective

The overall objective of this research was to evaluate the potential of bioelectrical impedance analysis to predict the energy content of juvenile rainbow trout (*Oncorhynchus mykiss).*

3.1.1 Specific Objectives

(1) Subject juvenile rainbow trout (*Oncorhynchus mykiss*) to one of three ration regimes, maintenance, optimum and satiation, over a three-month period, as a means to achieve three significantly different tiers of growth.

(2) Correlate the conductor-volume model, using transformed resistance and reactance data, with the actual energy, lipid and moisture content of the fish, determined through bomb calorimetry and proximate analysis, via linear regression analysis.

(3) Develop species specific indices to predict the proximate body composition and energy content of juvenile rainbow trout.

(4) Validate the use of BIA as a biomarker to accurately assess differential growth patterns of juvenile rainbow trout.

(5) Add to the growing body of evidence showing that BIA may become the next standard technique for determining the bioenergetics of fish.

4.0 MATERIALS AND METHODS

4.1 Laboratory Fish

A mixed sex natural population of 240 juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from the Rainbow Springs trout hatchery in Thamesford, Ontario, on June 6th, 2010. The trout were transported from the trout hatchery to UOIT in the aquatic holding tank of the Aquatic Toxicology F150 Pickup Truck. Upon arrival, the trout were transferred from the holding tank to a 1500 L coldwater tank (C-1000-7) containing 1000 L of water located in the Aquatic Toxicology Wet Laboratory. While in the Wet Laboratory, the trout were subjected to a 16-h light: 8-h dark photoperiod with a 30 minute simulated dawn and dusk included in the light phase.

4.2 Ration

4.2.1 Feed

Trout pellets fed to the fish were purchased from Martin Mills, Inc. Elmira, ON, on June 5th, 2010. Two different sizes of trout pellets (2 pt and 2.5 pt) were used in this study. The 2 pt pellets consisted of 45.0 % (min) crude protein, 22.0 % (min) crude fat, 1.5 % (max) crude fibre, 1.4 % (actual) calcium, 1.0 % (actual) phosphorus, 1.3 % (actual) sodium, 10,000 IU/kg vitamin A, 315 IU/kg vitamin C, 2800 IU/kg vitamin D3, and 320 IU/kg vitamin E. The 2.5 pt sized feed consisted of 44.0 % (min) crude protein, 16.0 % (min) crude fat, 3.0 % (max) crude fibre, 1.2 % (actual) calcium, 1.0 % (actual) phosphorus, 1.3 % (actual) sodium, 12,000 IU/Kg vitamin A, 210 IU/kg vitamin C, 2850 IU/kg vitamin D3, and 300 IU/kg vitamin E. The 2 pt sized pellets were used from the beginning of

the study to day 60, whereas the 2.5 pt sized pellets were used from day 60 to day 90 of the experiment. This change was necessary because the supply of 2 pt became seriously depleted during the second month of the experiment, and the only other suitable size of trout food available was the 2.5 pt sized pellets.

4.2.2 **Initial Ration Regimes**

Trout were fed either a maintenance ration that consisted of 0.4 % BW/day (Nourollahi-Fard & Woo, 2008; Russell, 1977), an optimum ration of 1.6 % BW/day (Russell, 1977; Nepal *et al.,* 2002) or a satiation ration of 3.4 % BW/day (Boujard & Medale, 1994; Glencross *et al.,* 2006). Ration regimes were based on both the actual and predicted tank biomass. The day 0 to day 30 ration regimes were based on the actual tank biomass, determined from the actual starting fish weights that were recorded during tagging.

Trout were fed their associated ration regimes using nineteen 150 ml plastic cups. The cups were marked with a fill line that reflected its associated tanks ration regime. The cups were labelled with their associated experimental replicate, ration regime, treatment replicate and tank number.

4.2.3 **Daily Feeding Frequency**

Trout were fed twice a day, at 9:00 AM and at 2:00 PM, from July 6th to July 15th. It was found, however, that feeding the trout three times a day instead of two would increase feeding efficiency and, therefore maximize growth potential (Ruohonen *et al.,* 1998). Thus, the trout were fed three times a day, at 9:00 AM, 12:00 PM and 3:00 PM, for the remainder of the experiment. The only time the fish were not subject to their daily feeding regimes was 24 hours prior to

sampling. Fish were starved for 24 hours before sampling to void their stomachs and bowels of excess food and fecal material. This 24 hour starvation period facilitated accurate weight determination during sampling.

4.2.4 **Ration Regime Revision**

Mean measurements of weight that were obtained from the previous sampling period were used to estimate remaining tank biomass. Ration regimes from day 30 to day 90 were based on the post-sampling predicted tank biomass. New feeding regimes for each tank were calculated by multiplying the tanks ration percentage (0.4 %, 1.6 %, or 3.4 %) by the predicted tank biomass. New fill lines were drawn on each of the 150 ml plastic cups to reflect these revised ration regimes. The trout were subjected to their new ration regimes the day after sampling.

4.3 Daily Maintenance and Records

4.3.1 **Vacuuming**

The tanks were vacuumed extensively prior to the first feeding of the day. Vacuuming the tanks prior to feeding helps to limit the potential for fecal material to be ingested by the fish while feeding. Once vacuumed, the tank lids were dismantled to gain access to the standpipe and standpipe filter. The standpipe filters were removed and cleared of blockages. Once all blockages were removed, the standpipe filters were replaced. The tank lids were then reassembled and inspected to make sure that they fit correctly. The time that the tanks were vacuumed was recorded on the daily monitoring form.

4.3.2 **Water Temperature**

A VWR Scientific water resistant digital thermometer was used to take daily measurements of temperature. Individual tank temperatures were recorded on the daily monitoring form.

4.3.3 **Water pH**

A Mettler Toledo SevenEasy bench-top pH meter was used to take daily measurements of pH. The pH meter was calibrated at three points (pH 4.0, pH 7.0 and pH 10.0) daily before measurements were taken. Individual tank pH measurements were recorded on the daily monitoring form.

4.3.4 **Air and Water Check**

Air-stones and water supply tubes were visually inspected on a daily basis to see if they were working properly. The time that the air-stones and water supply tubes were inspected was recorded on the daily monitoring form.

4.3.5 **Time of Feeding**

Trout were fed three-times a day for the duration of the experiment. The time(s) that the fish were fed were recorded on the daily monitoring form.

4.4 Stratified Tank Setup

4.4.1 **Tank Labeling**

Nineteen 70 L aquaria were randomly selected and labelled by experimental replicate, ration regime and treatment replicate using three different colours of masking tape. Yellow tape was chosen to represent the first experimental replicate, green tape was chosen to represent the second experimental replicate and blue tape was chosen to represent the Time Zero tank. Two experimental

replicates were performed in this study to assess the reproducibility of the results. The experimental tanks were labelled with either an “M” to represent the maintenance ration, an “O” to represent the optimum ration, or an “S” to represent the satiation ration (Figure 6). The Time Zero tank, however, was labelled simply “Time Zero” (Figure 6). In addition to the two different colours of masking tape, the experimental tanks were labelled with either an “R1” to represent the first experimental replicate or an “R2” to represent the second experimental replicate (Figure 6). The rations regimes in the first and second experiment were triplicated. The triplicate treatments were labelled either as “1” to represent the first triplicate, “2” to represent the second triplicate, or “3” to represent the third triplicate (Figure 6). The tanks were situated on top of one another in two rows of ten.

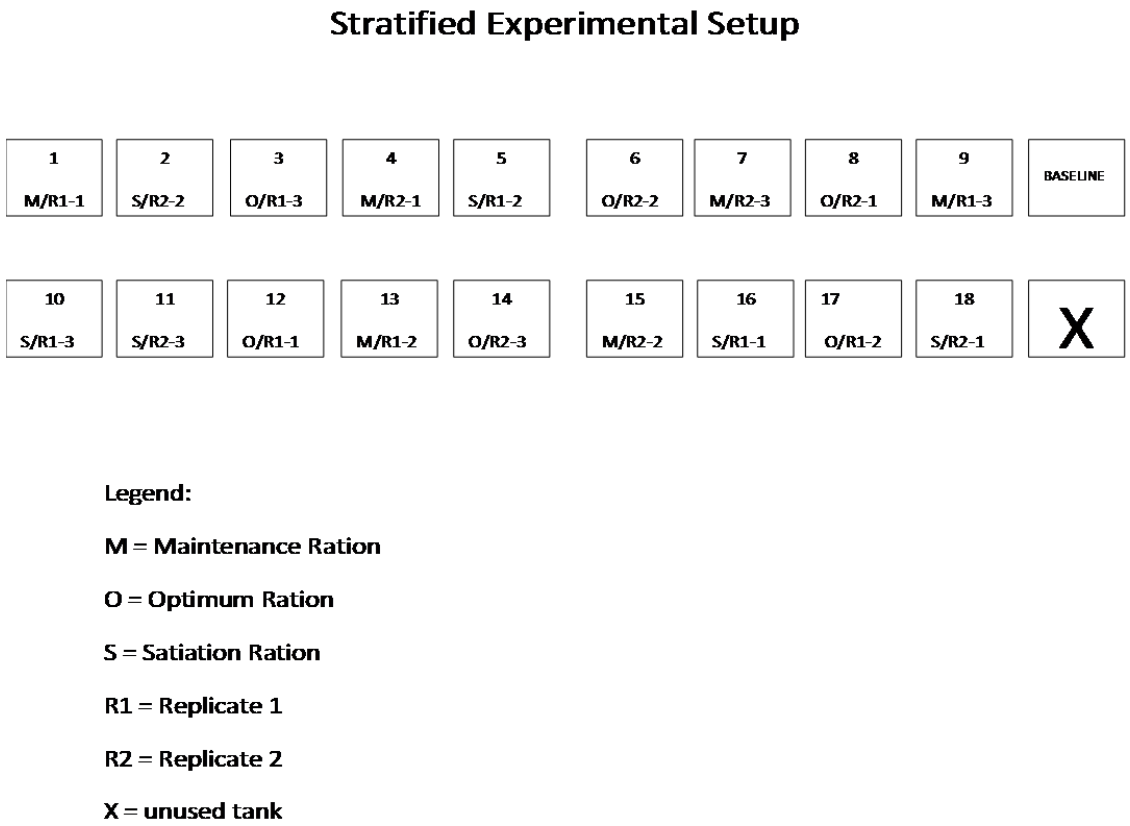

Figure 6: Stratified experimental tank setup. This figure illustrations the stratified and randomized positioning of experimental replicates, treatments, and replicates of treatments.

4.4.2 **Physical Tank Setup**

A black plastic divider was inserted between each tank to minimize tank interaction. All tanks were equipped with an air-stone and a water tube. The flow of 12^{o} C water into each tank was set at 3.4 ml/sec, which equates to four physical turnovers or 99 % molecular turnover per day. The tanks were equipped with a black plastic lid, fastened down with circular plastic clips made from standpipe tubing. The tanks were also equipped with a twelve-inch standpipe, elbow fitting and plastic filter. The tanks were filled with 70 litres of 12^{o} C water once the dry setup was complete.

4.5 1500 L Tank Acclimation

The rainbow trout were held in a 1500 L tank (labelled C-1000-7) containing 1000 L of water for one month until they were scheduled to be tagged and transferred to the 70 L aquaria. Trout were fed an optimum ration (1.6 % bw/day), three times a day, during the first week of acclimation. This initial feeding regime was designed to lessen the impact of their sudden environmental transition, and to wean them onto the feed. In the following weeks the trout were fed a maintenance ration (0.4 % bw/day) to ensure that growth was kept to a minimum.

4.6 Tagging

On July 14th and 15th, the experimental fish were removed from tank C-1000-7 and anesthetised using MS-222 in a 100 mg/L solution. Once anesthetised, the trout were weighed using a Mettler Toledo PB3002-S Delta Range Balance, measured for fork, standard and total length using an Aquatic Ecosystems Fish Measuring Board, and then tagged with numbered T-Bar anchor tags using an

Avery Dennison© tagging gun. Tags were injected in the muscle on the port side of the fish, just shy of the dorsal fin. Once tagged, trout were placed in a recovery bucket with an air-stone. Once recovered, the trout were transferred to one of the eighteen 70 L aquaria. Once all the experimental fish were successfully tagged, recovered and transferred to their associated tanks, an additional twelve fish were taken from tank C-1000-7 and placed in the 70 L Time Zero tank.

4.7 70 L Aquaria Acclimatization

The trout from the first and second experimental replicate were tagged and transferred to the 70 L aquaria on the 14th and 15th of July. All tanks were stocked with twelve rainbow trout, at an initial stocking density of 8.5 kg/m^3. The initial stocking density of 8.5 kg/m^3 avoided any adverse density-dependant growth impacts, while maintaining a statically relevant number of test organisms. The trout were left to acclimatize in the 70 L tanks for the duration of one week. During this time, the tanks were fed an optimum ration of 1.6 % bw/day. This ration percentage was used to help the trout reach a starting weight of approximately 50 g.

4.8 Growth Experiment

4.8.1 Time Zero

Five fish were sampled from the Time Zero tank on the 21st of July, one day prior to the onset of the study.

4.8.2 Experimental Population

The first and second growth experiment started on the 22nd and 23rd July. Four fish were sampled from each tank once every thirty days for three months. The

day 30 sampling for the first and second experimental replicates occurred on the 23rd and 24th of August; the day 60 sampling date for the first and second experimental replicate occurred on the 21st and 22nd of September; and the day 90 sampling date for the first and second replicate occurred on the 19th and 20th of October

4.9 Data Collection

4.9.1 Euthanasia

Rainbow trout were netted from each tank and euthanized in a 100 mg/L solution of MS-222. Samples from each tank were euthanized and sampled together as a means to avoid sampling errors from occurring.

4.9.2 Lengths and Weights

The trout were weighed on a Mettler Toledo PB2003-S Delta Range Balance to the nearest 0.01 g. Once weighed, the trout were measured for fork, standard and total length using an Aquatic Eco-Systems Fish Measuring Board. Weights and lengths were recorded on the data collection sheet for future analysis.

4.9.3 Bioelectrical Impedance Analysis

Bioelectrical Impedance Analysis (BIA) was performed on all sampled fish using a Quantum 3 Body Composition Bioelectrical Impedance Analyzer from RJL Systems Inc. The Quantum 3 Analyzer has two sets of needle electrodes (stainless steel, 28 gauge, 12mm). Each set of electrodes consists of one signal and one detecting electrode. The signal and detecting electrodes were positioned 1cm apart from one another. The first electrode set were positioned in the anterior dorsal region, and the second set of electrodes were placed in the caudal peduncle

region of the trout (Figure 7) (Cox and Hartman, 2005). The electrodes were consistently positioned in the same two locations on the trout throughout the study. Both sets of electrodes were positioned in series, midway between the lateral line and the dorsal midpoint (Cox and Hartman, 2005). The anterior set of electrodes were positioned so that they were situated at the anterior apex of the operculum (Cox and Hartman, 2005). The posterior electrodes were aligned with the anterior edge of the dorsal fin (Cox and Hartman, 2005). The electrode needles penetrated a depth of two millimeters into the tissue of the fish. The distance between the two sets of electrodes was measured with a set of Fisher Scientific calipers, and recorded on the data collection sheet. The distance between the electrodes was entered into the analyzer to help generate an accurate measure of resistance and reactance. A current was then introduced into the trout through the signal electrodes. The drop in voltage was instantly measured by the detecting electrodes. Accurate measures of resistance and reactance were generated by the analyzer and recorded onto the data collection sheet for future analysis.

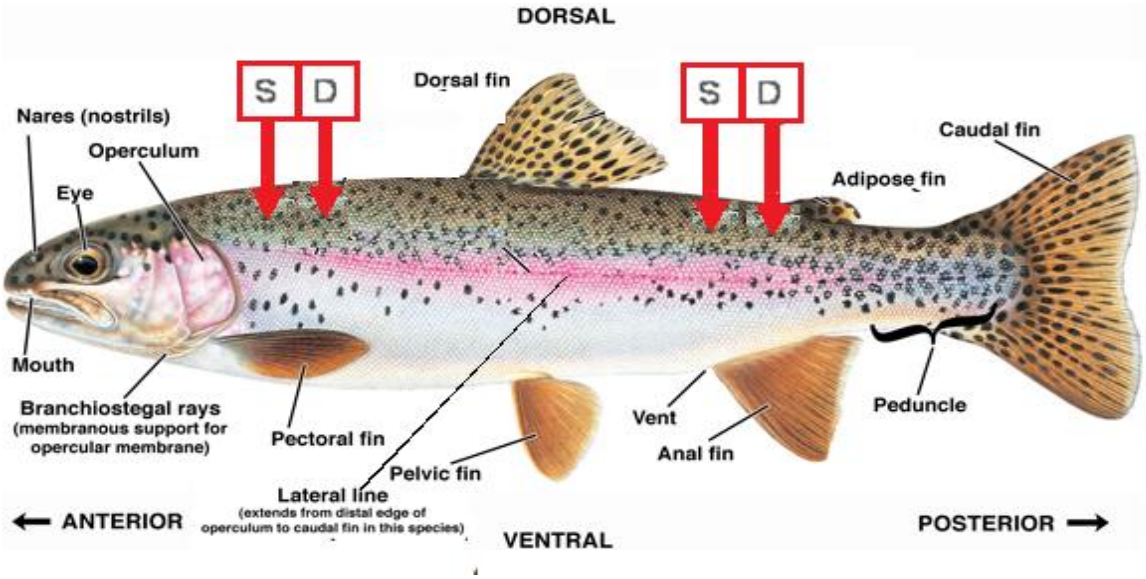

Figure 7: Diagram of locations for signal (S) and detector (D) electrodes used in measuring bioelectrical impedance values of rainbow trout (*Oncorhynchus mykiss*). The first set of electrodes were positioned in the anterior dorsal region, and the second set of electrodes were placed in the caudal peduncle region of the trout. (Adapted from Cox and Hartman, 2005).

4.10 Dissection

Trout were dissected longitudinally by inserting scissors into the anal vent and cutting towards the head, ending just after the operculum. The liver and gonads were excised and weighed to the nearest 0.001 g using a Mettler Toleto PB503-S Analytical Balance. Liver and gonad weights were recorded on the data collection sheet for future analysis. The gonads were visually examined as a means to determine the sex of the fish. The sex of the fish was recorded on the data collection sheet for future analyses involving gender. Duplicate one gram tissue samples were excised from the area between the BIA electrode placements.

The two tissue samples were excised by inserting the tip of the surgical scissors into the flesh of the fish, 1cm below the dorsal fin on the lateral line, and then cutting towards the operculum, stopping 0.5cm short of the electrode placement. The skin of the fish was peeled back using a pair of stainless steel tweezers. Once the skin was peeled all the way back exposing the tissue, both samples were excised using the pair of surgical scissors. The excised tissue samples were patted dry with paper towel and weighed to the nearest 0.001 g on the Mettler Toleto PB503-S Analytical Balance. Tissue weights were recorded on the data collection sheet for future analysis. The tissue samples were wrapped in pre-weighed Fisherbrand Aluminum Foil, and then immediately placed in a Styrofoam container filled with dry-ice. Once the sampling was complete, the tissue samples were stored in the Thermo Electron Corporation Forma -86^{o} C ULT Freezer at -80^{o} C.

4.11Freeze-Drying Tissue Samples

Tissue samples were taken out of the freezer and placed into a Styrofoam container filled with dry-ice. The samples were then transported to the room that houses the Thermo Electron Corporation ModulyoD Freeze Dryer. The frozen tissue samples were placed into several 100-500 ml flasks and freeze-dried for 72 hours. Once dried, the tissues were placed in a dessicator and transported back to the dry lab. The tissue samples remained in the dessicator until they were reweighed.

4.12 Reweighing and Storing Freeze-dried Tissue Samples

Tissue samples were taken out of the dessicator and reweighed on the PB503-S Mettler Toleto Analytical Balance to the nearest 0.001 g. Once reweighed, the samples were placed back into the dessicator to be stored until they were homogenized and compressed into pellets.

4.13 Homogenizing and Pelletizing Tissue Samples

Duplicate tissue samples were taken from the dessicator and homogenized using an IKA Works All Basic S1 tissue grinder. The pulverized tissue samples were compressed into 0.635 cm 0.2 g pellets using the dry lab's Parr 2812 Pellet Press, (Parr Instrument Company). The 216 experimental and five Time Zero samples were pelletized and stored in 15 ml plastic microtubes. The microtubes, now filled with pelletized tissue, were placed into the dessicator until they were scheduled for caloric determination via bomb calorimetry.

4.14 Bomb Calorimetry

4.14.1 **Standard Operating Procedure**

Individual samples were taken out of the dessicator and weighed to the nearest 0.001 g on the Mettler Toleto PB503-S Analytical Balance. The 10cm platinum fuse wire was then attached to one of the two hook terminals located on the head of the bomb (Parr, 2008). Once one end of the fuse wire was attached to the hook terminal, the other end was wrapped around an Allen key to form a five turn helical coil (Figure 8) (Parr, 2008). The coiled wire served to hold the sample in place while handling the bomb prior to ignition, and to concentrate the heat on the sample during combustion (Parr, 2008). Once the fuse wire was coiled and attached to both hook terminals, the sample was set in the capsule support loop and positioned so that the coiled fuse wire and sample made contact (Parr, 2008). Once contact was made between the sample and the coiled fuse wire, the head of the bomb and the screw cap were set into the bench socket. Once positioned in the bench socket, the body sleeve was slid up the head of the bomb and attached to the screw cap (Figure 8) (Parr, 2008). These parts were tightened firmly with an octagon wrench (Parr, 2008).

The conductivity of the electrical circuit was tested using an Equus 4320 voltage meter. This conductivity test detects bad electrical connections, which could cause the bomb to misfire. If the bomb passes the conductivity test it can then be filled with exactly 30 atmospheres of ultra high purity oxygen. To fill the bomb with oxygen, the hose from the Praxair 45.36 kg high purity oxygen tank was attached to the gas inlet tube on the bomb by sliding the A233A2 Slip

Connector downward onto the gas inlet tube until it rested firmly on the valve cap. The 10MB3 Pin Wrench was then inserted through the eye of the valve cap and turned one full revolution from the closed to the open position. Leaving the pin wrench in the cap, the bomb was filled with exactly 30 atmospheres of oxygen. The gas within the bomb was then purged by lifting the bleeder valve on the regulator of the oxygen tank. This step was repeated three times to ensure that the bomb contained no gas other than oxygen. The pin wrench was used to close the valve on the bomb at the end of the oxygen filling cycle. Residual pressure was released from the filling hose by lifting the bleeder valve on the oxygen tank regulator. The wrench pin was removed from the valve cap and the A233A2 Coupler was lifted off of the gas inlet tube.

The Dewar was filled with 450 ml of distilled water using the Mettler Toledo PB3002-S Delta Range Balance, and placed into the bomb calorimeter's oval air can. Following the filling of the Dewar, the calorimeter's positive ignition connecter was slid into the central terminal of the oxygen bomb. The calorimeter's negative connector was then slid into the ground terminal located on the oval buckets metal support bracket. The bomb was seated firmly in the buckets metal support bracket, and lowered into the Dewar. The stirrer and thermistor probe was lowered into the Dewar, and the cover of the calorimeter was closed and secured. Once the calorimeter cover was closed and secured, the test run could then be started (Figure 10).

Once the final reading of the test was obtained, the calorimeter was opened and the two ignition leads were removed. The bomb was then lifted out of the Dewar

and placed back into the bench socket. The pin wrench was inserted into the valve cap and turned slowly to depressurize the bomb. Once depressurized, the octagon wrench was used to loosen the head of the bomb from the screw cap. All unburned pieces of fuse wire were removed from the hook terminals using metal tweezers, and salvaged to be recycled. The hook terminals were thoroughly cleaned of excess carbon with a brass headed Dremel tool. Once cleaned, the bomb was ready to accept another sample.

4.14.2 **Calibrating the Oxygen Bomb Calorimeter**

The calibration (standardization) of the Parr 2725 Semimicro Calorimeter and the Parr 6772 Calorimetric Thermometer involved the introduction of a known amount of heat into the system through the combustion of a known amount of benzoic acid (Figure 9). This study employed two 1107 Semi-micro oxygen bombs. The energy equivalent for each oxygen bomb had to be determined before the tissue samples could be accurately tested (Parr, 2005). It was necessary to determine the energy equivalent of the oxygen bombs through the combustion of benzoic acid. The benzoic acid pellets that were used weighed approximately 0.2 g and were 0.635 cm in diameter (Parr, 2008). The energy equivalents associated with each bomb were determined by calculating the average of 10 benzoic acid tests (Parr, 2005). The standard deviation associated with each series of testing fell below 0.17%. Therefore the bombs were considered calibrated and ready for use (Parr, 2005).

4.14.3 **Bombing Freeze-dried Tissue Samples**

Following the standard operating procedure, bomb calorimetry, using a calibrated Parr 2725 Semi-micro Calorimeter and Parr 6772 Calorimetric Thermometer, was performed on 216 pelletized experimental freeze-dried trout tissue samples and five pelletized Time Zero samples in triplicate.

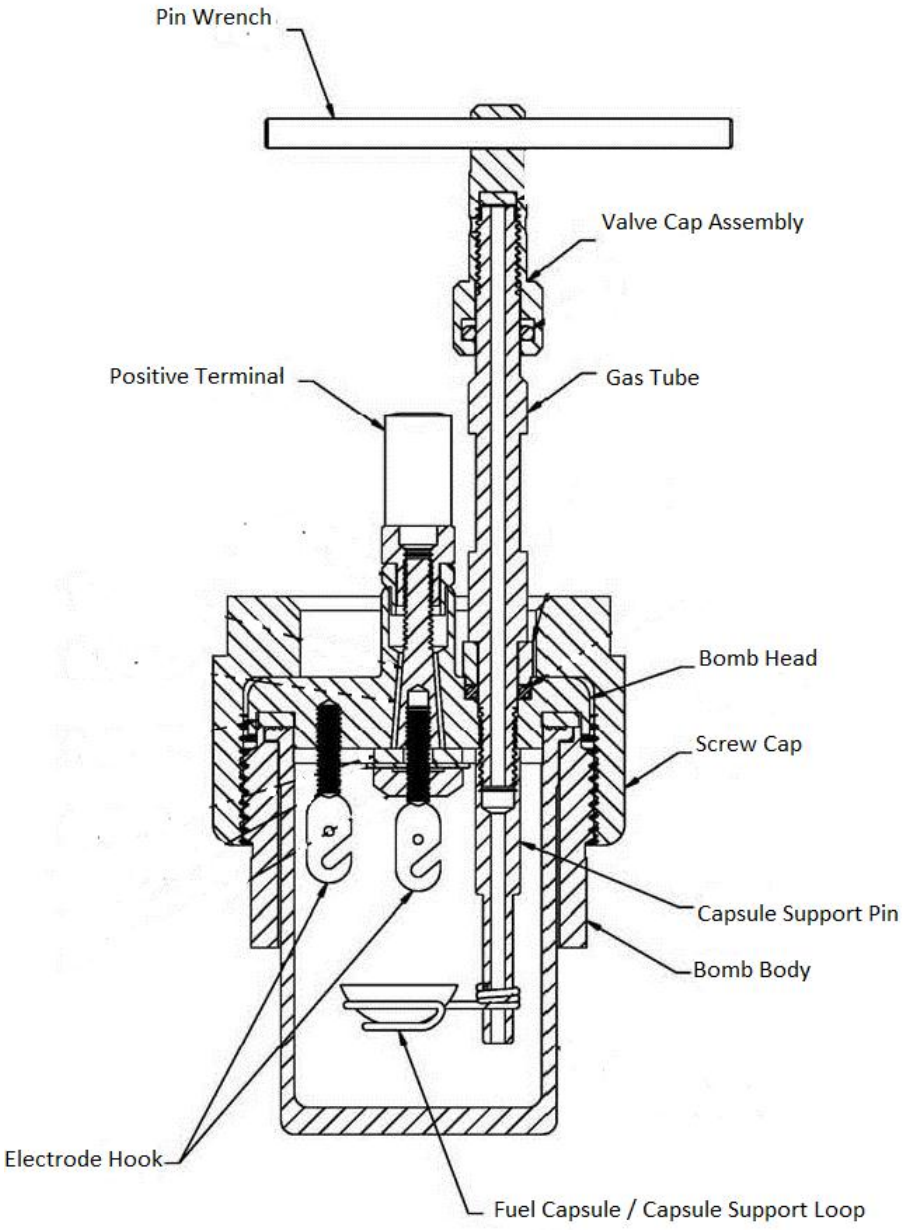

Figure 8: Schematic of the 1107 Semi-micro Oxygen Bomb parts. (Modified from Parr, 2008)

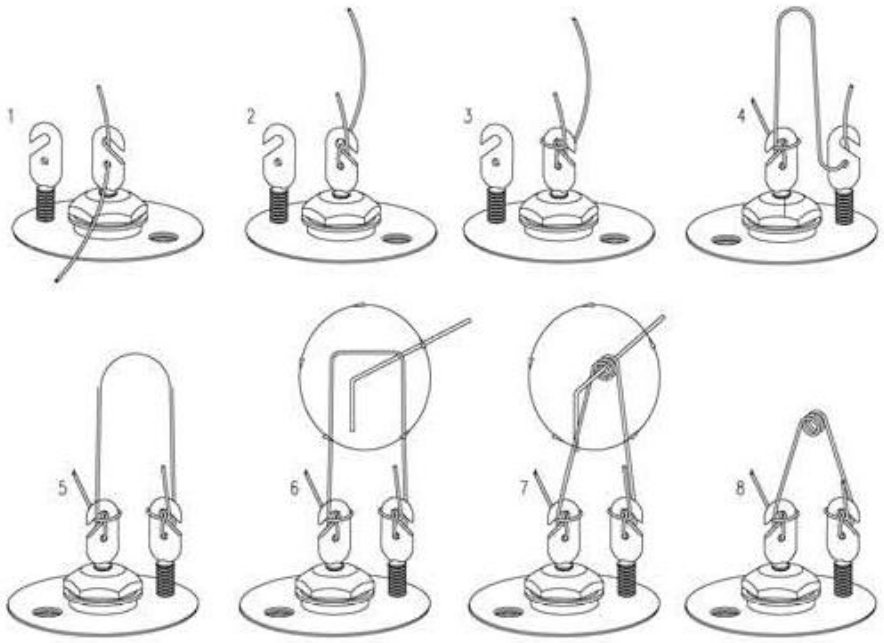

Figure 9: Diagram illustrating how to attach and coil the fuse wire to the hook terminals in the 1107 Semi-micro Oxygen Bomb. (Modified from Parr, 2008)

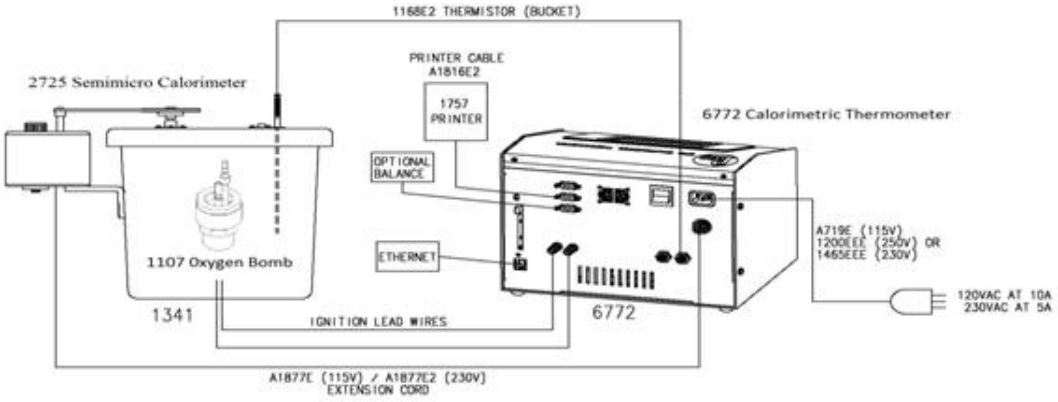

Figure 10: Schematic of the Parr 6772 Calorimetric Thermometer and the Parr 2725 Semimicro Calorimeter. (Modified from Parr, 2008)

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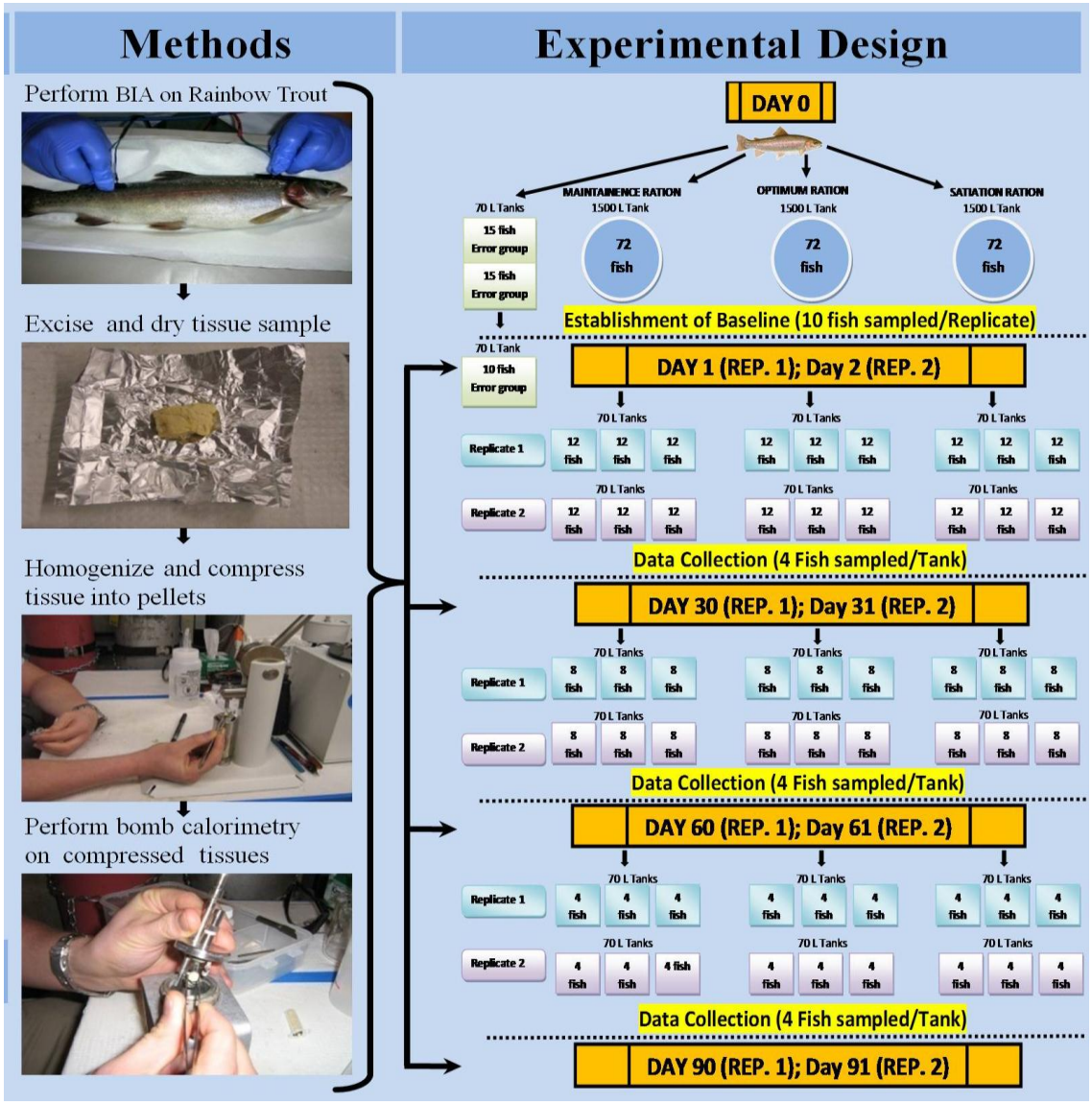

Figure 11: Outline of experimental design.

4.15 Data Analysis

4.15.1 **Statistics**

All proximate measurements are expressed in grams and were performed in triplicate and averaged for the final value. The mean proximate measurements were used as the dependent variables during linear regression analysis. The BIA model that resulted in the highest r^2 value, after the completion of linear regression analysis, was selected for each component of body composition (Table 3, 4, 5). As a result, a series of BIA indices were developed to predict each proximate component (Table 3, 4, 5). After using linear regression to determine each experimental replicates best-fit model for each body composition parameter, the similarity of the slopes and intercepts of the calibration model were compared using multiple regression to determine if there was a significant difference ($P \leq 0.05$) between the two experiments for pooling purposes (Figure 20, 25, 30). Normality was checked using the Shapiro-Wilk's test ($p \geq 0.05$) and homogeneity of variances was determined using the Levene's tests ($p \leq 0.05$). Data which failed these tests were logarithmically transformed using the equation log10(x). Once the conditions for parametric analysis were met, factorial analysis of variance (ANOVA), one-way ANOVA and repeated measures ANOVA were used to determine significant differences between experiments, treatments, and sampling times. When significant differences were detected, Tukey's Honest Significant Differences post-hoc test was used as a means to determine where those differences existed. A type one error (α) of 0.05 was used to increase the

statistical power of the analysis, and to reduce the potential for a type II error (false negative) to occur.

4.16 Equations

The equations that were employed in this study are as follows:

4.16.1 **Condition Factor:**

(Total Body Weight x 100) ÷ Total Length^3

4.16.2 **Hepatosomatic Index:**

(Weight of Liver ÷ Total Body Weight) x 100

4.16.3 **Gonadosomatic Index:**

(Gonad Weight ÷ Total Body Weight) x 100

4.16.4 **Phase Angle:**

Arctangent (Series Reactance ÷ Series Resistance) x ($180^O \div \pi$)

4.16.5 **% Moisture:**

(Wet Weight of Tissue ÷ Total weight of tissue) x100

4.16.6 **Actual Total Body Water:**

% Moisture x Total Body Weight

4.16.7 **Predicted Total Body Water:**

Total Length^2 ÷ Parallel Resistance

4.16.8 **% Dry Mass:**

(Dry Weight of Tissue ÷ Total Weight of Tissue) x 100

4.16.9 **Actual Dry Mass:**

% Dry Mass x Total Body Weight

4.16.10 **Predicted Dry Mass:**

Total Length^2 ÷ Parallel Resistance

4.16.11 **Predicted Energy Content:**

Total Length^2 ÷ Parallel Resistance

4.16.12 **Parallel Resistance:**

Series Resistance + (Series Reactance^2 ÷ Series Resistance)

4.16.13 **Parallel Reactance:**

Series Reactance + (Series Resistance^2 ÷ Series Reactance)

5.0 RESULTS

5.1 90-Day Growth Experiment

5.1.1 Abiotic Factors

Differences in temperature, pH, alkalinity, conductivity and water hardness over the duration of the experiment between treatments were not significant (Table 1).

5.1.2 Growth

Repeated measures ANOVAs showed no significant differences in wet weight between experimental replicates ($p = 0.01$), and thus the data were pooled for subsequent analysis. However, there were significant differences in wet weight between treatments (Figure 12). The maintenance (0.4 % bw/day) ration experienced the least amount of growth out of all three treatments (Figure 16), and maintenance fish had significantly lower wet weights than fish from both the optimum and satiation ration (Figure 12). Fish receiving the optimum ration (1.6 % bw/day) had wet weights midway between the maintenance and satiation rations (Figure 16), and were significantly different from both treatments (Figure 12). Fish receiving the satiation ration (3.4 % bw/day) were the largest by wet weight out of all the treatments (Figure 16), and were significantly heavier than both the optimum and maintenance ration fish (Figure 12).

Factorial ANOVAs showed no significant differences in wet weights between sampling times for trout receiving the maintenance ration, thus fish size did not change over the duration of the experiment (Figure 13). Furthermore, no significant differences in wet weight were found between fish receiving the

maintenance ration on day 30 and both fish receiving the optimum ration on day 30 and 60 (Figure 13). However, wet weight of fish at day 60 and 90 on the maintenance ration was significantly lower than the wet weight of fish receiving either the optimum or satiation ration (Figure 13). Weight of fish receiving the optimum ration at day 30 and 60 was not significantly different from the wet weight of trout receiving either the maintenance and satiation ration at day 30, but was statistically different than day 60 and 90 for both the maintenance (higher) and satiation (lower) rations (Figure 13). Fish fed the optimum ration had similar wet weights at day 90 relative to fish at day 60 receiving the same ration and day 30 fish receiving the satiation ration. Optimum ration fish at day 90 were significantly lighter than day 60 and 90 fish receiving the satiation ration and heavier than day 60 and day 90 fish receiving the maintenance ration (Figure 13). Wet weight of satiation ration fish on day 60 was significantly different from day 30 and day 90 fish on the same ration, and greater than fish receiving optimum and maintenance rations (Figure 13). Day 90 fish receiving the satiation ration were significantly heavier than day 30 and day 60 satiation ration fish (Figure 13).

Differences between experimental replicates with regard to growth rate (g/day) over the 90-day growth experiment were not significant, thus the data were pooled together for subsequent analyses. Fish receiving the maintenance (0.4 % bw/day) ration experienced the lowest growth rate out of all three treatments, significantly lower than both the optimum and satiation ration fish growth rates (Figure 14). Optimum ration (1.6 % bw/day) fish had growth rates midway between the maintenance and satiation ration, significantly different from both

treatments (Figure 14). Fish receiving the satiation ration (3.4 % bw/day) experienced the highest growth rate out of all the treatments, significantly higher than both the optimum and maintenance ration fish (Figure 14).

Factorial ANOVAs showed no statistical differences in growth rate within each treatment with respect to sampling time, which remained relatively constant over the duration of the experiment (Figure 15). The relatively constant growth rate of fish receiving the maintenance (0.4 % bw/day) ration was the lowest out of all three treatments, and was significantly different from the fish receiving the optimum and satiation rations at all sampling times (Figure 15). The relatively constant growth rate for fish receiving the optimum ration (1.6 % bw/day) was intermediate to the growth rates of fish receiving the maintenance and satiation rations, and was statistically different from both treatments (Figure 15). The growth rate for fish receiving the satiation ration (3.6 % bw/day) was the highest of all three treatments (Figure 15).

Table 1: Abiotic factors measured on lab water collected throughout the 90-day growth experiment. Values are written as means ± standard deviation (n = 90).

Abiotic Factors			
Parameter	**Mean ± Standard Deviation**	**Min**	**Max**
Temperature (^{o}C)	12.8 ± 0.54	10.8	16.3
pH	7.33 ± 0.33	7.87	6.43
Hardness (ppm of $CaCO_3$)	4	N/A	N/A
Alkalinity (ppm of $CaCO_3$)	12	N/A	N/A
Conductivity (µS/cm)	123 ± 20.5	N/A	N/A

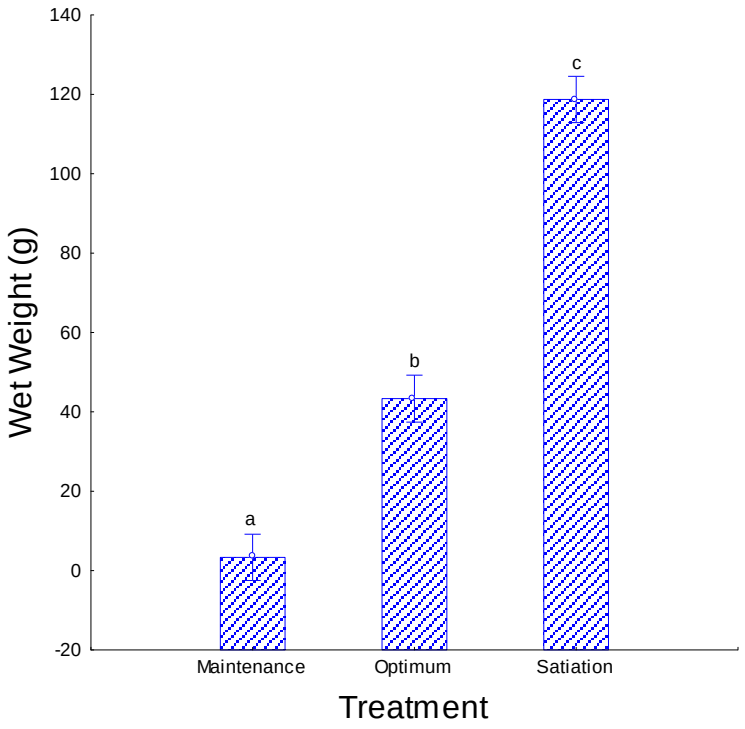

Figure 12: Rainbow trout wet weight by treatment ± standard error. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$).

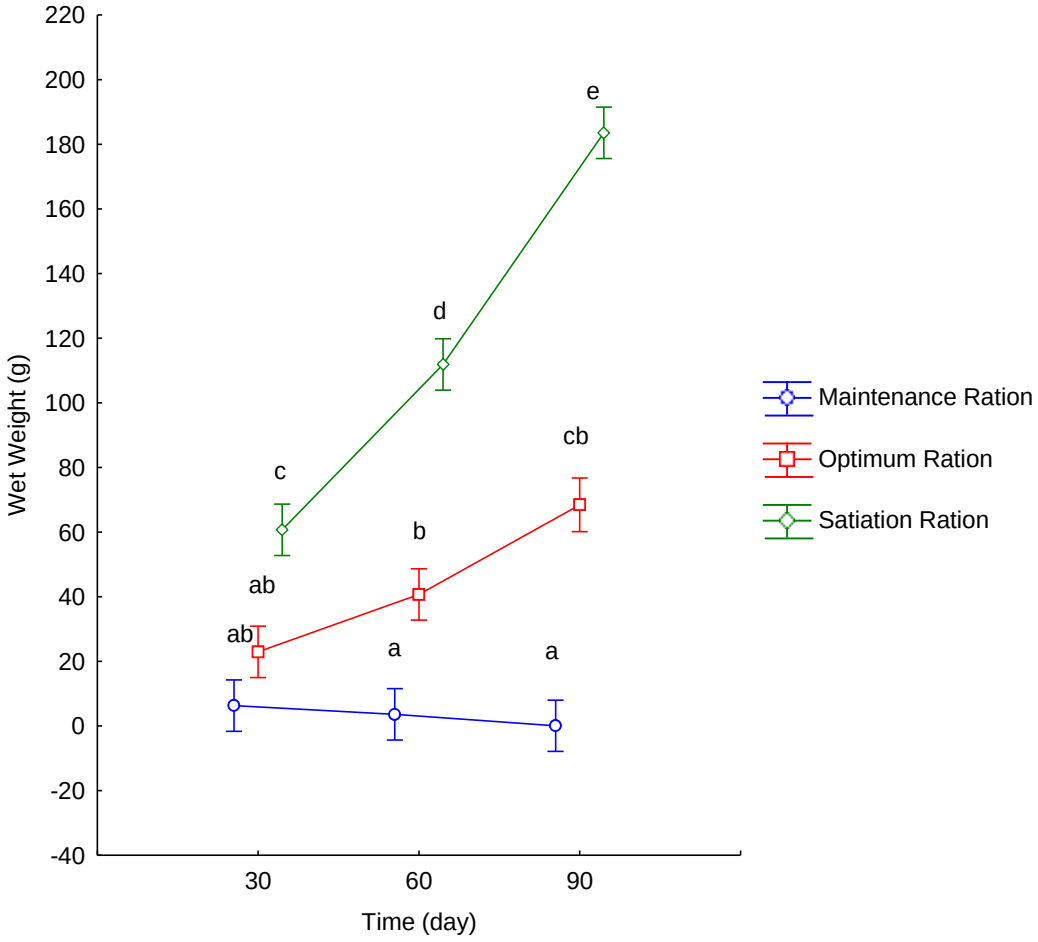

Figure 13: Rainbow trout wet weight by treatment ± standard error over time. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$).

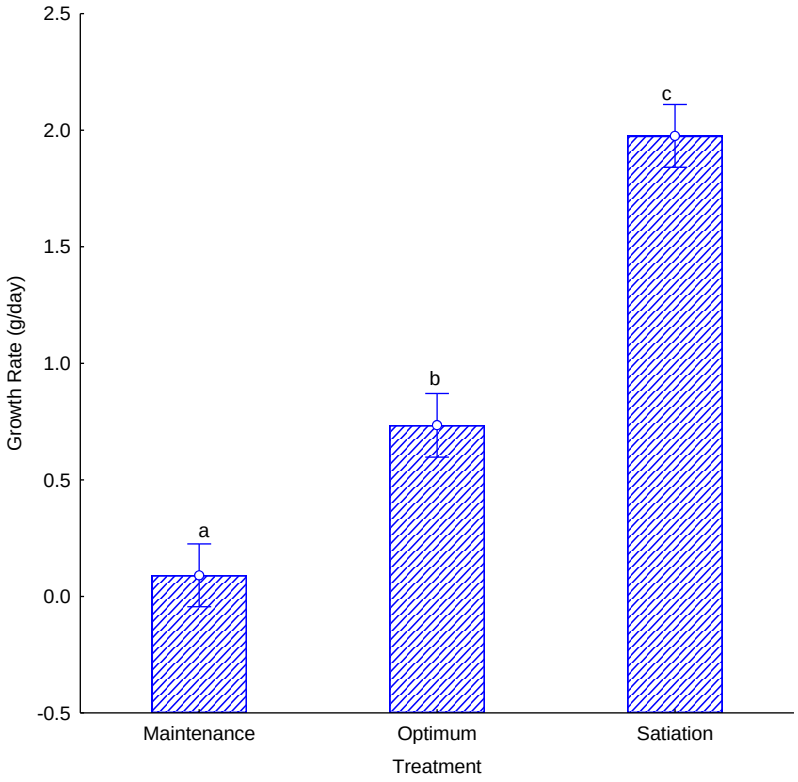

Figure 14: Rainbow trout growth rate by treatment ± standard error. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$).

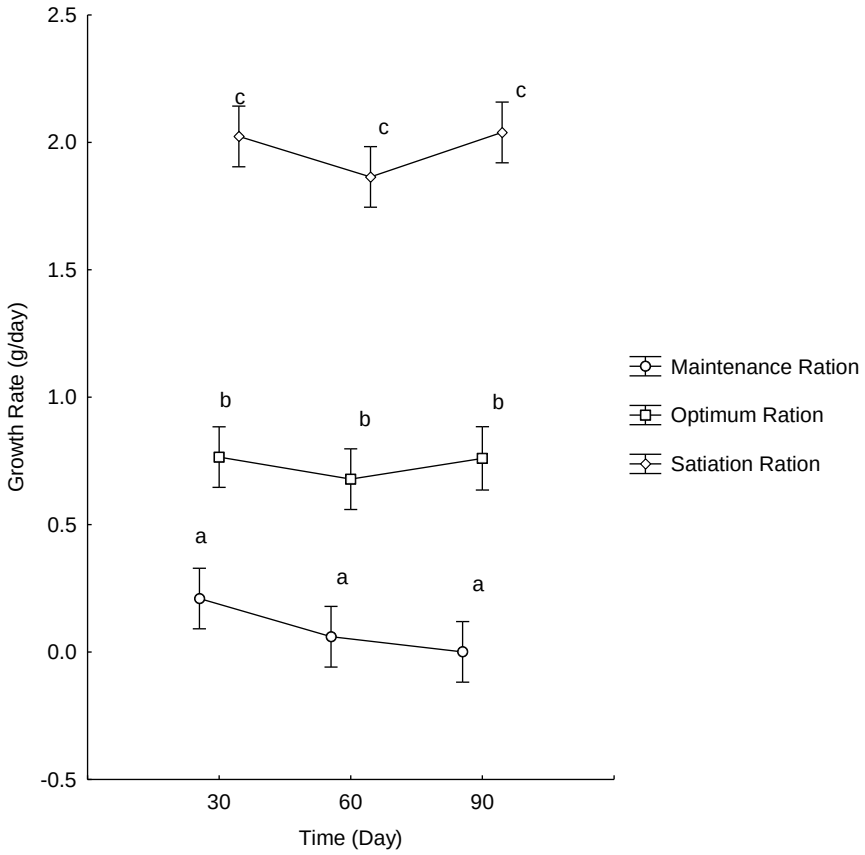

Figure 15: Rainbow trout growth rate by treatment ± standard error over time. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$).

Figure 16: Photograph of the three tiers of rainbow trout growth experienced between treatments. From the top to the bottom: satiation ration, optimum ration and maintenance ration. This photograph was taken during the final sampling date for experiment # 2 on October 23, 2010.

5.1.3 **Energy Density**

Repeated measures ANOVAs showed that differences between experimental replicates with regard to log transformed energy density (cal/g) over the 90-day growth experiment were not significant, thus the data were pooled for subsequent analyses. No statistical differences were found between energy density for fish receiving the maintenance ration and those measured at time zero. However, energy density of fish receiving the satiation and optimum ration were found to be significantly greater than time zero fish. Significant differences in energy density were found between treatments (Figure 17). Fish receiving the maintenance (0.4 % bw/day) ration had the lowest energy density out of all three treatments (Figure 19), and were found to be significantly lower than the energy densities of fish receiving both the optimum and satiation ration (Figure 17). Fish receiving the optimum ration (1.6 % bw/day) had an intermediate level of energy density between that of fish receiving the maintenance and satiation rations (Figure 19), significantly different from both treatments (Figure 17). Fish receiving satiation rations (3.4 % bw/day) had significantly higher energy density compared to all the treatments (Figures 17, 19). No statistical difference in energy density of fish was found between sampling times within each treatment (Figure 18). Furthermore, no statistical difference in energy density of fish was found between day 30 fish receiving the maintenance ration and day 30 fish receiving the optimum ration (Figure 18). However, energy density of day 60 and 90 fish receiving the maintenance ration was significantly lower than that of fish receiving both optimum and satiation rations at any time (Figure 18). The energy

density of optimum ration fish at day 60 and 90 and those receiving the satiation ration at day 60 and 90 were not significantly different. However, the energy density of fish receiving the optimum ration at day 60 and 90 was significantly lower than that of fish fed the satiation ration at day 30 (Figure 18).

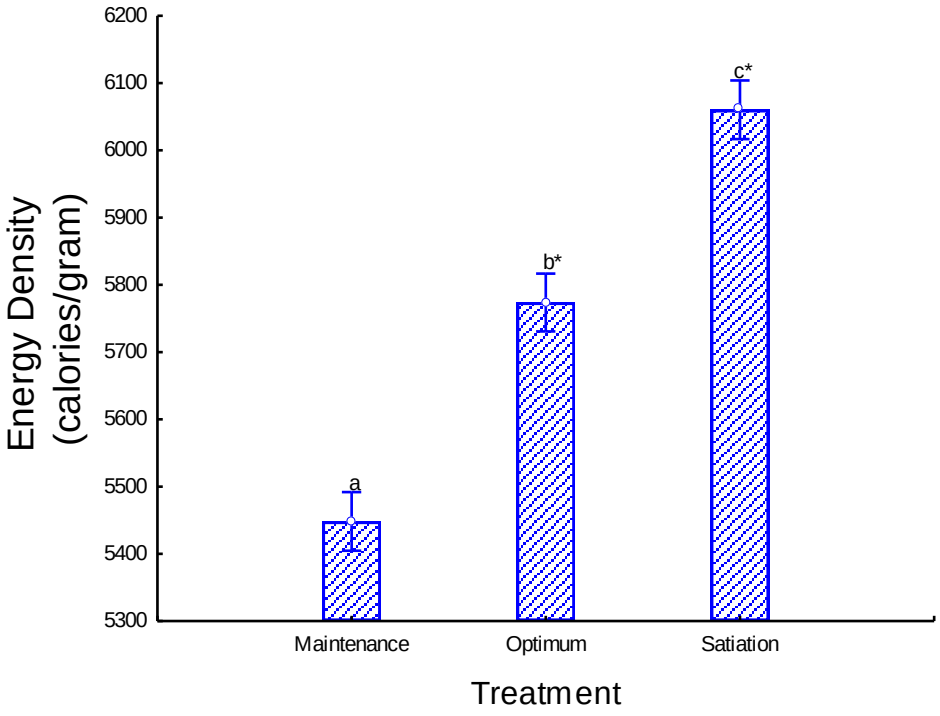

Figure 17: Energy density by treatment ± standard error. Subsampled over a 90-day standard growth experiment where juvenile rainbow trout were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

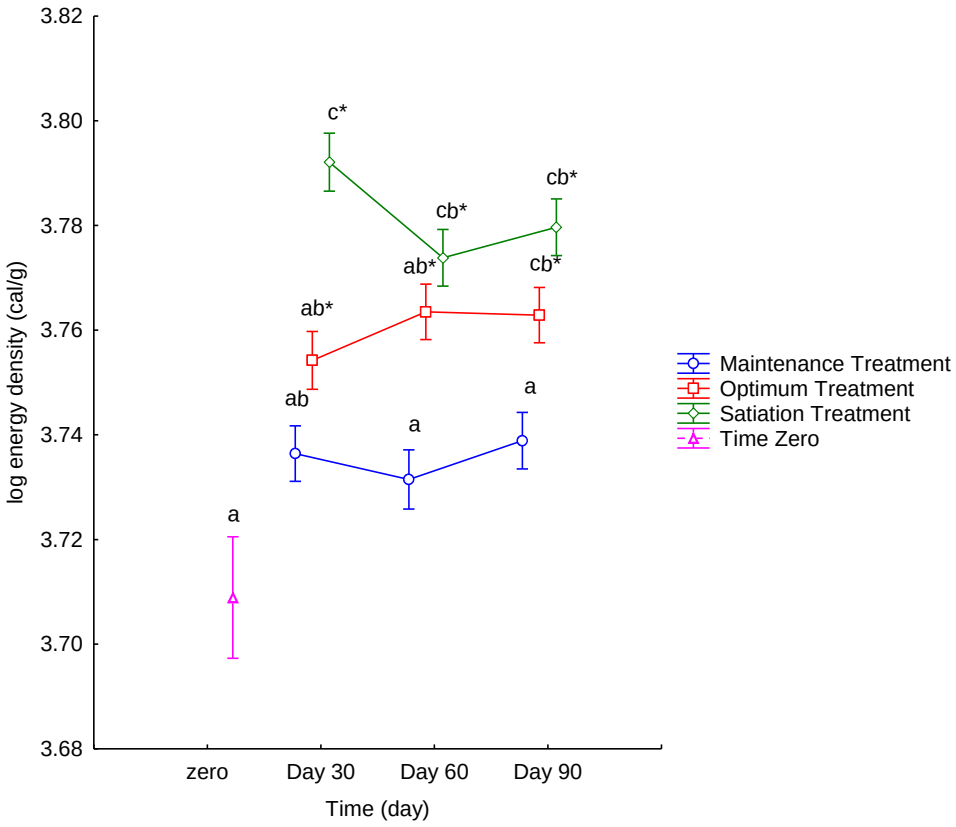

Figure 18: Log energy density by treatment ± standard error over time. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

Figure 19: Photograph of the three tiers of energy density in rainbow trout muscle tissue. From the left to right: maintenance ration, optimum ration and satiation ration. Yellowing of tissue samples with increasing ration is evident, and is indicative of increasing lipid content. This photograph was taken during the final sampling date for experiment # 2 on October 23, 2010.

5.1.4 **Caloric Content**

Repeated measures ANOVA showed differences in caloric content between experimental replicates over the 90-day growth experiment to be not significant, thus replicates were pooled for subsequent analyses (Figure 20). The pooled measured caloric content data were correlated against the measured BIA value (L^2R_P) data via linear regression analysis, which revealed a coefficient of determination $r^2 = 0.911$, and a slope equivalent to $82977e^{0.0224x}$ (Figure 21). The predicted caloric data, generated using the above equation, was correlated with the actual measured caloric data via linear regression analysis, which revealed a coefficient of determination $r^2 = 0.8981$ and a slope equivalent to $0.9619x + 10656$ (Figure 22).

Differences between the actual and predicted measurements of the log transformed caloric content data over the 90-day growth experiment were not significant (Figure 23). Furthermore, no statistical difference were found between the actual and predicted measurements for the fish fed the maintenance ration and time zero fish with regard to caloric content. However, the actual and predicted energy measurements for fish fed the satiation and optimum rations were statistically greater than time zero fish.

The actual and predicted energy measurements for fish fed the maintenance (0.4 % bw/day) ration were the lowest out of all three treatments (Figure 23). The actual and predicted energy measurements for fish fed the optimum ration (1.6 % bw/day) were intermediate to those of fish fed either the maintenance or satiation rations, and were statistically different from both

treatments (Figure 23). The actual and predicted energy measurements for fish fed the satiation ration (3.4 % bw/day) were significantly greater than all other treatments (Figure 23).

The actual and predicted monthly measurements of caloric content for fish fed the satiation and optimum ration were statistically greater than time zero fish. All of the actual and predicted monthly energy measurements for fish fed the maintenance (0.4 % bw/day) ration had the lowest caloric content of all three treatments (Figure 24). The actual and predicted monthly energy measurements for fish fed the optimum ration (1.6 % bw/day) were intermediate between the monthly energy values for fish fed the maintenance and satiation rations throughout the study. No significant differences in tissue energy were found between day 60 and day 90 fish fed the optimum ration. No significant differences in monthly tissue energy were found between day 30 fish fed the satiation ration and day 60 and 90 fish fed the optimum ration. Monthly energy measurements for fish fed the satiation ration were significantly greater than all other treatments.

Table 2: BIA resistance and reactance ± standard error by treatment. Juvenile rainbow trout were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment.

Parameter	**Treatment**	**n**	**Mean ± Standard Error**
Resistance (Ω)	Satiation	68	582± 11.6
	Optimum	70	520± 5.67
	Maintenance	72	535± 7.38
Reactance ^{o}	Satiation	68	5300± 738
	Optimum	70	6690 ± 2630
	Maintenance	72	2470 ± 1730

Table 3: Determination of best-fit BIA equation using linear regression analysis for caloric content.

Parameter	Equation	Coefficient of Determination (r^2)	Dependant variable (y)
Caloric Content (calories)	L^2/R_s	$r^2 = 0.846$	$y = 90003e^{0.021x}$
	L^2/R_p	$r^2 = 0.911$	$y = 82977e^{0.0224x}$
	L^2/Xc_S	$r^2 = 0.065$	$y = 7.1983e^{-17004.9x}$
	L^2/Xc_p	$r^2 = 0.084$	$y = 7.1793e^{-17074.7x}$

Total length of the fish squared is represented by L^2. R_p and R_s represent resistance in parallel and in series. Xc_p and Xc_S represent reactance in parallel and in series.

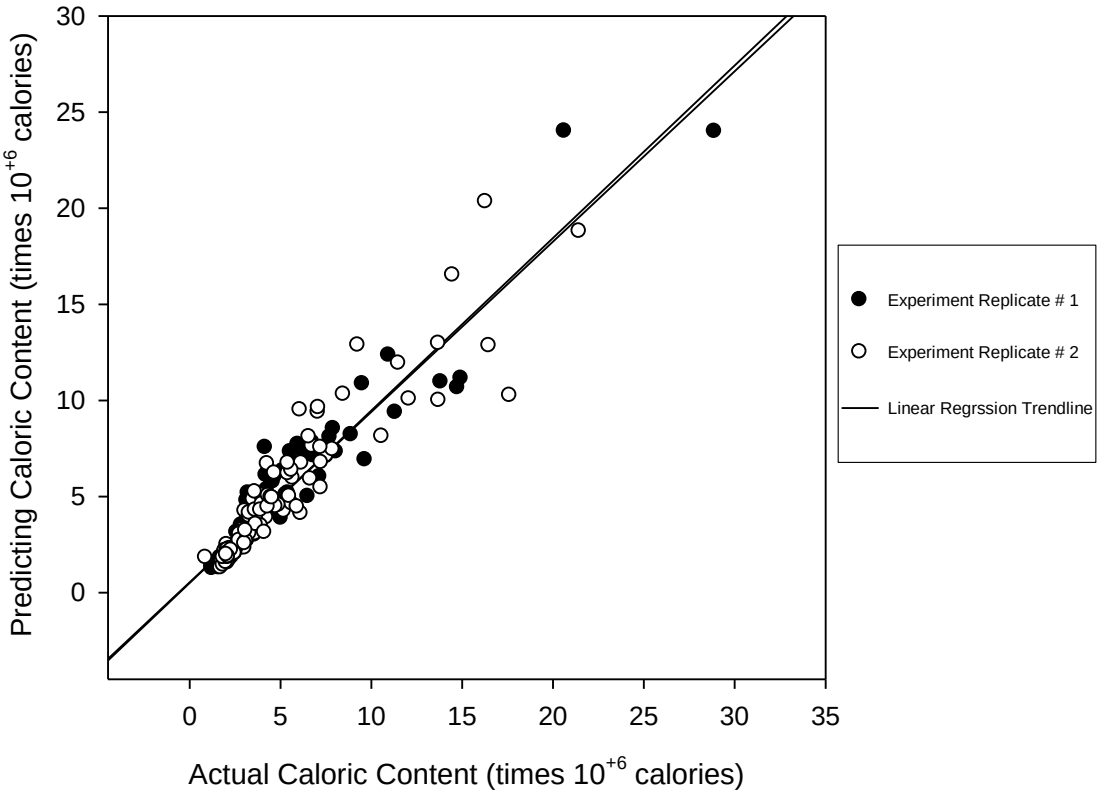

Figure 20: Correlation between the measured caloric content verses the measured BIA (equation L^2/R_P) for both experimental replicates via linear regression analysis.

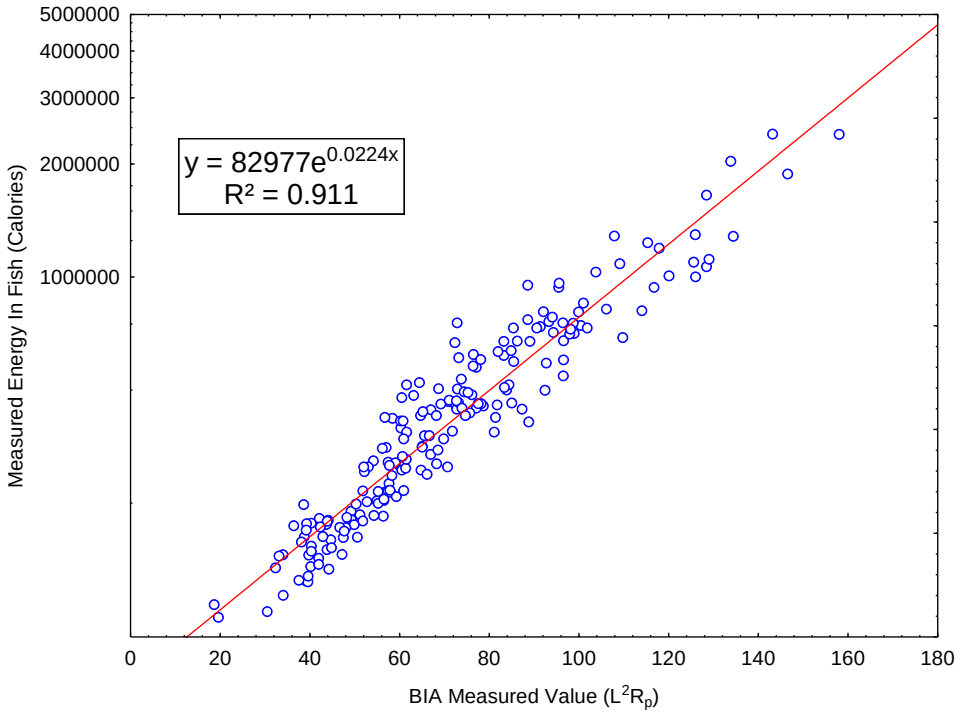

Figure 21: Correlation between the pooled measured caloric content values and the measured BIA value (L^2R_P) via linear regression analysis.

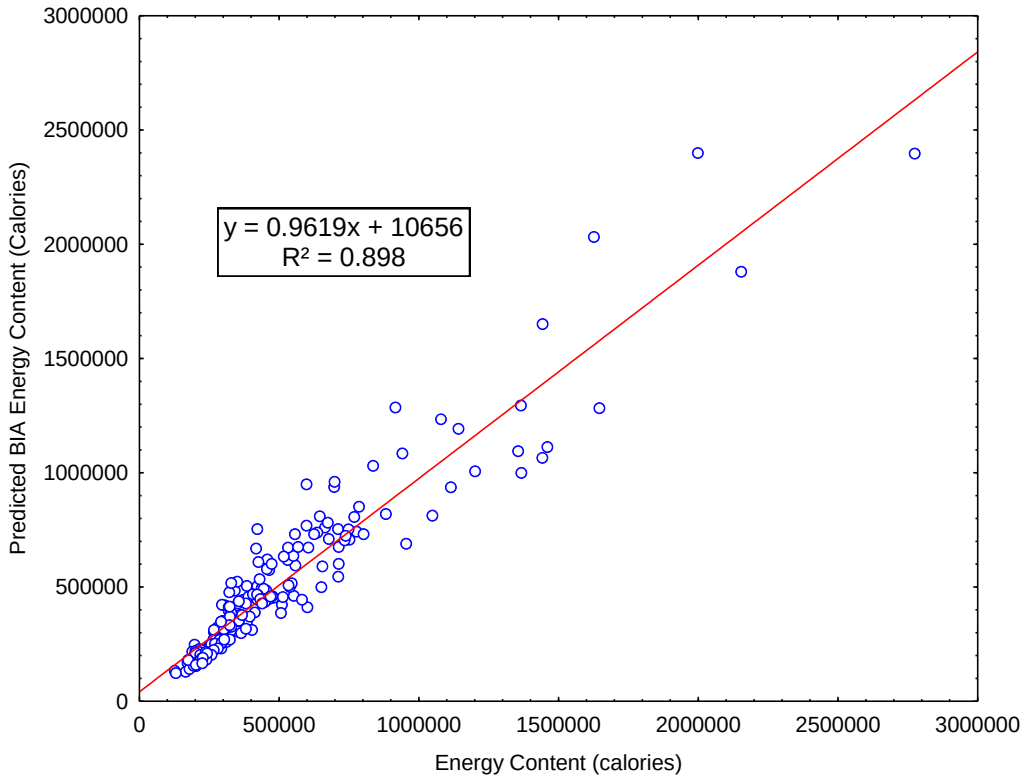

Figure 22: Correlation between the pooled measured caloric content data and the predicted caloric data.

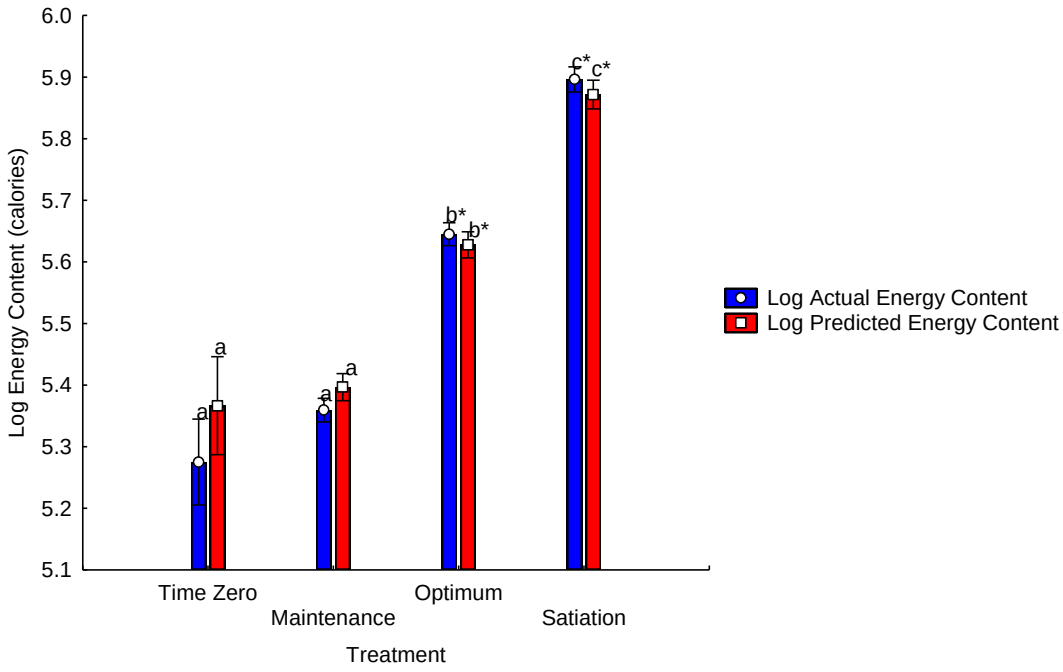

Figure 23: Actual vs. predicted energy content by treatment ± standard error. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

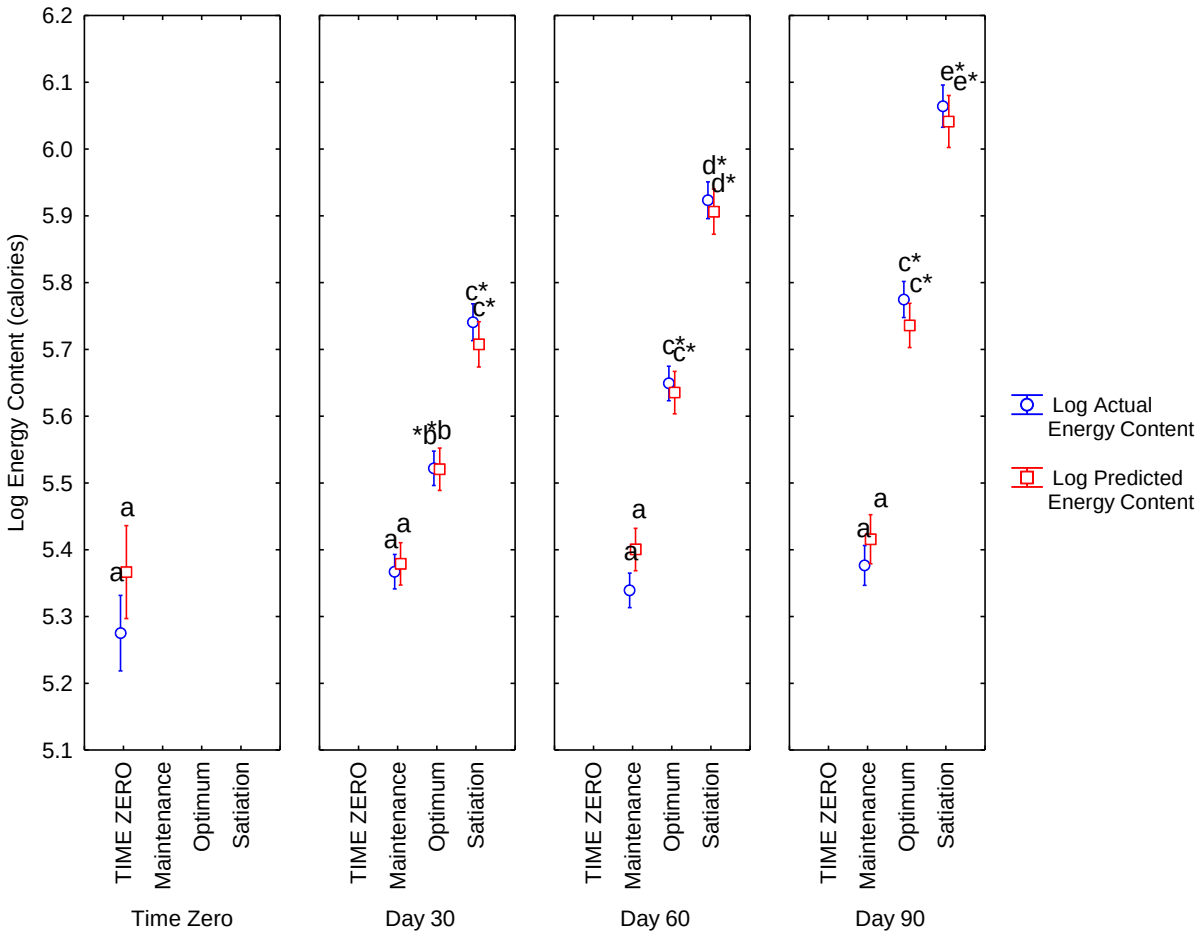

Figure 24: Actual vs. predicted Log energy content by treatment ± standard error over time. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

5.1.5 **Total Body Water**

There were no significant differences between experimental replicates with regard to total body water (ml) over the 90-day growth experiment, thus replicate data were pooled for subsequent analyses (Figure 25). Linear regression analysis of the measured total body water data vs. the measured BIA value (L^2/R_P) over the 90-day standard growth experiment revealed a coefficient of determination $r^2 = 0.917$, and a slope equivalent to $13.335e^{0.0198x}$ (Figure 26). The correlation between the measured total body water vs. the BIA predicted total body water via linear regression analysis revealed a coefficient of determination $r^2 = 0.8936$, and a slope equivalent to $0.9855x + 0.6346$ (Figure 26).

There were no significant differences between the actual and predicted measurements with regard to log transformed total body water (ml) over the 90-day growth experiment (Figure 27). There were no statistical difference between the total body water of fish fed the maintenance ration and time zero fish. However, the total body water of fish fed the satiation and optimum rations were found to be significantly higher than that of time zero fish. Moreover, fish fed the maintenance (0.4 % bw/day) ration had the lowest total body water content out of all three treatments (Figure 27).

Total body water of fish fed the optimum ration (1.6 % bw/day) was intermediate between total body water of fish fed the maintenance and satiation rations, and was statistically different from both treatments (Figure 27). Total body water of fish fed the satiation ration (3.4 % bw/day) was higher than that of all other treatments (Figure 27).

Table 4: Determination of best-fit BIA equation using linear regression analysis for total body water. Total length of the fish squared is represented by L^2. R_p and R_s represent resistance in parallel and in series. Xc_p and Xc_S represent reactance in parallel and in series.

Parameter	Equation	Coefficient of Determination (r^2)	Dependant variable (y)
Total Body Water (ml)	L^2/R_s	$r^2 = 0.882$	$y = 13.657e^{0.0191x}$
	L^2/R_p	$r^2 = 0.917$	$y = 13.335e^{0.0198x}$
	L^2/Xc_S	$r^2 = 0.021$	$y = 48.411e^{0.0123x}$
	L^2/Xc_p	$r^2 = 0.022$	$y = 48.242e^{0.0128x}$

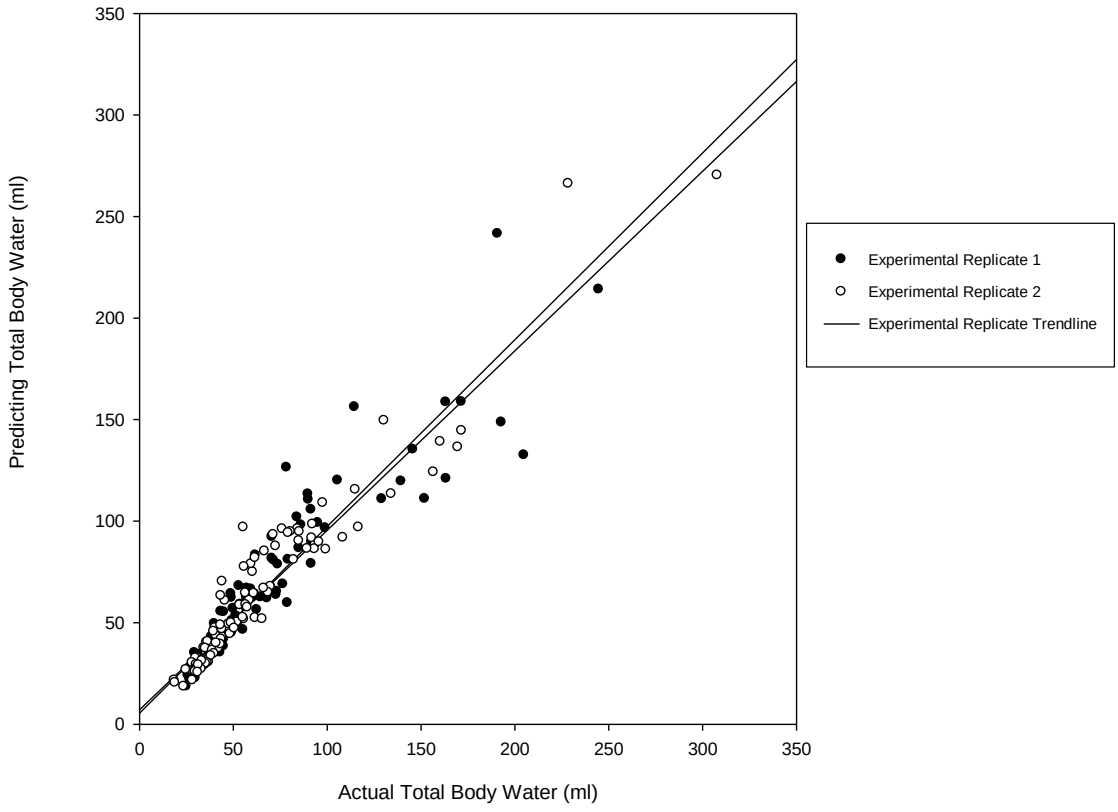

Figure 25: Correlation between the measured total body water and the BIA equation (L^2/R_P) for both experimental replicates via linear regression analysis.

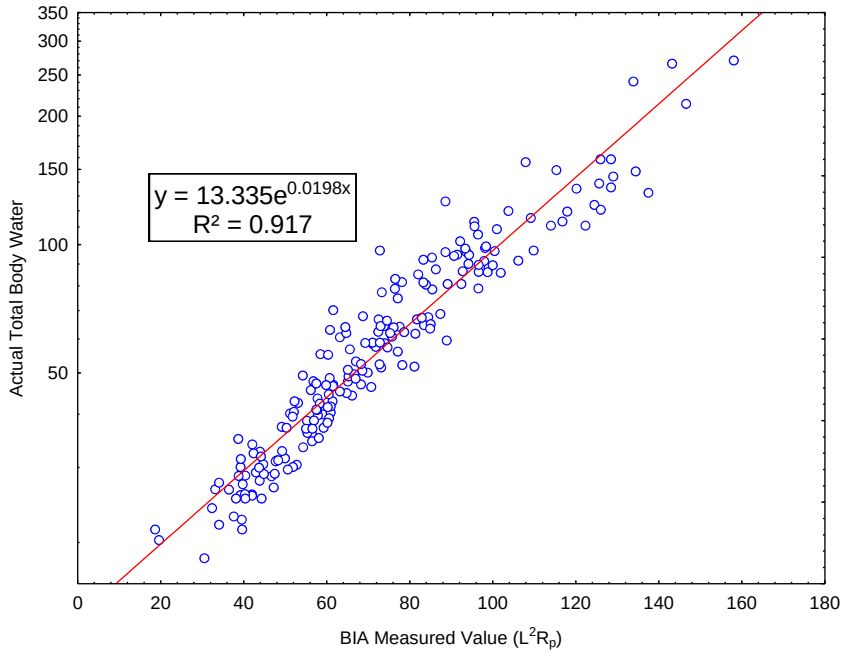

Figure 26: Correlation between the measured total body water content and the BIA equation (L^2R_P) via linear regression analysis.

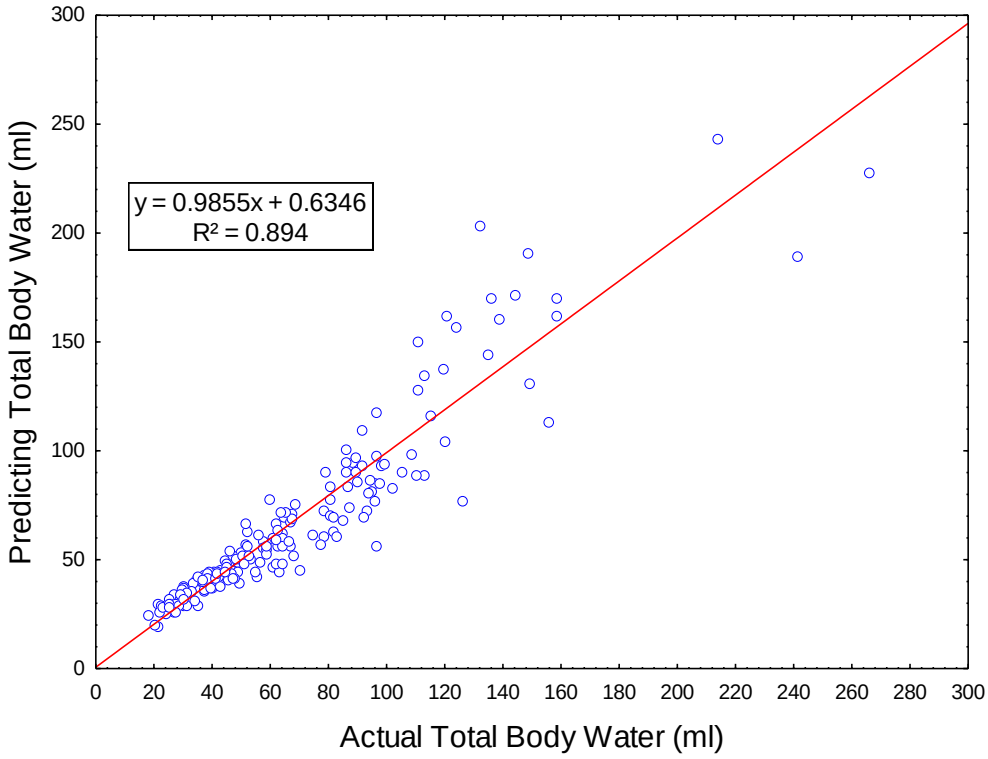

Figure 27: Correlation between the measured total body water content and the BIA predicted total body water via linear regression analysis.

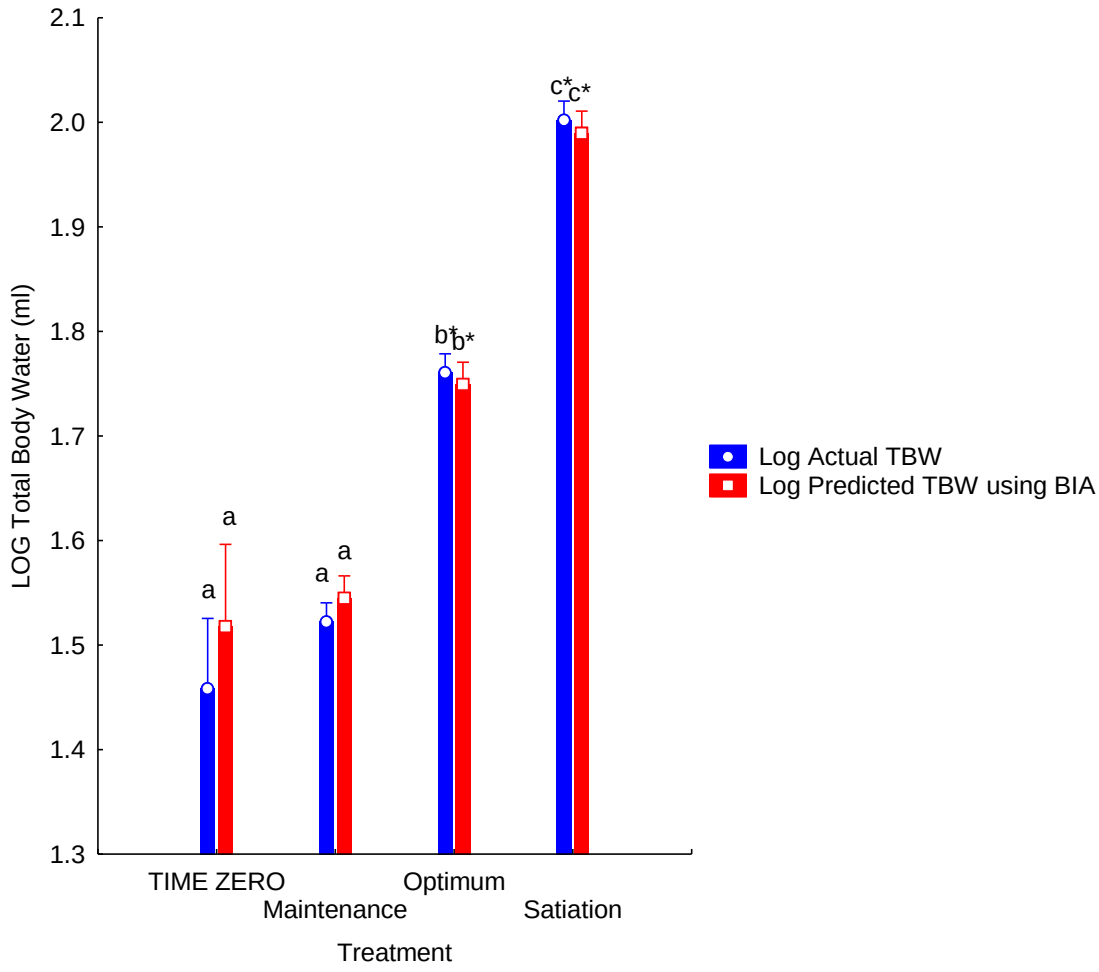

Figure 28: The actual vs. the predicted log total body water by treatment ± standard error. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

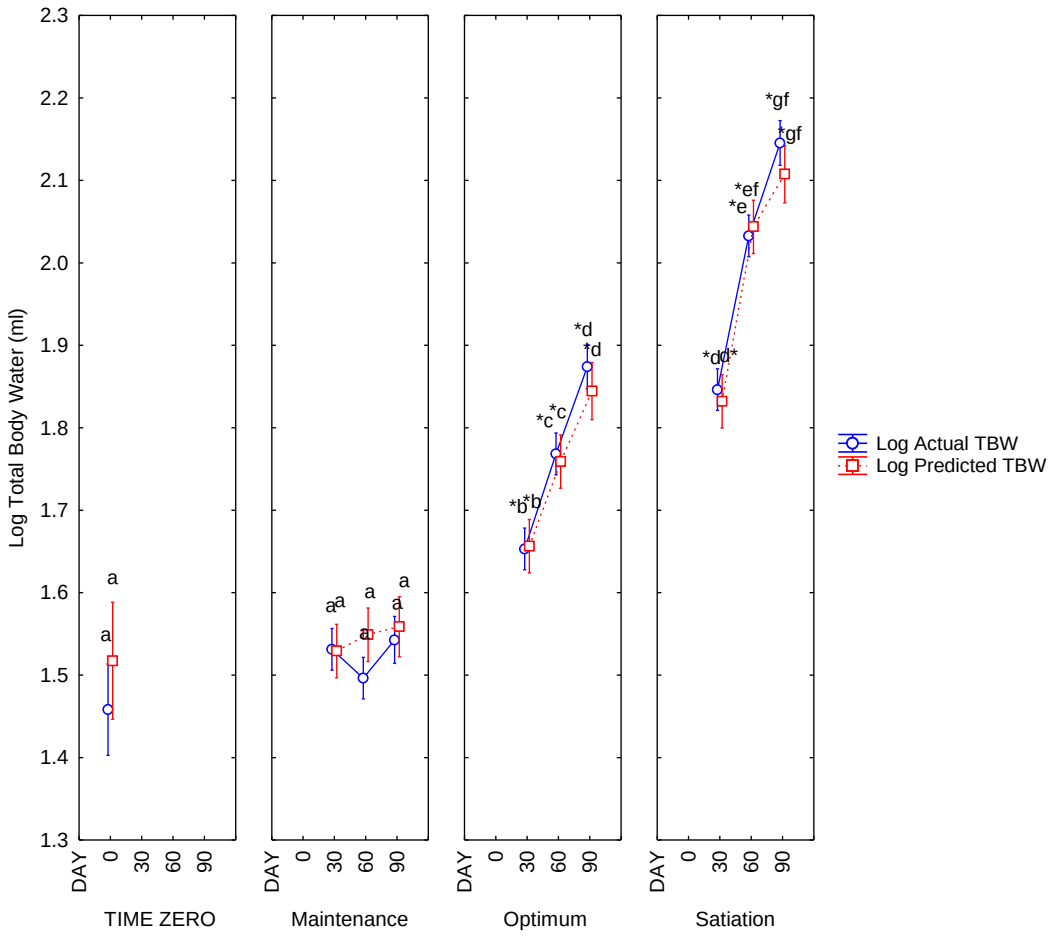

Figure 29: The actual vs. the predicted log total body water by treatment ± standard error over time. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

5.1.6 **Dry Mass**

Differences between experimental replicates with regard to dry weight (g) over the 90-day growth experiment were not significant, thus replicate data were pooled for subsequent analyses (Figure 30). Linear regression analysis of the measured dry weight data vs. the measured BIA value (L^2/R_P) over the 90-day standard growth experiment revealed a coefficient of determination $r^2 = 0.803$, and a slope equivalent to $3.7206e^{0.0212x}$ (Figure 31). The correlation between the measured dry weight vs. the predicted dry mass via linear regression analysis revealed a coefficient of determination $r^2 = 0.804$, and a slope equivalent to 0.2334 + 0.8119*x (Figure 32).

Differences between the actual and predicted measurements with regard to log transformed dry weight (g) over the 90-day growth experiment were not significant (Figure 33). There were no statistical difference between the dry weight of fish fed the maintenance ration and time zero fish. However, the dry weight of fish fed the satiation and optimum rations were found to be significantly higher than that of time zero fish. Moreover, fish fed the maintenance (0.4 % bw/day) ration had the lowest dry weight out of all three treatments (Figure 33, 34).

Dry weight of fish fed the optimum ration (1.6 % bw/day) was intermediate between dry mass of fish fed the maintenance and satiation rations, and was statistically different from both treatments (Figure 33, 34). Dry weight of fish fed the satiation ration (3.4 % bw/day) was higher than that of all other treatments (Figures 33, 34). No statistical differences were found involving the

dry weight of the fish within each treatment with respect to sampling time, which remained relatively constant over the duration of the experiment

Table 5: Determination of best-fit BIA equation using linear regression analysis for dry mass. Total length of the fish squared is represented by L^2. R_p and R_s represent resistance in parallel and in series. Xc_p and Xc_S represent reactance in parallel and in series.

Parameter	Equation	Coefficient of Determination (r^2)	Dependant variable (y)
Dry Mass (g)	L^2/R_s	$r^2 = 0.786$	$y = 3.4952e^{0.0217x}$
	L^2/R_p	$r^2 = 0.803$	$y = 3.7206e^{0.0212x}$
	L^2/Xc_S	$r^2 = 0.011$	$y = 19.276e^{-0.01x}$
	L^2/Xc_p	$r^2 = 0.015$	$y = 19.332e^{-0.04x}$

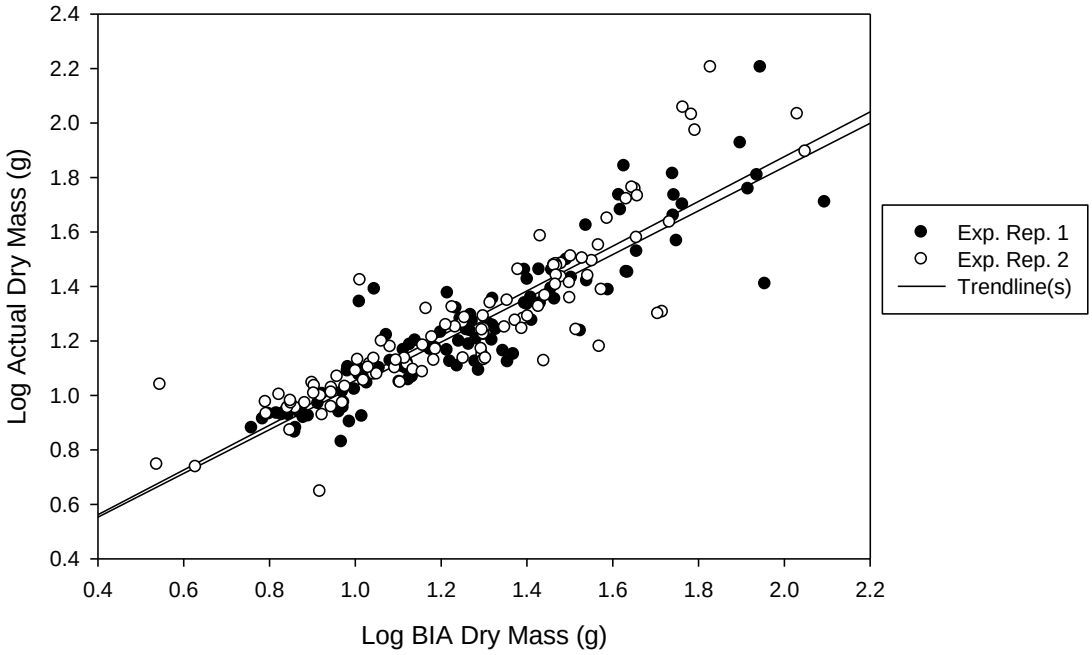

Figure 30: Correlation between the Log measured dry mass and the Log BIA equation (L^2/R_P) for both experimental replicates via linear multiple regression analysis.

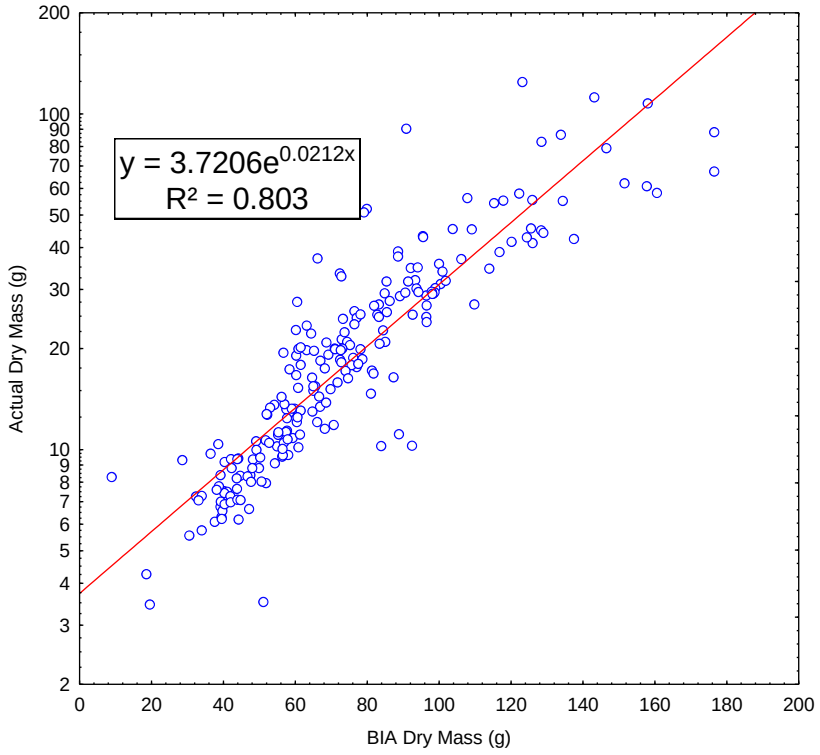

Figure 31: Correlation between the measured dry mass content and the BIA equation (L^2R_P) via linear regression analysis.

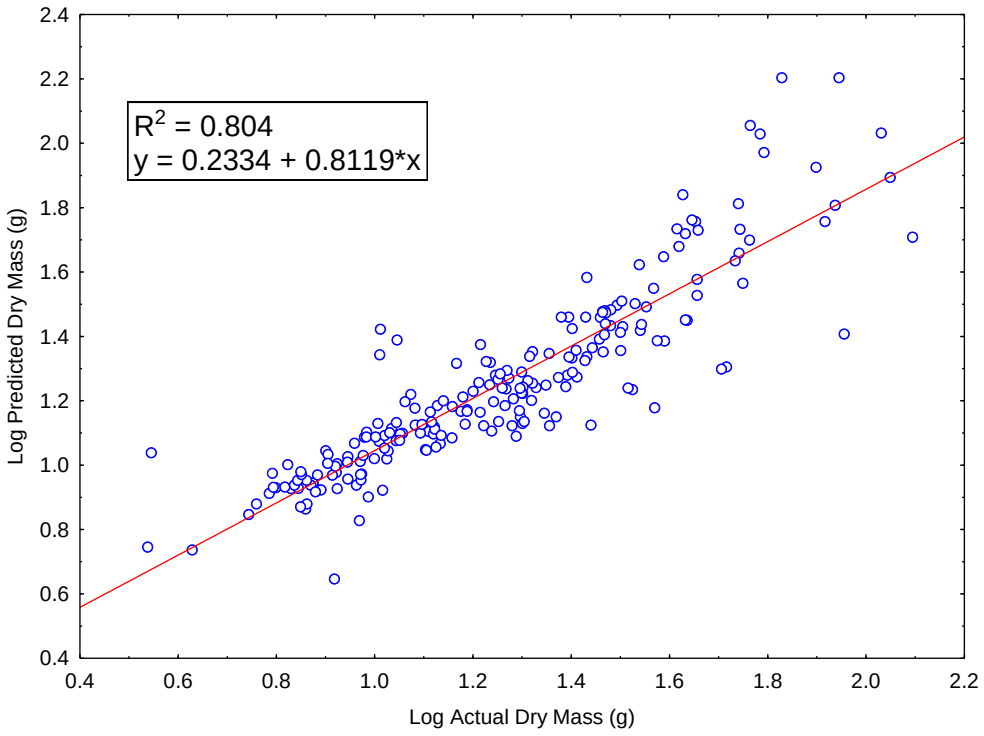

Figure 32: Correlation between the measured dry mass content and the BIA predicted total body water via linear regression analysis.

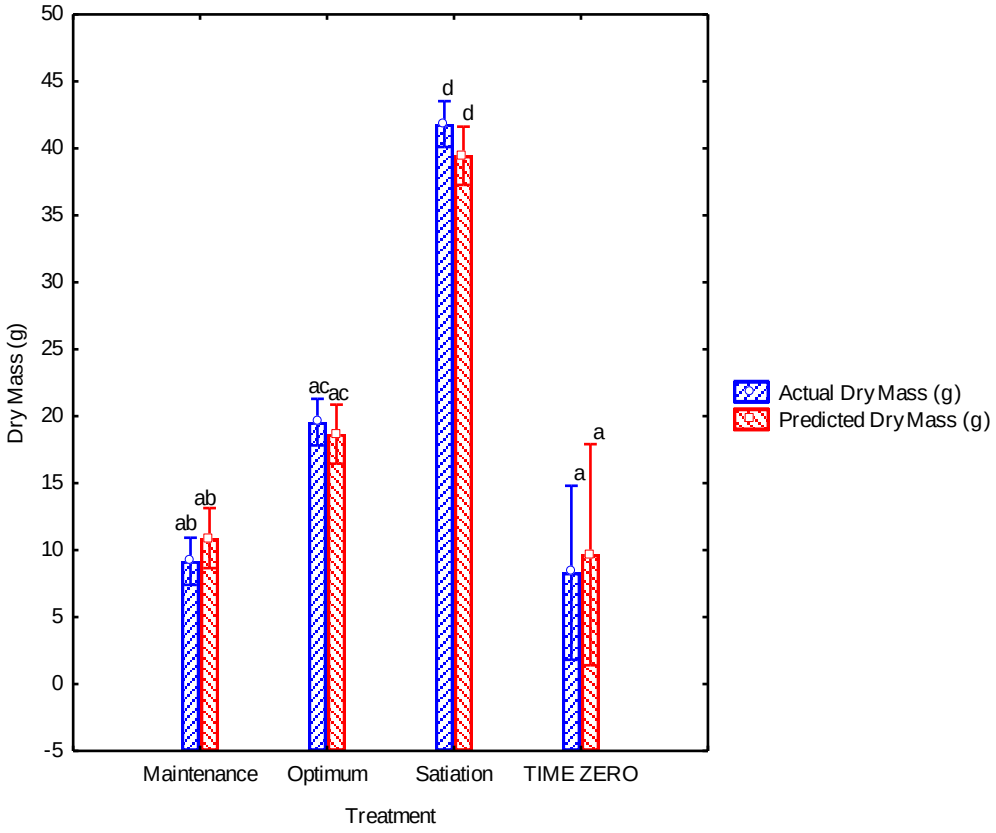

Figure 33: The actual vs. the predicted dry mass by treatment ± standard error. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

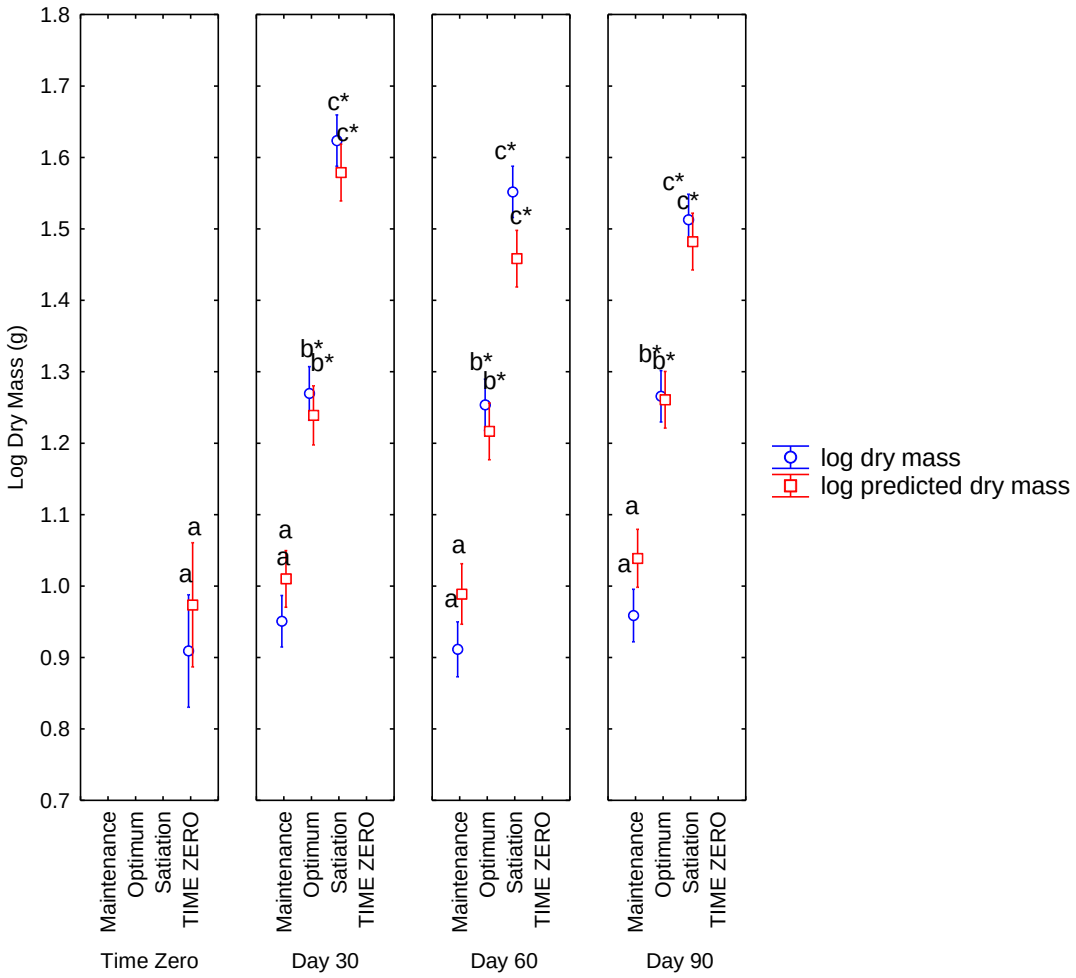

Figure 34: The actual vs. the predicted log dry mass by treatment ± standard error over time. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

5.1.7 Condition Indices

5.1.7.1 Condition Factor

There were no significant differences between experimental replicates with regard to condition factor (K) over the 90-day growth experiment, thus replicates were pooled for subsequent analyses. No significant differences were found in condition factor between fish fed the satiation and optimum rations and time zero fish. However, the condition factor of fish fed the maintenance ration was significantly lower than that of time zero fish.

There were significant differences in condition factor between treatments (Figure 31). The condition factor of fish fed the maintenance (0.4 % bw/day) ration was the lowest of all three treatments (Figure 30), and was significantly different from that of fish fed both the optimum and satiation rations (Figure 30). The condition factor of fish fed the optimum ration (1.6 % bw/day) was intermediate to that of fish fed the maintenance and satiation rations (Figure 30), and was statistically different from both treatments (Figure 30). The condition factor of fish fed the satiation ration (3.4 % bw/day) was significantly higher than all other treatments (Figure 30).

5.1.7.2 Gonadosomatic Index

There were no significant differences between experimental replicates with regard to the Gonadosomatic Index (GSI) over the 90-day growth experiment, thus replicates were pooled for subsequent analyses. The GSI of fish fed satiation rations (3.4 % bw/day) were significantly higher than fish fed maintenance rations (Figure 31). There were no significant differences in GSI

between treatments and time zero. Fish fed optimum rations did not show a significantly different GSI than fish fed the maintenance and satiation rations (Figure 31).

5.1.7.3 Hepatosomatic Index

There were no significant differences between experimental replicates with regard to the Hepatosomatic Index (HSI) over the 90-day growth experiment, thus replicates were pooled for subsequent analyses. Analysis showed significant differences between treatments The HSI of fish fed the maintenance (0.4 % bw/day) ration was the lowest of all three treatments (Figure 30), and was significantly different from that of fish fed both the optimum and satiation rations (Figure 30). The HSI of fish fed the optimum ration (1.6 % bw/day) was intermediate to that of fish fed the maintenance and satiation rations (Figure 30), and was significantly different from both treatments (Figure 30). The HSI of fish fed the satiation ration (3.4 % bw/day) was significantly higher than all other treatments (Figure 30).

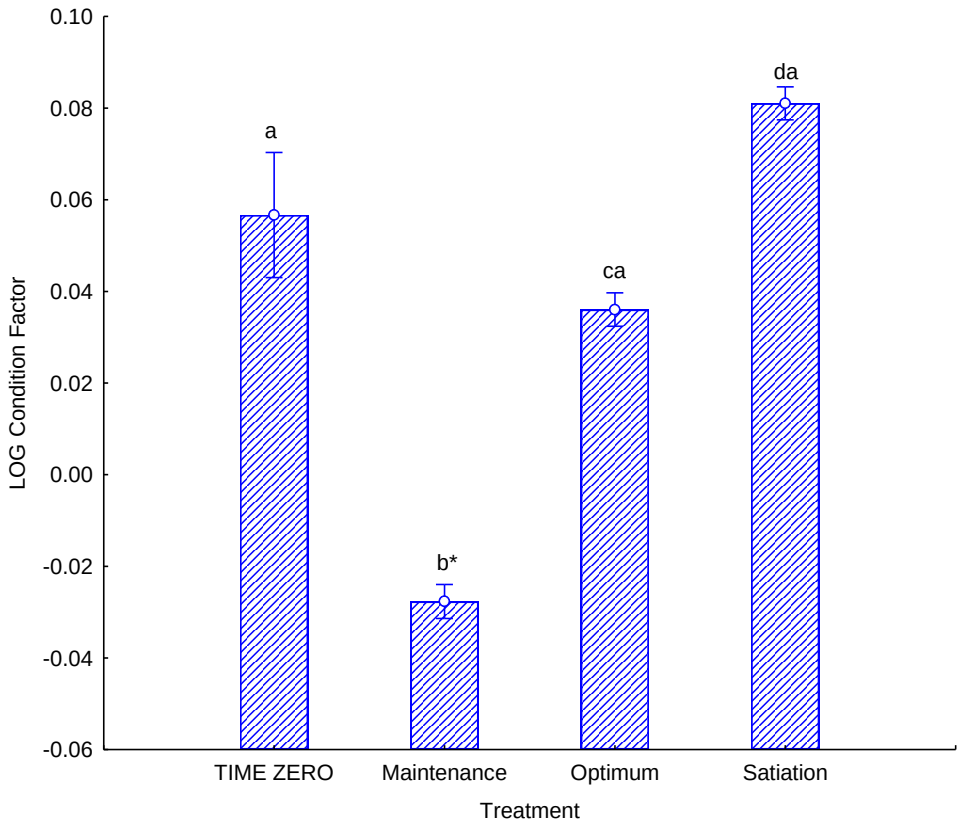

Figure 35: Log condition factor by treatment ± standard error. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

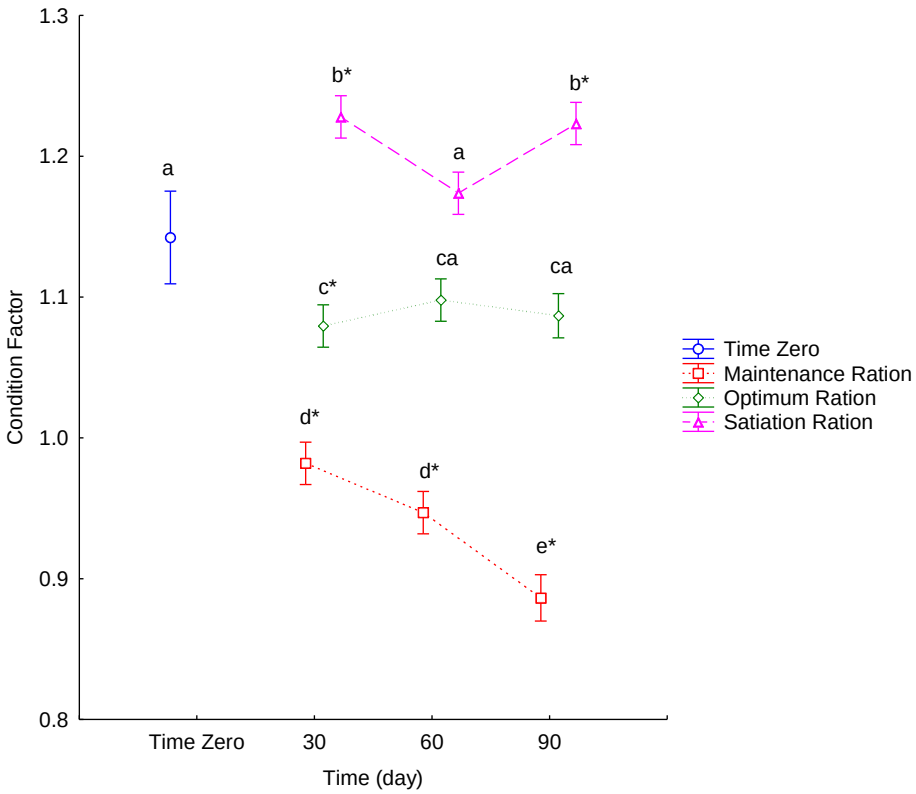

Figure 36: Condition factor by treatment ± standard error over time. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

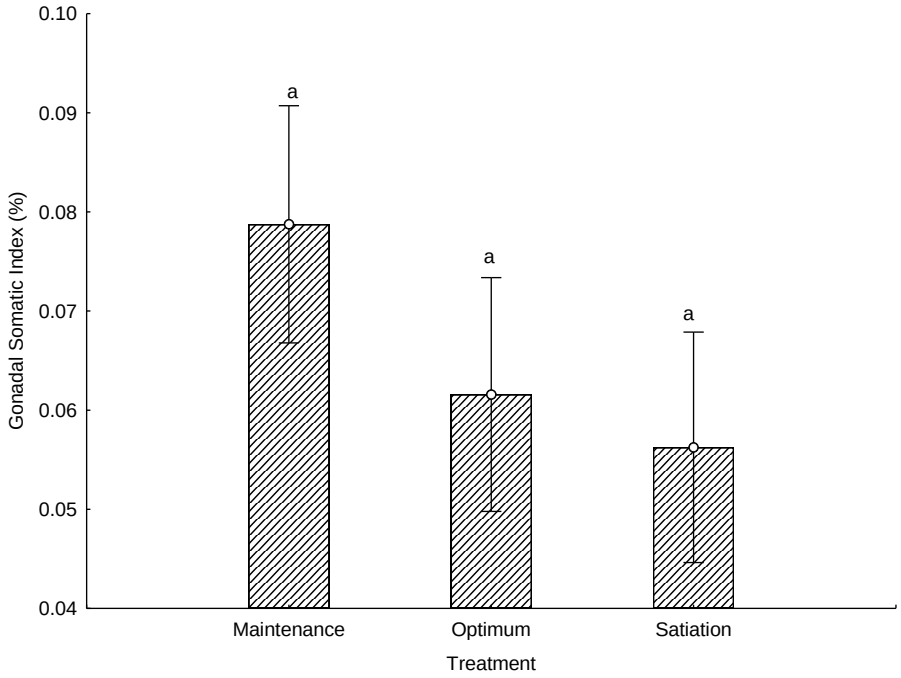

Figure 37: GSI by treatment ± standard error over time. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

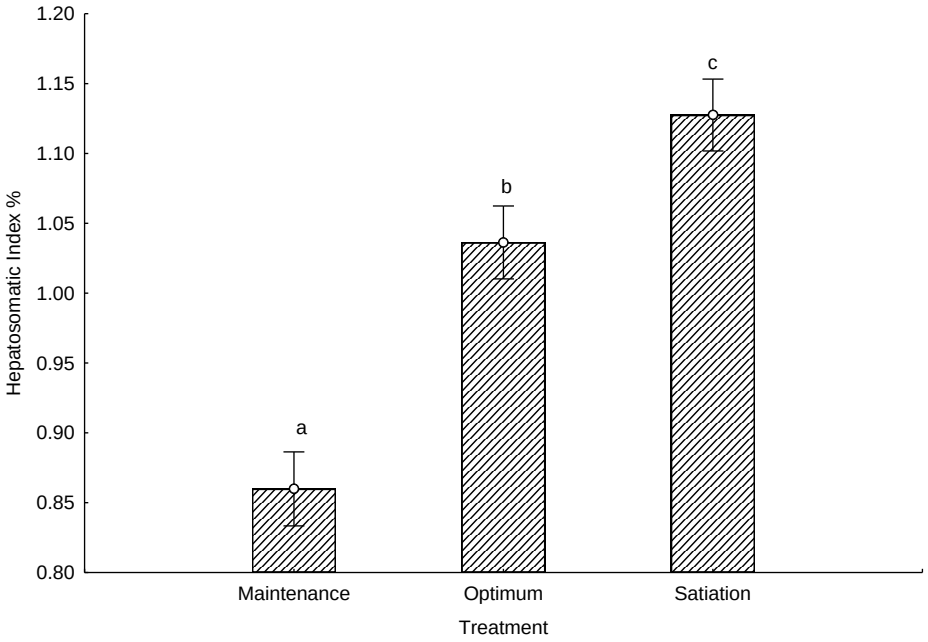

Figure 38: Hepatosomatic index by treatment ± standard error. Juvenile rainbow trout (n = 216) were fed either a maintenance (0.4 % bw/day), optimum (1.6 % bw/day), or satiation (3.4 % bw/day) ration, and subsampled once every thirty days over a 90-day standard growth experiment. Values without an alphabetical superscript in common are significantly different ($p \leq 0.05$). Values with an asterisk are significantly different than time zero.

6.0 DISCUSSION

In fish biology, energy is required for life, growth and reproduction (Jobling, 1994). The main source of energy for fish comes from their food (Moyle, 2004; Diana, 2004, Jobling, 1994). This energy is retained either through the production of new tissues, or is channelled into the production of gametes to advance reproduction (Moyle, 2004; Diana, 2004, Jobling, 1994). In fishery science, experimental studies focus on quantifying the different components of bioenergetics, such as growth, body composition and energy content, in order to produce as complete a picture as possible of the physiological transformations and pathways of energy partitioning occurring within the fish (Jobling, 1994).

It is important to understand the flow and transformation of energy in and between living organisms, and between living organisms and their environment as it can lead to the development of energy budgets, which can lead to predictions about fish populations (Diana, 2004). In this study, the bioenergetic components of interest were the total body water, dry mass and energy content in somatic tissues. The standard methodology to examine these bioenergetic components necessitate the killing of the individual, which negates the potential for repeated measures on the same individual and suppresses compositional studies involving endangered or threatened species (Cox and Hartman, 2005; Crossin and Hinch, 2005; Duncan *et al.,* 2007; Pothoven *et al.,* 2008; Hanson *et al.,* 2010). Furthermore, traditional measures can only be carried out in the laboratory, thus making bioenergetic studies in the field impossible (Cox and Hartman, 2005; Crossin and Hinch, 2005; Duncan *et al.,* 2007; Pothoven *et al.,* 2008; Hanson *et*

al., 2010). These limitations have increased interest to develop nonlethal techniques that are quicker, more portable and less expensive than the traditional bioenergetics methodology (Cox and Hartman, 2005; Crossin and Hinch, 2005; Duncan *et al.,* 2007; Pothoven *et al.,* 2008; Hanson *et al.,* 2010)

Recent research has shown bioelectrical impedance analysis to be a quick, easy-to-use, non-invasive, nonlethal, effective tool for estimating the proximate composition and energy content of fish, and is said to hold great promise by aquatic biologists as a means to provide researchers with a viable alternative to proximate analysis and bomb calorimetry (Cox and Hartman 2005; Duncan *et al.,* 2007; Pothoven *et al.,* 2008). The objective of this study was to evaluate the capability of BIA to assess the impact that ration has on the bioenergetics of juvenile rainbow trout (*Oncorhynchus mykiss*), and to develop species-specific indices to predict energy content, total body water and dry mass.

It was necessary to achieve differential growth patterns as a means to create as complete of a picture as possible of the physiological transformations and pathways of energy partitioning occurring within the juvenile rainbow trout test population. The three levels of ration used in this experiment successfully established three different growth and energy levels ranging from very low to high within the test population, and thus ensured the widest possible range of values to relate to BIA. The establishment of a wide range of growth and energy was necessary in order to create calibration curves relating BIA to the proximate composition and energy content of rainbow trout, and to assess the ability of BIA to successfully predict the energy of fish experiencing different growth patterns.

When compared against the traditional methods of compositional analysis, the utility of BIA as a method for the nonlethal assessment of juvenile rainbow trout was extremely high, evidenced by the predictions of total energy content (r^2 = 0.90), total body water (r^2 = 0.89) and total dry mass (r^2 = 0.80). Furthermore, BIA predictions and condition indices followed the same differential growth pattern throughout the study, which was indicative of their strong relationship to one another.

The precision achieved in this study is similar to that found by Cox and Hartman (2005) involving brook trout *Salvelinus Fontinalis*, Pothoven *et al.* (2008) for yellow perch *Perca flavescens*, walleye *Sander vitreus*, and lake whitefish *Coregonus clupeaformis*, Duncan *et al.* (2007) involving cobia *Rachycentron candadum* and Fitzhugh *et al.* (2010) concerning red hake *Urophycis chuss*, Acadian redfish *Sebastes fesiatus*, haddock *Melanogrammus aeglefinus*, Atlantic cod *Gadus morhua*, pollock *Pollachius virens*, winter flounder *Pseudopleuronectes americanaus*, American plaice *Hippoglossoides platessoides*, yellowtail flounder *Limanada ferruginea*, Atlantic herring *Cluppea harrengus*, tilefish *Lopholatitus chameleonticeps* and black sea bass *Centropristis striata.* Similar to the outcome of this study, these studies found that predictive relationships with BIA measures could explain more than 90% of the variation in total water content, total dry mass and total lipid content (Pothoven *et al.*, 2008; Duncan *et al.*, 2007; Cox and Hartman, 2005). Conversely, this study produced far better estimates of body composition than those by Bosworth and Wolters (2001) for channel catfish *Ictuluras Punctatus*, denoting the progress that has

been made in refining this technique over the last decade. The medical community, for instance, has been using BIA to assess the body composition and condition of people since the 1970s, and it is still being fine-tuned (Pothoven *et al.*, 2008).

One aspect of BIA that continues to be refined involves the selection of the best measure of impedance to be used in the conductor-volume model (Pothoven *et al.*, 2008). Reactance in series, reactance in parallel, resistance in series and resistance in parallel are the variables generally used in the conductor-volume model as a means to determine which measure of impedance can produce the most accurate estimation of proximate composition and caloric content (Cox and Hartman 2005; Duncan *et al.*, 2007; Pothoven *et al.*, 2008).

Similar to the findings of Pothoven *et al.* (2008), this study found that parallel resistance produced the best estimates of proximate composition and caloric content. Conversely, Cox and Hartman (2005) relied upon resistance in series for their assessments and found predictive relationships that could explain more than 95% of the variation in dry mass, lipid and total body water content. These results illustrate the continual refining of BIA models, thus illustrating the necessity for additional research to focus on developing better BIA models.

Similar to the findings reported by Cox and Hartman (2005) and Duncan (2009), the strong predictability and accuracy of this study was due primarily to the simple, uncomplicated body geometry of rainbow trout. Rainbow trout, like all fish, have a fusiform, cylindrically-shaped body that houses the majority of

mass in the thorax (Jobling, 1995; Cox and Hartman, 2005). Impedance measurements of this location would therefore produce an accurate approximation of the whole body of the fish (Cox and Hartman, 2005; Pothoven *et al.* 2008).

The strong linear relationships found in this study suggest that they might also be able to predict the body composition and energy content of other species similar in shape to rainbow trout. Since salmonids share a similar geometric shape (Quinn, 2004), the BIA prediction models established in this study may be applicable to other salmonids (Cox and Hartman, 2005; Duncan, 2009). Furthermore, the linear relationships found in this study were strong, regardless of fish size; therefore the relationships observed for rainbow trout should also hold true for other species, regardless of their size, as long as they share a similar geometric shape (Cox and Hartman, 2005, Duncan, 2009).

In this study, bioelectrical impedance analysis has proven to be a rapid, user-friendly, non-invasive, and most importantly, nonlethal technique that can be used as an effective tool for estimating the proximate composition and energy content of rainbow trout. The ability of BIA to accurately estimate the proximate composition of rainbow trout, and possibly other species of salmonids, will permit increased knowledge of proximate composition and bioenergetics on temporal and spatial scales that were previously thought impractical (Cox and Hartman, 2005). Furthermore, the robustness and portability of this technique can now allow biologists to accurately measure the bioenergetics of fish in the field at both the individual and population level (Cox and Hartman 2005; Duncan *et al.*, 2007;Duncan, 2009; Pothoven *et al.*, 2008).

At the individual level, BIA can work to improve the precision of bioenergetic models on rainbow trout by permitting repeated measures on the same individual over the course of a study (Pothoven *et al.,* 2008, Cox and Hartman, 2005). At the population level, BIA can help to provide detailed assessments of body composition, energy content, and condition on fish that reside within a particular ecosystem. Furthermore, BIA and condition indices can be used in combination to provide biologists with a new, more robust way of looking at data, provide new understanding of fish condition, and help to facilitate better management strategies and remedial actions (Pothoven *et al.,* 2008; Cox and Hartman, 2005; Duncan, 2009).

Future applications could include programs that use BIA, in combination with other condition indices such as GSI, to monitor the physiological and bioenergetic changes in fish during spawning, as previously suggested by Duncan (2009) and Fitzhugh (2010). BIA could also be used to generate reproduction models to determine and predict reproductive fitness, age of reproductive maturation, degree of fertility, fecundity and survivorship rates (Moyle and Cech, 2004; Duncan, 2009; Fitzhugh, 2010). BIA could also be used as a means to monitor the compositional and energetic changes of fish during migration, or be used to predict the migrational success of a particular species (Moyle and Cech, 2004; Duncan, 2009; Fitzhugh, 2010).

Aquaculture facilities can also use BIA as a tool to assess the composition and quality of their product, as suggested by Duncan *et al.,* (2007). The ability to monitor changes in body composition and energy content of aquacultured fish

would be a great asset to farming operations, as it would help eliminate the wasteful feeding programs and thus increase profit margins (Duncan et al, 2007; Duncan, 2009). Furthermore, BIA can allow farming operations to closely monitor the body composition of their products, which can help to create branding opportunities that would produce "designer fish", composed of highly sought after tissue composition properties (Bosworth and Wolters, 2001; Duncan et al, 2007; Duncan, 2009). High lipid levels, for instance, are highly desirable in salmon fillets by the sushi and sashimi trade (Duncan et al, 2007). Moreover, the portability, relative inexpensiveness and robustness of BIA equipment make well suited for the rigors associated with fish farming operations (Bosworth and Wolters, 2001l Duncan *et al.*, 2007; Duncan, 2009).

Subsequent experimental studies first must evaluate the reproducibility of this model using independent data sets of juvenile rainbow trout. If validated, future studies should then focus on testing the applicability of this model on mature, adult rainbow trout. If successful, energetic and compositional studies should then focus on assessing BIA's ability to determine sex and predict the age of reproductive maturation of juvenile rainbow trout. Further studies should also examine BIA's ability to assess the reproductive fitness, degree of fertility, and fecundity potential of mature adult rainbow trout. Furthermore, ecotoxicologists can use BIA to assess the impacts that environmentally relevant chronic and pulse contaminant exposure has on the bioenergetics of fish.

The amount of time allocated to conduct this study was relatively short, and thus limited the variety of proximate body components able to be examined.

Subsequent BIA studies involving juvenile rainbow trout should assess its predictive capabilities on measures of proximate analysis that were not addressed in this study such as free-fat mass (FFM), total body protein (TBP) total body ash (TBA) and total body fat mass (TBF). A larger arsenal of predictive models would help to form as complete of a picture as possible of the physiological transformations and pathways of energy partitioning occurring within the fish at various life stages.

Another possible limitation of this study, which could explain the 10% BIA prediction error, was that caloric and proximate analysis was performed on excised subsamples of muscle tissue instead of the whole-fish carcass. The correlation between whole-body electrical impedance with a subsample, instead of with the entire fish, may have attributed to some of the error associated with the prediction equations. To increase the predictive strength of BIA equations, future studies should correlate BIA with the actual energy content and body composition found in the whole fish.

One of the few problems encountered in this study was the occurrence of dispensatory growth. Although all treatments were affected by this phenomenon, it was more prominent involving fish fed maintenance rations, a finding commonly reported in the literature (Diana, 2004). One aspect known to attribute to the occurrence of depensatory growth is feeding rate (Moyle and Cech, 2004). The use of a high feeding rate, as was the case in this study, increases the chance for depensatory growth to occur (Diana, 2004). However, according to the findings of Ruohonen *et al.,* (1998), a relatively high frequency of feeding is

necessary to ensure that all food given is consumed, and to facilitate a wide spectrum of differential growth. To lessen the occurrence of depensatory growth, subsequent BIA growth studies should employ a reduced feeding regiment.

BIA was proven to be able to accurately predict the energy content and proximate composition of rainbow trout. Its predictions were shown to share the same differential growth pattern with condition indices, denoting a strong relationship to one another. BIA also proved to be an effective tool to monitor changes in body composition and caloric content over time. The results of this study, along with much of the existing literature, show BIA to be an accurate and nonlethal tool to estimate the bioenergetics and proximate composition of fish.

7.0 CONCLUSIONS

The manipulation of ration significantly impacted the growth, growth rate, energy density, caloric content, total body water, dry mass, condition factor and the hepatosomatic index of the juvenile rainbow trout. BIA was able to successfully reflect notable significant differences between treatments with regard to total energy content, energy density, total body water and dry mass, and thus mirror the impacts associated with ration manipulation. Furthermore, BIA was shown to accurately predict energy content ($r^2 = 0.90$), total body water ($r^2 = 0.89$) and dry mass ($r^2 = 0.80$) for juvenile rainbow trout. The level of precision achieved in this study is similar to those found in previous studies (Cox and Hartman, 2005; Pothoven *et al.,* 2008; Duncan *et al.,* 2007). The success of the conductor-volume model (L^2R_P) to estimate the proximate composition and energy content of juvenile rainbow trout is due in part to the suitability of the geometric shape of rainbow trout to BIA testing, and further illustrates the cross-species utility of BIA equations discussed in previous studies (Cox and Hartman, 2005; Pothoven *et al.,* 2008; Duncan *et al.,* 2007).

The BIA equations developed in this study can act to improve the precision of bioenergetic models on rainbow trout, and help to provide better assessments of body composition, energy content, and condition of rainbow trout populations. Furthermore, this BIA model can help to assess the bioenergetic impacts of chemicals on fish in a nonlethal manner. These results, along with much of the existing literature, indicate that BIA may be an accurate and reliable tool to estimate the bioenergetics and proximate composition of fish.

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