ABSTRACT

Title of Thesis: THE EFFECTIVENESS OF DUAL ENERGY X-RAY ABSORPTIOMETRY TO NON-INVASIVELY DETERMINE BODY COMPOSITION OF HYBRID STRIPED BASS

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The primary objective of this research project was to determine if dual energy x-ray absorptiometry was a valid method for non-invasively determining body composition of hybrid striped bass (*Morone chrysops* ♀ *x. Morone saxatilis* ♂). The first study utilized four dietary treatments to create a population with a broad range of body compositions. Fish were scanned using the Lunar model DPX-L dual energy x-ray absorptiometer in detailed medium and high resolution scan modes, manually analyzed for region of interest, in stacks, and scanned with a Hologic model QDR-4500A fan beam DXA. When compared to proximate analysis, results produced significant correlations and regression equations for: water and protein compared to lean, grams of fat, and ash compared to bone mineral composition. The second study utilized four dietary treatments of various energy:protein ratios to determine the ability of DXA to distinguish variations in body composition in accordance with dietary treatment. Fish were scanned with the Lunar model DPX-L DXA as well as the Lunar PIXImus DXA. The two scan values were compared to one another as well as to proximate analysis. Significant

correlations and regression equations were calculated for the same comparisons as listed above. Fan beam DXA was less reliable as a predictor of body composition than pencil beam, and there was no difference between values from scan modes for the pencil beam DXA. PIXImus DXA was limited by size of the scan area, but was as successful as the larger pencil beam method for predicting certain parameters of body composition.

# THE EFFECTIVENESS OF DUAL ENERGY X-RAY ABSORPTIOMETRY TO NON-INVASIVELY DETERMINE BODY COMPOSITION OF HYBRID STRIPED BASS

By

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LIST OF ABBREVIATIONS

| Abbreviation | Word Description |
|---|---|
| ANOVA | analysis of variance |
| ARS | Agriculture Research Station |
| BMC | bone mineral composition |
| BMD | bone mineral density |
| CV | coefficient of variation |
| DM | detailed medium mode |
| DO | dissolved oxygen |
| DPA | dual photon absorptiometry |
| DXA | dual energy x-ray absorptiometry |
| FM | fish meal |
| g | gram |
| HRM | high resolution mode |
| kg | kilogram |
| L | liter |
| LSD | least significant difference |
| mg | milligram |
| mL/g | milliliters per gram |
| ppt | parts per thousand |
| $r^2$ | correlation coefficient |
| SAS | Statistical Analytical Systems |
| SCA | sectional scan area |

| Abbreviation | Word Description |
| --- | --- |
| SEM | standard error of the mean |
| TBW | total body weight |
| USDA | United States Department of Agriculture |
| μSV | microsievert |

## Literature Review

The ability to accurately determine body composition is a primary focus in many scientific studies, particularly those of growth, aging, and nutrition, in both humans and animals. What is meant by body composition differs according to how the body is viewed. Anatomists may identify body composition in terms of the parameters of organs and/or tissues, physiologists may think in terms of intra and extra-cellular components, nutritionists in terms of nitrogen retention, lipid or energy contents, and breeders may identify body composition in terms of lean, fat, and bone. The methods selected to estimate body composition depend on these varying viewpoints (Fuller et al., 1990). There have been numerous studies on animals involving body composition, but most of these have relied on carcass analysis end points (Yasumura et. al., 1993).

Body composition methods can be divided into different groups. A possibility to group them is by dividing them into direct, indirect, and doubly indirect methods. A direct method includes chemical carcass analysis, which can only be applied to subjects in vitro. In vivo data can be determined directly by in vivo neutron activation analysis. With this method, the subject is bombarded with fast neutrons at a known energy level. The neutrons are captured in the body by specific chemical elements, depending on their energy level. This results in the formation of specific isotopes with an initially higher energy level. The energy is then emitted as gamma rays with a well-defined energy specific to the formed isotope. From its emitted energy, the amount of a specific element in the subject can be calculated. This technique is capable of determining the amounts of nitrogen, calcium, chlorine, and carbon in the subject. From this, body protein, bone mass, extra-cellular water, and fat, respectively, can be calculated. Unfortunately, this

method is expensive and requires the subject to be radiated (Deurenberg and Schutz, 1995).

Nearly all techniques available for determining body composition are indirect measurements. That is, these techniques measure a particular physical property of the body, which is related to body composition, and then use this data to calculate composition based on the assumption that there exists a consistency in the relationship of the chosen property. Indirect methods such as densitometry, $^{40}K$ measurements, and dilution techniques for the measurement of fat-free mass make use of data based on chemical carcass analysis. A major restraint for both densitometry and $^{40}K$ measurements is their reliance on the assumption that the chemical composition, including relative amounts of water, minerals, proteins, and potassium, remains constant in the body (Forbes, 1987). In truth, there remain many differences in the composition of fat-free mass in subjects of varying age, gender, fitness level, and even ethnicity. Computerized tomography or nuclear magnetic resonance imaging give a two-dimensional image of the body at the scanned body level. Analyzing the subsequent scans allows the calculation of the cross-sectional area of tissues. By making more scans of the subject, the volume of the tissues can be calculated and if the density of these tissues id known, the amount of tissue in kilograms can be calculated (Sjöström, 1991). In calculating the weight of tissues from their volume, assumed densities and chemical composition data are used which can result in errors. Dual-energy x-ray absorptiometry is also able to generate a two-dimensional image of the scanned subject, but one dimensional images are more commonplace. The use of x-rays at two different levels of energy to scan the subject results in quite a different image than the aforementioned

methods. The determination of bone density using this technique is very precise, whereas the figures for lean and fat tissues are less reliable due to the influence of tissue thickness and hydration (Deurenberg and Schutz, 1995).

Doubly indirect methods are those based on a statistical relationship between easily measured body parameters and data obtained using either direct or indirect methods. A popular doubly indirect method is bioelectrical impedance. A small alternating current is applied to the body, and resistance of the body to that current is then measured. Since only water and electrolytes are capable of conducting the current, total impedance is in truth a measure of total body water. When water is assumed to be a constant part of fat-free mass, the resulting impedance can also predict fat-free mass. This method tends to have a large prediction error, influenced by variables such as body build, body water distribution, extra-cellular water, and total body water. Other double indirect methods such as body mass index, body circumferences, creatinine, and 3-methylhistidine excretion in 24-hour urine also exhibit significant limitations (Deurenberg and Schutz, 1995).

Methods of determining body composition have been of two varieties, those which describe the chemical make-up of the body and those which describe the anatomical distribution of tissues. In the first, such as carcass analysis, the body is considered a homogeneous mixture of substances. This technique involves the sampling of small aliquots of homogenates from whole animals to chemically determine the body elements. Small variations in homogeneity and extrapolation from micro-sample analyses to whole body estimates of chemical composition can lead to large errors in determined body composition. Additionally, homogenization of an entire animal and

chemical analyses can be very time consuming (Yasumura et. al., 1993). For studies involving small or large animals, the destructive technique of comparative slaughter with dissection or homogenization of the carcass is very widely used and is often the most cost effective method.

The need for nondestructive techniques arises in human studies, with valuable animals, or when the sequential study of individuals is necessary or desired (Fuller et. al., 1990). Sequential measurements are of particular interest when following change in a subject over a given period of time. Growth and development is usually accompanied by change in human and animal subjects. This change can occur in a variety of ways, including shifts in the total and relative amounts of fat, lean and bone tissue mass (Mitchell et al., 1996). In vivo measurements are usually faster than traditional destructive analyses. Good accuracy and precision can be achieved by using size and weight matched phantom standards according to the method of measurement (Yasumura et. al., 1993).

A series of techniques for the in vivo measurement of body elemental composition have been developed. These techniques are suitable for a variety of studies where the effect of treatment can be studied longitudinally because the in vivo measurements can be repeated as often as necessary in the same animals (Yasumura et. al., 1993). Three approaches are being taken concerning the development of techniques used for measuring body composition: 1) improving current methods, 2) formulating and devising new measurement techniques for body composition, and 3) focusing on particular problems of changes in body composition (Kehayias, 1993).

A review by Topel and Kauffman (1988) reported on more than 30 techniques for estimating live animal or carcass composition. Traditionally, measurements of back fat and longissimus muscle area have been used to indicate the body composition of an entire live animal. Recently, research concerning body composition analysis has focused on a variety of instruments designed to obtain more detailed information from inside a live animal (Mitchell et al., 1996b).

The technique using x-ray absorptiometry was originally developed for the measurement of bone mineral content in humans. It evolved from the first single photon absorptiometers introduced in 1963, which used energy from a source such as iodine 125 or americium 241 to measure bone density at a single site. Dual photon absorptiometers were then developed to overcome the limitations of single photon technology, such as the inability to compensate for differences in thickness and shape of soft tissue inherent with use of in vivo applications. The method was based on the energy dependence of the atomic attenuation coefficients for photon absorption of bone mineral, which contains the high atomic number element calcium, and soft tissue, which contains mostly low atomic number elements--hydrogen, oxygen, and carbon. Comparing the attenuation of two photon beams produced by the energy source gadolinium provided more accurate values for bone density, no matter the thickness or various contours of the surrounding soft tissues. These absorptiometers also permitted scans of various anatomic sites in less time with greater accuracy and precision (Lauten et al., 2000). This dual-energy photon absorptiometry (DPA) was originally developed for the measurement of bone mineral mass and density in humans (Peppler and Mazess, 1981). The drawbacks of these first instruments were due to the limitations of their source of radioactivity producing the two

photon energies, and due to the assumption that the attenuation coefficient of soft tissue is constant (Roubenoff et al., 1993).

The first absorptiometers to use x-rays were introduced in 1987. An x-ray tube placed behind a filter that converts the x-ray beam into one that has two main energy peaks has now replaced radioactive sources of energy. This advancement in the technology further reduced scanning time and allowed decreased collimation, which in turn increased scan resolution. Since the technology's development, it has been known that x-ray attenuation data can also provide information concerning the fat and lean composition of soft tissue as a byproduct of the bone mineral measurement (Lauten et al., 2000). The attenuation of non-bone tissue is now measured rather than assumed to be constant.

With the evolution of the absorptiometry technology has come faster scanning times—10 to 20 minutes for adult human patients—and decreased levels of radiation exposure. While DXA technology uses x-rays, it differs significantly from traditional radiography, which is not a sufficiently sensitive tool for determination of bone mineral density. In order for change to be visualized on radiographic film, a 40 to 50% loss of mineral must have occurred. Quantification of bone density is not practical using conventional radiography, and fat and lean tissues are not able to be measured using this method. With the use of DXA, it has been possible to identify minute changes in bone mineral density of a human patient at any number of selected sites. It is also possible to perform sequential scans of a subject to quantify progressive changes in body composition, with a minimum exposure of radiation to the subject (Lauten et al., 2000). The radiation dose varies between manufacturers, machines and scan speeds but the

effective dose for a pre-menopausal woman is around 2μSv for whole body composition analysis. This is of a similar order of magnitude as a single day's background radiation (Jebb and Elia, 1993).

Although DXA systems differ slightly depending on their manufacturer, all systems consist of shared basic components and functions. There is an x-ray source with a collimator to direct the beam of x-rays through the body of the subject. There is also an x-ray detector system that is capable of measuring the intensity of the x-ray beam once it has passed through the body of the subject. This measurement is made at two distinct energy levels of 38 and 70 keV. Finally, there is a motorized drive system that is able to move the x-ray beam in a particular scanning pattern over the subject's body. In practice, the subject lies on the table while the scanning arm passes over them in a rectilinear manner. The actual time it takes varies depending on the machine employed. Some have variable scan times, but none exceed 20 minutes for a single adult human whole body composition analysis. The net result of the scan is that a measurement is made of the attenuation of the x-ray beam, at two energies, at every point in the defined scan area (Nord and Payne 1995).

Currently, the equipment for DXA is available from three different manufacturers, Hologic, Lunar, and Norland. Each manufacturer has a variety of instruments, not all of which are capable of performing whole body tissue composition analysis. Although the principle is the same for each, there are technical differences in the hardware and software such that measurements obtained using one instrument are not necessarily the same as measurements obtained by another. One of the most limiting factors for every machine concerning body composition analysis is the size of the scanning area, which is

approximately 190 x 60 cm. For this reason, DXA analysis cannot be used in the very obese, and the accuracy with which individual segments may be distinguished tends to decline as body size increases (Jebb and Elia, 1993). The largest manufacturers of DXA use different hardware and software arrangements for their calibration, data collection, and analysis. The DXA instruments from different manufacturers do not appear to offer directly comparable results (Pritchard et al., 1992; Tothill et al., 1994). There have been a few reports of comparisons of body composition results by DXA instruments from the different manufacturers that have tended to focus on healthy normal weight volunteers. This leaves matters of comparability of various DXA results somewhat unsolved (Kistrop and Svendsen, 1997).

The reliability of DXA depends on operator, subject, and apparatus factors. The precision of an individual DXA instrument for the assessment of bone is excellent, with coefficients of variation (CV) < 1.0% (Johnson et al., 1991; LeBlanc et al., 1990; Mazess et al., 1989; Nuti et al., 1987; Fuller et al., 1992; Compston et al., 1992). However, reported CVs for soft tissue measurements have been up to 3.1% for lean tissue, 8.6% for fat tissue, and 7.9% for percent body fat (Mazess et al., 1990; Johnson et al., 1991; Fuller et al., 1992; Compston et al., 1992; Heymsfield et al., 1989; Kelly et al., 1991; Slosman et al., 1992; Haarbo et al., 1991; Pritchard et al., 1993; Rico et al., 1994). The difference in precision between bone and soft tissue is not surprising primarily because DXA was originally designed to assess bone, and soft tissue was originally considered as heterogeneous. Assumptions do tend to be made that bone precision of DXA instrumentation extends to soft tissue precision. Very similar or identical DXA instruments may produce significant inter- and intra-site differences in soft tissue

measurements with both human subjects and whole body phantoms. Therefore, the need for both rigorous and standardized cross-calibration procedures for soft tissue measurement by DXA is needed (Economos et al., 1997).

The components of the body can be grouped into three classes with respect to their x-ray attenuation properties: bone mineral, fat (lipid), and lean (nonfat soft tissue). The x-ray properties of these materials are dissimilar primarily because of their differing proportions of high atomic number elements. Bone mineral contains a large percentage of calcium and phosphorus, whereas soft tissues are composed nearly completely of hydrogen, carbon, and oxygen. There is a slight but detectable difference between the lean and fat components of soft tissue. The lean compartment components contain traces of potassium, chlorine, sulfur, and calcium—primarily as electrolytes. The fat compartment contains none of these. The ratio of low to high energy attenuation (R-values) in soft tissue is used to make the distinction between fat and fat-free mass, minus the bone component (VanLoan and Mayclin, 1992). The soft tissue attenuation ratio ($R_{ST}$) values have been reported to range from 1.2 for fat to 1.4 for 100% lean in calibration studies at energies of 38 and 70 keV (Heymsfield et al., 1989).

The attenuation of pure fat ($R_F$) and of bone-free lean tissue ($R_L$) are known from both theoretical calculations and human experiments. Given the subject's $R_{ST}$ and the known $R_S$ for fat and lean, two equations can be solved—one at each x-ray energy—with two unknowns to calculate the proportion of fat ($\alpha$) and lean tissue ($\beta$) in each pixel (Roubenoff et al., 1993):

$$R_{ST}\ (38\ keV) = \alpha(R_F) + \beta(R_L)$$

$$R_{ST}\ (70\ keV) = \alpha(R_F) + \beta(R_L)$$

The DXA technique makes up for the limitation of two materials by making use of the fact that bone mineral in the body is concentrated in dense local regions, namely bones. Therefore it is possible to sort the pixels into those which contain bone and those which do not, and to analyze the two types accordingly. The pixels determined not to contain bone are analyzed per pixel (0.46 $cm^2$) for fat and lean as the two specific materials. The pixels containing bone are analyzed for bone and general soft tissue as their two components. The particular mix of fat and lean that is identified as 'soft tissue' in those bone-containing pixels must be estimated, since it cannot be specifically measured. In DXA regional scans, such as that of the lumbar spine, the soft tissue that is 'hidden' by the bone is simply assumed to be the same composition as the surrounding soft tissue. The surrounding soft tissue can be measured for composition, and remains to be a reasonable assumption of the 'hidden' tissue (Nord and Payne 1995).

To arrive at an unbiased and accurate measurement of body compartments it is necessary to avoid assumptions about these compartments. By measuring essentially three compartments—bone, bone-free lean, and fat—DXA is not free of the assumption of uniform hydration. Abnormal hydration due to illness, stress, or extremes of age can alter the scanned subject's $R_L$ value so that it deviates significantly from the $R_L$ assumed from calculations using DXA equations. This leads to an error in the amount of lean tissue attributed to each pixel. The degree to which DXA measurements of soft tissue are sensitive to changes in hydration in adult humans, let alone changes in any animal subject, has not been identified, and requires further investigation to clarify. Until such clarification, the hydration of lean body mass is assumed to be uniform and fixed at a value of 0.73 mL/g (Roubenoff and Kehayias, 1991). Currently, only multi-compartment

models that measure body cell mass or body protein as well as body water and body fat independently, are able to clearly avoid the need to assume a constant hydration of the lean mass (Roubenoff et al., 1993).

Computerized algorithms quantify the various components of body composition. Bone mineral content (BMC), expressed in grams, corresponds to 60% of hydrated bone tissue that is mineral and has been shown to correlate well with bone ash. Soft tissue, also expressed in grams, consists of all tissue not included in the category of bone and is composed of fat and lean mass. Fat tissue (essentially water-free tissue) may be separated from the lean mass, which includes all hydrated tissues other than bone mineral. The sum of BMC and soft tissue provides a DXA-derived body weight for the given test subject. Bone mineral density (BMD) is the bone mineral content value divided by the area computed to be bone by the DXA equipment, expressed as grams per square centimeter. Bone mineral density is a value computed from a 2-dimensional view of a 3-dimensional object, and therefore requires the assumption that the third dimension—bone thickness—is relatively constant, based on body weight and trunk thickness of the subject. Although BMD is the most commonly reported DXA value in research literature, the use of this particular calculated value is limited when applied to nonhuman patients due to the wide range of size, conformation, and bone geometry in various species of animals (Lauten et al., 2001).

Both DPA and DXA have received enormous attention for their abilities to determine human body composition and detect incremental changes within this composition. The DXA method is now considered by many in the scientific field to be the method of choice for measurement of human body composition. Studies have

included measurements of both pediatric (Venkataraman and Ahluwalia, 1992; Chan, 1992; Brunton et al., 1993) and adult subjects (VanLoan and Mayclin, 1992; Rico et al., 1994; Wellens et al., 1994), as well as studies involving patients at risks of malnutrition or metabolic changes due to dialysis (Borovnicar et al., 1996), diabetes (Kistorp and Svendsen, 1997), or even renal transplants (Hart et al., 1993) to name a few.

DXA has been used in numerous studies to determine the body composition of various animal species, including live pigs (Svendsen et al., 1993; Brunton et al., 1993; Pintauro et al., 1996; Mitchell et al., 1996a, 1996b, 1998a,b; Mitchell and Scholz, 1998), pig carcasses (Mitchell et al., 1998c), chickens (Mitchell et al., 1997a), beef carcass rib sections (Mitchell et al., 1997b), rats (Rose et al., 1998), mice (Sjögren et al., 2001), dogs (Lauten et al., 2001), and cats (Lauten et al., 2000). The accuracy and precision of DXA have been well validated both in vivo and in vitro in humans, pigs from 5 to 100 kg, sheep, and rodents. Pouilles et al. (2000) performed in vivo and ex vivo lumbar spine studies in sheep, using an absorptiometer, and then correlated this data with results of ashing the dissected bone ($r = 0.98$). Rose et al. (1998) compared DXA measurements to chemical analysis for body fat, lean body mass, and BMC in lean and obese female rats, as well as developed regression equations in order to relate these two methodologies. In these experiments, although it was found that DXA tended to overestimate absolute values for percent fat, good agreement between DXA and chemical analysis was concluded with correlation coefficients confirming this finding. The regression equations that were developed and used to estimate chemical values from DXA data indicate that DXA can be used as a tool to predict chemical analysis values. Also, the ability of DXA to detect differences due to dietary treatment similar to the ability of chemical analysis

indicates that DXA may also be an appropriate method for assessing body composition in such studies.

Mitchell et al. (1994) derived formulas for more accurate calculations of percentage body fat and lean mass on the basis of DXA scan output. Comparison with chemical analysis indicated that DXA scans of pigs can be used to accurately measure the percentage and total amount of fat in the body. Additionally, DXA values for lean body mass correlated well with total body protein, and with proper calibration could potentially offer a measurement of muscle mass. According to results from a Mitchell et al. study in 1996(a), additional calibration would be necessary before reliable information regarding the true fat or lean content could be obtained from either the ham or shoulder regions of a pig.

A modified DXA-based method for the in vivo determination of body fat in mice was used by Slögren et al. (2001). The researchers developed a simplified technique to estimate body fat in mice based on their current DXA image and software calculations. Their simplified approach used only the area of soft tissue scanned that was determined to contain 50% fat or more, thus circumventing some problems of point typing and assumptions of soft tissue distribution. The amount of fat determined with the DXA imaging procedure was well correlated with the amount of adipose tissue dissected in both male and female mice. A strong correlation was also found between the percentage of fat area and total body fat as determined by chemical extraction. The modified technique was used in order to make correlations and simplify complex calculations usually used for human total body scans. Application of calibration equations should optimize the accuracy of DXA measurements of body composition, and provide and

additional advantage of standardizing the DXA measurements to carcass analysis, which is a known laboratory standard when determining final body composition (Pintauro et al., 1996).

Measurements in many animal studies can be used as models for human studies, whether it concerns application to human growth studies or merely sequential measurement of body composition using DXA. Changes in the amount and distribution by body region of various components of body composition relative to body weight would be of interest with regard to various anthropometric comparisons. Measurements of total body and regional changes in fat, lean (water and protein), and bone mineral would also be applicable in human weight loss studies. Several measurements made in DXA studies involving animals tend to be more applicable to other animal studies, whether those be concerned with growth or changes in body composition due to a variety of factors such as diet, treatment, or merely time. Rates of fat, lean and bone mineral deposition tend to be more relevant to animal studies in which optimization of lean or protein deposition and minimization of fat deposition can be important factors—namely economic (Mitchell et al., 1996a,b).

Striped bass (*Morone saxatilis)* have received considerable attention in the past decade as an important commercial aquaculture species, an economic resource, and a research tool in the United States. With commercial production comes the need to better understand a species' environmental and dietary needs, so that the species of interest may be economically successful and practical. As an alternative to the striped bass in commercial aquaculture, the striped bass x white bass (*Morone chrysops*) hybrids have also been growing in interest in recent years. This has led to the development of many

new culture techniques for successful fingerling production as well as for grow-out purposes (Williams et al., 1981; Kerby et al., 1983a,b,1987; Woods et al., 1983, 1985; Liao, 1985; Smith et al., 1985). Despite significant efforts in management, culture, and domestication, there are still questions to be answered concerning the proper feed formulation and dietary requirements of the striped bass, and its hybrids. Development of diets specifically for striped bass and its hybrids could not only increase success of the species in a cultured situation, but also produce a healthier fish in all life stages (Hughes et al., 1992).

Striped bass and its white bass hybrids are all euryhaline teleosts which are carnivorous throughout all their life stages. Their digestive physiology suggests that they are able to efficiently utilize protein and lipids in their diet, but poorly utilize fiber and raw carbohydrates (Zeigler et al., 1984). The hybrids of the striped bass have exhibited hybrid vigor when compared to the striped bass through improved growth, survival in a cultured situation, disease resistance, and hardiness during the first two years of its life (Bishop, 1968; Logan, 1968; Williams, 1971; Ware, 1975; Bonn et al., 1976; Williams et al., 1981; Smith and Jenkins, 1984; Smith et al., 1985). The hybrid bass commonly called the sunshine bass (*M.* chrysops female x *M. saxatilis* male) have not only shown faster growth, but also tolerance to a wider range of salinities (Hodson, 1989) and lower metabolic energy needs when compared to either of their parent species (Tuncer et al., 1990). These studies, along with a few others, have tried to break down and explain why hybrid vigor has lead to better production of these fish.

In a study by Tuncer et al. (1990), it was found that growth rates increased significantly with increased feeding levels from 2.5% to 5% and ad libitum among striped

bass and hybrid (*M. saxatilis* female *x M. chrysops* male)—also known as palmetto bass--juveniles. This is consistent with the fact that an increase in ration size causes an increase in growth rate for almost all fish studied in such a manner (Brett and Groves, 1979). Tuncer et al. (1990) also found that assimilation efficiencies--the fraction of energy left for growth and metabolism--were similar among feeding levels and also for both the striped bass and hybrid palmetto bass. Although these assimilation efficiencies were similar, the energy diverted to growth and metabolism differed among feeding levels, and between the two juvenile groups studied (striped and palmetto bass). An increase in ration level generally results in a decrease in growth efficiency (Brett and Groves, 1979). So, the decrease in growth efficiencies with an increased ration size for the striped and palmetto bass was actually expected.

What was not as expected was that while having the same assimilation efficiency, compared to the striped bass, the better growth efficiencies, food conversion ratios and productive protein values were those of the hybrids. This revealed that the faster growth rates, and apparent hybrid vigor, of palmetto bass was not merely due to a better appetite but depended on some sort of metabolic mechanism. At either the 2.5% or 5% feeding level, striped bass juveniles had higher energy expenditures to metabolism, and therefore lower energy available for growth than did the palmetto bass. Growth efficiencies were shown to be between 10-14% higher and the fraction to metabolism was in turn 9-14% lower for palmetto than they were for striped bass. As a result of these metabolic partitions, the palmetto (hybrid) bass grew faster than the juvenile striped bass at either feeding level (Tuncer et al., 1990).

When comparing striped bass and palmetto bass, Tuncer et al. (1990) also found that the average value obtained for the growth component in the energy budget of striped bass (29.8%) was similar to general reported values for other carnivorous fish (29% ± 6%), while the value obtained for the palmetto (hybrid) bass was very high. When compared to reported metabolism and growth values of fish (Brett and Groves, 1979), Tuncer et al. (1990) also found that palmetto bass had a significantly higher growth fraction (41% vs. 29%) but a near average metabolism fraction (41% vs. 44%).

Overall comparison of striped bass and palmetto bass juveniles by Tuncer et al. (1990) has shown that the palmetto bass exhibit hybrid vigor, in terms of better production, as early as 80 days post-hatch. At higher feeding levels, this hybrid vigor could result in increased production than at lower feeding levels. It was shown that hybrid vigor among palmetto bass was not due to a better assimilation efficiency nor was it due to a better appetite. It was due to the fact that the palmetto bass were shown to have a lower metabolism than their striped bass counterparts, in turn allowing them to grow faster (Tuncer et al., 1990).

Protein is the most expensive component in many aquaculture diets, particularly when feeding naturally carnivorous fish species. Feed producers aim to provide a minimum level of protein in the diet while still satisfying the essential amino acids at a level that will result in acceptable growth (Webster et al., 1995). Protein utilization has been shown to improve when protein is replaced by lipid or carbohydrate in the diet. Unfortunately, this replacement may lead to an excess amount of energy available in the diet. Excess energy is undesirable because it may (1) reduce overall feed consumption (Lovell, 1979), (2) produce fatty fish (Page and Andrews, 1973; Reinitz et al., 1978), and

(3) may inhibit the optimal utilization of other components of the diet (Prather and Lovell, 1973; Winfree and Stickney, 1981). High lipid levels in specific feed ingredients has been shown to reduce the ability of a fish to digest protein (Kitamikado et al., 1964; Murray et al., 1977; Ufodike and Matty, 1983). The reason for this may, in part, be explained by a study conducted by Steffens (1989) that suggested the reduced protein digestibility in higher lipid feed ingredients may be related to the formation of protein and oxidized fat complexes during drying of the diet.

According to the literature, protein requirements of juvenile carnivorous fish species have varied. Smallmouth bass have been reported to require diets containing 45% protein, whereas largemouth bass require only 40% protein (Anderson et al., 1981). Protein requirements in the diet can vary among and within species depending on differences in conditions including specific diet composition, the quality of particular protein sources, the biological value of proteins in the diet, and the sources of non-protein energy—such as carbohydrate--included in the diet (NRC, 1983). Protein quality can be defined based on its palatability, essential amino acid composition, and particular digestibility (Webster et al., 1995).

Fish do not utilize carbohydrate in their diet as well as other domesticated animals, and among species (Bergot, 1979; Furuichi and Yone, 1981) and even subspecies (Austreng et al., 1977; Edwards et al., 1977; Refstie and Austreng, 1981) the ability to utilize carbohydrate greatly varies. Currently, there is no known requirement for dietary carbohydrate in fish (NRC, 1993); however, when fed carbohydrate-free diets some species have exhibited reduced growth rates (Anderson et al., 1984; Degani et al., 1986). The feeding of excess dietary carbohydrate to fish has also shown adverse effects

in numerous morphometric and physiological parameters used to determine nutrient utilization, growth, or physiological function (Hilton and Dixon, 1982; Dixon and Hilton, 1985). Many of the enzymes involved in the metabolism of dietary carbohydrate have been demonstrated to exhibit activity in the gastrointestinal tracts of fish (Shimeno, 1974; Cowey and Walton, 1989). Despite this, the mechanism responsible for some fish's inefficient utilization of carbohydrate has not been identified and the physiology of overall carbohydrate utilization in fish is not well understood (NRC, 1993). The relative utilization of dietary carbohydrate varies greatly among fish species, and in turn the optimal level of digestible carbohydrate in the diet also greatly varies (Small and Soares, 1999).

Some research has been conducted concerning the effects of different kinds and levels of dietary carbohydrate on striped bass and their hybrids. Dietary carbohydrate levels have been shown to adversely affect glucose metabolism in fish, as well as alter their liver composition. Small and Soares (1999) observed a prolonged hyperglycemia in striped bass similar to that seen in diabetic animals when plasma glucose concentrations were measured over a 24 hour period. This general hyperglycemic response has also been seen in sunshine bass (Hutchins et al., 1998), rainbow trout *Oncorhynchus mykiss* (Brauge et al., 1994), and Atlantic salmon *Salmo salar* (Hemre et al., 1995). In each of these studies, the plasma glucose levels of the fish peaked between 4 and 8 hours after the carbohydrate was administered to the individual. The results with striped bass (Small and Soares, 1999) were similar to results published for other species of fish in which there is a correlation between the level of carbohydrate in the diet and the plasma glucose concentration: an increase in the plasma glucose concentration occurs as the level of

carbohydrate in the diet also increases (Fynn-Aikins et al, 1992; Brauge et al., 1994; Woods et al., 1995). It was also found by Hutchins et al. (1998) that as glucose levels increased in the diets of sunshine bass, so did their plasma glucose concentrations. In contrast, and opposite effect was observed when the sunshine bass were fed increasing levels of either maltose, a disaccharide, or dextrin, a polysaccharide.

The apparent diabetic condition in fish has been studied and speculated about in numerous ways. In early studies involving the tolerance of glucose in fish, Furuichi and Yone (1982) and Wilson and Poe (1987) observed the prolonged hyperglycemic effect, and postulated it may be the result of low levels of endogenous insulin in fish. This was disproven when radioimmunoassay methods were developed to determine levels of insulin in fish. These results showed that insulin levels are often similar to and at times higher than the insulin levels shown in mammals (Wilson, 1994). It has since been suggested that a high sensitivity to glucose by the pancreatic cells that produce somatostatin may be the reason fish are not as tolerant of glucose (Ronner and Scarpa, 1987). In an experiment conducted by Sheridan et al. (1987), it was demonstrated that somatostatin actually functions to inhibit the release of insulin in a fish. It was also observed that there was no increase in concentrations of plasma insulin during the initial period following either oral or intraperitoneal glucose administration (Sheridan et al., 1991).

Utilization of high levels of digestible carbohydrates over an extended period of time has also been shown to cause enlargement of the liver (Hemre et al., 1989) as well as impaired liver function (Hilton and Atkinson, 1982). Many studies have observed that the hepatosomatic index (HSI) increases as a function of energy in the diet, especially

carbohydrate (Garling and Wilson, 1977; Hilton and Atkinson, 1982; Daniels and Robinson, 1986; Woods et al., 1995, Rawles and Gatlin, 1998; Small and Soares, 1999). Typically, striped bass fed diets containing lower levels of soluble carbohydrate have smaller livers than those fish fed diets containing higher levels of soluble carbohydrate. Liver glycogen values also tend to be significantly greater for fish fed diets containing soluble carbohydrate when compared to fish that did not have soluble carbohydrate available to them in the diet (Small and Soares, 1999). Rawles and Gatlin (1998) made the observation that as soluble carbohydrate was added to the diet, liver protein and liver lipid concentrations significantly decreased. Increasing the level of dietary carbohydrate has also been shown to increase the nonlipid fluid accumulation in the hepatocytes of striped bass. This may contribute to the observed increases in HSI values when dietary carbohydrate levels are increased for striped bass (Woods et al., 1995).

Previous research has shown that striped bass appear to use dietary carbohydrate more efficiently in general when compared to other carnivorous fish (Berger and Halver, 1987) and similar responses have also been observed with one of the striped bass hybrids, (the reciprocal cross) the sunshine bass (Nematipour et al., 1992a,b).

Rawles and Gatlin (1998) concluded that the growth of pure striped bass was apparently not related to the complexity of the carbohydrate included in the diet. Glucose tolerance in striped bass also appears to be unaffected by the complexity of carbohydrate in the diet. Not only are striped bass better able to tolerate glucose, is appears that they are also able to tolerate and utilize different forms of soluble carbohydrate in the diet, such as cornstarch, than are one of their hybrid counterparts the sunshine bass (Small and Soares, 1999). Berger and Halver (1987) showed that striped bass can effectively utilize

a high-protein, high-lipid diet with as much as 33% carbohydrate included in the form of dextrin, without a reduction in growth. A growth study conducted by Small and Soares (1999) indicated that juvenile striped bass are able to tolerate the maximal level of dietary carbohydrate at a proportion between 15 and 20% of the diet.

Dietary protein level and the correct ratio of protein to energy are both key factors when formulating diets to feed fish. The level of digestible energy available in the diet is also important because it affects the amount of diet that will be consumed by the fish. The protein: energy ratio in turn influences the efficiency to which that amount of consumed diet is converted (Reis et al., 1989).

Nematipour et al. (1992a) found that the juvenile sunshine bass (*M. chrysops x M. saxatilis*) were able to efficiently use up to 42% dextrin in their diet and that dietary lipid could be partially replaced by dietary carbohydrate without reducing the animal's productivity or carcass quality. Nematipour et al. (1992b) found that weight gain, feed efficiency, and protein efficiency of juvenile sunshine bass (9g initial average weight) peaked when fed a diet with a dietary energy: protein ratio of 8 kcal available energy / g protein. This ratio corresponds to a dietary dextrin level of 24%. Another study by Nematipour et al. (1992b) concluded that weight gain, feed efficiency, and protein efficiency of juvenile sunshine bass (1.0g average initial weight) were also relatively unaffected by changes in dietary carbohydrate: lipid ratio (% dextrin: % lipid) ranging from 25:10 to 42:2.5. However, when advanced juvenile sunshine bass were fed isocaloric diets containing 40% as compared to 20% soluble carbohydrate, weight gain, protein efficiency, and feed efficiency were significantly depressed—regardless of the complexity of the carbohydrate used (Hutchins et al., 1998).

Webster et al. (1995) continued work with protein to energy ratios of the diets fed to juvenile sunshine bass weighing an average of 125g initially. They found that the fish fed diets containing 116 mg protein/ kcal had a higher percentage dress-out (dressed carcass), a lower percentage of fat present in the abdominal cavity, a higher percentage of overall body protein, and a lower percentage of body lipid than hybrids fed any of their other experimental diets. It was also found in this study that a protein: energy ratio of less than 116 mg protein/ kcal (specifically 99 mg protein/ kcal) had the tendency to decrease lipid deposition and protein levels in sunshine bass. Conversely, both of these diets produced similar growth results in the fish.

Knowledge about the digestibility of the ingredients in a formulated diet is necessary to maximize what the animal gets out of the diet and the economic benefit of a lower cost feed formulation. Sullivan and Reigh (1995) determined the digestibility of several commonly used fish feedstuffs, including those that contain high levels of soluble carbohydrate, and concluded that the palmetto bass (*M. saxatilis x M. chrysops*) are able to utilize high protein, high lipid feedstuffs better than they are able to utilize those feedstuffs with high levels of carbohydrate. The energy digestibility was shown to greatly vary among the plant products examined, leading to the conclusion that when high-carbohydrate ingredients are included in the diet they should be chosen carefully. The specific protein digestibilities of feedstuffs of either plant or animal origin were high, making the selection of a dietary protein source able to be based on the specific amino acid content and the cost of that ingredient. Plant products, such as soybean meal and cottonseed meal, are commonly used as sources of dietary protein for omnivorous fish species. These results indicate that it may be possible to increase the level of plant-

protein sources within a palmetto bass diet. Increasing these protein sources over animal based sources would help decrease the cost of feed, as well as aid in reducing the current dependence on fish meals as the primary sources of protein in a formulated fish diet (Rumsey, 1993).

With an increase of the presence of plant-based ingredients again comes the concern of the fish's ability to digest other sources of energy, particularly one type of carbohydrate component—starch. Some plant materials can include high levels of starch. The ability to digest starch and utilize it as an energy source varies with species (Cowey and Walton, 1989). Fish with decidedly omnivorous or herbivorous feeding habits have been shown to digest starchy components of plant materials better than particular carnivorous fish, which appear to have a rather limited ability. Common carp have been shown to be able to digest as much as 85% of the starch in a high-starch diet (Chiou and Ogino, 1985), compared to a rainbow trout that is able to digest only 38 to 55% of dietary starch (Bergot and Breque, 1983). Some feeding experiments in ponds studying hybrid striped bass have indicated the possibility that the hybrid does have an effective mechanism to digest starch in the diet. These experiments showed that when there is some natural food available, high-starch plant products can be used effectively in the diets for production of hybrid striped bass. Zhang et al. (1994) showed that while feeding hybrid striped bass a low (4%) lipid diet that contained 67% plant materials—specifically 39% nitrogen-free extract (NFE) from soybean meal, corn, and wheat middlings, these pond-raised fish were able to grow as well as fish fed a comparable diet (37% NFE) with a higher amount of lipid (7%). The growth rates obtained in this study with a high-starch, low-lipid diet were comparable to and even better than studies conducted with both cage

and pond-reared hybrid striped bass that were fed higher levels of lipid in the diet at similar population densities (Williams et al., 1981; Woods et al., 1983; Kerby et al., 1987; Smith et al., 1989).

As the dietary requirements for nutrients such as protein, including specific essential amino acids, lipid, vitamins, and minerals become known through research for many fish species, including the striped bass and its hybrids, increasing attention can be focused on obtaining a more complete understanding of carbohydrate utilization in fish. A full understanding would help outline the limits of dietary inclusion of carbohydrate as an inexpensive source of energy (Wilson, 1994). In addition to the role of carbohydrate as a potential additional energy source in a practical fish diet, the ratio of protein to energy in the diet is also an important factor to consider for the optimal growth, body condition, and health of fish, in this case striped bass and their hybrids. Further research is needed to clarify the optimal protein to energy ratio for each subspecies at various life stages and management conditions.

An effective, non-invasive method to determine body composition in fish that could be used on the same fish at various life stages would be valuable tool in the field of teleost nutrition. With such a tool could come an even better understanding of effects of various dietary treatments, especially over time. DXA would be useful to nutritionists and producers alike, hopefully being both cost and time effective.

## Objectives

While research has been conducted concerning body composition determination using dual energy x-ray absorptiometry for many production animals, no published studies exist evaluating this method for use in fish. Due to the growing economic importance of these animals and the current trends in aquacultural research concerning dietary requirements and dietary effects on growth rates and overall body composition, it is surprising this has yet to be explored. The purpose of this project is to evaluate the use of DXA, both pencil beam and fan beam methodologies, as a method of accurately determining body composition for an important aquacultural species, the sunshine hybrid striped bass (*Morone chrysops x Morone saxatilis*), by comparing scan results to chemical analysis of carcasses of each species. This method will then be used to compare body composition of this species when various energy: protein ratios are employed in their diets.

The objectives are as follows:

1. To assess the ability of dual-energy x-ray absorptiometry (DXA) in determining body composition of hybrid striped bass with wide differences in body composition.
2. To compare two methods of dual-energy x-ray absorptiometry (DXA)—pencil beam and fan beam--in determining body composition of hybrid striped bass with wide differences in body composition.
3. To apply dual-energy x-ray absorptiometry (DXA) technology towards evaluating the effects diets with variable levels of protein and carbohydrate fed to hybrid striped bass have on body composition.

# CHAPTER 1

The Assessment of the Effectiveness of Two Types of Dual Energy X-Ray Absorptiometry to Non-Invasively Determine Body Composition of Hybrid Striped Bass (*Morone chrysops x Morone saxatilis*)

## Introduction

Dual energy x-ray absorptiometry (DXA) is now generally accepted as a standard method of non-invasively determining a measurement for bone mass in human beings, and is also becoming a preferred method for determining the composition of soft tissue in other subjects, including pigs (Koo, 2000). The majority of the studies involving the DXA technology utilize the pencil beam DXA technique for measurement of bone and soft tissue compositions (Fusch et al., 1999; Koo et al., 1995; Picaud et al., 1996). Studies have included measurements of both pediatric (Venkataraman and Ahluwalia, 1992; Chan, 1992; Brunton et al., 1993) and adult subjects (VanLoan and Mayclin, 1992; Rico et al., 1994; Wellens et al., 1994), as well as studies involving patients at risks of malnutrition or metabolic problems due to kidney dialysis treatment (Borovnicar et al., 1996), diabetes (Kistorp and Svendsen, 1997), or renal transplants (Hart et al., 1993) to name a few.

DXA has also been used in numerous studies to determine the body composition of various animal species, including live pigs (Svendsen et al., 1993; Brunton et al., 1993; Pintauro et al., 1996; Mitchell et al., 1996a, 1996b, 1998a,b; Mitchell and Scholz, 1998), pig carcasses (Mitchell et al., 1998c), chickens (Mitchell et al., 1997a), beef carcass rib sections (Mitchell et al., 1997b), rats (Rose et al., 1998), mice (Sjögren et al., 2001), dogs (Lauten et al., 2001), and cats (Lauten et al., 2000). The accuracy and precision of DXA have been validated both in vivo and in vitro in humans, pigs from 5 to 100 kg, and sheep. DXA was also validated for use with rodents utilizing a modified technique.

A more recent development in DXA technology has been the creation of a scanner that utilizes a fan beam x-ray technique. This technique offers many

improvements over the more traditional pencil beam technique, including the 5 to 10 times quicker run time of a scan and improved spatial resolution. To reduce the inherent error in this method due to the magnification of the fan beam itself, alterations have been made in the analytic algorithms of the software for human adult scans. Cross calibration with other methods of determining body composition, including the four compartment model, multislice tomography scans, and pencil beam DXA, has confirmed the equivalency of readings for bone mineral density, lean mass, and fat mass between these other methods and the fan beam DXA technique (Bouyoucef et al., 1996; Salamone et al., 2000).

Currently there is experimental software for scanning children and infants with the fan beam DXA, but the algorithms that calculate the components of body composition are actually based on the algorithm developed for the pencil beam DXA technique (Koo et al., 1995; Koo et al., 2000). These algorithms have yet to be validated for the use on children with the fan beam instrument (Koo et al., 2002). Koo et al. (2002) reported that the fan beam DXA technique can be adapted for use in scanning small subjects, such as studying piglets. It was concluded that fan beam DXA determines components of body composition with high accuracy and precision, including: scale weight, body contents of ash, calcium, lean and fat tissues. Each of these components was compared to their corresponding proximate value, and correlations were determined based on the extremely high adjusted $r^2$ calculated from the relationships. Due to its similar accuracy to the pencil beam DXA, Koo et al. (2002) recommended the fan beam DXA technique be utilized for studies involving body composition determinations for growing humans and animals.

While considerable research has been done using the pencil beam DXA technique to determine various components of body composition in humans and some animals, there is very little, if any, research involving the use of any DXA technology to non-invasively determine the body composition of any teleost species. The new methodology of the fan beam DXA technique has not been explored much beyond adult human subjects, and there is no research to date involving the use of this technique for determining body composition of teleosts either.

Striped bass (*Morone saxatilis)* have received considerable attention in the past decade as an important commercial aquaculture species, an economic resource, and a research tool in the United States. Striped bass and its hybrids are in intense production throughout the United States, as well as in Europe and Asia. Production of hybrid striped bass has grown an estimated 1400% between the years 1985 and 1995, and continues to increase in accordance to market demands (Harrell and Webster, 1997). Hybrid striped bass accounted for 11% of the state's food fish production in the year 2000, and are actually the second largest most commonly produced fish produced in Maryland in terms of volume (MDA, 2000). With commercial production comes the need to better understand a species' environmental and dietary needs, so that the species of interest may be economically successful and practical. This has led to the development of many new culture techniques for successful fingerling production as well as for grow-out purposes (Williams et al., 1981; Kerby et al., 1983a,b,1987; Woods et al., 1983, 1985; Liao, 1985; Smith et al., 1985).

Despite significant efforts in management, culture, and domestication, there are still questions to be answered concerning the proper feed formulation and dietary

requirements of the striped bass, and its hybrids. Development of techniques to assess a population during various stages of its life would be an invaluable tool to the aquaculture industry. Especially if techniques, such as DXA, could be utilized in longitudinal observations to give a producer a more accurate picture of how individuals are developing over time, and whether adjustments in feeding regimes or health procedures are necessary.

## Materials and Methods

### *Animal Husbandry*

Hybrid striped bass (*Morone chrysops x Morone saxatilis*), originally purchased from a commercial breeder, and currently from the fish population of the aquaculture nutrition research laboratory at the University of Maryland, College Park were utilized for the study. Twelve fish with an approximate age of two years old and an average starting weight of 700g were housed in each of twelve 1000-L circular fiberglass tanks that were part of a recirculating water system. The starting size of the population was a total of 144 fish. Water quality conditions for all recirculating water tank systems were maintained whenever possible at levels recommended by Nicholson et al. (1990) for striped bass. Initial, and replacement, fresh water was supplied to each tank system after municipal water passed through an in-line 25-micron particulate filter and an activated carbon filter for dechlorination. A salt solution consisting of calcium chloride, sodium chloride (9:1), magnesium chloride (5 mg/L), and sodium thiosulfate was continuously injected directly into incoming water in order to maintain a minimum concentration of 36 mg/L calcium hardness in the rearing tanks. Additional sodium chloride was added by hand to the systems to maintain salinity of approximately 5ppt. Sodium bicarbonate was also periodically added by hand to adjust pH levels within the systems to an approximate level of 7.5. Flow rates for all tanks were set for two complete turnovers per hour. Tanks were continuously aerated by air stone diffusers to maintain adequate dissolved oxygen (DO) levels of 6.5 to 7.0 mg/L. A photoperiod of 12 hours light: 12 hours dark was maintained. Water temperatures (average of 21°C) were recorded four times per day by a central computerized monitoring system (REES Scientific, Trenton, NJ, USA). Average

water quality parameters were: pH (7.5), DO (7.0 mg/L), total chlorine (<0.10 ppm), ammonia (<0.3 mg/L), nitrite (<0.7 mg/L), nitrate (<50 mg/L), salinity (5 ppt), and calcium hardness (221 mg/L), and each was measured weekly. DO and temperature were monitored using a YSI model 58 oxygen meter (model #58- Yellow Springs Instrument Co., Inc. Yellow Springs, Ohio, USA). pH was monitored with a Corning model 250 ion analyzer (Corning Incorporated, Corning, NY, USA). Chlorine (total), ammonia, nitrite, and nitrate were measured using a Hach DR850 colorimeter (Hach Company, Loveland, CO, USA). Calcium concentrations were measured with a Hach model HA-4P hardness test kit. Regular water changes (20% of total water volume, twice per week) and biofilter backwashes (once per week) were completed to maintain water clarity and to maintain nitrate levels below a level of 50 mg/L.

Groups of fish were fed one of two diets (Table 1.1) at various levels of energy intake, including one diet (low phosphorus-treatment 1) formulated to be low in phosphorus, and Diets 2 through 4 (basal) to produce a population of fish given a broad range of feed intake—from 0.5% of body weight to ad libitum--in order to produce a range of fat tissue. The diets were formulated to meet all known nutrient requirements for hybrid striped bass. When nutrient requirement information was not available for hybrid striped bass, the dietary requirements for salmonid fish were used (NRC, 1993). The experimental design was completely randomized with dietary treatments assigned to each tank, producing triplicate replication of each treatment.

*Sample Collection and Preparation*

After being maintained on the defined experimental regime for ten weeks, half of the population of fish from each tank—six per tank and 72 fish total--were randomly

Table 1.1. Composition of the two plant-based diets, basal and low Phosphorus, fed to hybrid striped bass to produce of a broad range of body compositions within the population.

| Ingredient | Basal Diet g/kg diet, as fed[a] | Low P Diet g/kg diet, as fed[b] |
|---|---|---|
| Soybean meal (48.5% protein) | 330.9 | 250 |
| Menhaden fish meal | 300 | 50 |
| Wheat flour | 256 | 0 |
| Menhaden fish oil | 90 | 100 |
| Wheat middlings | 0 | 241 |
| Corn gluten meal (60% protein) | 0 | 230 |
| Blood flour | 0 | 100 |
| Lysine HCl | 0 | 5 |
| Choline chloride (77%) | 0 | 4 |
| Vitamin premix 7-15 | 12.6 | 10 |
| Mineral premix | 10.5 | 10 |
| Total | 1000 | 1000 |

[a] Fed at three levels: highly restrictive—0.5% body weight once per day, restrictive—1% body weight twice per day, control—satiation 2-3 times per day.
[b] Fed to satiation twice per day.

selected to be sacrificed. Each fish was euthanized by immersion in an overdose solution of 8 mg/L tricaine methanesulfonate (MS-222). The fish were then weighed, individually bagged, and frozen in the lateral position at -20°C for subsequent analysis. The same euthanasia and storage procedures were done with the remaining fish in each tank after 16 weeks into the experimental regime.

*Primary DXA Validation-Experiment 1*

Frozen fish were then transported to the United States Department of Agriculture (USDA) Agriculture Research Station Growth Biology Laboratory of A. Mitchell in Beltsville, Md. Each fish was individually scanned in the lateral position, in the cranial to caudal direction by the Lunar model DPX-L #7618 dual energy x-ray absorptiometry (DXA) instrument. Each individual was scanned in two of the small animal modes: high resolution (HRM) and detailed medium (DM). Estimation of the tissue densities,

according to their x-ray attenuations, was performed by the associated computer small animal software Version 4.6d and produced body composition component values for each fish in each mode, including those defined as: DXA R-value (an attenuation coefficient of the two x-ray energies—38 and 70 kVp), percentage of fat, total grams of tissue, grams of fat, grams of lean, and bone mineral composition (BMC). Using the percent fat scan value, a simple estimated percent lean value was also determined.

*DXA Manual Sectional Analysis-Experiment 1B*

Of the previously performed scans using the Lunar model DPX-L #7618 DXA instrument, manual sectional analysis was performed on the scanned abdominal region of each fish in each mode, encompassing the region bordered by the distal portion of the skull, along the spine, and down to the proximal portion of the anal fin and the ventral edge of the abdominal cavity of the fish. Subsequent analysis of tissues resulted in the determination of the same body composition components as described above for the section of the body delineated in the scanned file.

*DXA Scan of Stacked Carcasses-Experiment 1C*

Frozen fish from the sixteen-week sampling were also scanned by the Lunar model DPX-L #7618 DXA instrument in stacks of three individuals per tank. The individuals were stacked in the same direction in the lateral position, and housed within a Styrofoam form to support the stack around the sides and keep the fish within position throughout the scans. These scans were done in both detailed medium (DM) and high resolution (HRM) small animal scan modes, in the cranial to caudal direction. Subsequent analysis of tissues resulted in the same body composition components as described above for each DXA scan mode.

*Fan Beam DXA Analysis-Experiment 1D*

After scanning with the pencil beam DXA instrument at the USDA's Agriculture Research Station Growth Biology Laboratory, frozen fish from the sampling after 16 weeks on the experimental regime were transported to the USDA's Agriculture Research Station Human Nutrition Laboratory of J. Conway in Beltsville, Md. Here fish were scanned with a Hologic model QDR-4500A fan beam DXA instrument. Each fish was positioned laterally on the instrument table and scanned in the whole body scan mode, from the cranial to the caudal region. Estimation of the tissue densities, according to their x-ray attenuations of x-rays at energies 100 and 140kVP, was performed by the associated computer software which predicted body composition components for each fish in each mode, including those defined as: percentage of fat, total grams of tissue, grams of fat, grams of lean, bone mineral composition (BMC), and grams of lean tissue plus BMC.

*Proximate Analysis*

After all scanning was completed, fish were returned to storage at -20°C until further analysis. Final analysis of each fish consisted of whole carcass proximate analysis, as individual crude protein (Nitrogen gas analysis x 6.25), ether extract, ash (combustion at 550°C), and moisture determinations by standard methods (AOAC 1995). For proximate analysis, each fish was first thawed overnight and macerated and ground until reduced to particles of less than 5mm in size. All tissues were collected and refrozen until analysis. For moisture analysis, a sub-sample of the homogenate was dried in an aluminum pan at 95°C for 48 hours. Sample weights were taken before and after drying, and the difference in weights was used to determine the moisture content. These

dried samples were then reground using a microgrinder (MicroMill; Pequannock, NJ) and stored in separate, labeled plastic tubes in a dessicator (Boekel; Philadelphia, PA) until additional analysis. Dry samples were analyzed for fat content by ether extraction, and these fat-free samples were then analyzed for crude protein and ash contents. In between all analyses, samples were stored in dessicators. Protein was determined via combustion and nitrogen gas analysis using a LECO FP-428 Nitrogen Analyzer (LECO; St. Joseph, MI). Ash contents were determined by overnight combustion in crucibles in a rectangular muffle furnace at 550°C, and the change in weight was used to calculate ash. All animal care and handling was done according to a protocol approved by the University of Maryland Animal Care and Use Committee (Protocol #R-00-59A and #R-00-59B).

*Statistical Analysis*

The data for all components of this experiment were analyzed for statistical differences using Statistical Analytical Systems (SAS) software (SAS Institute, 2001). The data was analyzed by one-way analysis of variance (ANOVA) as computed by the SAS mixed procedure which was used to determine if a significant treatment effect was present. The test of least significant difference (LSD) was used to test for significant differences between means. In all cases, a probability level of $p < 0.05$ was chosen for the determination of significant differences. The data conformed to the assumption of normality (Sokhal and Rohlf, 1987) necessary for proper statistical analysis by ANOVA and LSD. Correlation coefficients and regression equations were also calculated by methods described by SAS to compare values given by types of DXA and proximate analysis.

# Results

## *Proximate Analysis*

Proximate analysis values were used as the accepted values for the various components of body composition in all experiments. Fish were scanned with all types of DXA technology in all experiments as whole frozen carcasses, so to directly compare scan values to the proximate values back calculations were utilized to determine body composition on a whole body basis. The components of body composition are presented on a whole body wet basis for the fish involved in the DXA scans, and these values were used for comparison to scan values in both modes. Proximate analysis results are also presented as means for the DXA scans of stacks of fish carcasses. These scans involved selected fish from the sixteen week sampling period, so means for proximate analysis are different than those presented for the pooled sampling data. The same fish utilized in the stacked scans were also scanned with the Hologic fan beam DXA. Therefore, proximate analysis means are the same for these two different methodologies. The components of body composition are also presented on a whole body wet basis for the sets of fish involved in the stacked DXA scans.

## *Regression and Correlation*

### *Primary DXA Analysis*

Proximate analysis values were used to validate scan values for body composition estimated by the Lunar model DPX-L #7618 DXA instrument in two small animal scan modes—detailed medium and high resolution. DXA scan values and proximate analyses were correlated to one another. From the correlations of DXA scan values and proximate analysis values, regression equations were developed.

In detailed medium (DM) scan mode, some correlations and regression equations were unable to be calculated (Table 1.2). This is due to the poor variability in some estimations of body composition produced by this DXA scan mode. These poor estimations include: percent body fat and corresponding percent lean. The remaining correlations were all highly significant ($p< 0.0001$). Despite the non-existent correlation for percent body fat comparisons, proximate percent body fat did significantly correlate to the DXA DM r-value ($r^2= -0.349$). Therefore, the DXA r-value is able to provide a good estimation of percent body fat even though the DXA percent fat estimation is not.

In high resolution (HRM) scan mode, it was possible to calculate correlation coefficients for all comparisons (Table 1.3). Only one comparison was not statistically significant when hybrid striped bass were scanned in this mode—percent protein determined by proximate analysis compared to DXA percent lean. Percent carcass moisture compared to DXA percent lean and percent carcass fat compared to DXA percent fat were the only two significant comparisons that were significant at a lower level ($p<0.05$ vs. $p<0.0001$). All other correlations were highly significant ($p< 0.0001$).

*DXA Manual Sectional Analysis-Experiment 1B*

Correlation coefficients were also developed for the DXA scan values in abdominal sectional analysis when compared to proximate analysis values. Due to the fact that all scan values for sectional analysis were based on a whole frozen fish carcass, proximate analysis values were calculated to represent values on whole body wet basis. Regression equations were developed for the sectional DXA analysis, in each scan mode, when compared to proximate analysis values.

Table 1.2. Statistical comparisons of proximate analysis to DXA[a] estimates of body composition of hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | Proximate Analysis | DM DXA[a] |
| Weight/Tissue (g) | 967.3 ± 23.14 | 1044.3 ± 24.79 |
| Water (g) | 636.5 ± 14.59 | - |
| Moisture (% TBW[b]) | 66.0 ± 0.14 | - |
| Protein/Lean (g) | 166.8 ± 4.00 | 1002.5 ± 23.80 |
| Protein (% TBW[b])/ % Lean | 17.2 ± 0.07 | 96.0 ± 0.0 |
| DXA R-value | - | 1.410 ± 0.0007 |
| Fat (g) | 118.4 ± 3.88 | 41.8 ± 0.99 |
| Fat (% TBW[b]) | 12.1 ± 0.16 | 4.0 ± 0.0 |
| Ash/ BMC[c] (g) | 35.8 ± 0.99 | 15.85 ± 0.525 |
| Ash (% TBW[b]) | 3.7 ± 0.07 | - |

| Proximate Analysis vs. DM DXA[a] comparisons | | | |
|---|---|---|---|
| Comparison | Correlation Coefficient | P value | Regression Equation |
| Weight (g) vs. DM DXA[a] Tissue (g) | 0.990 | <0.0001 | y=1.0605x + 18.403 |
| Moisture (%) vs. DM DXA[a] Lean (%) | N/A | N/A | y= -3E-13x + 96 |
| Water (g) vs. DM DXA[a] Lean (g) | 0.988 | < 0.0001 | y=1.6111x - 22.872 |
| Protein (% TBW[b]) vs. DM DXA[a] Lean (%) | N/A | N/A | y= 6E-13x + 96 |
| Protein (g) vs. DM DXA[a] Lean (g) | 0.975 | <0.0001 | y=5.8002x + 34.906 |
| Fat (% TBW[b]) vs. DM DXA[a] R-Value | -0.349 | <0.0001 | y= -0.0016x + 1.4289 |
| Fat (% TBW[b]) vs. DM DXA[a] Fat (%) | N/A | N/A | y= 4 |
| Fat (g) vs. DM DXA[a] Fat (g) | 0.887 | <0.0001 | y=0.2264x + 14.957 |
| Ash (g) vs. DM DXA[a] BMC[c] (g) | 0.797 | <0.0001 | y=0.4239x + 0.6634 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total body weight (TBW)
[c] Bone Mineral Composition (BMC)

Table 1.3. Statistical comparisons of proximate analysis to DXA[a] estimates of body composition of hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | Proximate Analysis | HRM DXA[a] |
| Weight/Tissue (g) | 967.3 ± 23.14 | 1025.9 ± 24.47 |
| Water (g) | 636.5 ± 14.59 | - |
| Moisture (% TBW[b]) | 66.0 ± 0.14 | - |
| Protein/Lean (g) | 166.8 ± 4.00 | 981.0 ± 22.98 |
| Protein (% TBW[b])/ % Lean | 17.2 ± 0.07 | 95.7 ± 0.07 |
| DXA R-value | - | 1.396 ± 0.0008 |
| Fat (g) | 118.4 ± 3.88 | 44.9 ± 1.76 |
| Fat (% TBW[b]) | 12.1 ± 0.16 | 4.3 ± 0.07 |
| Ash/ BMC[c] (g) | 35.8 ± 0.99 | 21.23 ± 0.652 |
| Ash (% TBW[b]) | 3.7 ± 0.07 | - |

Proximate Analysis vs. HRM DXA[a] comparisons

| Comparison | Correlation Coefficient | P value | Regression Equation |
|---|---|---|---|
| Weight (g) vs. HRM DXA[a] Tissue (g) | 0.990 | <0.0001 | y=1.0463x + 13.721 |
| Moisture (%) vs. HRM DXA[a] Lean (%) | 0.246 | 0.004 | y= 0.1213x + 87.719 |
| Water (g) vs. HRM DXA[a] Lean (g) | 0.988 | < 0.0001 | y=1.5557x - 9.1418 |
| Protein (% TBW[b]) vs. HRM DXA[a] Lean (%) | -0.014 | 0.871 | y= -0.0129x + 95.948 |
| Protein (g) vs. HRM DXA[a] Lean (g) | 0.975 | <0.0001 | y=5.6001x + 46.817 |
| Fat (% TBW[b]) vs. HRM DXA[a] R-Value | -0.361 | <0.0001 | y= -0.0017x + 1.4156 |
| Fat (% TBW[b]) vs. HRM DXA[a] Fat (%) | 0.238 | 0.006 | y= 0.0983x + 3.0883 |
| Fat (g) vs. HRM DXA[a] Fat (g) | 0.796 | <0.0001 | y=0.3609x + 2.1417 |
| Ash (g) vs. HRM DXA[a] BMC[c] (g) | 0.801 | <0.0001 | y=0.5237x + 2.471 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total body weight (TBW)
[c] Bone Mineral Composition (BMC)

Sectional analysis was performed on the abdominal region of each scan image in each scan mode. The abdominal region was the area of interest because fish deposit the majority of their body fat in this area. In an attempt to get more representative fat readings with the DXA technology, the sectional analysis was performed and these values were compared to the same proximate values as previously mentioned.

For DM DXA scan data, percent fat estimations improved and significant ($p < 0.0001$) correlations were calculated when compared to percent carcass fat values (Table 1.4). For sectional analysis, the only comparison for which a significant correlation could not be calculated was the percent carcass protein to DXA percent lean comparison. All other comparisons were highly significant ($p < 0.0001$).

Sectional DXA analysis for the HRM scan mode produced the same results as the DM sectional analysis (Table 1.5). The only comparison for which a significant correlation could not be calculated was the percent carcass protein to DXA percent lean comparison. The significance level for the comparisons of percent carcass moisture compared to DXA percent lean and percent carcass fat compared to DXA percent fat improved with sectional analysis over whole body scan analysis. All other comparisons remained highly significant with sectional analysis ($p < 0.0001$).

Overall, sectional analysis was successful. Many of the new DXA estimations of body composition resulted in improved correlations to proximate analysis values for some comparisons, and the ability to actually calculate others completely. There were no statistical differences between scan modes in sectional analysis.

*DXA Scan of Stacked Carcasses-Experiment 1C*

For the stacked scan analysis (SCA) comparisons, a weighted average for

Table 1.4. Statistical comparisons of proximate analysis to sectional DXA[a] estimates of body composition of hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | Proximate Analysis | Sectional DM DXA[a] |
| Weight/Tissue (g) | 967.3 ± 23.14 | 290.8 ± 7.78 |
| Water (g) | 636.5 ± 14.59 | - |
| Moisture (% TBW[b]) | 66.0 ± 0.14 | - |
| Protein/Lean (g) | 166.8 ± 4.00 | 275.6 ± 7.11 |
| Protein (% TBW[b])/ % Lean | 17.2 ± 0.07 | 94.9 ± 0.16 |
| DXA R-value | - | 1.391 ± 0.0010 |
| Fat (g) | 118.4 ± 3.88 | 15.5 ± 0.89 |
| Fat (% TBW[b]) | 12.1 ± 0.16 | 5.1 ± 0.16 |
| Ash/ BMC[c] (g) | 35.8 ± 0.99 | 1.44 ± 0.048 |
| Ash (% TBW[b]) | 3.7 ± 0.07 | - |

Proximate Analysis vs. Sectional DM DXA[a] comparisons

| Comparison | Correlation Coefficient | P value | Regression Equation |
|---|---|---|---|
| Weight (g) vs. Sectional DM DXA[a] Tissue (g) | 0.953 | <0.0001 | y=2.8331x + 143.58 |
| Moisture (%) vs. Sectional DM DXA[a] Lean (%) | 0.478 | <0.0001 | y= 0.4072x + 27.327 |
| Water (g) vs. Sectional DM DXA[a] Lean (g) | 0.945 | < 0.0001 | y=1.9391x + 102.12 |
| Protein (% TBW[b]) vs. Sectional DM DXA[a] Lean (%) | 0.038 | 0.658 | y= 0.0173x + 15.605 |
| Protein (g) vs. Sectional DM DXA[a] Lean (g) | 0.931 | <0.0001 | y=0.524x + 22.412 |
| Fat (% TBW[b]) vs. Sectional DM DXA[a] R-Value | -0.565 | <0.0001 | y= -93.59x + 142.23 |
| Fat (% TBW[b]) vs. Sectional DM DXA[a] Fat (%) | 0.485 | <0.0001 | y= 0.4926x + 9.5759 |
| Fat (g) vs. Sectional DM DXA[a] Fat (g) | 0.842 | <0.0001 | y=3.6639x + 61.8 |
| Ash (g) vs. Sectional DM DXA[a] BMC[c] (g) | 0.749 | <0.0001 | y=15.614x + 13.383 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total body weight (TBW)
[c] Bone Mineral Composition (BMC)

Table 1.5. Statistical comparisons of proximate analysis to sectional DXA[a] estimates of body composition of hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | Proximate Analysis | Sectional HRM DXA[a] |
| Weight/Tissue (g) | 967.3 ± 23.14 | 286.6 ± 7.92 |
| Water (g) | 636.5 ± 14.59 | - |
| Moisture (% TBW[b]) | 66.0 ± 0.14 | - |
| Protein/Lean (g) | 166.8 ± 4.00 | 263.9 ± 6.70 |
| Protein (% TBW[b])/ % Lean | 17.2 ± 0.07 | 92.7 ± 0.30 |
| DXA R-value | - | 1.380 ± 0.0010 |
| Fat (g) | 118.4 ± 3.88 | 22.7 ± 1.52 |
| Fat (% TBW[b]) | 12.1 ± 0.16 | 7.3 ± 0.30 |
| Ash/ BMC[c] (g) | 35.8 ± 0.99 | 2.22 ± 0.073 |
| Ash (% TBW[b]) | 3.7 ± 0.07 | - |

Proximate Analysis vs. Sectional HRM DXA[a] comparisons

| Comparison | Correlation Coefficient | P value | Regression Equation |
|---|---|---|---|
| Weight (g) vs. Sectional HRM DXA[a] Tissue (g) | 0.962 | <0.0001 | y=2.8584x + 147.5 |
| Moisture (%) vs. Sectional HRM DXA[a] Lean (%) | 0.573 | <0.0001 | y= 0.2561x + 42.339 |
| Water (g) vs. Sectional HRM DXA[a] Lean (g) | 0.953 | < 0.0001 | y=2.1358x + 74.549 |
| Protein (% TBW[b]) vs. Sectional HRM DXA[a] Lean (%) | 0.013 | 0.880 | y= 0.005x + 16.724 |
| Protein (g) vs. Sectional HRM DXA[a] Lean (g) | 0.940 | <0.0001 | y=0.5714x + 15.718 |
| Fat (% TBW[b]) vs. Sectional HRM DXA[a] R-Value | -0.548 | <0.0001 | y= -95.236x + 143.5 |
| Fat (% TBW[b]) vs. Sectional HRM DXA[a] Fat (%) | 0.552 | <0.0001 | y= 0.2909x + 9.9284 |
| Fat (g) vs. Sectional HRM DXA[a] Fat (g) | 0.886 | <0.0001 | y=2.2262x + 66.457 |
| Ash (g) vs. Sectional HRM DXA[a] BMC[c] (g) | 0.769 | <0.0001 | y=10.154x + 13.075 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total body weight (TBW)
[c] Bone Mineral Composition (BMC)

proximate analysis values for the three fish in each stack was calculated and then correlated to corresponding scan values. Correlation coefficients for the sixteen-week sampling in detailed medium and high resolution scan modes compared to proximate analysis values were developed for all data available.

There were only a few statistically significant correlation coefficients able to be calculated for stacked comparisons. In DM scan mode, carcass water (g) and protein (g) correlated to DXA estimations of lean (g) for stacks of fish were statistically significant ($p < 0.05$) (Table 1.6). The remaining comparisons were not statistically significant ($p < 0.05$).

Stacked scans in the HRM scan mode did not produce any statistically significant correlations with proximate analysis values (Table 1.7). Even at a lower level of significance, only one comparison for stacked scans could be considered significant—carcass protein (g) compared to stacked DXA lean (g).

When hybrid striped bass carcasses were stacked in groups of three and scanned with the DXA technology, the DM DXA scan mode was more successful in estimating components of body composition than the HRM DXA scan mode.

*Fan Beam DXA Analysis-Experiment 1D*

For the fan beam dual energy x-ray absorptiometry analysis all scans are run in whole body scan mode. Correlations with body composition values from proximate analyses and regression equations were developed from the body composition component comparisons.

It was not possible to calculate a significant correlation for percent lean body

Table 1.6. Statistical comparisons of proximate analysis to DXA[a] estimates of body composition in stacks of hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | Proximate Analysis | Stacked DM DXA[a] |
| Weight/Tissue (g) | 1047.6 ± 40.42 | 2606.5 ± 338.70 |
| Water (g) | 689.3 ± 25.59 | - |
| Moisture (% TBW[b]) | 66.0 ± 0.23 | - |
| Protein/Lean (g) | 184.3 ± 6.84 | 2495.6 ± 325.50 |
| Protein (% TBW[b])/ % Lean | 17.6 ± 0.09 | 95.5 ± 0.24 |
| DXA R-value | - | 1.395 ± 0.0026 |
| Fat (g) | 121.9 ± 6.50 | 110.7 ± 13.46 |
| Fat (% TBW[b]) | 11.4 ± 0.25 | 4.5 ± 0.24 |
| Ash/ BMC[c] (g) | 38.3 ± 1.83 | 104.65 ± 7.713 |

Proximate Analysis vs. Stacked DM DXA[a] comparisons

| Comparison | Correlation Coefficient | P value | Regression Equation |
|---|---|---|---|
| Weight (g) vs. Stacked DM DXA[a] Tissue (g) | -0.212 | 0.449 | y= -1.1391x + 3813.7 |
| Moisture (%) vs. Stacked DM DXA[a] Lean (%) | -0.127 | 0.651 | y= -0.1037x + 102.34 |
| Water (g) vs. Stacked DM DXA[a] Lean (g) | 0.559 | 0.030 | y=4.5984x - 709.67 |
| Protein (% TBW[b]) vs. Stacked DM DXA[a] Lean (%) | 0.160 | 0.570 | y= 0.4078x + 88.314 |
| Protein (g) vs. Stacked DM DXA[a] Lean (g) | 0.575 | 0.025 | y=17.813x - 821.8 |
| Fat (% TBW[b]) vs. Stacked DM DXA[a] R-Value | -0.229 | 0.411 | y= -0.0018x + 1.415 |
| Fat (% TBW[b]) vs. Stacked DM DXA[a] Fat (%) | 0.116 | 0.681 | y= 0.0827x + 3.5608 |
| Fat (g) vs. Stacked DM DXA[a] Fat (g) | 0.074 | 0.795 | y=0.0964x + 98.952 |
| Ash (g) vs. Stacked DM DXA[a] BMC[c] (g) | 0.448 | 0.094 | y=1.3993x + 50.075 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total body weight (TBW)
[c] Bone Mineral Composition (BMC)

Table 1.7. Statistical comparisons of proximate analysis to DXA[a] estimates of body composition in stacks of hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | Proximate Analysis | Stacked HRM DXA[a] |
| Weight/Tissue (g) | 1047.6 ± 40.42 | 2421.8 ± 352.58 |
| Water (g) | 689.3 ± 25.59 | - |
| Moisture (% TBW[b]) | 66.0 ± 0.23 | - |
| Protein/Lean (g) | 184.3 ± 6.84 | 2192.3 ± 310.57 |
| Protein (% TBW[b])/ % Lean | 17.6 ± 0.09 | 90.7 ± 1.25 |
| DXA R-value | - | 1.374 ± 0.0029 |
| Fat (g) | 121.9 ± 6.50 | 229.5 ± 56.40 |
| Fat (% TBW[b]) | 11.4 ± 0.25 | 9.3 ± 1.25 |
| Ash/ BMC[c] (g) | 38.3 ± 1.83 | 131.95 ± 10.084 |
| Proximate Analysis vs. HRM DXA[a] comparisons | | |
| Comparison | Correlation Coefficient | P value | Regression Equation |
| Weight (g) vs. Stacked HRM DXA[a] Tissue (g) | -0.139 | 0.620 | y= -0.7812x + 3249.7 |
| Moisture (%) vs. Stacked HRM DXA[a] Lean (%) | 0.135 | 0.631 | y= 0.5922x + 51.626 |
| Water (g) vs. Stacked HRM DXA[a] Lean (g) | 0.417 | 0.122 | y=3.271x - 87.728 |
| Protein (% TBW[b]) vs. Stacked HRM DXA[a] Lean (%) | 0.176 | 0.531 | y= 2.3913x + 48.529 |
| Protein (g) vs. Stacked HRM DXA[a] Lean (g) | 0.443 | 0.099 | y=13.09x - 245.63 |
| Fat (% TBW[b]) vs. Stacked HRM DXA[a] R-Value | -0.294 | 0.287 | y= -0.0026x + 1.4029 |
| Fat (% TBW[b]) vs. Stacked HRM DXA[a] Fat (%) | 0.272 | 0.327 | y= 1.0268x - 2.3281 |
| Fat (g) vs. Stacked HRM DXA[a] Fat (g) | 0.204 | 0.466 | y=1.1199x + 92.418 |
| Ash (g) vs. Stacked HRM DXA[a] BMC[c] (g) | 0.431 | 0.109 | y=1.7585x + 63.365 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total body weight (TBW)
[c] Bone Mineral Composition (BMC)

tissue versus percent body moisture or percent protein, or for the comparison of percent fat tissue to percent carcass fat (Table 1.8). Grams of lean as determined by DXA as

compared to grams of water determined by proximate analysis produced a significant ($p < 0.0001$) correlation, as did the comparison of grams of lean tissue versus grams of body protein as determined by proximate analysis. The only comparison that was statistically significant lower that the $p < 0.0001$ level was that of grams of fat to grams of carcass fat, which was still highly significant ($p < 0.05$).

Comparison of the two different methods of dual energy x-ray absorptiometry scanning, fan beam DXA scan data were also compared to the pencil beam DXA scan data from both DM and HRM small animal modes. Some correlation coefficients could not be calculated due the lack of variability within the data. This included, in the DM scan mode, the comparison of the two methods' percent lean values and, in turn, for the comparison of the two methods' percent fat values (Table 1.9). There were comparisons of DXA detailed medium scan values to fan beam DXA values that were significant correlations ($p < 0.05$). For total tissue mass as determined by pencil beam versus total tissue mass as determined by fan beam, and grams of lean tissue correlation from either method. When bone mineral composition and grams of carcass fat values were compared across the two methods for the DM mode, significant correlations ($p < 0.05$) were also found.

Regression equations were also developed for the correlations of fan beam DXA scan values to both DM and HRM pencil beam scan values.

Correlation coefficients could be calculated for the same comparisons of data between the fan beam DXA and the pencil beam DXA in HRM scan mode as could be for the DM scan mode (Table 1.10). Those correlations that were significant ($p < 0.05$)

Table 1.8. Proximate analysis means ± SEM, fan beam DXA[a] scan means ± SEM, and statistical comparisons of proximate analysis to DXA[a] estimates of body composition of hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | Proximate Analysis | Fan Beam DXA[a] |
| Weight/Tissue (g) | 1047.6 ± 40.42 | 982.7 ± 39.08 |
| Water (g) | 689.3 ± 25.59 | - |
| Moisture (% TBW[b]) | 66.0 ± 0.23 | - |
| Protein/Lean (g) | 184.3 ± 6.84 | 941.2 ± 36.47 |
| Protein (% TBW[b])/ % Lean | 17.6 ± 0.09 | 96.3 ± 0.39 |
| DXA Lean + BMC (g) | - | 950.9 ± 36.79 |
| Fat (g) | 121.9 ± 6.50 | 31.9 ± 4.97 |
| Fat (% TBW[b]) | 11.4 ± 0.25 | 3.7 ± 0.39 |
| Ash/ BMC[c] (g) | 38.3 ± 1.83 | 9.67 ± 0.47 |

Proximate Analysis vs. Fan Beam DXA[a] comparisons

| Comparison | Correlation Coefficient | P value | Regression Equation |
|---|---|---|---|
| Weight (g) vs. Fan Beam DXA[a] Tissue (g) | 0.962 | <0.0001 | y=0.9946x + 70.167 |
| Moisture (%) vs. Fan Beam DXA[a] Lean (%) | -0.009 | 0.952 | y= -0.0054x + 66.482 |
| Water (g) vs. Fan Beam DXA[a] Lean (g) | 0.959 | < 0.0001 | y=0.6727x + 56.174 |
| Protein (% TBW[b]) vs. Fan Beam DXA[a] Lean (%) | 0.080 | 0.584 | y=0.0192x + 15.79 |
| Protein (g) vs. Fan Beam DXA[a] Lean (g) | 0.958 | <0.0001 | y=0.1797x + 15.165 |
| Fat (% TBW[b]) vs. Fan Beam DXA[a] Fat (%) | 0.068 | 0.643 | y= 0.0447x + 11.212 |
| Fat (g) vs. Fan Beam DXA[a] Fat (g) | 0.524 | 0.0001 | y=0.685x + 100.01 |
| Ash (g) vs. Fan Beam DXA[a] BMC[c] (g) | 0.558 | <0.0001 | y=2.1564x + 17.435 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total body weight (TBW)
[c] Bone Mineral Composition (BMC)

Table 1.9. Detailed medium mode DXA[a] scan means ± SEM, fan beam DXA[a] scan means ± SEM, and resulting correlation coefficients, probability values, and regression equations calculated from comparisons of both DXA[a] technologies in hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | DM DXA[a] | Fan Beam DXA[a] |
| Tissue (g) | 1135.0 ± 42.42 | 982.7 ± 39.08 |
| Lean (g) | 1089.6 ± 40.71 | 941.2 ± 36.47 |
| % Lean | 96.0 ± 0.0 | 96.3 ± 0.39 |
| Lean + BMC (g) | - | 950.9 ± 36.79 |
| DXA R-Value | 1.405 ± 0.0010 | - |
| Fat (g) | 45.4 ± 1.70 | 31.9 ± 4.97 |
| % Fat | 4.0 ± 0.0 | 3.7 ± 0.39 |
| Ash/ BMC[c] (g) | 17.29 ± 0.990 | 9.67 ± 0.47 |

DM Pencil Beam DXA[a] vs. Fan Beam DXA[a] comparisons

| Comparison | Correlation Coefficient | P value | Regression Equation |
|---|---|---|---|
| DM Pencil Beam vs. Fan Beam DXA[a] Tissue (g) | 0.961 | <0.0001 | y=0.9955x - 22.261 |
| DM Pencil Beam vs. Fan Beam DXA[a] Lean (%) | N/A | N/A | N/A |
| DM Pencil Beam vs. Fan Beam DXA[a] Lean (g) | 0.968 | < 0.0001 | y=0.8669x - 3.2993 |
| DM Pencil Beam R-value vs. Fan Beam DXA[a] Fat (%) | 0.031 | 0.830 | y=12.751x - 14.173 |
| DM Pencil Beam vs. Fan Beam DXA[a] Fat (%) | N/A | N/A | N/A |
| DM Pencil Beam vs. Fan Beam DXA[a] Fat (g) | 0.390 | 0.006 | y=1.1384x - 19.774 |
| DM Pencil Beam vs. Fan Beam DXA[a] BMC[c] (g) | 0.808 | <0.0001 | y=0.3869x + 2.9833 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Bone Mineral Composition (BMC)

included the comparison of total tissue mass as determined by pencil beam versus total tissue mass as determined by fan beam, and grams of lean tissue correlation from either method. As bone mineral composition values and fat values (in grams) were compared across the two methods for the HRM scan mode, significant correlations ($p < 0.05$) were also calculated. For the remaining comparisons the fan beam DXA data versus the pencil

Table 1.10. High resolution mode DXA[a] scan means ± SEM, fan beam DXA[a] scan means ± SEM, and resulting correlation coefficients, probability values, and regression equations calculated from comparisons of both DXA[a] technologies in hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | HRM DXA[a] | Fan Beam DXA[a] |
| Tissue (g) | 1116.9 ± 42.46 | 982.7 ± 39.08 |
| Lean (g) | 1064.8 ± 39.53 | 941.2 ± 36.47 |
| % Lean | 95.5 ± 0.15 | 96.3 ± 0.39 |
| Lean + BMC (g) | - | 950.9 ± 36.79 |
| DXA R-Value | 1.391 ± 0.0011 | - |
| Fat (g) | 52.2 ± 3.58 | 31.9 ± 4.97 |
| % Fat | 4.5 ± 0.15 | 3.7 ± 0.39 |
| BMC[b] (g) | 22.96 ± 1.202 | 9.67 ± 0.47 |

HRM Pencil Beam DXA[a] vs. Fan Beam DXA[a] comparisons

| Comparison | Correlation Coefficient | P value | Regression Equation |
|---|---|---|---|
| HRM Pencil Beam vs. Fan Beam DXA[a] Tissue (g) | 0.958 | <0.0001 | y=0.8817x - 1.999 |
| HRM Pencil Beam vs. Fan Beam DXA[a] Lean (%) | 0.055 | 0.709 | y=0.1368x + 83.199 |
| HRM Pencil Beam vs. Fan Beam DXA[a] Lean (g) | 0.966 | < 0.0001 | y=0.8911x - 7.6604 |
| HRM Pencil Beam R-value vs. Fan Beam DXA[a] Fat (%) | 0.053 | 0.717 | y=19.156x - 22.893 |
| HRM Pencil Beam vs. Fan Beam DXA[a] Fat (%) | 0.055 | 0.709 | y= 0.1368x + 3.1253 |
| HRM Pencil Beam vs. Fan Beam DXA[a] Fat (g) | 0.357 | 0.012 | y=0.4963x + 5.9754 |
| HRM Pencil Beam vs. Fan Beam DXA[a] BMC[b] (g) | 0.799 | <0.0001 | y=0.3155x + 2.4273 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Bone Mineral Composition (BMC)

beam HRM DXA, no significant correlations could be calculated. Although, when compared to HRM scan data correlations could at least be calculated due to more variable data that estimated by DM DXA scans.

## Discussion

Currently, there is no available literature that evaluates the use of dual energy x-ray absorptiometry (DXA) as a means to non-invasively determine body composition in any teleost species. However, DXA methodologies have been utilized to determine various aspects of body composition in humans and other types of animals. These species include, but are not necessarily limited to, live pigs (Svendsen et al., 1993; Brunton et al., 1993; Pintauro et al., 1996; Mitchell et al., 1996a, 1996b, 1998a,b; Mitchell and Scholz, 1998), pig carcasses (Mitchell et al., 1998c), chickens (Mitchell et al., 1997a), beef carcass rib sections (Mitchell et al., 1997b), rats (Rose et al., 1998), mice (Sjögren et al., 2001), dogs (Lauten et al., 2001), and cats (Lauten et al., 2000). Pouilles et al. (2000) performed in vivo and ex vivo lumbar spine studies in sheep, using an absorptiometer, and then correlated this data with results of ashing the dissected bone ($r = 0.98$). These results demonstrate highly significant correlations, and our results also show good correlations for selected parameters. Rose et al. (1998) compared DXA measurements to chemical analysis for body fat, lean body mass, and BMC in lean and obese female rats, as well as developed regression equations in order to relate these two methodologies. In these experiments, despite DXA's tendency to overestimate values for percent fat, a good correlation between DXA and chemical analysis was observed. The regression equations that were developed and used to estimate chemical values from DXA data indicate that DXA can be used as a tool to predict some chemical analysis values. Unfortunately, DXA for these experiments had the opposite tendency in estimating fat—DXA was not accurate in fat estimations and severely underestimated all fat readings. This may have been due to the fact that Rose et al. (1998) used a different

type of DXA machinery—the Hologic QDR 100W. Even though a Hologic instrument was used for a portion of this study, human software was used for the fish whereas Rat Whole Body V5.71P software was used in the study by Rose et al. (1998).

When scanning fish carcasses with the Lunar model DPX-L ID #7618 DXA instrument, certain logistical problems had to be overcome. To account for the fish's individual weight and overall size, the carcasses were scanned using the DXA's small animal scan mode. There are several options within this scan mode, and it was decided to scan each fish in both the detailed medium (DM) and high resolution (HRM) scan modes. In practice there are a few major differences between these modes, one being the overall time it takes to scan a single fish. The DM mode completed a fish scan in an average of ten minutes, while an HRM fish scan took almost 60 minutes. This time difference is due to the number of scan lines the instrument makes while scanning a carcass in each mode. DM mode scans over a carcass using less scan lines that are not as close together, and therefore not as many total lines when compared to the HRM mode. The sample size in the DM mode is 1.2 x 2.4 mm and in the HRM mode it is 0.6 x 1.2 mm. This results in more individual pixels, tissue points, and an overall clearer image for the HRM scan of a fish.

Of those compositional components produced by DXA computer absorptiometry analysis, R-value (an x-ray attenuation coefficient), percent fat, total grams of tissue, grams of fat, grams of lean, and bone mineral composition (in grams) were used for comparison to proximate analysis values. From the DXA percent fat value, a corresponding percent lean value was estimated and also used for comparison to proximate analyses. Since DXA scans do not produce protein values or moisture values

per se, the DXA values for lean—which is composed of protein and water or moisture—are compared to both the proximate analyses for protein and moisture.

Possibly due to their limited body thickness, and partially due to the programming of the DXA computer software, all fish scanned in the DM scan mode were determined to have 4.0% body fat. Consequently, estimated percent carcass lean values were all 96.0% in this scan mode. The DPX-L algorithms for determining body composition assume a minimum of 4.0% body fat in a viable carcass, so that is why the readings bottom out at 4.0% fat and are not estimated lower. Why the percent fat estimations do not vary above 4.0% may have also been due to the fact that the algorithms designed for these small animal modes were intended for use with mammalian species. This made correlations to proximate values impossible.

In HRM small animal mode, DXA percent fat values were more variable for individual whole body scans. Although they were not all 4.0%, they were still considerably lower than proximate analysis values. Variability in percent fat determinations caused better variability in estimated percent carcass lean values.

Due to such little variability in the percent fat readings for whole carcasses produced by DXA in any scan mode, an additional type of computer analysis was performed. Using the DXA computer software, it is possible to highlight a particular region on interest and analyze just that area for all of the calculated DXA values for body composition. Fish deposit the vast majority of their fat tissue in their abdominal cavity, instead of throughout the body like many other animals. DXA takes into account all pixels when calculating an overall percent body fat reading, which may play a part in the low variation among these readings on a whole body scan. For the manual sectional

analysis, a region of interest highlighting the abdominal cavity was defined for all fish and each scan image was analyzed for composition. The region was compared to the previously described whole body proximate values.

With the increased estimation of body fat via regional analysis, the correlations when compared to corresponding proximate analysis values were now significant.

Another possible reason the DXA tended to limit percent fat estimates to about 4.0% could be minimal tissue scanning depth. Stacking of three fish was done to determine if the tendency of DXA to underestimate fat was due the thickness of the fish carcass. Stacks were created using fish from select tanks sampled after sixteen weeks of being fed dietary treatments.

Unfortunately, many of the scans of the stacked fish in DM mode produced overall percent fat values of 4.0%. And the estimations of percent fat and lean tissue did not result in significant correlations with proximate analyses. This may have been due, in part, to the fact that due to stacking there was a much smaller sample size to utilize. The smaller amount of overall samples may have led to poorer correlations due to decreased data points to develop regression equations from. To further investigate the usefulness of stacking fish carcasses for scanning, it would be useful to have a much larger available sample pool to work from.

The results from these experiments are similar to other DXA experiments with small animals. Many find high correlations with proximate analysis when comparing lean and BMC (Pritchard et al., 1992, Jebb et al., 1996; Rose et al, 1998). None of these reports had the problem with low percent fat readings that was encountered in this

experiment. This may have been due to differences in software, manufacturers, or even species.

A different type of DXA scanning was also examined as a means to non-invasively determine body composition in fish. This instrument utilizes a fan beam of x-ray energies. The Lunar DXA scanner uses a pencil beam of x-ray energies, and scans a subject line by line as the pencil beam travels across the subject in a left to right motion. In contrast, the Hologic DXA scanner has a beam that emits as a fan from the x-ray source and covers a much wider area of the scan subject at once. This main difference between DXA technologies causes the Hologic fan beam scanner to complete a scan in significantly less time than the Lunar pencil beam scanner.

There was only one scan mode to utilize on this machine, so selected fish from the sixteen week sampling were all scanned in the whole body scan mode. The only non-significant correlations of the fan beam DXA values and proximate analysis values were those of the percent of lean tissue compared to percent moisture or protein, and the percent of fat tissue compared to percent carcass fat. All other comparisons were able to produce varying degrees of significant correlation coefficients ($p < 0.05$).

The fan beam DXA results were compared to the corresponding pencil beam scan values in detailed medium and high resolution scan modes. Of the comparisons made, about half significantly correlated. In both modes, the comparison of total grams of tissue as determined by each DXA, the comparison of grams of lean tissue as determined by each DXA, the comparison of both DXA's values for bone mineral composition (g), and the comparison of both DXA's grams of fat estimations were significantly correlated ($p < 0.05$).

In conclusion, it made no difference in pencil beam DXA scan mode when comparing the body composition values to those determined by fan beam DXA technology. However, overall the pencil beam technology was more successful for determining body composition in these hybrid striped bass.

In conclusion, pencil beam DXA technology was a useful tool for determining some body composition components non-invasively. There was high correlation for some scan data when compared to proximate analyses. The inability of DXA to determine some components may have more to do with the limitations of the computer software and its algorithms than the technology itself. Manual analysis by identifying specific regions of interest helped overcome the analysis problems for fat to some degree, so depending on carcass size, this may be the most viable analysis option until more technological changes are made. Important components of body composition for a producer were estimated well by the DXA, such as body protein or lean tissue.

Despite its speed, fan beam DXA technology does not appear to be as effective as pencil beam DXA technology for determining body composition in hybrid striped bass of this size. In terms of mode comparisons for the pencil beam DXA technology, there were no real differences in correlation coefficients. HRM scans do produce a clearer scan image, but the data the scans produce are not significantly different from one another. In simple interest of time, the detailed medium scan would be more beneficial as it only takes approximately ten minutes per fish while a high resolution scan can take closer to an hour for an individual. If DXA were to ever be used as an industry tool to determine the body composition of fish at different points in growth, and eventually utilize these scans on live animals, the detailed medium small animal scan mode would be the better

choice. Its speed would allow for the potential of a fish to survive out of water, under proper conditions, much better than the HRM scan speed. Also, in terms of accuracy, the scan modes are quite similar. This is especially true for grams of lean in the animal, which would be a key interest of a producer raising food fish.

# CHAPTER 2

## The Assessment of Pencil Beam Dual Energy X-Ray Absorptiometry to Non-Invasively Determine Body Composition of Juvenile Hybrid Striped Bass (*Morone chrysops x Morone saxatilis*) Fed Diets with Variable Protein: Energy Ratios

## Introduction

Protein is the most expensive component in many aquaculture diets, particularly when feeding naturally carnivorous fish species. Feed producers aim to provide a minimum level of protein in the diet while still satisfying the essential amino acids at a level that will result in acceptable growth (Webster et al., 1995). Protein utilization by the body for functions such as lean carcass retention has been shown to improve when protein is replaced by lipid. Unfortunately, this replacement may lead to an excess amount of energy available in the diet. Excess energy is undesirable because it may (1) reduce overall feed consumption (Lovell, 1979), (2) produce fatty fish (Page and Andrews, 1973; Reinitz et al., 1978), (3) may inhibit the optimal utilization of other components of the diet (Prather and Lovell, 1973; Winfree and Stickney, 1981), and (4) is an expensive component of a manufactured feed.

Dietary protein level and the correct ratio of protein to energy are both key factors when formulating diets to feed fish. The level of digestible energy available in the diet is also important because it affects the amount of diet that will be consumed by the fish. The protein: energy ratio in turn influences the efficiency to which that amount of consumed diet is converted (Reis et al., 1989). Variations in to which type of tissue a diet is converted also exist. Excesses in energy levels in a diet may result in the deposition of larger amount of fat in a fish carcass. This effect is not desirable for an aquaculture producer that aims to please a consumer that desires a lean fish product. Deposition of fat does not mean that as a result less lean tissue is being deposited, but a producer does not want to waste a high cost feed for a fish to simply store it as fat. Consequently many studies are done to optimize protein to energy ratios in a manufactured feed to improve

feed efficiency in the aquaculture industry. Studies have shown that excess energy in an aquaculture feed can produce fatty fish (Shiau and Lan, 1996), reduce feed consumption—reducing total protein intake, and reduce the utilization of other nutrients in the diet (Page and Andrews, 1973; Prather and Lovell, 1973; Takeda et al., 1975; Shiau and Huang, 1990). Excess dietary protein can also be used for energy and not for growth when inadequate energy is fed in the diet (Catacutan and Coloso, 1995). Therefore, a balance of protein and energy in the diet is important.

Determination of the progress of growing fish on diets of a variety of compositions, such as varying levels of protein: energy ratios, would be beneficial to a producer or developer of aquaculture feeds. Rates of fat, lean and bone mineral deposition tend to be more relevant to animal studies in which optimization of lean or protein deposition and minimization of fat deposition can be important factors—namely economic (Mitchell et al., 1996a,b). Hybrid striped bass are an important aquacultured food fish. Fish farmers and researchers strive to understand how to optimally raise a species, such as this, in captivity. Feeds are a large portion of this, and maximizing the utilization of a diet while minimizing wasted feed is important economically and environmentally.

A simple and quick method to non-invasively determine body composition of fish would be a useful tool feed manufacturers and researchers. Both dual energy photon absorptiometry (DPA) and dual energy x-ray absorptiometry (DXA) have received widespread attention as a means to determine human body composition and detect changes within this composition. The DXA method is now considered by many in the scientific field to be the method of choice for measurement of human body composition.

Studies have included measurements of both pediatric (Venkataraman and Ahluwalia, 1992; Chan, 1992; Brunton et al., 1993) and adult subjects (VanLoan and Mayclin, 1992; Rico et al., 1994; Wellens et al., 1994). DXA has been used in numerous studies to determine the body composition of various animal species, including live pigs (Svendsen et al., 1993; Brunton et al., 1993; Pintauro et al., 1996; Mitchell et al., 1996a, 1996b, 1998a,b; Mitchell and Scholz, 1998), pig carcasses (Mitchell et al., 1998c), chickens (Mitchell et al., 1997a), beef carcass rib sections (Mitchell et al., 1997b), rats (Rose et al., 1998), mice (Sjögren et al., 2001), dogs (Lauten et al., 2001), and cats (Lauten et al., 2000).

To date, DXA has not been used in any published research to determine various components of body composition in fish—whether or not they are subject to dietary treatments. For juvenile fish, only a few analytical DXA techniques are available to determine body composition. A PIXImus table-top scanner, developed for use primarily in smaller laboratory animals, can scan a specified scan area; while the Lunar pencil beam DXA technology scans a subject line by line in a much larger possible area. The objectives of this experiment is to assess the ability of various methods of DXA technology to determine body composition in juvenile hybrid striped bass fed diets with various energy: protein ratios.

## Materials and Methods

### *Animal Husbandry*

Juvenile hybrid striped bass originally from Keo Fish Farms (Keo, AR, USA) and currently of the fish population of the aquaculture nutrition research laboratory at the University of Maryland, College Park were utilized for the study. Thirty fish with an average starting weight of 75.6g were housed in each of twelve 1000-L circular fiberglass tanks that were part of a recirculating water system, for a total of 360 fish in the experimental population. Salinity was maintained at approximately 5ppt throughout the experiment. Water quality conditions for all recirculating water tank systems were maintained whenever possible at levels recommended by Nicholson et al. (1990) for striped bass. Initial and replacement fresh water was supplied to each tank system after municipal water passed through an in-line 25 micron particulate filter and an activated carbon filter for dechlorination. A salt solution consisting of calcium chloride, sodium chloride (9:1), magnesium chloride (5 mg/L), and sodium thiosulfate was continuously injected directly into incoming water, which allowed the maintenance of a minimum concentration of 36 mg/L calcium hardness in the rearing tanks. Additional sodium chloride was added by hand to the systems to maintain salinities of approximately 5ppt. Sodium bicarbonate was also periodically added by hand to adjust pH levels within the systems to approximately 7.5. Flow rates for all tanks were set for two complete turnovers per hour. Tanks were continuously aerated by air stone diffusers to maintain adequate dissolved oxygen levels. A photoperiod of 12 hours light :12 hours dark was maintained. Water temperatures (average of 22°C) were recorded four times per day by a central computerized monitoring system (REES Scientific, Trenton, NJ, USA). Water

quality parameters of pH (7.5), dissolved oxygen (DO: 7.0 mg/L), total chlorine (<0.10 ppm), ammonia (<0.3 mg/L), nitrite (<0.7 mg/L), nitrate (<50 mg/L), salinity (5 ppt), and calcium hardness (221 mg/L) were measured weekly. DO and temperature were monitored using a YSI model 58 oxygen meter (model #58- Yellow Springs Instrument Co., Inc. Yellow Springs, Ohio, USA). pH was monitored with a Corning model 250 ion analyzer (Corning Incorporated, Corning, NY, USA). Chlorine (total), ammonia, nitrite, and nitrate were measured using a Hach DR850 colorimeter (Hach Company, Loveland, CO, USA). Calcium concentrations were measured with a Hach model HA-4P hardness test kit. Regular water changes (20% of total water volume, twice per week) and biofilter backwashes (once per week) were completed to maintain nitrate levels below a level of 50 mg/L.

Groups of fish were fed one of four diets (Table 2.1) at various ratios of energy: protein intake—9.6, 8.6, 7.9, and 7.1. Digestible energy in the diets was also held to approximately 3400 kcal/kg as fed. The diets were formulated to meet all known nutrient requirements for hybrid striped bass, with lysine, the most limiting essential amino acid, being kept above the amino acid requirement of 1.81% in all four diets (Small and Soares, 1998). When nutrient requirement information was not available for hybrid striped bass, the dietary requirements for salmonid fish were used (NRC, 1993). The experimental design was completely randomized with dietary treatments assigned to each tank, producing three replicates of thirty fish per treatment.

Table 2.1. Composition of the four diets with various ratios of energy: protein ratios fed to hybrid striped bass.

| Ingredient | Diet 1 g/kg diet, as fed | Diet 2 g/kg diet, as fed | Diet 3 g/kg diet, as fed | Diet 4 g/kg diet, as fed |
|---|---|---|---|---|
| Soybean meal (48.5% protein) | 165 | 194.7 | 220 | 243 |
| Menhaden fish meal | 220 | 259.6 | 287.5 | 325 |
| Wheat flour | 190 | 120.9 | 80 | 60 |
| Menhaden fish oil | 100 | 90 | 80 | 70 |
| Wheat middlings | 200 | 170 | 139.5 | 79.5 |
| Corn gluten meal (60% protein) | 100 | 129.8 | 146 | 162.5 |
| Lysine HCl | 5 | 2 | 0 | 0 |
| Cellulose | 0 | 13 | 27 | 40 |
| Vitamin premix 7-15 | 10 | 10 | 10 | 10 |
| Mineral premix | 10 | 10 | 10 | 10 |
| Total | 1000 | 1000 | 1000 | 1000 |
| Energy:Protein Ratio | 9.6:1 | 8.6:1 | 7.9:1 | 7.1:1 |
| Digestible Energy (Kcal/kg) | 3465 | 3471 | 3443 | 3391 |
| % Protein | 34.3 | 38.5 | 41.3 | 44.9 |
| Arginine | 1.97 | 2.21 | 2.39 | 2.60 |
| Histidine | 0.79 | 0.89 | 0.96 | 1.04 |
| Isoleucine | 1.42 | 1.61 | 1.74 | 1.89 |
| Leucine | 2.99 | 3.49 | 3.80 | 4.13 |
| Lysine | 2.25 | 2.28 | 2.32 | 2.56 |
| Methionine | 0.74 | 0.85 | 0.93 | 1.02 |
| Cystine | 0.46 | 0.52 | 0.55 | 0.59 |
| Phenylalanine | 1.58 | 1.80 | 1.95 | 2.11 |
| Tyrosine | 1.20 | 1.38 | 1.50 | 1.63 |
| Threonine | 1.25 | 1.42 | 1.54 | 1.68 |
| Tryptophan | 0.37 | 0.41 | 0.44 | 0.48 |
| Valine | 1.70 | 1.93 | 2.09 | 2.27 |

*Sample Collection and Preparation*

Before beginning the proposed experimental regime, a baseline sampling of the fish population was taken. Two fish from each tank, twenty- four fish total, were euthanized by immersion in an overdose solution of tricaine methanesulfonate (MS-222). Each fish was individually weighed and measured for standard length, individually

bagged, and frozen in the lateral position at -20°C for subsequent analysis. After being maintained on the experimental regime for five weeks, the population of fish from each tank was weighed together and fish were measured individually for standard length. Measurements were taken starting at the tip of the nose and continuing to the posterior end of the fleshy caudal peduncle (USGS, 2000). The caudal fins are not included in this type of measurement, resulting in data more indicative of the true body length of the fish. Before sampling, the fish were anesthetized by immersion in a 4mg/L solution of tricaine methanesulfonate (MS-222). Fish were then returned to their respective tanks and maintained on the same feeding regime for another five weeks. At the end of the ten weeks, fish were sampled again. Weights per tank and individual standard lengths were recorded as they were at five weeks, and three fish were randomly selected from the population of each tank, making thirty six fish total, and euthanized by immersion in an overdose solution of 8mg/L tricaine methanesulfonate (MS-222). The fish were then individually weighed, stored in plastic bags, sealed, and frozen in the lateral position at -20°C for subsequent analysis.

*Lunar PIXImus DXA Analysis-Experiment 2A*

Frozen fish were then transported to the United States Department of Agriculture (USDA) Agriculture Research Station Growth Biology Laboratory of A. Mitchell in Beltsville, Md. Each fish from the baseline and ten week samplings was individually scanned in the lateral position by the Lunar model PIXI table-top dual energy x-ray absorptiometry (DXA) instrument. This type of DXA instrument has a limited scan area, and images are obtained by exposing the entire area to the two x-ray energies. For this reason fish were arranged within the scan window so the scan would at minimum include

their abdominal region, since many fish were too long for their entire body to fit within the scan area. Scan images showed all of the abdominal cavity, and depending on the size of the fish, included many other components of the body. Computer absorptiometry analysis estimated body composition by calculations for programmed parameters, including: percent fat, grams of tissue, grams of fat, grams of lean, and bone mineral composition (BMC). Due to the fixed scan area of the PIXImus DXA, a different proportion of each fish was scanned depending on the size of the individual. For consistency between scans of all fish, a common area was defined for body composition analysis. To do this, manual sectional analysis was performed on the scanned abdominal region of each fish, encompassing the region from the posterior side of the swim bladder, along the ventral side of the spine, straight down to the anal fin of the fish, and along the ventral aspect of the abdomen. The same body composition components as described above were estimated for the designated region. From these body composition components, a simple percent lean value was also determined.

*Lunar Pencil Beam DXA Analysis-Experiment 2B*

The individual fish were also scanned in the lateral position, in the cranial-caudal direction, in high resolution small animal scan mode by the Lunar model dual energy x-ray absorptiometry (DXA) instrument, provided by A. Mitchell in the Growth Biology Laboratory of the USDA/ARS in Beltsville, Md. Due to the larger scale of this instrument, fish could be scanned in batches of nine individuals at a time. Manual analysis of the computer images allowed for analysis of individual fish, and produced estimates of various body composition components: a DXA R-value (an attenuation coefficient of the two x-ray energies), percent fat, grams of tissue, grams of fat, grams of

lean, bone mineral composition (BMC), and bone mineral density. Using the percent fat scan value, a simple estimated percent lean value was also determined.

*Proximate Analysis-Experiment Series 2*

After each scanning, fish were returned to storage at -20°C until further analysis. Final analysis of each fish consisted of whole carcass proximate analysis, as individual crude protein (Nitrogen gas analysis x 6.25), ether extract, ash (combustion at 550°C), and moisture determinations by standard methods (AOAC 1995). For proximate analysis, each fish was first thawed overnight and macerated and ground until reduced to particles of less than 5mm in size. All tissues were collected and refrozen until analysis. For moisture analysis, the homogenate was dried in an aluminum pan at 95°C for 48 hours. The homogenate sample was weighed before and after drying, and the difference in weights was used to determine the moisture content. These dried samples were then reground using a microgrinder (MicroMill; Pequannock, NJ) and stored in separate, labeled plastic tubes in a dessicator (Boekel; Philadelphia, PA) until additional analysis. Dry samples were analyzed for fat content through ether extract, and these fat-free samples were then analyzed for crude protein and ash contents. In between all analyses, samples were stored in dessicators. Protein was determined via combustion and nitrogen gas analysis using a LECO FP-428 Nitrogen Analyzer (LECO; St. Joseph, MI). Ash contents were determined by overnight combustion in crucibles in a rectangular muffle furnace at 550°C, and the change in weight was used to calculate ash. All animal care and handling was done according to a protocol approved by the University of Maryland Animal Care and Use Committee (Protocol #R-00-59A and #R-00-59B).

*Statistical Analysis*

The data for all components of this experiment were analyzed for statistical differences using Statistical Analytical Systems (SAS) software (SAS Institute, 2001). In the experiment, one-way analysis of variance (ANOVA) as computed by the SAS mixed procedure was used to determine if a treatment effect was present. The test of least significant difference (LSD) was used to test for significant differences between means when the ANOVA analysis indicated that significant differences were present. In all cases, a probability level of $p \leq 0.05$ was chosen for the detection of significant differences, and the data conformed to the assumption of normality (Sokhal and Rohlf, 1987) necessary for proper statistical analysis by ANOVA and LSD. Correlation coefficients and regression equations were also calculated by methods described by SAS to compare values given by each DXA methodology and the appropriate proximate analysis.

## Results

*Proximate Analysis-Experiment Series 2*

Analyses values are given as back-calculated whole body values in order to directly compare these values to either of the DXA scan values. Proximate analyses are considered the reference standard for actual body composition of the fish in these experiments and DXA values were compared to the latter to estimate the accuracy of the different types of scan technology.

Proximate analyses were also used to determine the effects the four dietary treatments had on body composition in juvenile hybrid striped bass (Table 2.2). All baseline samplings are presented per dietary treatments for comparison purposes, even

Table 2.2. Mean ± SEM proximate analysis values per dietary treatment, on a whole body wet basis, for juvenile hybrid striped bass sampled at baseline (n=6 per treatment) and after 10 weeks (n=9 per treatment) on dietary treatments.

| | Diet 1-Ratio 9.6 kcal Energy/ g Protein | Diet 2-Ratio 8.6 kcal Energy/ g Protein | Diet 3-Ratio 7.9 kcal Energy/ g Protein | Diet 4-Ratio 7.1 kcal Energy/ g Protein |
|---|---|---|---|---|
| | Baseline Sampling | | | |
| Weight (g) | 66.5[a] ± 5.73 | 84.6[b] ± 12.89 | 66.6[a] ± 7.13 | 67.1[a] ± 4.83 |
| % Moisture | 71.1 ± 0.86 | 71.2 ± 0.72 | 70.2 ± 0.17 | 70.4 ± 0.32 |
| Water (g) | 47.0 ± 3.54 | 59.8 ± 8.74 | 46.8 ± 5.11 | 47.3 ± 3.51 |
| % Fat | 8.1 ± 0.24 | 8.2 ± 0.69 | 8.6 ± 0.27 | 8.3 ± 0.63 |
| Fat (g) | 5.4 ± 0.50 | 7.2 ± 1.31 | 5.7 ± 0.47 | 5.6 ± 0.54 |
| % Protein | 14.8 ± 0.96 | 14.9 ± 0.74 | 16.3 ± 0.55 | 16.1 ± 0.88 |
| Protein (g) | 10.1 ± 1.46 | 13.0 ± 2.38 | 10.9 ± 1.27 | 10.8 ± 0.99 |
| % Ash | 2.8 ± 0.51 | 2.8 ± 0.32 | 3.0 ± 0.56 | 3.2 ± 0.23 |
| Ash (g) | 2.2 ± 0.32 | 2.4 ± 0.65 | 1.7 ± 0.18 | 2.2 ± 0.29 |
| | 10 Weeks on Dietary Treatments | | | |
| Weight (g) | 162.7 ± 11.72 | 185.2 ± 14.14 | 170.3 ± 10.88 | 178.1 ± 7.72 |
| % Moisture | 66.3 ± 0.94 | 66.8 ± 2.52 | 68.1 ± 0.14 | 67.7 ± 0.34 |
| Water (g) | 107.1 ± 7.04 | 122.6 ± 9.15 | 116.0 ± 7.34 | 120.4 ± 4.78 |
| % Fat | 11.4[a] ± 0.95 | 10.2[ab] ± 0.82 | 9.2[b] ± 0.29 | 9.7[b] ± 0.33 |
| Fat (g) | 19.0 ± 2.48 | 19.3 ± 2.47 | 15.8 ± 1.25 | 17.4 ± 0.20 |
| % Protein | 18.0 ± 0.46 | 18.8 ± 1.29 | 18.7 ± 0.39 | 18.2 ± 0.45 |
| Protein (g) | 29.2 ± 2.14 | 35.1 ± 3.91 | 31.9 ± 2.13 | 32.6 ± 1.91 |
| % Ash | 3.4 ± 2.87 | 2.7 ± 0.12 | 3.5 ± 0.92 | 3.4 ± 0.37 |
| Ash (g) | 4.6[a] ± 0.59 | 8.3[b] ± 1.85 | 4.9[a] ± 0.33 | 5.2[a] ± 0.43 |

[ab] Means within rows with different letters are significantly different at $p < 0.05$

though none of these fish were subjected to the assigned dietary treatments. Separation was done to also determine any difference between populations before fish were fed the various dietary treatments. The only difference between projected dietary treatments before the start of the experiment was the starting weight of the fish (n= 24). Baseline fish that would be on dietary treatment 2 (n= 6) weighed significantly more than the other

fish ($p < 0.05$). According to the proximate analyses, all baseline fish were statistically similar for all other major components of body composition ($p < 0.05$).

Calculations from proximate analysis values were also completed for the ten-week sampling period, and values for proximate components were determined on a whole body wet basis for these fish ($n = 36$) as well. Most treatment means for fish sampled after ten weeks of dietary treatments were not statistically different from one another on a whole body wet basis ($p > 0.05$) (Table 2.2). Percent fat values, on a whole body wet basis, were significantly different ($p<0.05$) between treatments 1 and 3 as well as between treatments 1 and 4, with the mean of treatment 1 being the highest percent fat of them. Mean grams of carcass ash for fish fed dietary treatment 2 ($n = 9$) was significantly higher than all other dietary treatment means ($p<0.05$).

From all fish in the experimental population, growth data, including individual lengths and tank weights, and daily dietary intakes were recorded (Table 2.3). Feed conversion ratios (FCR) were calculated from fish weight gains and feed intake between each sampling period. The specific calculation is:

$$FCR = (\text{grams of feed intake})/(\text{grams of tissue gain})$$

For the baseline sampling, all fish were statistically similar in lengths, total tank weights, and individual weights. After ten weeks on dietary treatments, fish fed the dietary treatments with an energy: protein ratio of 7.1 gained more weight overall than fish fed the dietary treatments with energy: protein ratios of 9.6 or 8.6 ($p < 0.05$). While on experimental treatments, fish fed the diet with an energy: protein ratio of 9.6 gained the least in body mass and grew the least in length. After ten weeks on experimental diets, fish fed the diet with an energy: protein ratio of 7.1 also had the most efficient feed

Table 2.3. Growth sampling data mean ± SEM per dietary treatment for all juvenile hybrid striped bass from samplings at baseline and after 10 weeks on dietary treatments.

| | Diet 1- E/P Ratio 9.6 | Diet 2- E/P Ratio 8.6 | Diet 3- E/P Ratio 7.9 | Diet 4- E/P Ratio 7.1 |
|---|---|---|---|---|
| | Baseline Sampling | | | |
| n | 90 | 90 | 90 | 90 |
| Total Fish Weight (g) | 2069.0 ± 102.1 | 2070.7 ± 69.23 | 2122.3 ± 110.92 | 2027.3 ± 123.47 |
| Mean Weight (g) per Fish | 73.9 ± 3.65 | 76.7 ± 2.51 | 78.6 ± 4.11 | 73.3 ± 4.16 |
| Length (cm) per Fish | 16.8 ± 1.78 | 15.2 ± 0.19 | 15.3 ± 0.23 | 15.2 ± 0.05 |
| | 10 Weeks on Dietary Treatments | | | |
| n | 78 | 78 | 78 | 78 |
| Total Fish Weight (g) | $4279.4^{b}$ ± 36.42 | $4695.7^{c}$ ± 204.03 | $4666.3^{bcd}$ ± 459.08 | $5329.0^{d}$ ± 275.29 |
| Mean Weight (g) per Fish | $171.2^{b}$ ± 7.39 | $201.2^{c}$ ± 3.21 | $194.4^{c}$ ± 7.10 | $216.4^{d}$ ± 4.51 |
| Overall Tank Gain (g) | $2392.1^{b}$ ± 153.01 | $2814.8^{c}$ ± 216.09 | $2753.3^{bc}$ ± 359.80 | $3494.8^{d}$ ± 254.02 |
| Length (cm) per Fish | $19.1^{b}$ ± 0.02 | $20.4^{c}$ ± 0.36 | $19.8^{c}$ ± 0.50 | $20.3^{c}$ ± 0.32 |
| FCR[a] | $1.29^{b}$ ± 0.06 | $1.22^{b}$ ± 0.01 | $1.22^{b}$ ± 0.04 | $1.09^{c}$ ± 0.03 |

[a] Feed conversion ratio (FCR)
[bcd] Means within rows with different letters are significantly different at $p < 0.05$.

conversion ratio of all treatments. There were no mortalities for any of the dietary treatments over the course of the experiment.

*Regression and Correlation of DXA Data to Proximate Analysis*

*Proximate Analysis*

For comparison to DXA values and to validate DXA as an appropriate method to determine body composition in fish, the proximate values determined in the nutritional study were used. These values were correlated to their appropriate DXA compositional value, and the overall means for each body composition component are also presented (Table 2.4).

*Lunar PIXImus DXA Analysis-Experiment 2A*

Proximate analysis values were used to validate the scan values for body composition estimated by the Lunar PIXImus DXA scanner. This technology utilizes a small fixed scan window, so fish were individually scanned and a common region of interest was defined in the scan image for all fish. This method was used to create consistency among fish scans. These estimations of components of body composition were compared to and correlated with proximate values to validate this technology's ability to determine body composition (Table 2.4).

From the correlations of PIXImus DXA scan values and proximate analyses values, regression equations were developed (Table 2.4). The data from PIXImus DXA instrumentation to determine body composition in juvenile hybrid striped bass showed correlations to the proximate analyses. Some correlation coefficients were high, and those that were highly statistically significant ($p < 0.0001$) included the PIXI DXA grams of total tissue versus the actual weight, in grams, of the fish carcass ($r^2 = 0.949$), PIXI DXA grams of lean versus the amount of water in grams determined by proximate analysis ($r^2 = 0.929$), DXA grams of lean versus the amount of protein in grams determined by proximate analysis ($r^2 = 0.926$), PIXI DXA grams of fat versus the grams of fat determined by proximate analysis ($r^2 = 0.833$), and PIXI DXA grams of bone mineral composition (BMC) versus the grams of ash determined by proximate analysis ($r^2 = 0.570$). PIXI DXA percent fat versus the percent total body fat determined by proximate analysis ($r^2 = 0.266$) was also statistically significant at a lower probability level ($p < 0.05$). Correlation coefficients calculated for other PIXI DXA and proximate analysis comparisons, including the DXA percent lean versus both percent moisture and

Table 2.4. Proximate analysis means ± SEM, PIXImus DXA[a] scan means ± SEM, and resulting correlation coefficients, probability values, and regression equations calculated from comparisons of proximate analysis to PIXImus DXA[a] values for body composition in juvenile hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | Proximate Analysis | PIXI DXA[a] |
| Weight/Tissue (g) | 132.9 ± 7.55 | 51.7 ± 2.85 |
| Water (g) | 90.0 ± 4.88 | - |
| Moisture (% TBW[b]) | 68.6 ± 0.47 | - |
| Protein/Lean (g) | 23.8 ± 1.58 | 41.5 ± 2.23 |
| Protein (% TBW[b])/ % Lean | 17.3 ± 0.32 | 80.8 ± 0.70 |
| Fat (g) | 13.1 ± 0.97 | 10.3 ± 0.76 |
| Fat (% TBW[b]) | 9.40 ± 0.25 | 19.2 ± 0.70 |
| Ash/ BMC[c] (g) | 4.29 ± 0.41 | 0.43 ± 0.03 |
| Ash (% TBW[b]) | 3.14 ± 0.42 | - |

| Proximate Analysis vs. PIXI DXA[a] comparisons | | | |
|---|---|---|---|
| Comparison | Correlation Coefficient | P value | Regression Equation |
| Weight (g) vs. PIXI DXA[a] Tissue (g) | 0.949 | <0.0001 | y=2.5113x + 3.0068 |
| Moisture (%) vs. PIXI DXA[a] Lean (%) | 0.168 | 0.198 | y=0.1461x + 56.794 |
| Water (g) vs. PIXI DXA[a] Lean (g) | 0.929 | < 0.0001 | y=2.0319x + 5.7403 |
| Protein (% TBW[b]) vs. PIXI DXA[a] Lean (%) | -0.155 | 0.236 | y=-0.0714x + 23.032 |
| Protein (g) vs. PIXI DXA[a] Lean (g) | 0.926 | <0.0001 | y=0.6579x - 3.493 |
| Fat (% TBW[b]) vs. PIXI DXA[a] Fat (%) | 0.266 | 0.040 | y=0.0961x + 7.5542 |
| Fat (g) vs. PIXI DXA[a] Fat (g) | 0.833 | <0.0001 | y=1.0564x + 2.26 |
| Ash (g) vs. PIXI DXA[a] BMC[c] (g) | 0.570 | <0.0001 | y=9.0916x + 0.4115 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total Body Weight (TBW)
[c] Bone Mineral Composition (BMC)

percent body protein as determined by proximate analysis, were not significant ($p > 0.05$).

*Lunar Pencil Beam DXA Analysis-Experiment 2B*

Proximate analysis values were also used to validate the scan values for body composition estimated by the Lunar pencil beam DXA scanner. This technology scans a much larger area, so fish were able to be scanned in groups of nine individuals. Scans were done in the high resolution small animal scan mode, and computer regional analysis was used to individually analyze scanned fish (Table 2.5).

From the correlations of the pencil beam DXA scan values and proximate analysis values, regression equations were developed (Table 2.5). The correlation coefficients that were highly statistically significant ($p< 0.0001$) included: the DXA grams of total tissue versus the actual weight, in grams, of the fish carcass ($r^2 = 0.982$), DXA grams of lean versus the amount of water in grams determined by proximate analysis ($r^2 = 0.969$), DXA grams of lean versus the amount of protein in grams determined by proximate analysis ($r^2 = 0.949$), the DXA R-value, an attenuation coefficient, versus the percent fat as a percent of total body weight as determined by proximate analysis ($r^2 = -0.565$), DXA grams of fat versus the grams of fat determined by proximate analysis ($r^2 = 0.875$), and DXA bone mineral composition (g) versus grams of ash determined by proximate analysis ($r^2=0.667$). The non-significant ($p< 0.05$) correlation coefficients calculated for pencil beam DXA scans and proximate analysis comparisons were DXA lean (%) versus percent moisture determined by proximate analysis ($r^2=0.168$), DXA lean (%) versus percent body protein determined by proximate analysis ($r^2= -0.117$), and DXA fat

Table 2.5. Proximate analysis means ± SEM, high resolution mode Pencil beam DXA[a] scan means ± SEM, and resulting correlation coefficients, probability values, and regression equations calculated from comparisons of proximate analysis to Pencil beam DXA[a] values for body composition in juvenile hybrid striped bass.

| Variable | Mean ± SEM | |
|---|---|---|
| | Proximate Analysis | Pencil Beam DXA[a] |
| Weight/Tissue (g) | 132.9 ± 7.55 | 149.3 ± 8.45 |
| Water (g) | 90.0 ± 4.88 | - |
| Moisture (% TBW[b]) | 68.6 ± 0.47 | - |
| Protein/Lean (g) | 23.8 ± 1.58 | 143.1 ± 8.06 |
| Protein (% TBW[b])/ % Lean | 17.3 ± 0.32 | 95.9 ± 0.05 |
| Fat (g) | 13.1 ± 0.97 | 6.26 ± 0.41 |
| Fat (% TBW[b]) | 9.40 ± 0.25 | 4.12 ± 0.05 |
| R-value | - | 1.404 ± 0.002 |
| Ash/ BMC[c] (g) | 4.29 ± 0.41 | 1.32 ± 0.10 |
| Ash (% TBW[b]) | 3.14 ± 0.42 | - |

Proximate Analysis vs. Pencil Beam DXA[a] comparisons

| Comparison | Correlation Coefficient | P value | Regression Equation |
|---|---|---|---|
| Weight (g) vs. Pencil Beam DXA[a] Tissue (g) | 0.982 | <0.0001 | y=0.8767x + 2.0243 |
| Moisture (%) vs. Pencil Beam DXA[a] Lean (%) | 0.168 | 0.198 | y=1.6699x - 91.507 |
| Water (g) vs. Pencil Beam DXA[a] Lean (g) | 0.969 | <0.0001 | y=0.5869x + 6.0368 |
| Protein (% TBW[b]) vs. Pencil Beam DXA[a] Lean(%) | -0.117 | 0.371 | y=-0.8027x + 94.222 |
| Protein (g) vs. Pencil Beam DXA[a] Lean (g) | 0.949 | <0.0001 | y=0.1865x - 2.8967 |
| Fat (% TBW[b]) vs. Pencil Beam DXA[a] R-value | -0.565 | <0.0001 | y=-79.133x + 120.55 |
| Fat (% TBW[b]) vs. Pencil Beam DXA[a] Fat (%) | 0.216 | 0.097 | y=1.1588x + 4.6256 |
| Fat (g) vs. Pencil Beam DXA[a] Fat (g) | 0.875 | <0.0001 | y=2.0462x + 0.286 |
| Ash (g) vs. Pencil Beam DXA[a] BMC[c] (g) | 0.667 | <0.0001 | y=2.6956x + 0.7209 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total Body Weight (TBW)
[c] Bone Mineral Composition (BMC)

(%)versus percent body fat determined by proximate analysis ($r^2$=0.216). Their corresponding regression equations were also not significant ($p > 0.05$).

*PIXImus and Pencil Beam DXA Scan Technologies-Experiment Series 2*

PIXImus DXA values and pencil beam DXA scan values were compared to one another to determine if the two instruments produced similar results. The scan values from the common region of analysis for the PIXImus scans were directly compared to the individual scan values from the pencil beam DXA analysis. From the correlations of the PIXImus DXA scan values and the pencil beam DXA scan values, regression equations were developed (Table 2.6). Some correlation coefficients and their regression equations were not statistically significant ($p> 0.05$).

Correlation coefficients were high and all statistically significant ($p< 0.05$), including the comparison of both types of DXA scans for grams of total tissue of the fish carcass ($r^2 = 0.966$), grams of lean tissue ($r^2 = 0.964$), grams of fat ($r^2 = 0.764$), and grams of bone mineral composition ($r^2 = 0.863$). All other comparisons between the two types of DXA scan methods; including percent fat, DXA R-value versus percent fat, and percent lean tissue, were not significant ($p> 0.05$).

*DXA Accuracy*

To evaluate the ability of DXA to accurately determine aspects of body composition in juvenile hybrid striped bass, regression equations were used to back calculate various proximate values. Data for twenty percent of the sampled population (n=11) was removed and regression equations were developed for this population subset. These new equations were then used to predict proximate values of the fish that were

Table 2.6. Correlation coefficients, probability values, and regression equations calculated from comparisons of PIXImus DXA[a] values to high resolution pencil beam DXA[a] values for body composition in juvenile hybrid striped bass.

| Comparison | Correlation Coefficient | P value | Regression Equation |
|---|---|---|---|
| | Both DXA[a] Scans | | |
| PIXI DXA[a] Tissue (g) vs. Pencil Beam DXA[a] Tissue (g) | 0.966 | <0.0001 | y=2.8625x + 1.2183 |
| PIXI DXA[a] Lean (%) vs. Pencil Beam DXA[a] Lean (%) | 0.074 | 0.576 | y=0.005x - 95.478 |
| PIXI DXA[a] Lean (g) vs. Pencil Beam DXA[a] Lean (g) | 0.964 | <0.0001 | y=3.4851x - 1.4742 |
| PIXI DXA[a] Fat (%) vs. Pencil Beam DXA[a] R-value | -0.201 | 0.124 | y= -0.0005x + 1.4146 |
| PIXI DXA[a] Fat (%) vs. Pencil Beam DXA[a] Fat (%) | 0.074 | 0.576 | y=0.005x + 4.0245 |
| PIXI DXA[a] Fat (g) vs. Pencil Beam DXA[a] Fat (g) | 0.764 | <0.0001 | y=0.4144x + 2.0104 |
| PIXI DXA[a] BMC[b] (g) vs. Pencil Beam DXA[a] BMC[b] (g) | 0.863 | <0.0001 | y=3.4079x - 0.1298 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Bone Mineral Composition (BMC)

removed from the population. This was done for the PIXI DXA and pencil beam DXA data.

The ability of DXA to predict proximate values can be determined, in part, by the percent difference calculated between the actual composition value determined by proximate analysis and the value predicted using the regression equation. Modified

regression equations were used to predict the body composition values that were excluded. For the PIXI DXA data, only one of the mean percent differences between actual and calculated body composition values was under five percent—the comparison of percent moisture to PIXI DXA percent lean (Table 2.7). The highest mean percent difference from the actual proximate values was for the PIXI DXA scan value comparison to grams of ash. This extreme in differences is likely due to the limited scan area of the PIXI DXA technology, and the DXA's poor ability to determine total ash in the carcasses due to this limitation. All other mean differences were between 7.4 and 28.0 for the comparison of PIXI DXA scan values proximate analysis values.

Modified regression equations were also developed for the comparisons of pencil beam DXA scans to proximate analyses (Table 2.8). As with the PIXI DXA comparisons, when the regression equations were used to calculate corresponding body composition values only one of the mean percent differences was below five percent—percent carcass moisture vs. pencil beam DXA percent lean. Also similar to the PIXI DXA data, the mean percent difference between grams of ash determined by proximate analysis and grams of ash determined by the modified regression equation was the highest. This difference also corresponds with the inability of the pencil beam DXA to accurately determine proximate ash when the original regression equations and correlations were developed.

For both types of DXA scan technologies, the ability of regression equations to predict proximate body composition values corresponded to the ability of the DXA technology to generally determine these components of body composition. The mean percent differences for grams of fat and percent fat for the pencil beam DXA equations

Table 2.7. Modified regression equations, corresponding mean ± SEM proximate value determined by the equation, and mean ± SEM percent difference from actual proximate values for comparisons of proximate analysis to PIXI DXA[a] values for juvenile hybrid striped bass.

| Comparison | Modified Regression Equation | Determined Mean Proximate Value | Mean % Difference From Actual Value |
|---|---|---|---|
| | PIXI DXA[a] Scans | | |
| Moisture (%) vs. PIXI DXA[a] Lean (%) | y =0.164x + 55.303 | 68.8 ± 0.30 | 2.2 ± 0.87 |
| Water (g) vs. PIXI DXA[a] Lean (g) | y =2.0293x + 6.0224 | 79.9 ± 7.58 | 8.0 ± 1.97 |
| Protein (% TBW[b]) vs. PIXI DXA[a] Lean (%) | y = -0.0683x + 22.973 | 17.4 ± 0.13 | 11.5 ± 4.22 |
| Protein (g) vs. PIXI DXA[a] Lean (g) | y =0.6576x - 3.2147 | 20.7 ± 2.46 | 14.5 ± 4.20 |
| Weight (g) vs. PIXI DXA[a] Tissue (g) | y =2.5149x + 2.7556 | 114.9 ± 12.09 | 7.4 ± 2.11 |
| Fat (% TBW[b]) vs. PIXI DXA[a] Fat (%) | y =0.1047x + 7.4031 | 9.3 ± 0.19 | 15.2 ± 3.80 |
| Fat (g) vs. PIXI DXA[a] Fat (g) | y =1.0854x + 1.9505 | 10.8 ± 1.54 | 28.0 ± 8.37 |
| Ash (g) vs. PIXI DXA[a] BMC[c] (g) | y =9.4386x + 0.3972 | 4.2 ± 0.46 | 49.8 ± 11.51 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total Body Weight (TBW)
[c] Bone Mineral Composition (BMC)

were statistically similar. The original correlation coefficients for these comparisons were not similar. Correlation between pencil beam DXA percent fat and proximate percent carcass fat was not statistically significant ($p < 0.05$), while the correlation between pencil beam DXA fat in grams and proximate carcass fat in grams was highly significant ($p < 0.001$).

Table 2.8. Modified regression equations, corresponding mean ± SEM proximate value determined by the equation, and mean ± SEM percent difference from actual proximate values for comparisons of proximate analysis to high resolution pencil beam DXA[a] values in juvenile hybrid striped bass.

| Comparison | Modified Regression Equation | Determined Mean Proximate Value | Mean % Difference From Actual Value |
|---|---|---|---|
| | Pencil Beam DXA[a] Scans | | |
| Moisture (%) vs. Pencil Beam DXA[a] Lean (%) | y =1.1898x - 45.568 | 68.6 ± 0.06 | 2.3 ± 0.69 |
| Water (g) vs. Pencil Beam DXA[a] Lean (g) | y =0.5885x + 5.6975 | 78.3 ± 7.77 | 5.8 ± 1.82 |
| Protein (% TBW[b]) vs. Pencil Beam DXA[a] Lean(%) | y = -0.6785x + 82.521 | 17.4 ± 0.04 | 11.8 ± 4.11 |
| Protein (g) vs. Pencil Beam DXA[a] Lean (g) | y =0.1868x - 2.7424 | 20.3 ± 2.47 | 13.5 ± 2.47 |
| Weight (g) vs. Pencil Beam DXA[a] Tissue (g) | y =0.8778x + 1.7665 | 114.7 ± 12.12 | 5.3 ± 0.83 |
| Fat (% TBW[b]) vs. Pencil Beam DXA[a] R-value | y = -79.561x + 121.15 | 9.1 ± 0.35 | 14.0 ± 3.41 |
| Fat (% TBW[b]) vs. Pencil Beam DXA[a] Fat (%) | y =0.7321x + 6.4243 | 9.4 ± 0.04 | 16.0 ± 3.55 |
| Fat (g) vs. Pencil Beam DXA[a] Fat (g) | y =1.9903x + 0.6906 | 11.2 ± 1.21 | 16.4 ± 3.39 |
| Ash (g) vs. Pencil Beam DXA[a] BMC[c] (g) | y =2.7092x + 0.7345 | 3.7 ± 0.40 | 35.4 ± 9.04 |

[a] Dual energy x-ray absorptiometry (DXA)
[b] Total Body Weight (TBW)
[c] Bone Mineral Composition (BMC)

*DXA Sensitivity Analysis*

Significant differences between groups of fish is primarily of interest to determine whether DXA was able to detect incremental changes in composition. To check this, fish were separated according to their dietary treatment and sampling period. DXA estimations of the various components of body composition were averaged per treatment and sampling, and compared to determine any significant differences among them. These differences between treatments as determined by DXA were compared to differences determined by proximate analysis.

*Lunar PIXImus DXA Analysis-Experiment 2*A

Baseline data was separated according to the projected dietary treatments. These fish were sampled before the tanks were fed the experimental diets, so the division of data is primarily for statistical comparison to later samplings and proximate values. From each tank, two fish were randomly selected to be sampled and were euthanized. After individuals were weighed and measured, they were individually bagged and stored frozen until scanning. After scanning, proximate analysis was performed on each fish. The proximate values were back-calculated to give an indication of wet, whole body composition. Mean percent body fat values determined by DXA were not statistically different ($p > 0.05$) for the baseline sampling group of fish. As with mean percent fat values, mean percent lean values were also not statistically different between dietary treatments. Mean total grams of fat ranged from 4.32g to 6.77g for these fish, with no statistical differences between treatment means ($p > 0.05$). The fish from the baseline sampling had mean total tissue values from 23.25g to 36.2g, and mean lean tissue amounts ranging from 18.92g to 29.43g. There were statistical differences ($p < 0.05$) in

the amount of total tissue as determined by the PIXI data. Fish from dietary treatment 2 and dietary treatment 3 had statistically higher total tissue values than did fish from dietary treatment 1 or dietary treatment 4 ($p < 0.05$). This pattern continued with the mean lean tissue amounts as determined by the PIXI DXA. The means for dietary treatments 2 and 3 were not statistically different from one another, but were statistically higher from the means for dietary treatments 1 and 4. The fish also had mean bone mineral composition values in a range of 0.18g to 0.28g. In this case, the mean BMC value for fish fed dietary treatment 2 was statistically higher than those fed dietary treatments 1 and 4, and fish fed dietary treatment 3 had significantly higher BMC values than those fish fed dietary treatment 1 ($p < 0.05$). The only statistical difference between baseline fish among their projected dietary treatments according to proximate analyses was in their total body weight. Fish intended for dietary treatment 2 started the experimental feeding regime with the highest overall body weight when compared to all other fish ($p < 0.05$). The fact that these fish started he experiment at a higher body weight allows for clarification of final growth results and dietary response, depending on their overall growth throughout the experiment. PIXI DXA did determine that dietary treatments 2 and 3 were statistically higher in total tissue than treatments 1 and 4 ($p < 0.05$). The fact that PIXI DXA did not determine that baseline fish from dietary treatment 2 were not the highest of all treatments may have been due to the limited scan area of the PIXI DXA. The entire fish was not analyzed, as it was for proximate analysis.

After ten weeks on dietary treatments, PIXI DXA detected differences in body composition of the fish sampled. Mean percent body fat values for fish fed dietary treatment 1(21.7%) were statistically higher than for fish fed dietary treatment 3(17.4%)

($p<0.05$) (Table 2.9). These differences in treatment means correspond to differences detected by proximate analysis (Table 2.2). According to proximate analyses, fish fed dietary treatment 1 had statistically higher percent carcass fat values than fish fed dietary treatment 3, but the proximate analyses for % fat were also different between fish fed treatment 1 and 4 ($p< 0.05$). The PIXI DXA was unable to detect the latter differences between treatments. The PIXI DXA mean percent lean tissue values were 79.91% for dietary treatment 2 and 82.53% for dietary treatment 3, which was the only statistical difference between treatment means ($p< 0.05$) for percent lean tissue. Therefore, according to PIXI DXA an energy: protein ratio of 7.9 appeared to maximize lean tissue growth in hybrid striped bass. Percent protein values according to proximate analyses did not indicate any significant differences between treatment means ($p> 0.05$). Total tissue and mean lean tissue weights were statistically lower for treatment 3 ($p< 0.05$) as compared to other dietary treatments. No differences were detected between treatment means for grams of protein or carcass weight as determined by proximate analyses ($p> 0.05$). There were no statistical differences in PIXI DXA BMC between any of these treatment means; however, proximate grams of ash were statistically higher for fish fed dietary treatment 2 than any other dietary treatment ($p< 0.05$). For some components of body composition, PIXI DXA did not seem to be as effective in determining differences between the dietary treatments as proximate analyses. This may be due to the limited scan area of PIXI DXA.

*Lunar Pencil Beam DXA Analysis-Experiment 2B*

When individual baseline fish from each sampling period were scanned in the lateral position, in the cranial-caudal direction, by the Lunar model #7618 pencil beam

Table 2.9. Mean ± SEM PIXI DXA[a] scan results for juvenile hybrid striped bass from the baseline sampling and after 10 weeks on dietary treatments. (Experiment 2A)

| | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|
| | | Baseline Sampling | | |
| Tissue (g) | $24.30^{c} \pm 2.003$ | $36.20^{d} \pm 7.056$ | $33.87^{d} \pm 4.517$ | $23.25^{c} \pm 2.517$ |
| Lean (%) | $80.63 \pm 2.625$ | $82.67 \pm 3.130$ | $82.78 \pm 1.148$ | $81.83 \pm 2.774$ |
| Lean (g) | $19.4^{c} \pm 1.34$ | $29.4^{d} \pm 5.13$ | $27.9^{d} \pm 3.68$ | $18.9^{c} \pm 1.99$ |
| Fat (%) | $19.37 \pm 2.625$ | $17.33 \pm 3.130$ | $17.22 \pm 1.148$ | $18.17 \pm 2.774$ |
| Fat (g) | $4.87 \pm 0.999$ | $6.77 \pm 2.459$ | $5.95 \pm 0.953$ | $4.32 \pm 0.789$ |
| BMC[b] (g) | $0.18^{ce} \pm 0.014$ | $0.28^{d} \pm 0.047$ | $0.24^{de} \pm 0.036$ | $0.20^{e} \pm 0.012$ |
| | | 10 Week Sampling | | |
| Tissue (g) | $13.94 \pm 2.087$ | $14.77 \pm 1.587$ | $11.08 \pm 1.212$ | $14.03 \pm 1.612$ |
| Lean (%) | $63.2^{cd} \pm 4.79$ | $73.0^{c} \pm 5.59$ | $63.7^{d} \pm 2.88$ | $66.7^{cd} \pm 2.90$ |
| Lean (g) | $49.2^{c} \pm 3.39$ | $58.3^{d} \pm 4.34$ | $52.6^{cd} \pm 2.72$ | $52.6^{cd} \pm 1.90$ |
| Fat (%) | $21.7^{c} \pm 1.75$ | $20.1^{cd} \pm 1.09$ | $17.5^{d} \pm 1.93$ | $20.7^{cd} \pm 1.86$ |
| Fat (g) | $78.3^{c} \pm 1.75$ | $79.9^{cd} \pm 1.09$ | $82.5^{d} \pm 1.93$ | $79.3^{cd} \pm 1.86$ |
| BMC[b] (g) | $0.52 \pm 0.043$ | $0.56 \pm 0.043$ | $0.59 \pm 0.046$ | $0.57 \pm 0.031$ |

[a] Dual Energy X-Ray Absorptiometry (DXA)
[b] Bone Mineral Composition (BMC)
[cde] Means within rows with different letters are significantly different at $p<0.05$

dual energy x-ray absorptiometry instrument, there were no statistical differences between treatment means for any of the components of body composition ($p< 0.05$). Aside from fish whole body weights, this corresponds with proximate analysis determinations that showed there were no differences between baseline fish. Scans of fish fed the dietary treatments (Table 2.10) once again presented as 4.0% body fat for the baseline fish.

The limitation in determining percent body fat was also observed after ten weeks on dietary treatments (Table 2.10). There were no statistical differences among treatment means ($p< 0.05$) for most components of body composition as determined by

Table 2.10. Mean ± SEM Pencil Beam DXA[a] scan values for fish from the baseline sampling and after 10 weeks on dietary treatments. (Experiment 2B)

| | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|
| | | Baseline Sampling | | |
| R-Value | 1.416 ± 0.007 | 1.423 ± 0.017 | 1.414 ± 0.004 | 1.422 ± 0.005 |
| Tissue (g) | 70.9 ± 11.07 | 92.2 ± 31.26 | 90.5 ± 22.95 | 69.1 ± 10.70 |
| % Fat | 4.0 ± 0 | 4.0 ± 0 | 4.0 ± 0 | 4.0 ± 0 |
| Fat (g) | 2.9 ± 0.45 | 3.7 ± 1.23 | 3.6 ± 0.94 | 2.8 ± 0.43 |
| % Lean | 96.0 ± 0 | 96.0 ± 0 | 96.0 ± 0 | 96.0 ± 0 |
| Lean (g) | 68.0 ± 10.64 | 88.5 ± 29.98 | 86.9 ± 22.05 | 66.4 ± 10.28 |
| BMC[b] (g) | 0.364 ± 0.095 | 0.824 ± 0.480 | 0.496 ± 0.170 | 0.375 ± 0.040 |
| | | 10 Week Sampling | | |
| R-Value | 1.389[c] ± 0.005 | 1.393[cd] ± 0.008 | 1.399[d] ± 0.004 | 1.401[d] ± 0.003 |
| Tissue (g) | 187.1 ± 40.71 | 209.7 ± 50.09 | 189.3 ± 36.06 | 194.3 ± 24.46 |
| % Fat | 4.4 ± 0.41 | 4.4 ± 0.76 | 4.0 ± 0 | 4.0 ± 0 |
| Fat (g) | 8.2 ± 2.24 | 9.6 ± 4.04 | 7.6 ± 1.42 | 7.8 ± 0.98 |
| % Lean | 95.6 ± 0.41 | 95.6 ± 0.76 | 96.0 ± 0 | 96.0 ± 0 |
| Lean (g) | 178.9 ± 38.62 | 200.1 ± 48.22 | 181.7 ± 23.48 | 186.5 ± 34.60 |
| BMC[b] (g) | 1.654 ± 0.514 | 2.058 ± 0.536 | 1.829 ± 0.386 | 1.901 ± 0.367 |

[a] Dual Energy X-Ray Absorptiometry (DXA)
[b] Bone Mineral Composition (BMC)
[cd] Means within rows with different letters are significantly different at $p<0.05$

the pencil beam DXA. The only component of body composition as determined by the pencil beam DXA scans that had statistically different treatment means was the DXA R-value attenuation coefficient. The mean DXA R-value for dietary treatment 1 was statistically different than the mean for dietary treatment 3 and dietary treatment 4 ($p< 0.05$), but was not statistically different than the mean DXA R-value of dietary treatment 2. This directly corresponds to results from proximate analyses. Ether extract results showed statistical differences between fish fed dietary treatment 1 and those fed dietary treatments 3 and 4. The remaining estimates of body composition were all statistically similar ($p< 0.05$) according to pencil beam DXA. No other differences detected by proximate analyses were detected by these DXA scans.

## Discussion

Many studies have been conducted to determine the effects various levels of protein and energy in the diet have on body composition of fish. Webster et al. (1995) concluded from their work with sunshine bass that the ideal energy: protein ratio was 10.1, or 99 mg protein/kcal, when fish meal comprises 56% of this dietary protein. Webster et al. (1995) found that weight gain of sunshine bass was highest when fish were fed diets containing between 99 and 116 mg protein/kcal (energy: protein ratios from 10.1 to 8.6). Fish fed diets containing 116 mg protein/kcal had a higher percentage dressed carcass, a lower percentage of abdominal lipid, a higher percentage of body protein, and a lower percentage of body lipid than other fish in the study. The fish fed a diet with a protein: energy ratio lower than 116 mg protein/kcal tended to increase lipid deposition and decrease protein levels in these sunshine bass, but according to results from Webster et al. (1995) growth of fish fed diets containing 99 and 116 mg protein/kcal was similar. Nematipour et al. (1992) also reported that optimal growth of sunshine bass was achieved with diets containing between 110 and 167 mg protein/kcal (energy: protein ratios from 9.09 to 5.99).

Since fish can meet part of their energy requirement from dietary protein, the protein to energy ratio, or vice versa, is highly influential on the efficiency of protein and energy utilization (Webster et al., 1995). The level of digestible energy in a diet affects the amount of food consumed by fish and the energy: protein ratio of the diet will influence the feed conversion efficiency (Reis et al., 1989).

According to the experimental results in our study, sunshine bass grew best when fed a diet with an energy: protein ratio of 7.1. The FCR values were also consistently the

most efficient after ten weeks on dietary treatments for this group. Weight gains were also the highest for these fish, and average lengths were also higher than the highest energy: protein ratio (9.6). The only difference in components of body composition determined by proximate analysis between dietary treatment means was between percent carcass fat and grams of ash. Despite their increased growth, fish fed dietary treatment 4 (energy: protein ratio 7.1) had significantly lower ($p < 0.05$) percent carcass fat than fish fed dietary treatment 1 (energy: protein ratio 9.6). Therefore, fish fed dietary treatment 4 had increased growth not simply due to excess fat deposition, and that in this type of rearing situation an energy: protein ration of 7.1 is ideal for growth.

The size of these hybrid striped bass allowed, both Lunar dual energy x-ray absorptiometry technologies to be utilized to determine the various carcass body composition components. The PIXImus technology was primarily designed to scan smaller subjects. The larger Lunar DXA scanner was originally intended for use with large subjects. Our hybrid striped bass were longer than the set scan window for the PIXI DXA, but the both types of scans could include the abdominal areas of all fish.

Few studies have been conducted utilizing small animals, especially fish. Many DXA studies with smaller animals involve rats (Bertin et al., 1998; Rose et al., 1998) or mice (Sjögren et al., 2001) as the scan subject. Similar to results in Rose et al. (1998), good correlations were able to be calculated between standard proximate analyses and lean. However, Rose et al. (1998) was also able to calculate high correlations with percent carcass fat values. This was not the case for these experiments. However, an inability of DXA to determine amounts of carcass fat was also found in studies with mice done by Sjögren et al. (2001) and with rats in a study by Bertin et al. (1998). Sjögren et

al. (2001) developed a modified technique to estimate body fat in mice based on the DXA/image procedure of their DXA technology combined with an image analysis. The values estimated by the DXA software did not correlate with values from either the amount of adipose tissue dissected from the mice or their serum leptin levels. To account for this, Sjögren et al. (2001) choose a setting in which tissue containing >50% fat appeared as white on the scan image. This was regarded as the fat area in the mice, and a percentage fat area was then calculated. From this modification, Sjögren et al. (2001) found a high correlation between the percentage of fat area in DXA and adipose tissue ($r = 0.88$) and a good correlation between serum leptin levels and DXA percentage of fat area ($r = 0.84$). This type of modification in technique could not be done in this experiment to try to overcome the poor percent fat estimations because of the differences in imaging of the technology. Sjögren et al. (2001) used the Norland pDEXA Sabre and the Sabre Research software (Version 3.9.2), which allowed for changes in settings to employ this modified technique. Neither Lunar DXA scanner and its corresponding software allowed for these types of changes.

Bertin et al. (1998) also found significant differences in fat mass measurements obtained with DXA when they were compared to fat mass determined by chemical extraction. Residual variance was also only 5%, so Bertin et al. (1998) were able to adjust the DXA data to the chemical extraction data using a mean correction factor of 0.75. This adjustment allowed for linear regression analysis of fat mass percentage from chemical analysis to percent fat mass determined by DXA. The correlation between these values was highly significant ($p< 0.0001$) at $r^2 = 0.95$. In our experiment, the y-intercept for the PIXI DXA comparisons was significantly different from zero so a

modification like this was not performed. Also, the pencil beam DXA estimates produced a majority of percent fat values of 4.0%. This poor sensitivity did not allow for a similar modification.

Differences in the abilities of DXA in our study compared to others could have been due to the difference of the subjects—hybrid striped bass as compared to a small mammal. DXA algorithms in the instrument software were originally formulated based on mammalian data, so the calculations may not be as effective for estimating body composition in a non-mammalian species. It could have also been due to the fact that in either of these experiments, DXA machines made by different manufacturers were used. Various manufacturers utilize different types of computer programs and imaging methods. Some also develop more or less scan modes according to size and/or weight. Others even modify the x-ray beam itself so that it is not focused in a pencil beam shape, but is rather a fan beam that sweeps over a greater area during the scanning.

In conclusion, the DXA scan results were better correlated to proximate analysis results when they were produced by the Lunar PIXImus DXA technology. While the pencil beam and PIXImus DXA scanners were similarly successful for determining various components of body composition, the PIXImus DXA produced one more significant ($p < 0.05$) correlation to proximate values. From the scan values, PIXImus is sensitive to changes in mass in fish of this size. The major downfall with the pencil beam DXA scans was that all baseline fish were determined to have similarly low percent body fat values of 4.0%. While the PIXI DXA did not have such a problem, it was still very inaccurate for determining percent body fat in these fish. The PIXI DXA is slightly faster, on average per individual, than the pencil beam DXA so may be more desirable in

more time sensitive situations. The PIXI DXA was also able to detect more of the differences in treatment means between dietary treatments that proximate analysis detected, so may be more effective in detecting incremental changes in body composition due to dietary treatments fed to hybrid striped bass of this size. However, according to the PIXI DXA estimations of body composition the diet with an energy: protein ratio of 7.1 did not necessarily improve fish body composition. From the Lunar pencil beam scanning data, fish fed dietary treatments 3 and 4 had the highest R-values and therefore the lowest amounts of carcass fat. This is the only indication from pencil beam DXA that these diets were more beneficial for hybrid striped bass this size. The PIXI DXA is limited in its size of scan area, so the size of a fish would also need to be taken into consideration.

Both of these DXA technologies present limitations for use in the aquaculture industry. They are both expensive pieces of equipment for producers to individually purchase. They then require training to use, and room to keep them. As of now, they have only been validated for euthanized fish so a producer would still have to sacrifice part of their fish population, and in turn profit, in order to determine the status of a growing population. Extensive research would need to be done to eventually utilize this technology on a live fish, due to their physiological requirements for circulation, oxygen supply, and waste removal.

# General Discussion and Conclusions

# GENERAL DISCUSSION AND CONCLUSIONS

Aquaculture has been the world's fastest growing food production industry over the past decade and accounts for more than a quarter of the world's total fish production (FAO, 1998). Striped bass (*Morone saxatilis*) and its hybrids are in intense production throughout the United States, as well as in Europe and Asia. Standard hybrids of striped bass are with other species of the *Morone* genus, particularly the white bass (*Morone chrysops*). Their production has grown an estimated 1400% between the years 1985 and 1995, and continues to increase in accordance to market demands (Harrell and Webster, 1997). Hybrid striped bass accounted for 11% of Maryland's food fish production in the year 2000, and are the second largest group of fish produced in Maryland in terms of volume (MDA, 2000).

Body composition is an important indicator of nutritional status and health, and nutritional assessment of an individual is enhanced by the accurate measurement of various components of body composition (Economos et al., 1997). Dual energy x-ray absorptiometry (DXA) is a method that is being used increasingly for the non-invasive assessment of body composition in certain species, humans and swine in particular (Rose et al., 1998). Currently, there is no available literature that explores the use of dual energy x-ray absorptiometry (DXA) as a means to non-invasively determine body composition in any teleost species.

Species with which DXA technology has been utilized include, but are not necessarily limited to, live pigs (Svendsen et al., 1993; Brunton et al., 1993; Pintauro et al., 1996; Mitchell et al., 1996a, 1996b, 1998a,b; Mitchell and Scholz, 1998), pig carcasses (Mitchell et al., 1998c), chickens (Mitchell et al., 1997a), beef carcass rib

sections (Mitchell et al., 1997b), rats (Rose et al., 1998), mice (Sjögren et al., 2001), dogs (Lauten et al., 2001), and cats (Lauten et al., 2000). The accuracy and precision of DXA have been well validated both in vivo and in vitro in humans, pigs from 5 to 100 kg, and sheep. Pouilles et al. (2000) performed in vivo and ex vivo lumbar spine studies in sheep, using an absorptiometer, and then correlated this data with results of ashing the dissected bone ($r = 0.98$). The high correlation coefficient indicates accuracy of the absorptiometry technique. Rose et al. (1998) compared DXA measurements to chemical analysis for body fat, lean body mass, and BMC in lean and obese female rats, as well as developed regression equations in order to relate these two methodologies. In these experiments, although it was found that DXA tended to overestimate absolute values for percent fat, good agreement between DXA and chemical analysis was concluded with correlation coefficients confirming this finding. The regression equations that were developed and used to estimate chemical values from DXA data indicate that DXA can be used as a tool to predict chemical analysis values. Also, the ability of DXA to detect differences due to dietary treatment similar to the ability of chemical analysis indicates that DXA may also be an appropriate method for assessing body composition in such studies. Unfortunately, DXA for these experiments had the opposite tendency in estimating fat—DXA was not accurate in fat estimations and severely underestimated all fat readings. This may have been due to the fact that Rose et al. (1998) used a different type of DXA machinery—the Hologic QDR 100W. Even though a Hologic instrument was used for a portion of this study, human software was used for the fish whereas Rat Whole Body V5.71P software was used in the study by Rose et al. (1998). The animal specific software was developed using rats, so the body composition estimates in the

study by Rose et al. (1998) would be more in tune to their actual scan subjects than scanning a fish with human software. If fish had been scanned with fish, or even small animal software, the algorithms may have been able to more accurately estimate body composition.

DXA has been evaluated against the chemical analysis of whole carcasses that have been homogenized (Lander Svendsen et al. 1993). It has been concluded that there are at least three flaws with this technique: 1) a truly representative sample from the homogenate of the carcass may be difficult to obtain (Ellis et al. 1994, Lander Svendsen et al. 1993); 2) the ash obtained from the subsequent analysis of the homogenate sample contains both bone and non-bone mineral components (Ellis et al. 1994, Lander Svendsen et al. 1993, Heymsfield et al. 1989a); and 3) whole carcasses may also include bone marrow fat. This last concern is not applicable to fish carcass homogenates, as fish utilize their kidney tissue as a homopoetic organ and do not have a marrow component to their bones. When evaluating DXA, it may be more accurate to do a carcass dissection that was then followed by a homogenization of the separate tissues. Then, the mineral mass should be analyzed in the skeleton and the fat mass could be analyzed in only the soft tissue, if applicable (Elowsson et al. 1998). This dissection and analysis may lead to more accurate body composition estimates, which would result in more accurate regression equations. These could later be applied to better estimate body composition in whole carcasses.

When scanning fish carcasses with the Lunar model #7618 DXA machinery, some logistical problems did arise. To account for the fish's individual weight and overall size, the carcasses were scanned using the technology's small animal scan mode.

There are several options within this scan mode, and again it was decided according to the fish size, to scan each fish in both the detailed medium (DM) and high resolution (HRM) scan modes. There are a few major differences between these modes, including the overall time it takes to scan a single fish. The detailed medium mode completed a fish scan in an average of ten minutes, while a high resolution fish took almost 60 minutes. This time difference is due to the number of scan lines the machinery makes while scanning a carcass in each mode. Detailed medium mode scans over a carcass, and the pencil beam scans in lines that are not as close together; therefore, there are not as many total lines when compared to the high resolution mode. The sample size in the DM mode is 1.2 x 2.4 mm and in the HRM mode is 0.6 x 1.2 mm. This results in more individual pixels, tissue points, and an overall clearer image for the high resolution scan of a fish. Based on correlation to proximate analysis, the increased time was not justified. DM scan estimates resulted in the same significant correlations as HRM scan estimates when compared to proximate analysis values.

The DPX-L algorithms for determining body composition assume a minimum of 4.0% body fat in a viable carcass. For DM scans all fish were estimated to have 4.0% body fat, and HRM scans produced very little variability in this estimation. Similar to this study, difficulties were encountered in determining percent carcass fat in other studies, including DXA scans of chickens weighing less than 2000g (Mitchell et al., 1997) and transgenic mice (Sjögren et al., 2001). This reinforces the need for refinement of the DXA technique or algorithms for accurate determination of percent fat in smaller scan subjects.

Most other correlations were highly significant ($p < 0.0001$) in either scan mode. Similar correlations were found in other published studies, such as those comparing the DXA lean mass of chickens to proximate total body water ($r = 0.93$) and protein ($r = 0.90$) (Mitchell et al., 1997). Mitchell et al. (1996b) found that DXA estimations for total fat mass were not significantly different ($p > 0.05$) from direct or chemical measurements of growing pigs with a starting weight of 26.3kg. Bertin et al. (1998) also found excellent accuracy ($p < 0.0001$) for DXA in reference to chemical extraction ($r = 0.95$) for fat mass in rats weighing over 200g.

Due to such little variability in the percent fat readings for whole carcasses produced by DXA in either scan mode, an additional type of computer analysis was performed. Using the DXA computer software, it is possible to highlight a particular region on interest and analyze just that area for all of the calculated DXA values for body composition. Fish deposit the vast majority of their fat tissue in their abdominal cavity, instead of throughout the body like many other animals. DXA takes into account all detection pixels when calculating an overall percent body fat reading, which may play a part in the low variation among these readings on a whole body scan. For the manual sectional analysis, a region of interest was defined for all fish and each scan image was analyzed for this region's particular composition. The region was designed to include the entire abdominal cavity.

Regional analyses did result in percent fat values that were different than those determined for the whole body scans. This caused estimated percent lean values to also be more variable than were determined for the whole body scans of the same fish. With the increased estimation of body fat via regional analysis, the correlations when

compared to corresponding proximate analysis values were now significant at $p < 0.0001$, as compared to no correlation in the whole body DM scans and an $r^2$ value of 0.004 for the whole body HRM scans. When a percent carcass fat reading is desired, regional analysis of the scan image provides a better estimation in fish of this size.

Stacking was done to determine if the tendency of DXA to produce readings of 4.0% fat was, in fact, due the thickness of the fish carcass. Despite stacking, many of the scans in detailed medium mode produced overall percent fat values of 4.0%. There was slight variation in the percent fat reading, so correlation coefficients and regression equations could be developed. However, none of these were statistically significant relationships. Despite the result that none of the percent fat values in high resolution scan mode were determined to be 4.0%, a significant correlation could not be developed. This may have been due, in part, to the fact that due to stacking there was a much smaller sample size to utilize. The small amount of samples may have led to poorer correlations due to decreased data points with which regression equations could be developed. To further investigate the usefulness of stacking fish carcasses for scanning, it would be useful to have a much larger available sample pool to work from. No published reports could be found in which subjects were stacked and then scanned with DXA technologies.

When the Hologic DXA scanner which has a beam that emits as a fan from the x-ray source and covers a much wider area of the scan subject at once was used, there were very few significant correlations developed from this scan data when compared to proximate analysis. Koo et al. (2002) used fan beam DXA to scan piglets with weights between 1.95 and 21.1kg, and compared scan data to scale weights, carcass ash and calcium, and chemical lean and fat contents. Scans in this study and the current study

were done using the same type of DXA absorptiometer. Significant correlations ($p < 0.001$) were calculated for DXA total body weight compared to scale weight ($r = 0.999$), bone mineral content compared to carcass ash ($r = 0.937$), and DXA lean and fat mass compared to chemical lean ($r = 0.994$) and fat ($r = 0.994$) contents. The weights of the pigs were much higher than the fish scanned in our experiment, suggesting that as the size of an animal decreases with this type of DXA technology, so does its ability to accurately determine some aspects of body composition. However, the study by Koo et al. (2002) did not find any relation between DXA's ability to estimate body composition and size of the pig.

To further investigate the fan beam DXA technology, the values produced via fan beam scans were compared to the corresponding pencil beam scan values in detailed medium and high resolution scan modes. Only a few comparisons for both scan modes produced significant correlation coefficients ($p<0.05$) and corresponding regression equations. When comparing the body composition values to those determined by fan beam DXA technology to those determined by pencil beam DXA, there was no difference in correlations between pencil beam scan modes—DM or HRM.

In conclusion, pencil beam DXA technology was a useful tool for non-invasively determining some parameters of body composition in hybrid striped bass. The inability of DXA to determine specific parameters of body composition in fish may have more to do with the limitations of the computer software than the technology itself. Manual analysis by identifying specific regions of interest helped overcome the analysis problems for fat to some degree, so depending on carcass size, this may be the most viable analysis option until more technological changes are made. Important components of body

composition for a producer were estimated well by the DXA, such as body protein or lean tissue.

Despite its speed, fan beam DXA technology does not appear to be as effective as pencil beam DXA technology for determining body composition in hybrid striped bass of this size.

In terms of mode comparisons for the pencil beam DXA technology, there were few differences in correlation coefficients. High resolution scans did estimate percent carcass fat so that a significant correlation could be calculated ($p<0.05$). They also produce a clearer scan image due to the higher number of lines included in a scan image. However, in the simple interest of time the detailed medium scan would be more beneficial as it only takes approximately ten minutes per fish while a high resolution scan can take closer to an hour for an individual. The DXA r-value for DM scan mode significantly correlates to carcass fat as determined by proximate analysis, and can be used as the percent fat indication instead of the specific DXA estimation for percent fat.

If DXA were to ever be used as an industry tool to determine the body composition of fish at different points in growth, and eventually utilize these scans on live animals, the detailed medium small animal scan mode would be the better choice. Its speed would allow for the potential of a fish to survive out of water, under proper conditions, much better than the HRM scan speed. Also, in terms of accuracy, the scan modes are quite similar. This is especially true for grams of lean in the animal, which would be a key interest of a producer raising food fish.

Due to the size of the juvenile hybrid striped bass used, both Lunar dual energy x-ray absorptiometry technologies were able to determine the various carcass body

composition parameters. Few studies have been conducted utilizing small animals such as this. Many DXA studies with smaller animals involve rats (Rose et al., 1998) or mice (Sjögren et al., 2001) as the scan subject, with success but an observed overestimation of amounts of fat or soft tissues by DXA when values were compared to those determined by proximate or chemical analysis.

The PIXImus Lunar DXA scanner functions in a slightly different manner than the Lunar model DPX-L ID #7618 pencil beam scanner. Instead of scanning an individual line by line with a beam of two x-ray energies, it exposes a designated scan area to a continuous amount of the x-rays in two different energies (38 and 70 keV)—more comparable to a photograph than a traditional scan. The x-ray source from the PIXImus machinery does not move, so the scan area is also fixed in size and locality. As a result, only a portion of the fish carcass could be scanned. Due to the variable sizes of these fish, the amount of the fish that was able to be scanned was also variable. To ensure consistency among the scans for all samplings, an area was defined that was available to analyze by computer in all scan files. The same area of interest was utilized for all scans performed on the PIXImus DXA machinery.

The high correlations between proximate data and PIXI DXA scan data mirror other studies comparing proximate analyses to DXA data in other small animals. Rose et al. (1998) used DXA to scan rats weighing 200-600g, and then compared scans to chemical body composition analysis. High correlations for percent fat ($r = 0.99$), percent protein ($r = 0.96$), and percent BMC ($r = 0.81$) were found between the two methods. These results indicate DXA could be used to estimate body composition in rats of this size. Koo et al. (2002) used piglets to compare DXA scan results to proximate analyses.

Significant correlations ($p < 0.001$) were calculated for DXA total body weight compared to scale weight ($r = 0.999$), bone mineral content compared to carcass ash ($r = 0.937$), and DXA lean and fat mass compared to chemical lean ($r = 0.994$) and fat ($r = 0.994$) contents. Again, these results indicate the ability of DXA to estimate body composition in smaller animals. However, these experiments were done using Hologic DXA scanners. This may explain why these results have higher correlations than the current experiment. Different software and technologies were utilized, possibly improving results for these animals.

Fish were individually analyzed with the Lunar pencil beam DXA technology after being scanned in batches using high resolution small animal scan mode.

While some studies have shown DXA's ability to estimate aspects of body composition, there are studies that bring to light the limited applications of the technology. Sjögren et al. (2001) used DXA to quantify the amount of fat in transgenic mice. Similar to our studies, they were unable to obtain any correlation between the amount of fat given by the software and the amount of adipose tissue later dissected from the animal. Grams of fat were able to be correlated with proximate analyses here, and for the mice a modified technique using the DXA image itself was used to determine adipose tissue (Sjögren et al., 2001).

In addition to each type of scan being compared to the results from proximate analysis, the two types of scans were also compared to one another. The DXA scan results were better correlated to proximate analyses when they were produced by the pencil beam Lunar DXA technology. This was despite the hybrid striped bass' smaller size as compared to more traditional subjects scanned with this technology. While the

PIXImus DXA was successful for determining various components of body composition, overall the pencil beam DXA produced better results. This conclusion is based on the fact that the correlation coefficients created through comparison to proximate values tended to be higher, with many being significant at a higher probability level. Either technology is recommendable to determine body composition in hybrid striped bass of this size. The PIXI DXA is slightly faster, on average per individual, than the pencil beam DXA so may be more desirable to those involved in the aquaculture industry. Webster et al. (1995) found that juvenile hybrid striped bass fed diets with an energy: protein ratio of 8.6 had a higher percentage of protein and lower percentage of abdominal fat. In the experiment reported here, diet 2 was formulated to have this same energy: protein ratio, but the body composition results were different. Diets 3 and 4, with lower energy: protein ratios—7.9 and 7.1 respectively, resulted in fish with lower carcass fat values. However, according to PIXI DXA scans the dietary treatment with an energy: protein ratio of 8.6 produced fish with the highest amounts of percent and grams of lean tissue ($p < 0.05$). This is similar to the results from the study by Webster et al. (1995). In the case of mean PIXI DXA BMC values, a diet with an energy: protein ratio of 7.9 was statistically lower than the mean for diets with energy: protein ratio of 8.6 and 7.1 ($p < 0.05$). According to proximate analyses, fish fed a diet with an energy: protein ratio of 8.6 had higher amounts of carcass ash than fish fed any other treatment. These differences in treatment means for the ten-week sampling show that, according to the PIXI DXA analysis, dietary treatments cased significant changes in body composition.

*Conclusion*

The effect of treatment on body composition parameters is, in most cases, studied via chemical analysis of a laboratory animal—i.e., after the animal has been euthanized. This means of using carcass analysis to study body composition severely limits the design of a particular study. To determine such carcass data, longitudinal studies with more than one data collection point are not possible. The only way to get close to 'longitudinal data' would be with repeated samplings from a larger representative population (Deurenberg, 2001).

The development of a method to non-invasively determine body composition in an animal so that repeated measures could be taken over time would be highly advantageous to researchers and producers alike. Dual energy x-ray absorptiometry has been studied for such use in a number of animal species, both large and small. DXA is unique in that it allows an accurate determination of the body composition of live animals through the use of low-dose radiation and without subject discomfort. The extremely low doses of radiation exposure allow for serial scans and analyses over extended periods, with minimal risk to the subject being scanned (Lauten et al., 2001).

The purpose of these studies was to facilitate the adaptation of this new technology to the field of aquaculture research, and eventually industry. There have been no published studies employing this type of technology with finfish species, so these experiments also examined various types of DXA technologies and their abilities to determine body composition parameters in hybrid striped bass of various sizes and life stages.

The experimental data from these experiments suggest that DXA can be applied to aquaculture situations, and can be a useful technology for determining certain aspects of body composition. Although there were significant drawbacks, overall DXA could prove useful. According to this data, DXA R-values from the pencil beam analysis are the most reliable data values to distinguish fat accumulation in an individual fish carcass. This is probably due to the thickness of the fish carcass, and a fish's tendency to accumulate the majority of its body fat in the abdominal cavity. Regional analysis of scans proved helpful, and were able to determine better values for the percent fat readings as compared to proximate analysis data. The best solution to the percent fat, and in turn percent lean, problem may involve the creation of new algorithms for this machinery specific to fish. Total tissue mass and lean tissue amounts were able to correlate to proximate data well, and these parameters of composition are a major concern in fish nutrition and industry. Bone mineral composition is also a concern, but is simply not as important to a producer or for a fish being raised for food. Optimization of lean tissue while keeping a healthy population of fish on a reasonably priced diet is one of a food fish producer's primary concerns. The ability to determine the progress of a population of fish as it grows through longitudinal data acquisitions would be very useful in the field to allow a producer to make adjustments if needed.

In interest of time, detailed medium small animal scan mode is the more highly recommended type of scan for this technology. Hologic fan beam scans are slightly faster—approximately 3 minutes as compared to ten minutes—but Lunar pencil beam values are more reliable and create better correlations. For smaller fish, the PIXImus DXA is quicker than the pencil beam DXA. These two scan values were quite similar to

one another, producing similar correlations to proximate analysis values. When determining the body composition of juvenile hybrid striped bass either method could be quite useful. The decisive factor would probably be which, if either, technology was available to a researcher or producer. The portability of the PIXI DXA machinery may make that scan technology more beneficial to more producers if it could be transported among aquaculture and research facilities.

DXA technologies do present limitations for use in the aquaculture industry. They are both expensive pieces of equipment for producers to individually purchase. They then require training to use, and room to keep them. As of now, they have only been validated for euthanized fish so a producer would still have to sacrifice part of their fish population, and in turn profit, in order to determine the status of a growing population. Extensive research would need to be done to eventually utilize this technology on a live fish, due to their physiological requirements for circulation, oxygen supply, and waste removal.

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