2004

The Influence of Diet on Stable Carbon Isotope Composition in Otoliths of Juvenile Red Drum (Sciaenops Ocellatus)

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THE INFLUENCE OF DIET ON STABLE CARBON ISOTOPE COMPOSITION IN OTOLITHS OF JUVENILE RED DRUM (*SCIAENOPS OCELLATUS*)

By

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**A Thesis submitted to the
Department of Oceanography
in partial fulfillment of the
requirements for the degree of
Master of Science**

**Degree Awarded
Spring Semester, 2004**

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ACKNOWLEDGEMENTS

I am tremendously thankful for the guidance of my advisor, Jeff Chanton, who demonstrated utmost patience in coaxing me along through what often seemed like a snail's pace to complete this experiment and manuscript. He tolerated my mode of independence but kept me going when I strayed too far.

It would not have been possible to work on this study without the persuasion of Chris Koenig, who directed me to the Oceanography Department and my advisor. He also provided guidance on setting up this experiment and logistics of seeing it through.

Personnel at the Florida State University Marine Lab, under Dr. John Hitron, could not have been more helpful. The staff at the Lab, including Mark Daniels, Butch Perring, Dennis Tinsley, and Marlene Buller was extremely cooperative and assisted in feeding my specimens on the few days when I could not make the journey to the Lab. A warm appreciation is also deserved for Ms. Patricia Jackson who helped with much of the administrative logistics at the Lab.

I am indebted to the personnel at both of the fish hatcheries that provided fish for the several trials of this experiment. Eric Latimer and Earnie Olsen from the Progress Energy Crystal River Mariculture Center supplied the initial stock of juvenile red drum during what turned into the trial and error stage. Staff at the Florida Marine Research Institute's (FMRI) Stock Enhancement Research Facility (SERF), under Dr. Bill Hatfield, were most gracious in supplying numerous batches of redfish.

Field staff at the FMRI Apalachicola Field Lab, including Steve Rider, Troy Tuckey, Todd Brackin, Jeff Wren, Kevin Grant, Christa Pierini, Stephanie Fahrny, supplied much of the pinfish through their Fisheries Independent Monitoring sampling program. Several batches of pinfish were purchased from Captain Don Davis when the supply in the Bay was dwindling. During the coldest part of the year when most fish left

the Bay, the stored supply of pinfish depleted and had to be purchased from Raffields Fisheries in Port St. Joe, Florida. Folks that provided technical and field assistance during this experiment included Pete and Sandy Malcombsen, Lia Chasar, Jake Stalder, Terry Petrosky, Emily Fairbanks, Harmon Harden and Jim Prater.

And lastly, a sincere and heartfelt appreciation must be given to my wife, Della Parker-Hanson who had to endure the longevity and stress of this project. She provided much advice, prodding, and field and technical support especially during the numerous revisions of this manuscript. But most of all, the greatest virtue she supplied was extreme patience.

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ABSTRACT

To evaluate the influence of dietary carbon on fish otoliths, juvenile red drum (*Sciaenops ocellatus*) were raised for 6 and 9 months in tanks with flow-through ambient seawater and fed diets differing by 2.12‰ ($p < 0.001$) in carbon isotope composition ($\delta^{13}C$). Muscle tissue from the two treatment groups also differed by 2.12‰ ($p < 0.001$) at the end of the experiment and both were enriched by 1.51‰ in ^{13}C relative to the respective diets. Otolith $\delta^{13}C$ displayed similar isotopic distinction between the two groups and were enriched (~16-17‰) from their respective diets. The carbon isotope signatures of otoliths between the two treatment groups were significantly different ($p < 0.001$).

There was a detectable difference in red drum otolith weights between the diet groups. Regression analysis between otolith weight and carbon isotope composition was not correlated. Overall red drum growth and growth rates were plotted against otolith $\delta^{13}C$ and no strong relationships emerged. Since both treatment groups were subjected to the same environmental conditions, while carbon consumption was manipulated, it is postulated that sources of carbon through diet control overall otolith $\delta^{13}C$ in juvenile red drum. Results from this study indicate the potential of evaluating fish dietary patterns by analyzing carbon isotopic signatures recorded in otoliths.

INTRODUCTION

Stable isotope analysis is a valuable tool for investigation of ecological patterns (reviewed by Peterson and Fry, 1987; Lajtha and Michener, 1994). This approach can be implemented at scales that vary from the single species level to entire ecosystems. In utilizing a stable isotope approach for ecological studies, it is essential to understand the origination of the particular isotope and how isotopic signatures in natural systems are transferred between and within organisms. Additionally, one must determine any isotopic fractionations that may occur in association with these transfers.

Stable isotope research was pioneered in the 1950's in examination of the carbon isotopes of plant material (Lajtha and Marshall, 1994). Since that early work, the approach has been applied to a wide range of ecological problems. Seminal studies of marine ecosystems during the late 1970's examined carbon isotopic composition of salt marsh systems (Haines, 1976; Haines and Montague, 1979) and fauna of inshore bays and seagrass beds (Fry and Parker, 1979). These early studies focused on how carbon sources are incorporated into organisms and how carbon flows through ecosystems, particularly marine environments (Fry and Sherr, 1984; Rounick and Winterbourn, 1986; Michener and Schell, 1994).

Stable Isotopes Notation and Terminology

Atoms with the same atomic number but different atomic masses are referred to as isotopes. The number of protons for isotopes is fixed while the number of neutrons varies leading to different atomic masses (Atkins & Beran, 1990). Examples of the elements of "life" that fit this definition are C, N, O, S, and H. These five isotopes are widely studied to answer biogeochemical and ecological patterns (Peterson & Fry, 1987).

An isotope ratio mass spectrometer (IRMS) is used to measure stable isotope ratios by comparing the heavy/light element ratio in a sample relative to a known standard. Isotopic ratios are typically expressed using the "del" (δ) notation with the general equation:

$$\delta X\ (‰) = (R_{sample}/R_{standard} - 1) * 1000$$

where: X = sampled isotope ratio (e.g. $^{13}C/^{12}C$)

R_{sample} = isotope ratio of the sample

$R_{standard}$ = isotope ratio of the standard

The δ X (‰), is considered to be either enriched (more positive) or depleted (more negative) in the heavier isotope relative to a standard (Lajtha & Michener, 1994). The standard used for carbon isotopes is typically Pee Dee Belemnite (PDB) from a marine limestone fossil, *Belemnitella americana*.

Organic matter and organisms obtain their isotopic signatures ($\delta^{13}C$, $\delta^{14}N$, $\delta\ ^{18}O$, $\delta^{34}S$) from a variety of processes. For example, carbon isotopes are ultimately determined by the $\delta^{13}C$ of precursor CO_2, in addition to the photosynthetic pathway. Differences in photosynthetic pathway result from isotopic fractionation at specific steps (Lajtha & Marshall, 1994). Fractionation refers to the altering of heavy/light isotopic ratios (e.g. $^{13}C/^{12}C$) as a chemical reaction proceeds. Compounds containing a higher proportion of heavy isotopes are "enriched" and those with a surplus of light isotopes are termed "depleted." Knowledge of isotopic fractionation is fundamental for understanding how these ratios transfer through an ecosystem.

Natural Chemical Tracers

Stable isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$) have been used as chemical tracers to quantify the flow of organic matter through and within aquatic food webs and to evaluate trophic levels. For instance, Fry (1991) examined several freshwater ecosystems throughout the United States to demonstrate isotopic differences ($^{13}C/^{12}C$, $^{15}N/^{14}N$) between lakes and streams. Peterson and Howarth (1991) employed a multiple stable isotope approach ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$) to follow the flow of organic matter

through salt marshes in Georgia. Aquatic food webs in the Arctic were characterized by tracing carbon isotopes through various freshwater and marine ecosystems (Schell and Ziemann, 1989).

Though there remain uncertainties with the stable isotope approach, this type of analysis for food web ecology has distinct advantages over conventional methods, such gut content analysis, direct observation, and radiotracer techniques (Michener and Schell, 1994). These advantages include simpler collection techniques, automated and relatively inexpensive analysis, and the ability to examine numerous trophic levels simultaneously (Michener and Schell, 1994). In addition, carbon isotope ratios reflect long-term diet relative to the type of tissue examined and that tissue is a record of assimilated and incorporated material from the digested food source (Rounick and Winterbourn, 1986). Thus, an important consideration in food web ecology is how isotopic signatures are reflected within specific animal tissues and among organisms in an ecosystem.

Research investigating predator-prey relationships or feeding behaviors typically examines carbon isotope composition of animal tissues. Studies have demonstrated that organisms are enriched in ^{13}C relative to their respective diets. For instance, DeNiro and Epstein (1978) evaluated the carbon isotopic composition of the whole body from various types of animals raised in the laboratory and determined that animals were enriched by 1‰ on average relative to their diet. Their research also revealed differing relationships between the various types of tissue examined and the nature of the diet. Fry & Arnold (1982) studied isotopic turnover of brown shrimp fed diets differing in carbon isotopic signatures and showed that the shrimp achieved an isotopic resemblance within 1‰ of their respective diets as a function of body weight. Gearing (1991) reviewed several studies and compared the various tissues and organ carbon isotopic ratios concluding that soft body parts ranges are generally within 3‰ of each other.

Stable Isotopes in Fish Research

Fish species are important members of ecosystems; some have additional commercial and or recreational value. Fisheries research is often geared towards economically important species that have been over exploited or have received significant pressure. Employing a stable isotope approach, researchers can quantitatively investigate an assortment of ecological questions pertinent to certain fish species and populations.

Stable isotopes have been used to investigate specific food web ecology, movement patterns, and life history dynamics of various species of fish. Feeding studies often employ carbon and nitrogen isotopic analysis to study diet, trophic levels and food web structure (Fry and Parker, 1979; Fry, 1988; Harrigan, Zieman, and Macko, 1989; Hesslein et al., 1991; Sholto-Douglas et al., 1991; Gu et al., 2001; Kline and Willette, 2002). Migration and movement patterns of fish species may be examined by using sulfur, carbon, and oxygen isotopes (Hesslein et al., 1991; Kline, Wilson, and Goering, 1998; Bilby et al., 2001; Gu et al., 2001). Multiple stable isotopes have also been used to determine habitat utilization of fish species (Dufour, Pierre, and Rancher, 1998; Herzka and Holt, 2000; Das et al., 2000).

Muscle tissue of organisms, especially fish species, is typically enriched in ^{13}C by 1-2‰ relative to diet (DeNiro and Epstein, 1978; Sholto-Douglas, James and van der Merwe, 1991). Generally, muscle tissue is analyzed to demonstrate an isotopic relationship between consumers and diet. Biogenic carbonates, such as coral and fish otoliths (ear bones), are typically much more enriched in ^{13}C than soft tissue, and are often more similar to the dissolved inorganic carbon (DIC) in seawater (Degens, Deuser, and Haedrich, 1969; Mulcahy et al., 1979; McConnaughey, 1989a). However, it is not clear whether the carbon in fish otoliths are a reflection of diet through assimilation of respired CO_2 in the bloodstream or reflect the signal of dissolved inorganic carbon incorporated from seawater (Degens et al., 1969; Mulcahy et al., 1979; Radtke, et al., 1987; Kalish, 1991a; Iacumin, Bianucci, and Longinelli, 1992; Radtke, et al, 1996; Thorrold et al., 1997; Schwarz et al., 1998; Weidman and Millner, 2000; Gao

et al., 2001; Begg and Weidman, 2001; Chasar, 2002; and Lenanton et al., 2003). This uncertainty confounds the use of otolith $\delta^{13}C$ as an indicator of either feeding history or environmental parameters.

Fish Otoliths and Carbon Isotopes

Fish otoliths are ear stones which are primarily composed of calcium carbonate (aragonite). They are found in the cranial cavity and are extensively used in fisheries research. Typically, otoliths are exploited to determine age and growth relationships of fish species. Because of their chemical composition, otoliths are permanent records and contain valuable information regarding life history and environmental conditions experienced by the fish (et al., 1979; Kalish, 1991a; Iacumin, 1992; Schwarcz, et al., 1998; Radtke et al., 1996; Radtke et al., 1998).

Analyzing stable isotopes in otoliths ($\delta^{13}C$ and $\delta^{18}O$) have led to recent advances in fisheries research. Environmental conditions, such as temperature, that the fish have experienced can be determined by measuring $\delta^{18}O$ in otolith aragonite (Mulcahy et al., 1979; Kalish, 1991a; Radtke et al., 1996; Radtke et al., 1998). Since the oxygen in calcium carbonates is precipitated in isotopic equilibrium with the surrounding seawater (Degens et al., 1969; Thorrold et al, 1997; Radtke et al., 1998; Weidman and Millner, 2000), the temperatures of the ambient seawater during the lifetime of the fish can be determined (Kalish, 1991a). Oxygen isotope data, coupled with carbon isotope data ($\delta^{13}C$), can be employed to determine habitat utilization and site-specificity for fish species (Schwarz et al., 1998; Thorrold et al., 1998; Dufour et al., 1998; Begg and Weidman, 2001). Many recent studies have used oxygen and carbon isotopes to delineate fish populations and stocks (Gao and Beamish, 1999; Stephenson et al., 2000; Newman et al., 2001; Bastow, Jackson, and Edmonds, 2002). However, there are factors that complicate interpretation of $\delta^{13}C$ data from otoliths.

Fish otolith calcium carbonate is precipitated as aragonite into fish otoliths from two possible sources. It may be derived from CO_2 in the blood through catabolic processes, and it may be incorporated from dissolved inorganic carbon (DIC) in

seawater passing through the gills, mainly as bicarbonate (Degens et al., 1969; Kalish, 1991a; Iacumin et al., 1992; Radtke et al., 1996; Thorrold et al., 1997; Schwarcz et al., 1998; Weidman and Millner, 2000). Early work by Degens et al. (1969) indicated that aragonite is deposited in isotopic equilibrium with surrounding seawater. More recent work suggests that carbon isotopes in fish otoliths are not in equilibrium with seawater (Mulcahy et al., 1979; Radkte et al., 1987; Kalish, 1991a; Iacumin et al., 1992; Radtke et al., 1996; Thorrold et al., 1997; Schwarcz et al., 1998; Dufour et al., 1998; Gao et al., 2001). Alternatively, there may be a mixture of carbon from both seawater and metabolically derived carbon from respiration. Estimates of the proportion of metabolic carbon input range from 20% to over 30% (Kalish, 1991a; Weidman and Millner, 2000; Hoie, Folkvord, and Otterlei, 2003). Overall, it is not clear in the literature whether changes or differences in otolith $\delta^{13}C$ are derived from metabolic differences (growth rates, age, diet) or variation in physical/chemical parameters of seawater (depth, temperature, salinity).

Changes in seawater salinity and depth of water alter $\delta^{13}C$ of seawater DIC, and have been suggested as possible explanations for shifts of otolith $\delta^{13}C$ (Mulcahy et al., 1979; Thorrold et al., 1998). Other explanations for otolith $\delta^{13}C$ differences among fish populations and in individual fish include dietary shifts and variation in $\delta^{13}C$ of food supply (Kalish, 1991a; Radtke et al., 1996; Schwarcz et al., 1998; Weidman and Millner, 2000; Gao et al., 2001; Bastow et al., 2002). In addition, changes in metabolism and growth rates associated with age and sexual maturity may also influence otolith $\delta^{13}C$ (Schwarcz et al., 1998; Begg and Weidman, 2001; Hoie et al., 2003). There is not yet a consensus in the literature as to the major cause of otolith $\delta^{13}C$ variation.

The objective of my work was to evaluate the influence of dietary sources on the $\delta^{13}C$ of carbonate within fish otoliths and to assess the potential to differentiate diet and feeding histories using stable carbon isotopes. The stable carbon isotope relationship between the diet of a particular fish species (*Sciaenops ocellatus*) and its otoliths and

muscle tissue was examined. The hypothesis that diet controls the $\delta^{13}C$ of the juvenile red drum otoliths was tested by measuring the carbon isotope signatures of otoliths from red drum raised in similar conditions fed diets differing in carbon isotopic signatures.

MATERIALS AND METHODS

Experimental Fish and Conditions

Juvenile red drum, *Sciaenops ocellatus*, (Pisces: Sciaenidae), were raised in flow-through saltwater aquaria at the Florida State University Marine Laboratory (FSUML) in Franklin County, Florida. Red drum were selected as the experimental fish because of their rapid growth rate (Peters & McMichael, 1987), large otoliths at small size, consumption of a variety of food, including commercial hatchery diets, and availability at fish hatcheries in the state. Fish for this study were obtained from the Stock Enhancement Research Facility (Florida Fish and Wildlife Conservation Commission) in Port Manatee, Florida.

The red drum were raised in eight 210-liter rectangular flow-through saltwater tanks. These tanks had average flow rates of 782.0 L hr^{-1} for the individual (range = 599.9 to 851.7 L hr^{-1}) and exchange rates of 3.7 times hr^{-1} (range = 2.8 times hr^{-1} and 4.3 hr^{-1}). Temperature (°C) and salinity (ppt) measurements were taken several times daily from seawater flowing into the aquaria. The water entering the flow-through system was first stored in 5,000-gallon reservoirs prior to disbursement, which exposed the water to air temperature fluctuations.

Experimental Design

The red drum were divided into two groups according to diet treatments and placed in four tanks for each diet group. Approximately 500 fish were obtained 39 days after the hatching date at the Port Manatee facilities. They averaged 26.5 millimeters (SD ± 1.34 mm) in standard length (SL) upon arrival. Standard length is measured from nose tip to base of caudal peduncle. Fish were acclimated and placed into holding tanks for several weeks until they were able to consume their prescribed diets. On day 1 of the experiment (September 17, 2001), approximately 50 fish (mean length = 64.1 ± 2.34

mm SL) were placed into each of the eight tanks. Group A fish had mean lengths of 65.0 ± 2.3 mm SL (n = 204) while Group B fish had mean lengths of 63.2 ± 2.2 mm SL (n = 189).

Growth rates of red drum were calculated by subtracting the mean standard length of each diet group (A = 65.0 ±0 2.3 mm; B = 63.2 ± 2.2 mm) at the start of the experiment from the individual standard lengths at the time each fish was sampled and divided by the number of experiment days. The equation $(F_n - x_s) / T_n$ results in millimeters of growth per day (mm day $^{-1}$) where F_n is the individual fish standard length (mm) when it was sampled, x_s is the mean standard length (mm) of red drum in their respective group at the start of the experiment, and T_n is the experiment day that individual fish were sampled.

During the first week of the acclimation period after their arrival on August 10, 2001, the red drum were fed their accustomed commercial diet (Ziegler Salmon Starter, Burris, Inc.; $\delta^{13}C \sim -21‰$). Fish were then fed another commercial hatchery feed (AquaMax™ Fingerling Starter 300, Purina Nutritional International, Inc.) for thirty-nine days until they would consume ground fish tissue. On September 17, 2001 (Day 1), the fish began the diet treatment of two diets with differing carbon stable isotope signatures. The fish in diet group A continued with the AquaMax feed, which had a preliminary $\delta^{13}C$ of -21 to -22‰. The AquaMax Fingerling Starter 300 was purchased as a 50-pound bag from a local feed store. Diet B consisted of muscle tissue from pinfish, *Lagodon rhomboides* (Sparidae), with an expected $\delta^{13}C$ of approximately -17 to -18‰ (Chanton and Lewis, 1999). The majority of the pinfish used were collected from Apalachicola Bay in the Big Bend region of Florida using otter trawl gear and 183-meter beach seines. Some of the pinfish were purchased from local seafood and bait dealers who harvested in the Bay and nearby waters.

During the experiment, fish were fed to satiation once each day. The amount varied with actual consumption throughout the experiment. Equal amounts of AquaMax were poured into each of the four tanks in Group A. Pinfish were frozen whole until they were needed. The pinfish were thawed and filleted while any leftovers were stored

in refrigeration. Initially the muscle tissue was ground with a coffee grinder, but was eventually cut into pieces, as the red drum grew large enough to consume bigger particles.

Sample Processing

Random samples (n = 4) were taken from the batch of AquaMax commercial feed during the course of the experiment. The AquaMax pellets were ground with a standard coffee grinder and a Crescent Wig-L-Bug. Processed samples were shipped to Isotope Services, Inc. in Los Alamos, New Mexico for determination of the carbon stable isotopes.

Samples from each batch (n= 23) of pinfish were collected and frozen until further processing. Batches differed by date and location harvested. Thawed muscle tissue was rinsed and soaked in tap water. All bones and scales were removed. The samples were then dried in an oven at low temperature (approximately 70°C) for several days until muscle consisted of dry flaky material. The drying time varied for each individual muscle sample depending on amount. Upon removal from the oven, the tissue was ground with mortar and pestle and with a standard coffee grinder into a fine, homogenous material. The tissue was then further ground with a Crescent Wig-L-Bug. Processed samples were shipped to Isotope Services, Inc in Los Alamos, New Mexico.

Seawater samples from the tanks were collected to measure $\delta^{13}C$ of dissolved inorganic carbon (DIC). Approximately 10 milliliters (ml) of seawater from three randomly-selected tanks were injected into pre-evacuated glass vials once each month. The vials were stored in refrigeration until further processing. Approximately 0.2 milliliters of 30% phosphoric acid was injected into each water sample. Head space (~ 0.2 ml) from the vials were then direct-injected into a Finnigan Mat Isotope Ratio Mass Spectrometer (IRMS) at Florida State University.

Red drum were sampled from each of the tanks at day 160 and day 261 of the experiment. On day 160, five fish from each tank for a total of twenty fish from each diet group were removed and measured. The fish were then overdosed with an anesthetic (MS-222) according to FSU Animal Care and Use Committee (ACUC)

guidelines and frozen until further processing. All fish from one batch in Group A suffered premature mortality on day 159 though five specimens from the batch were used in the analysis. And one fish in Group A died on day 157 and was also included in the data set. The remaining fish (n = 34) were sampled on or about day 261: fifteen in the commercial diet group (A) and nineteen in the pinfish diet group (B).

Thawed fish were measured to the standard length in millimeters (SL mm), and weighed in grams to nearest tenth. Muscle from one side of the fish was filleted and weighed in grams to nearest tenth. Bones were extracted from the muscle with forceps and scales rinsed off in tap water. The muscle was then soaked in tap water for several minutes and dried in a drying oven (~70°C) for several days varying with tissue amount. Muscle tissue was then coarse ground with a mortar and pestle, and further ground with a standard coffee grinder, then fine ground with a Crescent Wig-L-Bug. Samples were shipped to Isotope Services, Inc. in New Mexico to determine carbon isotopic signatures.

The sagittal otoliths were extracted with forceps, rinsed in tap water, and cleansed with paper towel to remove the membranous sac surrounding the otoliths. Pairs of otoliths were separated into left and right and placed into evacuation vials, then flushed with nitrogen gas and dissolved with 30% phosphoric acid. The samples were then direct-injected into a Finnigan Mat Isotope Ratio Mass Spectrometer (IRMS) at Florida State University to obtain carbon isotopic signatures.

Data Analysis

Mean $\delta^{13}C$ values were analyzed by analysis of variance (ANOVA) and 2-sample T-test (Minitab 1998 statistical software). Linear regressions were performed using either Microsoft Excel or Minitab (1998). Grubbs Test was used to test for outliers in the data set (Sokal and Rohlf, 1995). All significance levels were set at $\alpha = 0.05$.

Fish reared on the commercial AquaMax diet are annotated by “A” while red drum fed the pinfish diet were designated by “B”. Each diet group has two age groups:

"a" refers to the younger fish and "b" indicates the older fish. Red drum raised in the same tank are referred to as "subgroups" and are replicates within each diet group from both age classes. For example, there are four subgroups (replicates) of younger fish from both diet groups individually annotated (A1a, A2a, A3a, A4a; B1a, B2a, B3a, B4a) and are collectively designate as Aa and Ba. The individual subgroups (replicates) of older fish from both diet groups are similarly annotated (A3b, A4b, A5b; B1b, B2b, B4b, B5b) and the combined replicates (Ab and Bb) indicate all older fish in each diet group. Individual fish from each replicate (subgroup) have been assigned a fish code number (e.g. A1a-1, Aa1-2, etc.). See Figure 1 below for clarification.

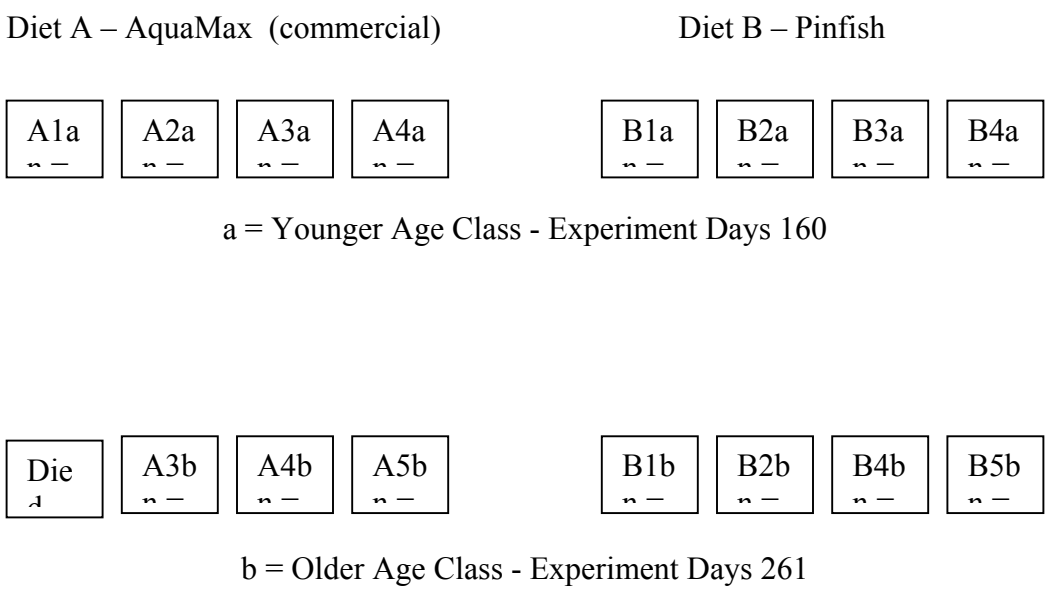


Figure 1. Schematic of labeling system used for two treatment groups and the age group within each diet group. Each square represents individual tanks. One tank in the AquaMax treatment group died prematurely and were not included in the data set.

RESULTS

Temperature and Salinity of Seawater

The daily mean temperature for the entire experiment was 19.4 ± 5.1 °C. The temperatures of the seawater ranged from a low of 7.1°C on day 110 (January 4) and a high of 29.4 °C on day 237 (May 11). The five winter months (November – March) had mean daily temperatures below 20°C while the other five months had daily mean temperatures above 20 °C (Figure 2). The highest monthly mean temperatures (26.9 ± 2.2 °C) were in May while the lowest mean monthly temperatures (13.7 ± 3.8 °C, SD = 3.80) were in January (Table 1).

The mean daily salinity of the ambient seawater during the experiment was 30.1 ± 1.4 ppt (Figure 2). A low salinity of 25.0 ppt was recorded on day 29 (October 15) and the highest salinity, 32.5 ppt, was recorded on day 257 (May 31). Mean monthly salinity ranged from 29.0 ± 1.7 ppt in September to 31.4 ± 0.5 ppt in November (Figure 3).

Dissolved Inorganic Carbon Isotopic Composition

The $\delta^{13}C$ of dissolved inorganic carbon (DIC) in the seawater was enriched relative to atmospheric CO_2 (Figure 4). Carbon isotopic composition values of monthly seawater samples combined ranged from -5.80‰ to -2.32‰ with a mean $\delta^{13}C$ of -3.70 ± 0.79‰ (Table 2). Monthly DIC isotopic compositions ranged from -4.82‰ to -2.59‰ and had a mean of -3.83 ± 0.81‰. These values were similar to DIC isotopic composition of higher saline waters of nearby Apalachicola Bay (Chanton and Lewis, 1999).

Chanton and Lewis (1999) demonstrated that seawater is more enriched in ^{13}C than brackish water and DIC approaches isotopic equilibrium with atmospheric CO_2 as salinity increases. The relationship between salinity and $\delta^{13}C$ DIC in this study is

somewhat weaker ($r^2 = 0.32$) than they observed ($r^2 = 0.88$) for Apalachicola Bay. (Figure 5).

Growth of Red Drum

The red drum exhibited similar growth throughout the experiment (Figure 6). On around day 160 of the experiment, five specimens were sampled from each of four subgroups (Table 3). The mean length for all fish combined was 138.1 ± 16.1 mm SL (n = 40) with a range of 103 to 172 mm SL. There was not a significant difference in ending standard lengths between the two groups (T-test, p = 0.43). The range in lengths for Group Aa fish at day 160 was 104 to 172 mm SL compared to the range of Group Ba fish 103 to 162 mm SL (Table 4).

The younger fish subgroups from both diet groups combined had similar ending standard lengths (Figure 7a). Group Aa red drum lengths were more variable ($F_{3,16} = 0.35$, p = 0.788) than Group Ba (ANOVA, $F_{3\ 16} = 0.08$, p = 0.969) and the younger fish of Group A also had a wider range of means (134.4 ± 11.7 to 145.0 ± 16.6 SL mm) than younger fish in Group B (133.2 ± 22.7 to 138.0 ± 10.0 SL mm). An analysis of variance test on all individual subgroups detected no difference of lengths (ANOVA, $F_{7,32} = 0.26$, p = 0.966).

The remaining fish were sampled on day 261 of the experiment and had a combined mean standard length of 203.1 ± 24.9 mm with a range of 140 to 250 mm (Figure 7b). The mean standard length of red drum in Group Ab (206.7 ± 15.5 mm, n = 15) was not significantly different (T-test, p = 0.42) from that of Group Bb (200.2 ± 30.5 mm, n = 19). The standard lengths at the end of the experiment for red drum raised on AquaMax ranged from 177 to 239 mm. The standard lengths of the red drum raised on pinfish ranged from 140 to 250 mm.

The standard lengths of fish from the three subgroups of the Ab Group had means that were not dissimilar (ANOVA, $F_{2,12} = 1.40$, p = 0.283). Likewise, Group B had similar standard lengths at the end of the experiment that were not significantly different (ANOVA, $F_{3,15} = 1.69$, p = 0.212). Comparing all subgroups from this age

group to each other (Table 5), the null hypothesis of similar means is not rejected (ANOVA, $F_{6,27} = 1.55$, $p = 0.202$).

Red Drum Growth Rates

Growth rates of all red drum within the two groups were similar (T-test, $p = 0.57$) and among the fifteen subgroups varied but were not significantly different ($F_{15,59} = 1.29$, $p = 0.240$) Mean growth rates from all fish in Group A (mean = 0.51 ± .09 mm/d) fish combined were higher than the pooled Group B fish (mean = 0.49 ± .11 mm/d), and had a difference of 0.02 mm/d (Table 6).

The younger fish sampled on day 160 of the experiment had a slightly slower growth rate (mean = 0.47 ± 0.10 mm/d, n = 40) than the fish sampled at the end of the experiment (mean = 0.54 ± 0.10 mm/d, n = 34). This difference in means (T-test, $p = 0.0026$) was significantly different (Figure 8a). Fish from Group Aa sampled at day 160 grew slower (mean = 0.47 ± 0.10 mm/d, n = 20) than fish sampled at the end of the experiment from Group Ab (mean = 0.55 ± 0.06 mm/d, n = 15). A statistical comparison shows a significant difference (T-test, $p = 0.011$) in the growth rates. Growth rates between the two age classed from Group B (Ba = 0.46 ± 0.10 mm/d, n = 20; Bb m = 0.53 ± 0.12 mm/d, n = 19) had a detectable but not significant difference (T-test, $p = 0.064$; Figure 8b).

Growth rates were not significantly different (T-test, $p = 0.68$) between groups from the initial sampling (Figure 8). The difference in growth rates between the older fish of both treatment groups was not significant (T-test, $p = 0.47$). When subgroups of each age class of fish were compared to each other, there were no statistically different growth rates among and between subgroups.

Otolith Weights

Pairs of sagittal otoliths were weighed individually to a tenth of a milligram (mg) for individual fish from the younger age class (Table 3) and the older age class (Table 4). Otolith pairs within the same age class of each diet group had similar

weights. The left otolith weights (mean = 63.0 ± 9.2 mg, n = 20) from the younger fish fed the commercial diet were similar (T-test, p = 0.87) to the right otolith weights (mean = 63.4 ± 8.5 mg, n = 20). The group fed pinfish also had similar (T-test, p = 0.78) weights between the otolith pairs: left otolith mean = 53.2 ± 11.4 mg; right otolith mean = 53.6 ± 14.0 mg; n = 20. A simple linear regression (Figure 9) revealed very strong positive correlation between combined otolith pairs from the commercial diet group (r^2 = 0.966, $p < 0.001$) and significant agreement in the pinfish diet group ($r^2 = 0.878$, $p < 0.001$). There were two individuals in the pinfish diet group (B1b-2, B1b-4; Table 4) that had otoliths with very different weights which affected the relationship.

Red drum otoliths from the commercial diet (Group A) had accumulated more material, and were therefore heavier, than Group B red drum otoliths. Statistical comparison between the mean weights of left otoliths from the younger fish in each diet groups revealed significant differences (T-test, p = 0.0007). The right otoliths between the younger fish also were also not equal (T-test, p = 0.0003), differing by 13.0 mg (Figure 10a). The older fish had similar disparities between otolith weights (Figure 10b). The weights of the left otoliths (T-test, p = 0.019) and the weights of the right otoliths (T-test, p = 0.0004) were significantly greater in the red drum raised on AquaMax .

The older fish from the two diet groups also had more variable weights between the pairs of otoliths than the younger age class (Table 7). The weights of Group Ab left otoliths (n = 15) had a mean of 120.7 ± 21.8 mg and were not different (T-test, p = 0.49) than the right otoliths with a mean of 126.1 ± 20.3 mg. Similarly, fish in Group Bb (n = 19) had left otolith weights (mean = 95.7 ± 34.6 mg) and right otolith weights (mean = 89.5 ± 32.2 mg) that were not statistically different (T-test, p = 0.63). Otolith weights from the younger fish in the AquaMax group (left: 63.0 ± 9.2 mg, n = 20; right: 63.4 ± 8.5 mg, n = 20) were similar (T-test, p = 0.87). Group B otoliths (left: 51.4 ± 10.6 mg, n = 20; right: 50.4 ± 11.7 mg, n = 20) also had similar means (T-test, p = 0.78).

Red drum in the commercial diet treatment showed a stronger correlation (r^2 = 0.84) between growth (SL mm) and otolith weights (Figure 11a) than red drum in the

pinfish treatment group ($r^2 = 0.73$; Figure 11b). The fish in the B diet group had much more scatter in the bigger specimens (> 200 mm SL) than fish raised on AquaMax when otolith weights were plotted against ending standard lengths.

Carbon Isotopic Signatures of the Diets

The two diet treatments used in the experiment differed significantly (T-test, $p < 0.001$) in carbon isotopic composition by 2.12‰ (Figure 12a). The $\delta^{13}C$ values of the commercial diet (n = 4) ranged from -19.87 to -19.46‰ and had a mean $\delta^{13}C$ of -19.64 ± 0.21‰. The carbon isotopic signatures from the pinfish diet (n = 23) ranged from -22.64 to -15.07‰ with a mean $\delta^{13}C$ of -17.52 ± 1.62‰ (Table 8a).

The commercial diet fed to the younger fish (Group Aa) was slightly depleted (mean $\delta^{13}C$ = -19.82 ± 0.08‰; n = 2) relative to the older fish (Group Ab: mean $\delta^{13}C$ = -19.47 ± 0.01‰; n = 2). The difference between these two means was not significantly different (T-test, p = 0.10). The $\delta^{13}C$ values of the commercial pellets used later in the experiment ($\delta^{13}C$ = -19.47‰ and -19.46‰) were slightly more positive relative to the pellets fed earlier in the experiment ($\delta^{13}C$ = -19.87‰ and -19.76‰; Figure 12b).

The pinfish fed to treatment Group B were harvested periodically throughout the experiment and showed a wider range of $\delta^{13}C$ values than the commercial diet (Figure 13). There was greater difference in ^{13}C between batches of pinfish fed to the younger fish than to the older fish. The pinfish fed to Group B during the first part of the experiment (n = 16) had a range of $\delta^{13}C$ (-22.64 to -15.31‰) with a mean $\delta^{13}C$ of -17.61 ± 1.84‰. Pinfish fed during the second part of the experiment (n = 7) had a carbon isotopic signature range from -18.09 to -15-07‰ with a mean $\delta^{13}C$ of -17.31 ± 1.05‰. The 0.30‰ difference between these two means was detectable, but not statistically significant (T-test, p = 0.62).

Carbon Isotopic Composition of Red Drum Muscle Tissue

Carbon isotopic signatures of red drum muscle tissue were significantly different ($p < 0.001$) between the two treatment groups (Figure 14a). The $\delta^{13}C$ values for individual specimens of the two groups are listed in Tables 8 and 9. Carbon signatures of Group A muscle tissue (mean $\delta^{13}C = -18.13 \pm 0.15$; $n = 32$) had a range of -18.36 to -17.63‰ and tissue from Group B (mean $\delta^{13}C = -16.01 \pm 0.24$; $n = 38$) ranged from -16.49 to -15.39‰ (Table 11). The difference in $\delta^{13}C$ values between the treatment groups was 2.12‰. Three outliers (Grubbs test; $\alpha = 0.05$) were not included in the data set (A1a-3: -16.46‰, A5b-4: -16.29‰ and B4b-2: -16.88‰) and one muscle tissue sample (A4b-3) did not get processed.

Muscle tissue between the two diet groups at both age classes was significantly different in isotopic signature (Figure 14b). Tissue from the younger fish in Group A (mean $\delta^{13}C = -18.18 \pm 0.10$‰) were depleted 2.03‰ relative to their Group B counterparts (mean $\delta^{13}C = -16.14 \pm 0.17$‰) and that difference was significant (T-test, $p < 0.001$). Likewise, the older fish from the two groups had a significantly different means of carbon isotopic signatures (T-test, $p < 0.001$) where Group A (mean $\delta^{13}C = -18.06 \pm 0.20$‰) was depleted 2.20‰ relative to Group B (mean $\delta^{13}C = -15.86 \pm 0.21$‰).

There was detectable enrichment of ^{13}C in older fish from Group A (Figure 15a) and in tissue from Group B (Figure 15b). The $\delta^{13}C$ values from the older fish fed the commercial diet (range = -18.26 to -17.63‰; $\delta^{13}C$ mean = -18.18 ± 0.10‰) was slightly ^{13}C enriched compared to younger fish (range = -18.36 to -18.01‰; mean $\delta^{13}C = -18.06 \pm 0.20$‰). A statistical comparison revealed that this enrichment of 0.12‰ was not substantial (T-test, $p = 0.064$). Tissue from the older red drum raised on pinfish (range = -16.29 to -15.39‰; mean $\delta^{13}C = -15.86 \pm 0.21$‰) were also more enriched in ^{13}C relative to younger fish from group B (range = -16.49 to –15.86‰; mean $\delta^{13}C = -16.14 \pm 0.17$‰). The 0.28‰ enrichment of ^{13}C between the two age classes of Group B fish differed significantly (T-test, $p = 0.0001$)

Red drum muscle tissue from both groups was enriched in ^{13}C with respect to the $\delta^{13}C$ of the diet (Figure 16). Group A's carbon signature of muscle tissue from all subgroups combined was enriched 1.51‰ relative to the AquaMax diet signature (Table 12). The combined data of muscle tissue from the red drum raised on pinfish was also isotopically lighter by 1.51‰ relative to the carbon signature of diet B. The difference in isotopic composition of the two diets and muscle tissue from both groups was 2.12‰.

The individual subgroups (replicates) within each diet group exhibited variation in carbon isotopic composition of muscle tissue (Figure 17). The four younger subgroups in Group A (Figure 18a) had similar isotopic means (ANOVA, $F_{3,15} = 0.55$, $p = 0.659$) while the three older fish subgroups of that group (Figure 18b) had means that were more varied but not of significance (ANOVA, $F_{2,10} = 3.13$, $p = 0.088$). The younger red drum raised on pinfish (Figure 19a) had $\delta^{13}C$ means that were significantly different (ANOVA, $F_{3,16} = 8.71$, $p = 0.001$) while the older fish in that group (Figure 19b) had $\delta^{13}C$ means with less variation and were not substantially different (ANOVA, $F_{3,14} = 2.48$, $p = 0.104$). This isotopic variation in muscle tissue between the two age classes of both treatment groups mimics the signatures of the diets (Figure 20), though there was much more scatter in the $\delta^{13}C$ signal of the pinfish diet.

Carbon Isotopic Signatures of Red Drum Otoliths

Carbon isotopic signatures were measured for the sagittal otolith pairs from individual fish from both diet groups (Tables 9 and 10) and each age class within the diet groups (Table 13). The mean $\delta^{13}C$ for the pair of otoliths from the red drum raised on the commercial diet (Group A: $n = 33$) was -2.94 ± 0.23‰ within a range of -3.50 to -2.44 ‰. Red drum fed pinfish (Group B: $n = 38$) had a mean $\delta^{13}C$ of -1.68 ± 0.23‰ and ranged from -2.19 to -1.25‰ for the otolith pairs combined (Table 13). Two outliers from Group A (A1a-3: -1.29‰ and A5b-4: -1.88‰) were not included in the otolith data set and one outlier from Group B (B4b-2: -2.93 ‰) was not included in the data (Grubbs Test, $\alpha = 0.05$). These were the same outliers from the muscle data (Table 9, Table 10) and it was presumed that they were a result of processing error.

Otolith pairs combined from fish of both age classes in Group A had a lighter carbon isotope signature (mean $\delta^{13}C$ = -2.94 ± .24‰; n = 66) than the otoliths from the combined Group B (mean $\delta^{13}C$ = -1.68 ± .25‰; n = 76). This difference of 1.26‰ between the two pooled means was significantly different (T-test, $p < 0.001$; Figure 21a). Each otolith pair between the two diet groups exhibited similar disparity (Figure 21b). Left otoliths between the two treatment groups had different means (Group A: -2.93 ± 0.25‰, n = 33; Group B: -1.69 ± 0.25‰, n = 38; T-test, $p < 0.001$). The two treatment groups also had unequal means in the right otoliths (Group A: -2.95 ± 0.23‰, n = 33; Group B: -1.67 ± 0.25‰, n = 38; T-test, $p < 0.001$). An analysis of variance for subgroups of combined age classes within each group (Figure 22a) also showed evidence of dissimilarity (ANOVA, $F_{9,61} = 58.06$, $p < 0.001$). The differences between the mean $\delta^{13}C$ values of each age class were similar within both diet groups (Figure 22b).

There were no significant differences, as shown in Table 14, between pairs of otoliths from either group combined (Figure 23a) or split into the two age classes (Figure 23b). A simple linear regression showed strong agreement ($r^2 = 0.917$, $p < 0.001$) between all otolith pairs (Figure 24). The isotopic composition for the left otoliths from Group A exhibited a wider range of values (-3.51 to –2.33‰) than the right otoliths (-3.49 to -2.55‰), but had similar means (T-test, $p = 0.69$). The left otoliths from Group B fish had a slightly more enriched $\delta^{13}C$ range (-2.33 to –1.23‰) than their right counterpart (-2.29 to –1.17‰) and also had similar means (T-test, $p = 0.76$).

Comparing the carbon signature of left otoliths between the age classes (Figure 25a), both diet groups had a detectable but not significant difference in ^{13}C (T-test, $p = 0.17$). The left otoliths from the older fish of the commercial diet group (mean $\delta^{13}C$ = -2.99 ± 0.22‰) were 0.11‰ lighter in ^{13}C than the younger fish of that group (mean $\delta^{13}C$ = -2.88 ± 0.26). The carbon isotopic composition of the younger fish in the pinfish diet group had more depleted left otoliths (mean $\delta^{13}C$ = -1.65 ± 0.24‰) than the older

fish (mean $\delta^{13}C$ = -1.76 ± 0.27‰). A 2-sample T-test showed no evidence (p = 0.38) that this 0.11‰ enrichment was substantial.

There was a difference between right otoliths from age classes of the two diet groups (Figure 25b) which was significant for the red drum fed AquaMax (T-test, p = 0.010) but not for the pinfish fed group (T-test. p = 0.83). The right otoliths of the older fish in Group A (mean $\delta^{13}C$ = -3.07 ± 0.24‰) were depleted by 0.21‰ relative to the younger fish (mean $\delta^{13}C$ = -2.86 ± 0.19‰). The ^{13}C depletion of 0.02‰ in the right otoliths of the older Group B (mean $\delta^{13}C$ = - 1.66 ± 0.28‰) fish was not substantially different (T-test, p = 0.83) from the younger fish raised on pinfish (mean $\delta^{13}C$ = -1.68 ± 0.23‰).

Statistical comparisons of otolith pairs of the individual subgroups from both age classes also revealed significant differences (ANOVA, $F_{14,56} = 39.89$, $p < 0.001$) between the two diet groups (Figure 26). Within each diet group, there were differences of $\delta^{13}C$ means but not of any significance when individual subgroup means were tested. The isotopic differences of the subgroups for the each age class raised on AquaMax (Figure 27a) were significantly different when tested as individual means (ANOVA, $F_{13,52} = 1.91$, p = 0.05;), and were significantly different when the means of the two age classes were pooled (T-test, p = 0.035; Figure 25b). The otolith isotopic composition of red drum raised on pinfish (Figure 27b) were similar between the two age classes when subgroups were tested as individual means ($F_{15,60}$= 0.65, p = 0.823) and when age class data was pooled (T-test, p = 0.71).

The otoliths from both diet groups were similarly enriched in ^{13}C with respect to the isotopic signature of their diet and muscle tissue (Figure 28a). Otoliths from red drum raised on AquaMax were enriched 16.70‰ from the signature of the diet while otoliths from the red drum raised on pinfish were enriched 15.84‰ from the diet (Table 12). The otoliths from Group A were enriched 15.19‰ from the muscle carbon signature and Group B otoliths were enriched 14.33‰ with respect to the muscle tissue (Figure 28b). When separated into age classes, the otoliths from the two diet groups demonstrate similar isotopic enrichment (Table 12).

A regression analysis between otolith $\delta^{13}C$ and muscle $\delta^{13}C$ for all data points combined exhibited a strong relationship ($r^2 = 0.842$, $p < 0.001$) as shown in Figure 29. This relationship was similar to Chasar (2002), who compared muscle tissue $\delta^{13}C$ and otolith $\delta^{13}C$ from adult red drum (*Sciaenops ocellatus*) and snook (*Centropomus undecimalis*) and found a positive correlation ($r^2 = 0.84$).

There were no strong relationships between otolith weights and stable isotopic composition of otoliths. Otolith weights from all fish combined were plotted against the individual $\delta^{13}C$ values from paired otoliths (Figure 30) and the correlation was extremely weak ($r^2 = 0.087$) but significant ($p = 0.013$). Regressions between weights and $\delta^{13}C$ of left and right otoliths from both groups combined revealed some relationship. The strongest correlation was of right otoliths from Group A ($r^2 = 0.232$, $p = 0.005$), though the correlation is quite weak. Neither Group B left otoliths ($r^2 = 0.001$, $p = 0.549$) nor right otoliths ($r^2 = 0.048$, $p = 0.189$) exhibited in relationship between weights and $\delta^{13}C$. Correlation in left otoliths of the A Group were also nonexistent ($r^2 = 0.059$, $p = 0.173$).

When otolith weight data was examined against $\delta^{13}C$ by subgroups and age classes, there were some general trends (Table 16). There was a gradual $\delta^{13}C$ increase with otolith weight in the younger age classes from both groups and only one of the four tests (Ba right otoliths) revealed significance ($r^2 = 0.255$, $p = 0.023$). The older fish from Group B also exhibited similar relationship in the right otoliths ($r^2 = 0.359$, $p = 0.09$). The left otoliths in Group B had a detectable relationship but not statistically significant ($r^2 = 0.212$, $p = 0.055$). The older fish in Group A displayed a decrease in ^{13}C with increasing otolith weight with only the right pair having a positive relationship ($r^2 = 0.232$, $p = 0.005$). A recent study on somatic growth effects in fish on otolith $\delta^{13}C$ revealed similar trends with otolith weights less than 100 mg (Bastow, Jackson, and Edmonds, 2002). Our data did not reveal a distinct relationship of otolith weights under 100 mg, and all correlations between otolith weight and $\delta^{13}C$ were not particularly strong.

A regression analysis did not reveal any relationships between red drum standard lengths and $\delta^{13}C$ of otoliths. Plots of lengths and otoliths combined ($r^2 = 0.000$, $p = 0.950$), of Group A ($r^2 = 0.128$, $p = 0.041$), and of Group B ($r^2 = 0.084$, $p = 0.078$) indicate that the two variables are not related. Similar regression analysis was performed to test the relationship between growth rate and $\delta^{13}C$ of the otoliths. For Group B, there was a significant but extremely weak correlation ($r^2 = 0.241$, $p = 0.002$) between the two variables. Data from all fish combined ($r^2 = 0.005$, $p = 0.533$) and A Group data ($r^2 = 0.036$, $p = 0.291$) showed there was not a compelling correlation between growth rates and otolith $\delta^{13}C$.

DISCUSSION

The source and mechanisms of biogenic carbonate deposited in teleost fish otoliths in the form of aragonite have been and continues to be debated. The general consensus from previous studies is that carbon isotopes are not deposited in equilibrium in otoliths with the surrounding seawater (Mulcahy et al., 1979; Kalish, 1991a; Radtke et al., 1996; Thorrold et al., 1997). Isotopic fractionation of otoliths results from two possible effects: kinetic or metabolic (Thorrold et al., 1997). Kinetic effects, which result in discrimination against the heavier isotope (^{13}C) during the hydration and hydroxylation of carbon dioxide (McConnaughey, 1989a), do not seem to play a prominent role in otolith carbonate. There is increasing evidence that the carbon isotopic signal in sagittal otoliths is largely due to metabolic effects (Mulcahy et al., 1979; Radtke et al., 1987; Kalish, 1991a; Radtke et al., 1996; Thorrold et al., 1997; Schwarcz et al., 1998; Dufour et al., 1998; Newman et al., 2000; Begg and Weidman, 2001; Gao et al., 2001; Bastow et al., 2002; Hoie et al., 2003). However, there is still conflicting evidence as to what extent and how metabolic effects (e.g. growth, age, and nutrition) control or influence carbon isotopic signatures in otoliths, which complicates interpretation of isotope signals. Nutritional carbon seems to be contributing, at least in part, to juvenile red drum otolith carbon isotope composition and significantly influencing the overall otolith $\delta^{13}C$.

In this experiment, red drum from the two treatment groups exhibited similar growth patterns, though fish reared on the commercial diet (Group A) grew somewhat longer in standard length than fish weaned on a pinfish diet (Group B). The Group A fish also grew at a slightly faster rate than fish in Group B, and all older fish grew significantly faster than the younger age class of fish (Figure 7b). Increasing ambient seawater temperature during the latter portion of the experiment (February to June; Figure 1) presumably could account for this increased growth. The relationship between

growth rates and carbon isotope composition in Group B otoliths was weak, but significant. There was not a distinguishable correlation between Group A otolith $\delta^{13}C$ and growth rates. Radtke et al, (1996) report similar results in a diet experiment rearing Atlantic cod (*Gadus morhua)*. In that study, juvenile cod were raised for three months on two diets differing in carbon isotopic composition. Cod otoliths had a significant but weak correlation between final total lengths and isotopic composition ($\delta^{13}C$ otolith: $r^2 = 0.184$, $p = 0.007$) in one treatment group and no significant correlation in the other ($\delta^{13}C$ otolith: $r^2 = 0.005$, $p = 0.639$). Kalish (1991a) raised juvenile salmon (*Arripis trutta*) in various temperature regimes and did not find a relationship between otolith carbon and somatic growth. Results from our experiment concur with Radtke et al. (1996) and Kalish (1991a) that growth and growth rates appear to have negligible effects on $\delta^{13}C$ of otoliths on age 0 (young of the year) juvenile fish. However, Hoie et al. (2003) found some significant differences in cod (*Gadus* morhua) otoliths ($\delta^{13}C$) from fish raised at different temperatures and growth rates. In that experiment, juvenile cod were raised at four different water temperatures and growth rates were further manipulated by varying nutrition sources and concentrations. Hoie et al. (2003) concluded that the ^{13}C enrichment in the cod otoliths could not have resulted from increased metabolism with ambient water temperature. Metabolic effects from growth were not substantial and diet was postulated as the major control of carbon isotopic signals in juvenile cod otoliths. Begg and Weidman (2001) found declining otolith $\delta^{13}C$ values with increased length of one-year-old haddock (*Melanogrammus aeglefinus*) in Georges Bank. This positive shift in carbon was attributed to reduction in metabolic rates with increasing size in haddock. In the present experiment, there was not an appreciable relationship between ending standard length and otolith $\delta^{13}C$ for either diet group (Figure 31), indicating carbon isotopic composition in the juvenile red drum otoliths was not greatly affected by somatic growth.

Though the variation in overall growth and growth rates were not different statistically in our experiment, it was presumed the two groups would exhibit similar growth. The commercial diet (Aquamax Fingerling Starter 300, Purina Mills

International, Inc.) fed to the faster growing fish is a feed that has been formulated to produce fast growth in an aquaculture setting. Coincidentally, there was a higher mortality rate in the AquaMax diet group, which resulted in one less subgroup in the older age class. The pinfish diet (*Lagodon rhomboides*) mainly consisted of filleted muscle tissue and was probably deficient in sufficient nutrients needed for faster growth. However, as the red drum aged and matured, pinfish were fed as chunks (which did contain some bone, scales, and skin) rather than fillets. Nonetheless, bioenergetics was not a part of this experiment and conclusions can not be drawn without further experimentation.

There was an appreciable disparity between otolith weights among the treatment groups (Figure 9). The otoliths at both age classes of Group B fish were substantially lighter than the otoliths from red drum reared on AquaMax. Though pinfish are a potential prey item for red drum in the wild (which was one particular reason for choosing pinfish as a diet treatment for this experiment) they are available in nature as whole fish. There may have been a nutrient deficiency from being reared on a near homogenous diet of muscle tissue, especially in the early stages of the experiment. Since otoliths are made of aragonite, a form of calcium carbonate ($CaCO_3$), it may be plausible that the red drum fed pinfish fillets were deficient in providing sufficient calcium resulting in less $CaCO_3$ deposited in their otoliths even though there is ample Ca^{2+} available in seawater. This deficiency may explain the significantly lighter otolith weights in the pinfish diet treatment group. However, otolith weight and stable carbon isotope composition did not have a strong relationship in either group or age class (Figure 29) though some regression analyses were significant (Table 16). Similar studies by Kalish (1991a) and Hoie et al. (2003) also did not find strong, significant relationships with otolith carbon isotopes and otolith weight. Incidentally, there was a general trend for $\delta^{13}C$ to become more positive with increasing otolith weight for the younger red drum and all Group B fish, but the trend became negative in older Group A fish. There was not a clear, distinct agreement between the two variables, suggesting

that somatic growth in terms of otolith weights is not a reliable predictor of carbon isotopic composition in juvenile red drum otoliths.

The mixed results correspond with data from a recent study on growth effects to otolith carbon composition in young juvenile pink snapper (*Pagrus auratus*) otoliths (Bastow, Jackson and Edmonds 2002). Bastow et al. (2002) showed an increase in $\delta^{13}C$ values in otoliths with weight between 13 and 100 mg (caudal fork length 62-250 mm). This enrichment was attributed to a decreasing metabolic rate with age of the younger fish. The trend towards enrichment of ^{13}C with otolith weight for smaller fish is comparable to our results since the younger red drum in both treatment groups had similar otolith weights (35.1 to 75.7 mg) to the juvenile pink snapper. However, the older red drum in our experiment had a wider spread of otolith weights (44.7 to 161.0 mg), especially in the older Group B fish, which had a lower range of otolith weights than Group A fish (Table 7) and evidently skewed the relationship. Bastow et al. (2002) did not find a significant relationship with carbon isotope composition of the otoliths that weighed more than 100 mg (> 250 mm caudal fork length). In our study, there were no appreciable relationships for either treatment group when data was partitioned at 100 mg for otolith weights. The data does not show clear evidence of somatic growth effects on otolith $\delta^{13}C$ values, however metabolic effects from growth seem to have some influence on $\delta^{13}C$ signals in juvenile red drum otoliths.

The carbon isotopic composition of red drum muscle tissue from both treatment groups in this experiment was positively enriched (1.51‰) relative to the respective diet (Table 12). These results are consistent with previous studies that detail isotopic enrichment (1-2‰) of whole animals relative to diet (DeNiro and Epstein, 1978; Sholto-Douglas et al., 1991). Similar tissue enrichment existed for both age classes of fish, though the younger red drum were more varied between the two treatment groups. An isotopic separation (2.12‰) between the two diets used in the experiment was also recorded in the muscle tissue demonstrating that both treatment groups exhibited similar fractionation of $^{13}C/^{12}C$ ratios between dietary carbon and muscle tissue (Table 12).

Muscle tissue in fish from the older groups was more enriched with respect to the muscle tissue of the younger fish. The enrichment (0.28‰) in Group Bb muscle tissue was similar to the positive increase (0.30‰) in the diet from the latter portion of the experiment. Pinfish fed during the early part of the experiment were more depleted in ^{13}C, but also had more variation (SD = 1.84‰) compared to the pinfish fed latter (SD = 1.05‰). The commercial diet was more constant in terms of $\delta^{13}C$ (SD = 0.21‰) but did exhibit similar enrichment (0.35‰) in isotopic composition of AquaMax fed to the older fish relative to the pellets fed to the younger fish. The older red drum muscle tissue from Group A were ^{13}C enriched (0.12‰) with respect to the younger muscle tissue. The more constant muscle tissue $\delta^{13}C$ in Group A may be due to less variability in the diet (compared to pinfish diet $\delta^{13}C$) throughout the course of the experiment. Since there was a consistent 1-2‰ agreement between all red drum muscle and the diet, it is reasonable to conclude that turnover of ^{13}C in the muscle tissue occurred within the first 160 days of the experiment. The further positive increase of muscle tissue in both treatment groups reflected a slight enrichment ^{13}C in conjunction with the food source.

There was a distinct relationship in $\delta^{13}C$ between red drum muscle and otoliths in both treatment groups (Figures 27, 28, and 29). Both muscle tissue and otoliths, with respect to dietary $\delta^{13}C$, became more enriched in ^{13}C. It is evident from the results that the carbon in the red drum muscle tissue directly relates to carbon through consumption and is within the established degree of enrichment (DeNiro and Epstein, 1978). The otolith ^{13}C fractionation from the dietary $\delta^{13}C$ was in much less but similar in proportion to the muscle fractionation between the two treatment groups (Table 12, Figure 27). This difference between muscle and otolith $\delta^{13}C$ values of the two treatment groups demonstrated comparable trends between the two portions of the experiment.

Similar fractionation from nutrition sources exists in other published work (Kalish, 1991a; Radtke et al., 1996; Hoie et al., 2000). These authors report that carbon from the nutrition source plays a prominent role in controlling the overall isotopic signature in otoliths. Radtke et al. (1996) demonstrated a fractionation of approximately 15-19‰ between Atlantic cod otolith $\delta^{13}C$ and respective diets. The red drum otoliths in

our experiment were enriched in ^{13}C by 16.70‰ and 15.84‰ for combined Group A and Group B fish, respectively (Figure 32). Similar fractionation was displayed when data was split into age classes. However, otoliths from all older fish of were slightly ^{13}C depleted than the younger fish. Group Ab fish were depleted (0.17‰) and group B fish were depleted (0.03‰) compared to the younger fish in their respective treatment group. This depletion between the age classes occurred while both diets from the two portions of the experiment became slightly enriched (0.30-0.35‰). The otolith depletion was within the standard deviations of the mean $\delta^{13}C$ from both diet groups suggesting this shift in ^{13}C may be within the normal range of otolith $\delta^{13}C$ and inconsequential.

The reported $\delta^{13}C$ of otolith carbon in this experiment were within a similar range as reported by others for species of marine fish (Iacucim et al., 1992; Kalish, 1991; Gao et al., 2001). Degens et al. (1969) point out freshwater fish exhibit much more negative $\delta^{13}C$ values (-11.0 to -13.0) than marine species, which may be a reflection of a higher consumption of respiratory CO_2 or reflect lower DIC values of freshwater relative to seawater (Iacucim et al., 1992). Striped mullet, *Mugil cephalus*, a species that traverses between freshwater and seawater had $\delta^{13}C$ values of otoliths ranging from –8.89 to -0.10‰ possibly illustrating salinity effects on otolith $\delta^{13}C$ (Iacucim et al., 1992). However, it also may be plausible that this wide range of salinities reflects the variation in the primary food sources from these areas of vastly differing salinities (Weidman and Millner, 2000).

During the course of this experiment the monthly salinities were relatively constant maintaining approximately 30ppt, though there was some fluctuation in daily salinities most likely corresponding to localized rain fall. Typically, seawater DIC becomes more positive with increasing salinity (Chanton and Lewis, 1999). With the overall variation in seawater salinities minimal, the range in DIC $\delta^{13}C$ remained relatively constant hovering around the mean (3.73 ± 0.67%) with some apparent fluctuations most likely corresponding to daily salinity changes. Also, DIC $\delta^{13}C$ values

faintly increased in the second half of the experiment, primarily toward the end of the experiment in which the salinity decreased somewhat.

Some evidence exists that demonstrates that variations in otolith $\delta^{13}C$ corresponds to differences in salinity and seawater DIC (Schwarcz et al., 1998; Bastow et al., 2002). For instance, Schwarcz et al. (1998) observed an increase in otolith $\delta^{13}C$ of Atlantic cod (*Gadus morhua*) with age through the first six years of life. This increase was attributed, in part, to cod migration towards deeper waters where DIC levels are lower and slowdown of metabolism with age. However, the majority of the change in otolith $\delta^{13}C$ was suggested to be reflecting an increase in dietary carbon, which indicates an upward shift in trophic position. Bastow el al. (2002) observed more positive otolith $\delta^{13}C$ values of pink snapper (*Pagrus auratus*) with increasing salinity. This ^{13}C enrichment was explained to be a reflection of prey organisms rather than just simply on differences in the salinities. Similarly, Gao et al. (2001) suggested that varying values of $\delta^{13}C$ in Norwegian cod (*Gadus morhua*) could be attributed to differing dietary sources.

Metabolic contributions to otolith $\delta^{13}C$ have been estimated to be approximately 20-30% of the overall carbon (Kalish, 1991a; Weidman and Millner, 2000; Hoie et al., 2003). These studies indicate otoliths are not in isotopic equilibrium with ambient conditions, but much of the otolith carbon is derived from dissolved inorganic carbon in seawater resulting in otolith $\delta^{13}C$ values closer to seawater DIC. These studies have suggested that changes in otolith carbon isotopic signatures are controlled by either growth, metabolism, and/or diet. Thus, the overall carbon isotopic composition of fish otoliths is from metabolically derived sources and from inorganic carbon species in seawater. The present study exhibited similar results between juvenile red drum otoliths and ambient seawater. Otoliths were in close proximity with DIC $\delta^{13}C$, though both otolith groups were more positive than the surrounding seawater which is contrary to Weidman and Millner (2000) who report DIC is typically more positive than otolith aragonite. Nonetheless, juvenile red drum otoliths appear to be a mixture of seawater DIC and metabolic carbon, but the controlling factor of the overall carbon signal is

juvenile red drum otoliths apparently comes from dietary sources as postulated by Weidman and Millner (2000) and indicated in results from this experiment.

CONCLUSION

The primary objective of this study was to determine if dietary carbon sources are reflected in the isotopic signature of juvenile red drum otoliths. Results from this experiment illustrate strong relationships between dietary carbon and otolith carbon composition. The otolith $\delta^{13}C$ fractionation is somewhat less than that of red drum muscle tissue enrichment among the diet treatment groups and within the subgroups (individual tanks). Fish muscle tissue is known to directly correlate to dietary carbon, as is the case in this experiment, while the red drum otoliths displayed similar enrichment patterns from the dietary carbon source though at more positive values.

Metabolic effects seem to be playing a significant role in the incorporation of biogenic carbon in red drum otoliths. Somatic growth relationships, such as ending lengths, growth rates, and otolith weights, showed little overall influence in the deposition of otolith $\delta^{13}C$. Fish metabolism was not specifically tested but has been demonstrated to influence otolith isotopic signals, especially with increasing fish age (Schwarcz et al., 1998; Begg and Weidman, 2001; Hoie et al., 2003). Though otolith $\delta^{13}C$ is a mixture of carbon from seawater and metabolic sources (Hoie et al., 2003), the controlling factor in the carbon signal of otoliths is metabolically derived through consumption. In the current study, the isotopic distinctions between red drum otoliths from the two diet treatment groups can be best explained by the significant differences in dietary isotopic compositions while the background carbon is mainly derived from seawater dissolved inorganic carbon sources.

The findings of this study compliment existing data that examine differing isotopic composition in fish otoliths. Though there is not yet definitive mechanisms reported for the processes involved with isotope incorporation into the otoliths, there seems to be evidence mounting that otolith $\delta^{13}C$ is controlled by dietary sources. Since carbon compositions of fish otoliths can vary between individuals, across geographical

regions, and ambient conditions, it is possible to distinguish populations and behavioral patterns, such as movement and feeding, of fish species. The results from this experiment indicate that all-else being equal, differences in otolith carbon signals are controlled by the carbon composition of dietary sources. Thus, analyzing otolith carbon signatures from wild specimens may give insight to feeding patterns and behaviors of individuals and among populations.

Table 1. Monthly averages of temperature (°C) and salinity (ppt) during experiment from daily measurements recorded at Florida State University Marine Laboratory.

Month	Temp (°C)	SD (°C)	Salinity (ppt)	SD (ppt)
Sep 2001	26.3	2.5	29.4	0.9
Oct 2001	22.5	2.6	30.3	1.2
Nov 2001	20.2	1.4	31.1	1.2
Dec 2001	18.3	3.5	30.9	2.8
Jan 2002	15.5	3.3	31.2	1.3
Feb 2002	16.8	2.5	31.0	1.1
Mar 2002	19.2	3.2	29.1	2.2
Apr 2002	24.4	2.2	30.1	1.1
May 2002	26.4	2.5	30.2	1.7
Jun 2002	28.7	0.8	32.0	0.1

Table 2. Monthly $\delta^{13}C$ values (‰) of Dissolved Inorganic Carbon (DIC) from seawater extracted from tanks during experiment. Data are pooled as combined monthly averages (n = 10) and combined individual samples (n =29).

			Mean	StDev	Min	Max
		N	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)
DIC pooled		10	-3.59	0.68	-4.82	-2.59
DIC all		29	-3.69	0.72	-5.42	-2.32
Sample	Month					
DIC 5	Sep	3	-4.54	0.79	-5.42	-3.92
DIC 6	Oct	3	-4.01	0.54	-4.59	-3.53
DIC 7	Nov	3	-3.31	0.29	-3.59	-3.01
DIC 8	Dec	3	-3.30	0.53	-3.87	-2.83
DIC 9	Jan	3	-3.76	0.03	-3.79	-3.73
DIC 11	Feb	3	-3.80	0.84	-4.60	-2.93
DIC 12	Mar	3	-3.33	0.36	-3.56	-2.91
DIC 13	Apr	2	-3.66	0.21	-3.81	-3.51
DIC 15	May	3	-4.57	0.41	-4.83	-4.10
DIC 17	Jun	3	-2.59	0.24	-2.75	-2.32

Table 3. Somatic growth of the red drum younger age class. "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish; "a" = younger age class.

Fish Code	Day Sampled	Age Of Fish (days)	Standard Length (mm)	Growth Rate (mm/d)	Left Otolith wt (mg)	Right Otolith wt (mg)
A1a-1	157	235	104	0.25	59.1	59.5
A1a-2	160	238	143	0.49	66.1	65.0
A1a-3	160	238	172	0.67	65.4	65.3
A1a-4	160	238	150	0.53	68.6	68.3
A1a- 5	160	238	130	0.41	56.1	56.2
A2a-1	160	238	153	0.55	67.1	66.8
A2a-2	160	238	125	0.38	43.0	47.1
A2a-3	160	238	131	0.41	59.7	59.5
A2a-4	160	238	125	0.38	60.4	68.0
A2a-5	160	238	138	0.46	56.8	57.8
A3a-1	159	237	145	0.51	67.5	66.7
A3a-2	159	237	145	0.51	77.3	76.7
A3a-3	159	237	145	0.51	68.2	68.0
A3a-4	159	237	142	0.48	65.0	63.6
A3a-5	159	237	129	0.40	43.0	44.0
A4a-1	160	238	131	0.41	67.8	68.3
A4a-2	160	238	140	0.47	66.7	65.6
A4a-3	160	238	131	0.41	54.2	54.1
A4a-4	160	238	153	0.55	69.9	71.1
A4a-5	160	238	170	0.66	77.1	76.4
B1a-1	160	238	127	0.40	45.1	44.1
B1a-2	160	238	147	0.53	54.7	55.0
B1a-3	160	238	132	0.43	51.1	48.5
B1a-4	160	238	134	0.45	48.8	47.4
B1a-5	160	238	150	0.55	75.5	74.8
B2a-1	160	238	150	0.55	56.7	56.3
B2a-2	160	238	123	0.38	44.4	43.0
B2a-3	160	238	135	0.45	57.6	56.0
B2a-4	160	238	162	0.62	64.1	64.2
B2a-5	160	238	119	0.35	39.0	30.8
B3a-1	160	238	137	0.46	52.1	51.8
B3a-2	160	238	115	0.33	35.7	34.7
B3a-3	160	238	105	0.26	35.1	29.1
B3a-4	160	238	157	0.59	61.5	61.0
B3a-5	160	238	152	0.56	56.0	57.6
B4a-1	160	238	103	0.25	38.0	39.3
B4a-2	160	238	134	0.45	43.6	43.9
B4a-3	160	238	142	0.50	48.2	49.1
B4a-4	160	238	145	0.51	58.2	59.7
B4a-5	160	238	151	0.55	62.1	61.6

Table 4. Somatic growth of older age class of red drum as measured by standard lengths in millimeters (SL mm), growth rates in millimeters per day (mm/d), and otolith weights in milligrams (mg) of individual fish that were culled on approximately Day 261 of the experiment. "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish; "b" = older age class.

Fish Code	Day Sampled	Age Of Fish (days)	Standard Length (mm)	Growth Rate (mm/d)	Left Otolith wt (mg)	Right Otolith wt (mg)
A3b-1	261	339	177	0.43	111.6	110.3
A3b-2	261	339	210	0.56	118.2	139.9
A3b-3	261	339	206	0.54	138.9	138.1
A3b-4	261	339	217	0.58	115.6	129.5
A3b-5	261	339	212	0.57	117.6	119.2
A4b-1	257	335	230	0.64	148.2	151.4
A4b-2	257	335	239	0.68	161.0	163.8
A4b-3	257	335	198	0.52	126.3	128.0
A4b-4	257	335	203	0.54	136.8	143.8
A4b-5	257	335	208	0.56	137.7	133.4
A5b-1	258	336	208	0.56	120.7	126.6
A5b-2	261	339	204	0.54	101.8	105.2
A5b-3	261	339	193	0.49	83.2	111.0
A5b-4	261	339	210	0.56	85.0	85.8
A5b-5	261	339	186	0.47	107.3	105.4
B1b-1	261	339	233	0.65	138.7	138.3
B1b-2	261	339	250	0.72	146.1	91.4
B1b-3	261	339	176	0.43	80.6	84.0
B1b-4	261	339	233	0.65	151.4	115.1
B2b-1	261	339	196	0.51	67.5	70.4
B2b-2	261	339	189	0.48	100.5	100.6
B2b-3	261	339	147	0.32	57.4	41.0
B2b-4	261	339	238	0.67	97.4	99.6
B2b-5	261	339	199	0.52	63.6	66.0
B4b-1	261	339	179	0.45	76.5	66.6
B4b-2	261	339	196	0.51	125.1	128.0
B4b-3	261	339	140	0.30	44.7	46.9
B4b-4	261	339	195	0.51	88.9	88.6
B4b-5	261	339	198	0.52	74.8	90.3
B5b-1	261	339	240	0.68	131.7	132.2
B5b-2	261	339	194	0.50	97.7	94.3
B5b-3	261	339	211	0.57	120.4	122.1
B5b-4	261	339	170	0.41	70.2	65.5
B5b-5	261	339	219	0.60	122.1	123.7

Table 5. Growth to standard length in millimeters of red drum subgroups during experiment. "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish; "a" = younger age class; "b" = older age class.

Subgroup	N	Mean SL mm	StDev SL mm	Min SL mm	Max SL mm
Aa all	20	140.1	15.8	104.0	172.0
A1a	5	139.8	25.1	104.0	172.0
A2a	5	134.4	11.7	125.0	153.0
A3a	5	141.2	7.0	129.0	145.0
A4a	5	145.0	16.6	131.0	170.0
Ba all	20	136.0	16.7	103.0	162.0
B1a	5	138.0	10.0	127.0	150.0
B2a	5	137.8	18.1	119.0	162.0
B3a	5	133.2	22.7	105.0	157.0
B4a	5	135.0	18.9	103.0	151.0
Ab all	15	206.7	15.5	177.0	239.0
A3b	5	204.4	15.8	177.0	217.0
A4b	5	215.6	17.9	198.0	239.0
A5b	5	200.2	10.3	186.0	210.0
Bb all	19	200.2	30.5	140.0	250.0
B1b	4	223.0	32.3	176.0	250.0
B2b	5	193.8	32.4	147.0	238.0
B4b	5	181.6	24.5	140.0	198.0
B5b	5	206.8	26.4	170.0	240.0

Table 6. Growth rates (millimeters per day) of red drum subgroups during experiment. "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish; "a" = younger age class; "b" = older age class.

Subgroup	N	Mean mm/d	SD mm/d	Min mm/d	Max mm/d
A all	35	0.51	0.09	0.25	0.68
Aa all	20	0.47	0.10	0.25	0.67
A1a	5	0.47	0.15	0.25	0.67
A2a	5	0.44	0.07	0.38	0.55
A3a	5	0.48	0.05	0.40	0.51
A4a	5	0.50	0.11	0.41	0.66
Ab all	15	0.55	0.06	0.43	0.68
A3b	5	0.54	0.06	0.43	0.58
A4b	5	0.59	0.07	0.52	0.68
A5b	5	0.52	0.04	0.47	0.56
B all	39	0.49	0.11	0.25	0.72
Ba all	20	0.46	0.10	0.25	0.62
B1a	5	0.47	0.06	0.40	0.55
B2a	5	0.47	0.11	0.35	0.62
B3a	5	0.44	0.14	0.26	0.59
B4a	5	0.45	0.13	0.25	0.55
Bb all	19	0.53	0.12	0.30	0.72
B1b	4	0.62	0.12	0.43	0.72
B2b	5	0.50	0.12	0.32	0.67
B4b	5	0.46	0.09	0.30	0.52
B5b	5	0.55	0.10	0.41	0.68

Table 7. Weights of red drum otoliths from diet treatment groups, subgroups and age classes. "A" = Group A fish raised on Aquamax; "B" Group B fish raised on pinfish; "a"= younger age class; "b" = older age class.

		Left Otoliths				Right Otoliths			
Subgroup	N	mean wt (mg)	SD (mg)	min (mg)	max (mg)	mean wt (mg)	SD (mg)	min (mg)	max (mg)
Aa all	20	62.95	9.17	43.00	77.30	63.40	8.50	44.00	76.70
A1a	4	63.06	5.23	56.10	68.60	62.86	4.89	56.20	68.30
A2a	5	57.40	8.89	43.00	67.10	59.84	8.39	47.10	68.00
A3a	5	64.20	12.73	43.00	77.30	63.80	12.09	44.00	76.70
A4a	5	67.14	8.29	54.20	77.10	67.10	8.29	54.10	76.40
Ab all	15	120.66	21.77	83.20	161.00	126.09	20.34	85.80	163.80
A3b	5	120.38	10.67	111.60	138.90	127.40	12.60	110.30	139.90
A4b	5	142.00	13.15	126.30	161.00	144.08	14.28	128.00	163.80
A5b	4	99.60	15.74	83.20	120.70	106.80	14.62	85.80	126.60

Subgroup	N	mean wt (mg)	SD (mg)	min (mg)	max (mg)	mean wt (mg)	SD (mg)	min (mg)	max (mg)
Ba all	20	51.38	10.55	35.10	75.50	50.40	11.72	29.10	74.80
B1a	5	55.04	11.96	45.10	75.50	53.96	12.30	44.10	74.80
B2a	5	52.36	10.32	39.00	64.10	50.06	13.18	30.80	64.20
B3a	5	48.08	12.05	35.10	61.50	46.84	14.17	29.10	61.00
B4a	5	50.02	10.02	38.00	62.10	50.72	9.73	39.30	61.60
Bb all	18	97.65	32.09	44.70	151.40	92.87	28.70	41.00	138.30
B1b	4	129.20	32.80	80.60	151.40	107.20	103.20	84.00	138.30
B2b	5	77.28	20.14	57.40	100.50	75.50	70.40	41.00	100.60
B4b	5	82.00	29.10	44.70	125.10	84.10	88.60	46.90	128.00
B5b	5	108.40	24.70	70.20	131.70	107.60	122.10	65.50	132.20

Table 8a. The overall carbon isotopic composition ($\delta^{13}C$) of two diets fed to the red drum throughout the experiment.

Diet	N	MEAN $\delta^{13}C$ (‰)	SD (‰)	MIN (‰)	MAX (‰)
Pinfish (B)	23	−17.52	1.62	−22.64	−15.07
Aquamax (A)	4	−19.64	0.21	−19.87	−19.46

Table 8b. Carbon isotopic composition ($\delta^{13}C$) of two diets fed to the red drum during the two portions of experiment. “a” = first five months; “b” = final four months.

Diet	N	MEAN $\delta^{13}C$ (‰)	SD (‰)	MIN (‰)	MAX (‰)
Pinfish Ba	16	−17.61	1.84	−22.64	−15.31
Pinfish Bb	7	−17.31	1.05	−18.09	−15.07
Aquamax Aa	2	−19.82	0.08	−19.87	−19.76
Aquamax Ab	2	−19.47	0.01	−19.47	−19.46

Table 9. Stable isotope data ($\delta^{13}C$) of individual red drum muscle tissue and otoliths from diet Group A (Aquamax). * = outliers; "na" = data not available; "a" = younger fish; "b"= older fish.

	Muscle tissue		Otoliths					
Fish			Left		Right		Both	
Code	$\delta^{13}C$ (‰)	SD (‰)	$\delta^{13}C$ (‰)	SD (‰)	$\delta^{13}C$ (‰)	SD (‰)	$\delta^{13}C$ (‰)	SD (‰)
A1a-1	-18.12	0.12	-2.49	0.15	-2.61	0.16	-2.55	0.08
A1a-2	-18.30	0.02	-2.75	0.02	-2.84	0.23	-2.79	0.06
A1a-3	-16.46*	0.06	-1.17*	0.04	-1.42*	0.06	-1.29*	0.18
A1a-4	-18.22	0.11	-2.71	0.14	-2.86	0.09	-2.78	0.10
A1a-5	-18.03	0.12	-3.08	0.20	-3.07	0.33	-3.07	0.01
A2a-1	-18.24	0.04	-3.10	0.07	-2.98	0.11	-3.04	0.09
A2a-2	-18.36	0.07	-3.20	0.06	-3.11	0.22	-3.15	0.07
A2a-3	-18.16	0.04	-3.25	0.05	-3.19	0.13	-3.22	0.04
A2a-4	-18.05	0.06	-2.71	0.01	-2.75	0.06	-2.73	0.03
A2a-5	-18.20	0.04	-2.62	0.11	-2.59	0.21	-2.60	0.02
A3a-1	-18.26	0.01	-3.02	0.13	-2.60	0.16	-2.81	0.29
A3a-2	-18.14	0.02	-3.26	0.13	-3.07	0.21	-3.16	0.14
A3a-3	-18.11	0.1	-2.98	0.18	-2.99	0.01	-2.98	0.01
A3a-4	-18.14	0.06	-3.00	0.00	-2.73	0.05	-2.86	0.19
A3a-5	-18.01	0.06	-3.00	0.12	-2.80	0.11	-2.90	0.14
A4a-1	-18.17	0.05	-2.71	0.03	-2.85	0.06	-2.78	0.10
A4a-2	-18.16	0.03	-2.99	0.13	-2.90	0.00	-2.95	0.06
A4a-3	-18.12	0.02	-2.81	0.12	-2.88	0.28	-2.84	0.05
A4a-4	-18.32	0.01	-2.63	0.08	-2.94	0.10	-2.78	0.22
A4a-5	-18.23	0.06	-2.33	0.10	-2.55	0.12	-2.44	0.15
A3b-1	-18.08	0.01	-2.82	0.04	-2.97	0.01	-2.89	0.10
A3b-2	-18.16	0.03	-3.21	0.11	-3.21	0.11	-3.21	0.00
A3b-3	-18.13	0.04	-3.13	0.07	-2.98	0.03	-3.06	0.11
A3b-4	-18.23	0.01	-2.91	0.01	-2.84	0.03	-2.87	0.05
A3b-5	-18.19	0.02	-2.69	0.08	-2.63	0.21	-2.66	0.04
A4b-1	-17.82	0.03	-3.51	0.22	-3.49	0.02	-3.50	0.01
A4b-2	-17.85	0.15	-2.94	0.19	-3.09	0.30	-3.01	0.11
A4b-3	na	na	-3.08	0.08	-3.32	0.04	-3.20	0.17
A4b-4	-17.63	0.05	-2.97	0.01	-2.92	0.13	-2.94	0.04
A4b-5	-18.25	0	-2.97	0.11	-3.12	0.24	-3.04	0.11
A5b-1	-17.91	0.06	-2.96	0.13	-3.40	0.04	-3.18	0.31
A5b-2	-18.15	0.06	-2.61	0.01	-2.86	0.07	-2.73	0.18
A5b-3	-18.26	0.03	-3.14	0.21	-3.26	0.10	-3.20	0.09
A5b-4	-16.29*	0.01	-1.80*	0.07	-1.95*	0.04	-1.88*	0.11
A5b-5	-18.11	0.01	-2.97	0.12	-2.95	0.04	-2.96	0.01

Table 10. Stable isotope data ($\delta^{13}C$) of individual red drum muscle tissue and otoliths from diet Group B (pinfish). * = outliers; "a" = younger fish; "b" = older fish.

	Muscle tissue		Otoliths					
Fish Code			Left		Right		Both	
	$\delta^{13}C$ (‰)	SD (‰)	$\delta^{13}C$ (‰)	SD (‰)	$\delta^{13}C$ (‰)	SD (‰)	$\delta^{13}C$ (‰)	SD (‰)
B1a-1	-15.88	0.01	-1.47	0.06	-1.60	0.06	-1.53	0.09
B1a-2	-16.18	0.01	-1.29	0.16	-1.46	0.45	-1.37	0.12
B1a-3	-15.86	0.00	-1.66	0.06	-1.67	0.01	-1.66	0.00
B1a-4	-16.07	0.03	-1.76	0.05	-1.59	0.14	-1.67	0.12
B1a-5	-16.05	0.02	-1.31	0.12	-1.47	0.09	-1.39	0.11
B2a-1	-16.15	0.02	-1.58	0.13	-1.83	0.21	-1.70	0.18
B2a-2	-16.28	0.08	-1.87	0.00	-1.75	0.28	-1.81	0.09
B2a-3	-16.38	0.04	-1.63	0.10	-1.89	0.12	-1.76	0.18
B2a-4	-16.19	0.12	-1.74	0.09	-1.56	0.08	-1.65	0.13
B2a-5	-16.13	0.00	-1.31	0.16	-1.50	0.01	-1.40	0.13
B3a-1	-16.39	0.01	-1.57	0.29	-1.57	0.03	-1.57	0.00
B3a-2	-16.49	0.04	-1.70	0.37	-1.96	0.20	-1.83	0.19
B3a-3	-16.25	0.01	-1.74	0.12	-2.29	0.05	-2.01	0.39
B3a-4	-16.26	0.02	-1.30	0.29	-1.37	0.12	-1.33	0.06
B3a-5	-16.17	0.02	-1.85	0.13	-1.70	0.08	-1.78	0.11
B4a-1	-15.92	0.08	-1.71	0.07	-1.63	0.21	-1.67	0.06
B4a-2	-15.94	0.02	-1.66	0.03	-1.74	0.30	-1.70	0.06
B4a-3	-16.12	0.03	-2.18	0.09	-1.90	0.13	-2.04	0.19
B4a-4	-16.07	0.05	-2.04	0.05	-1.81	0.02	-1.92	0.16
B4a-5	-16.05	0.07	-1.69	0.01	-1.27	0.06	-1.48	0.30
B1b-1	-15.81	0.02	-1.31	0.06	-1.25	0.14	-1.28	0.04
B1b-2	-16.00	0.05	-1.93	0.20	-1.38	0.11	-1.65	0.39
B1b-3	-16.06	0.06	-1.91	0.21	-2.02	0.05	-1.96	0.08
B1b-4	-16.02	0.04	-1.64	0.11	-1.78	0.01	-1.71	0.10
B2b-1	-15.77	0.03	-1.54	0.14	-1.56	0.24	-1.55	0.01
B2b-2	-15.51	0.02	-1.95	0.08	-1.71	0.23	-1.83	0.17
B2b-3	-15.83	0.04	-2.06	0.38	-2.16	0.01	-2.11	0.07
B2b-4	-15.75	0.01	-1.81	0.07	-1.66	0.09	-1.73	0.11
B2b-5	-15.78	0.03	-1.54	0.01	-1.58	0.23	-1.56	0.02
B4b-1	-16.01	0.06	-1.57	0.19	-1.60	0.12	-1.58	0.02
B4b-2	-16.88*	0.06	-2.80*	0.20	-3.07*	0.32	-2.93*	0.19
B4b-3	-15.39	0.05	-2.33	0.04	-2.05	0.06	-2.19	0.20
B4b-4	-15.86	0.04	-1.61	0.25	-1.17	0.29	-1.39	0.31
B4b-5	-15.74	0.01	-1.73	0.11	-1.71	0.05	-1.72	0.01
B5b-1	-16.05	0.04	-1.23	0.08	-1.27	0.10	-1.25	0.03
B5b-2	-16.02	0.01	-1.84	0.30	-1.83	0.05	-1.83	0.01
B5b-3	-15.71	0.02	-1.55	0.01	-1.52	0.07	-1.54	0.02
B5b-4	-16.29	0.01	-1.80	0.07	-1.95	0.04	-1.88	0.11
B5b-5	-15.86	0.00	-1.71	0.09	-1.68	0.04	-1.69	0.02

Table 11. Carbon isotope composition ($\delta^{13}C$) of red drum muscle from diet groups and subgroups. "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish; "a" = younger age class; "b" = older age class.

Subgroup	N	Mean $\delta^{13}C$ (‰)	SD (‰)	Min $\delta^{13}C$ (‰)	Max $\delta^{13}C$ (‰)
A all	32	-18.128	0.153	-18.360	-17.630
Aa all	19	-18.176	0.096	-18.360	-18.010
A1a	4	-18.168	0.118	-18.300	-18.030
A2a	5	-18.202	0.113	-18.360	-18.050
A3a	5	-18.132	0.089	-18.260	-18.010
A4a	5	-18.200	0.078	-18.320	-18.120
Ab all	13	-18.059	0.195	-18.260	-17.630
A3b	5	-18.158	0.057	-18.230	-18.080
A4b	4	-17.887	0.261	-18.250	-17.630
A5b	4	-18.108	0.146	-18.260	-17.910
B all	38	-16.008	0.237	-16.490	-15.390
Ba all	20	-16.142	0.171	-16.490	-15.860
B1a	5	-16.008	0.136	-16.180	-15.860
B2a	5	-16.226	0.104	-16.380	-16.130
B3a	5	-16.312	0.127	-16.490	-16.170
B4a	5	-16.020	0.086	-16.120	-15.920
Bb all	18	-15.859	0.212	-16.290	-15.390
B1b	4	-15.973	0.111	-16.060	-15.810
B2b	5	-15.728	0.125	-15.830	-15.510
B4b	4	-15.750	0.264	-16.010	-15.390
B5b	5	-15.986	0.218	-16.290	-15.710

Table 12. Isotopic fractionation between diet, muscle tissue, and otoliths for all data combined, for data from first portion of experiment, and for data last portion of experiment. "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish; "a" = younger age class; "b" = older age class.

All Groups	Diet $\delta^{13}C$ (‰)	Muscle $\delta^{13}C$ (‰)	Enrichment (‰)	Otoliths $\delta^{13}C$ (‰)	Enrichment diet (‰)	Enrichment muscle (‰)
A	**-19.64**	**-18.13**	1.51	**-2.94**	16.70	15.19
B	**-17.52**	**-16.01**	1.51	**-1.68**	15.84	14.33
difference	2.12	2.12		1.26		
Groups	Diet $\delta^{13}C$ (‰)	Muscle $\delta^{13}C$ (‰)	Enrichment (‰)	Otoliths $\delta^{13}C$ (‰)	Enrichment diet (‰)	Enrichment muscle (‰)
Aa	**-19.82**	**-18.18**	1.64	**-2.86**	16.96	15.32
Ba	**-17.61**	**-16.14**	1.47	**-1.66**	15.95	14.48
difference	2.21	2.04		1.20		
Groups	Diet $\delta^{13}C$ (‰)	Muscle $\delta^{13}C$ (‰)	Enrichment (‰)	Otoliths $\delta^{13}C$ (‰)	Enrichment diet (‰)	Enrichment muscle (‰)
Ab	**-19.47**	**-18.06**	1.41	**-3.03**	16.44	15.03
Bb	**-17.31**	**-15.86**	1.45	**-1.69**	15.62	14.17
difference	2.16	2.20		1.34		

.

Table 13. Carbon isotope signatures of red drum otoliths from commercial diet treatment group, subgroups and age classes. "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish; "a"= younger age class; "b" = older age class.

		Left Otolith				Right Otolith			
Subgroup	N	δ^{13}C ‰	SD ‰	min ‰	max ‰	δ^{13}C ‰	SD ‰	min ‰	max ‰
A all	33	-2.93	0.25	-3.51	-2.33	-2.95	0.23	-3.49	-2.55
Aa all	19	-2.88	0.26	-3.26	-2.33	-2.86	0.19	-3.19	-2.55
A1a	4	-2.76	0.24	-3.08	-2.49	-2.85	0.19	-3.07	-2.61
A2a	5	-2.98	0.29	-3.25	-2.62	-2.92	0.25	-3.19	-2.59
A3a	5	-3.05	0.12	-3.26	-2.98	-2.84	0.19	-3.07	-2.60
A4a	5	-2.70	0.24	-2.99	-2.33	-2.82	0.16	-2.94	-2.55
Ab all	14	-2.99	0.22	-3.51	-2.61	-3.07	0.24	-3.49	-2.63
A3b	5	-2.95	0.22	-3.21	-2.69	-2.93	0.21	-3.21	-2.63
A4b	5	-3.09	0.24	-3.51	-2.94	-3.19	0.22	-3.49	-2.92
A5b	4	-2.92	0.22	-3.14	-2.61	-3.12	0.26	-3.40	-3.37

		Left Otolith				Right Otolith			
Subgroup	N	δ^{13}C ‰	SD ‰	min ‰	max ‰	δ^{13}C ‰	SD ‰	min ‰	max ‰
B all	38	-1.69	0.25	-2.33	-1.23	-1.67	0.25	-2.29	-1.17
Ba all	20	-1.65	0.24	-2.18	-1.29	-1.68	0.23	-2.29	-1.27
B1a	5	-1.50	0.21	-1.76	-1.29	-1.56	0.09	-1.67	-1.46
B2a	5	-1.63	0.21	-1.87	-1.31	-1.71	0.17	-1.89	-1.50
B3a	5	-1.63	0.21	-1.85	-1.30	-1.78	0.36	-2.29	-1.37
B4a	5	-1.86	0.24	-2.18	-1.66	-1.67	0.24	-1.90	-1.27
Bb all	18	-1.76	0.27	-2.33	-1.23	-1.66	0.28	-2.16	-1.17
B1b	4	-1.70	0.29	-1.93	-1.31	-1.61	0.36	-2.02	-1.25
B2b	5	-1.78	0.24	-2.06	-2.01	-1.73	0.25	-2.16	-1.56
B4b	4	-1.81	0.35	-2.33	-1.57	-1.63	0.36	-2.05	-1.17
B5b	5	-1.73	0.25	-1.84	-1.23	-1.65	0.27	-1.95	-1.27

Table 14. T-test results of otolith $\delta^{13}C$ within and between subgroups. "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish; "a"= younger age class; "b" = older age class; ∗ = significant difference.

Subgroup	Otoliths	*df*	T-test	P-value
Among				
A	L v R	63	0.41	0.690
Aa	L v R	32	-0.24	0.820
Ab	L v R	25	0.92	0.370
B	L v R	73	-0.31	0.760
Ba	L v R	37	0.34	0.740
Bb	L v R	33	-0.72	0.480
Aa v Ab	Left	30	1.39	0.170
Aa v Ab	Right	23	2.79	0.010*
Aa v Ab	Pair	57	2.93	0.005*
Ba v Bb	Left	34	0.88	0.380
Ba v Bb	Right	33	-0.21	0.830
Ba v Bb	Pair	69	0.47	0.640

Subgroup	Otoliths	*df*	T-test	p-value
Between				
A v B	Left	67	-20.81	<0.000*
A v B	Right	68	-22.12	<0.000*
A v B	Pair	138	-30.54	<0.000*
Aa v Ba	Left	36	-15.21	<0.000*
Aa v Ba	Right	36	-17.52	<0.000*
Aa v Ba	Pair	76	-23.50	<0.000*
Ab v Bb	Left	29	-14.71	<0.000*
Ab v Bb	Right	29	-15.32	<0.000*
Ab v Bb	Pair	61	-21.34	<0.000*

Table 15. One-way analysis of variance (ANOVA) of otolith $\delta^{13}C$ within subgroups. "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish; "a"= younger age class; "b" = older age class; ∗ = significant difference.

Subgroup	Otoliths	*df*	F-stat	P-value
Among				
Aab	pair	13,52	1.91	0.050*
Aa	pair	7,30	1.40	0.243
Ab	pair	5,22	1.20	0.342
Aab	left	6,26	1.96	0.109
Aa	left	3,15	2.69	0.088
Ab	left	2,11	0.79	0.478
Aab	right	6,26	2.18	0.078
Aa	right	3,15	0.25	0.859
Ab	right	2,11	1.79	0.218
Bab	pair	15,60	0.65	0.823
Ba	pair	7,32	1.29	0.288
Bb	pair	7,28	0.29	0.950
Bab	left	7,30	1.11	0.384
Ba	left	3,16	2.35	0.111
Bb	left	3,14	0.40	0.749
Bab	right	7,30	0.33	0.932
Ba	right	3,16	0.75	0.538
Bb	right	3,14	0.15	0.928

Table 16. Regression analysis between otolith weights and otolith $\delta^{13}C$. "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish; "a"= younger age class; "b" = older age class; * = significant relationship.

Subgroup	Otolith	Trend	R^2	P-value
AB	pair	negative	10.4	<0.000*
a -all	pair	negative	16.1	<0.000*
b -all	pair	negative	18.2	<0.000*
Aab	pair	negative	13.1	0.003*
Aa	pair	positive	4.2	0.218
Ab	pair	negative	11.7	0.074
Aab	left	negative	5.9	0.173
Aa	left	negative	5.9	0.318
Ab	left	negative	10.3	0.263
Aab	right	negative	23.2	0.005*
Aa	right	negative	2.3	0.540
Ab	right	negative	10.9	0.248
Bab	pair	negative	0.2	0.726
Ba	pair	positive	12.9	0.023*
Bb	pair	positive	25.8	0.002*
Bab	left	positive	2.3	0.361
Ba	left	positive	4.0	0.395
Bb	left	positive	21.2	0.055
Bab	right	positive	17.2	0.100
Ba	right	positive	25.5	0.023*
Bb	right	positive	35.9	0.009*

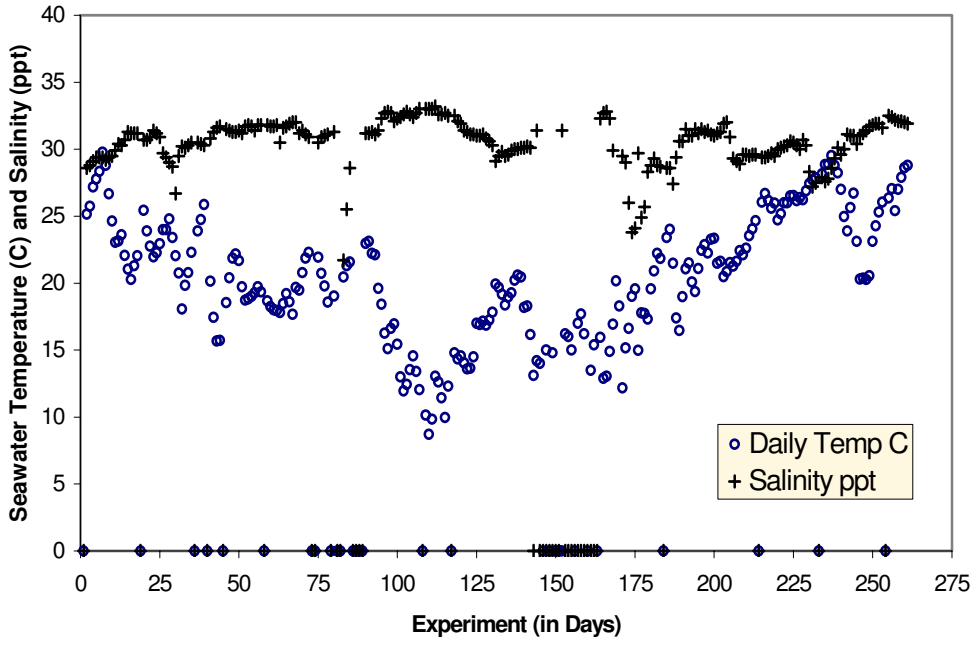


Figure 2. Daily temperature (°C) and salinity (ppt) measurements recorded at Florida State University Marine Laboratory during the experiment. Symbols on y-axis represent gaps in data. o = daily temperature; + = daily salinity.

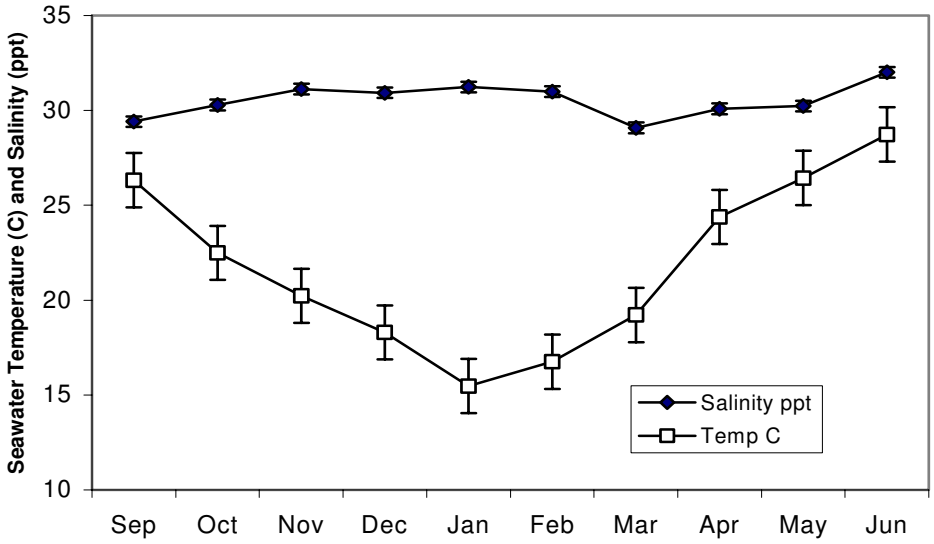


Figure 3. Average monthly temperature (°C) and salinity recordings at FSUML during experiment. • = temperature and □ = salinity.

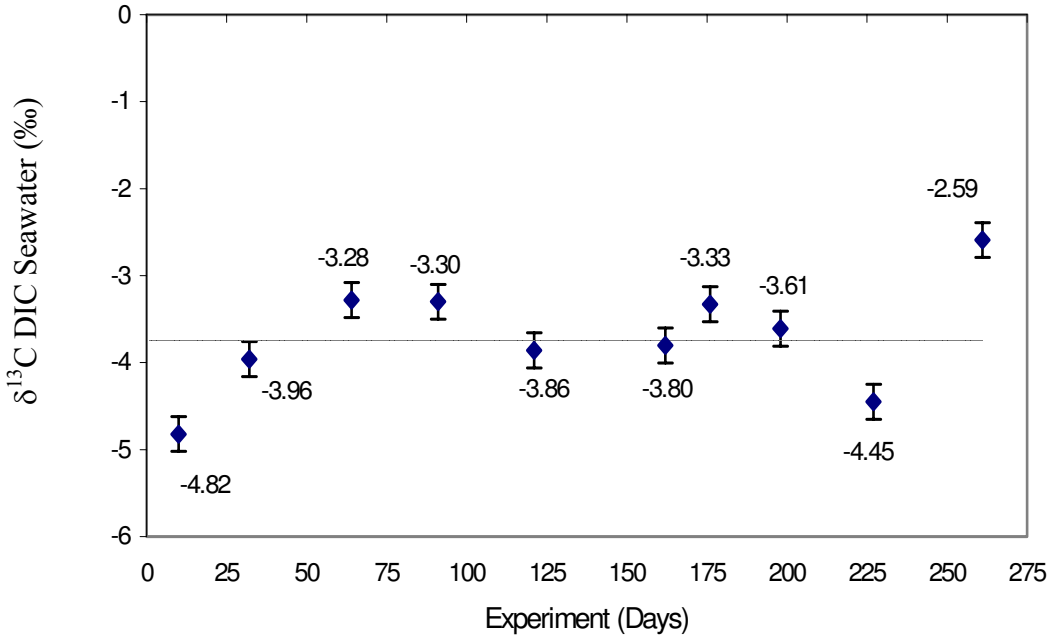


Figure 4. Carbon stable isotopic composition ($\delta^{13}C$) of seawater dissolved inorganic carbon (DIC) taken monthly over the course of the experiment. Dotted line shows mean $\delta^{13}C$ (-3.83 ± 0.81‰).

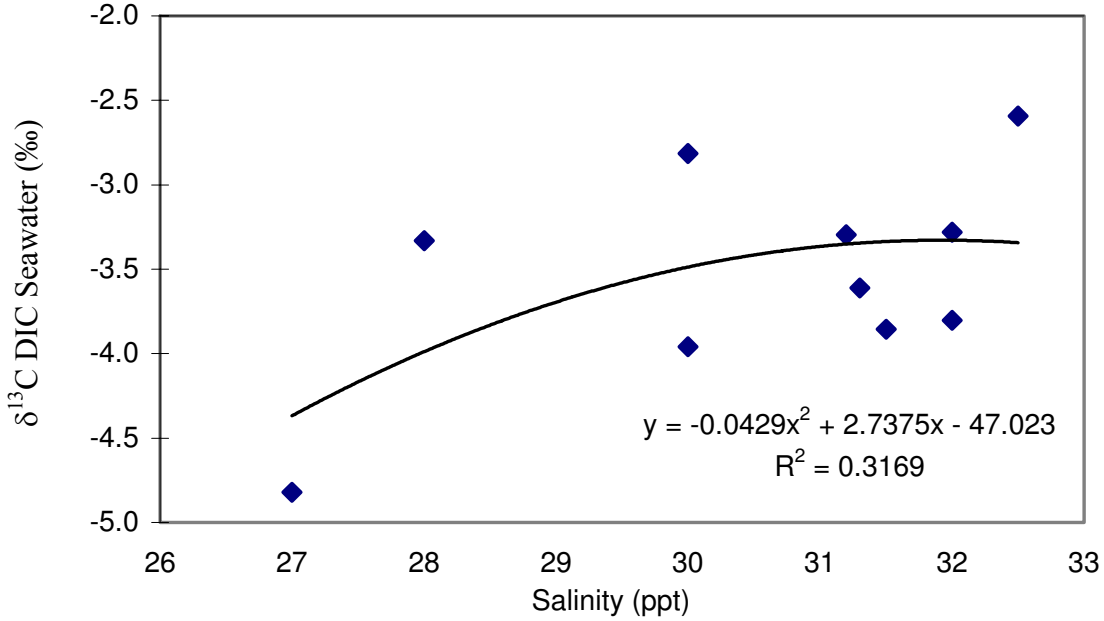


Figure 5. Regression analysis of seawater dissolved inorganic carbon (DIC) plotted against salinty (ppt) of ambient during experiment demonstrates a somewhat weak relationship ($r^2 = 0.32$).

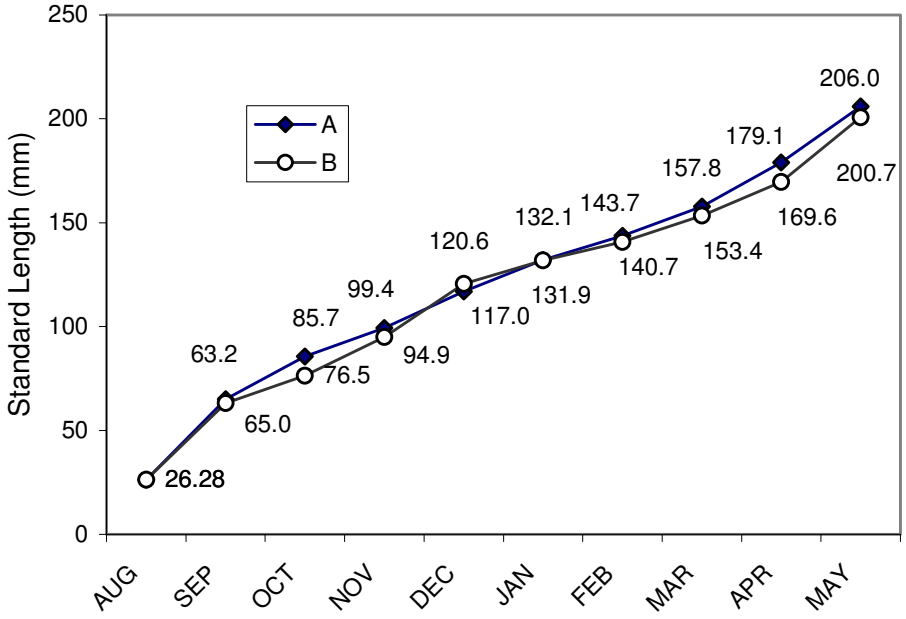


Figure 6. Ending standard lengths in millimeters (mm) of red drum from each diet group. ♦ = Group A fish raised on Aquamax; o = Group B fish raised on pinfish.

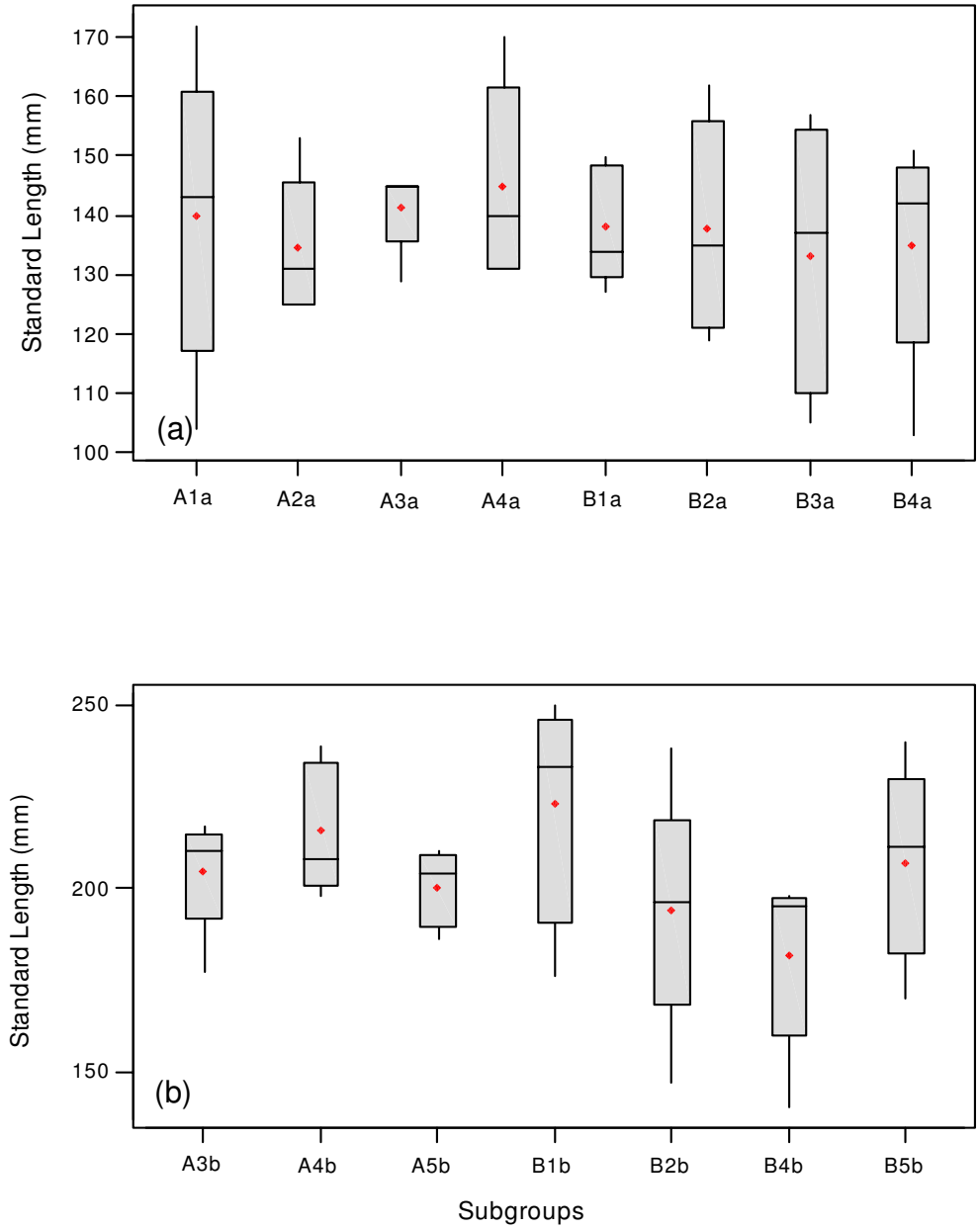


Figure 7. Ending standard lengths (SL) in millimeters (mm) of red drum from both treatment groups of the a) the younger age class (Day 161) were similar between subgroups (p = 0.966) and b) older age class (day 261) did not differ significantly (p = 0.202) between subgroups. (♦) mean standard length; "A" = Group A fish raised on Aquamax; "B" = Group B fish raised on pinfish.

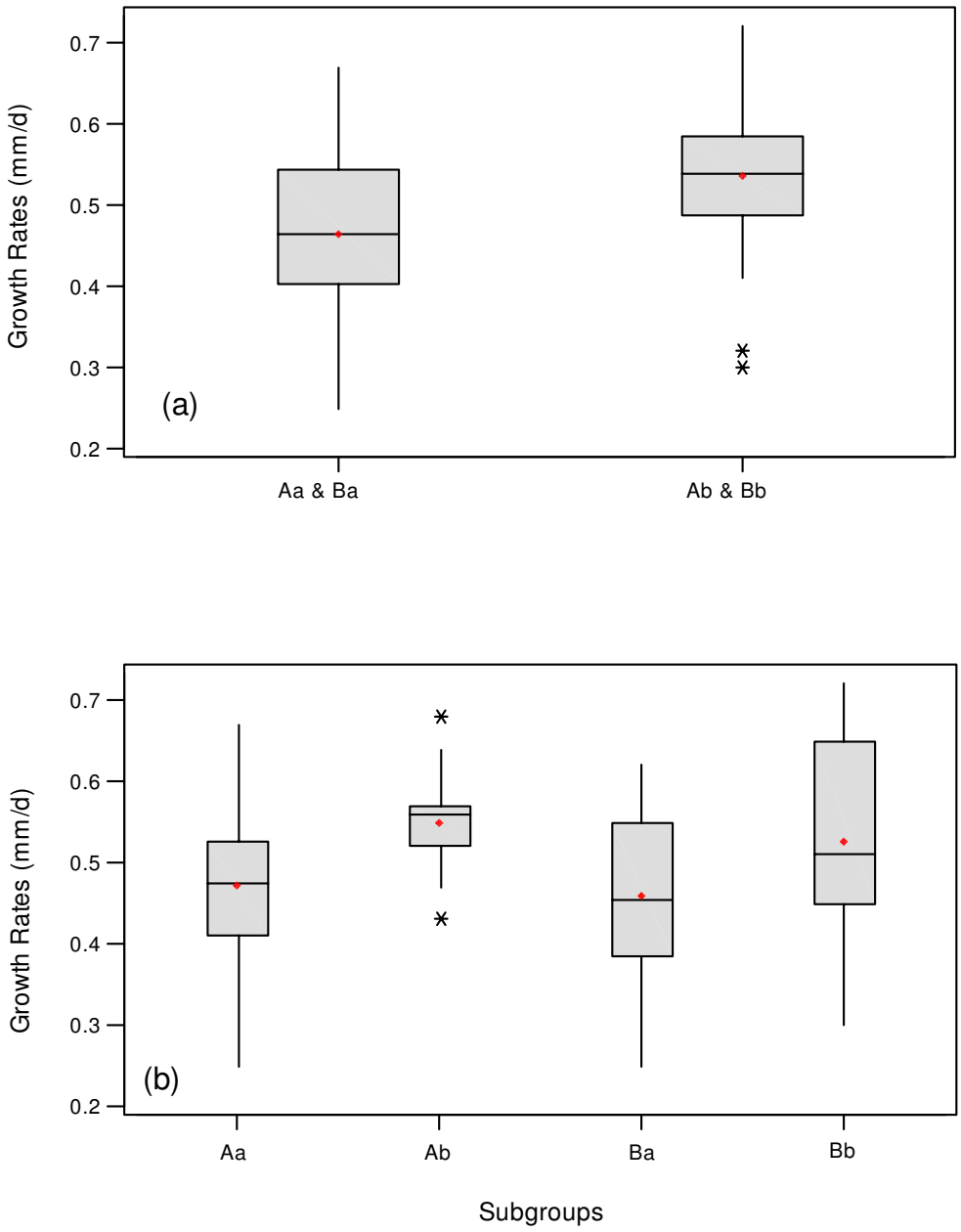


Figure 8. Growth rates in millimeters per day (mm/d) of the two age classes from diet treatment groups. (a) The younger fish from both groups combined (mean = 0.47 mm/d ± 0.10) grew significantly slower (p = 0.007) than the older fish from the two groups combined (mean = 0.54 mm/d ± 0.10). The younger fish from Group A grew slightly faster than the younger Group B fish (p = 0.677). The older fish of Group A had a somewhat faster growth rate than the older fish of Group B (p = 0.495). ∗ = data points outside lower/upper quartile, ♦ = mean growth rates.

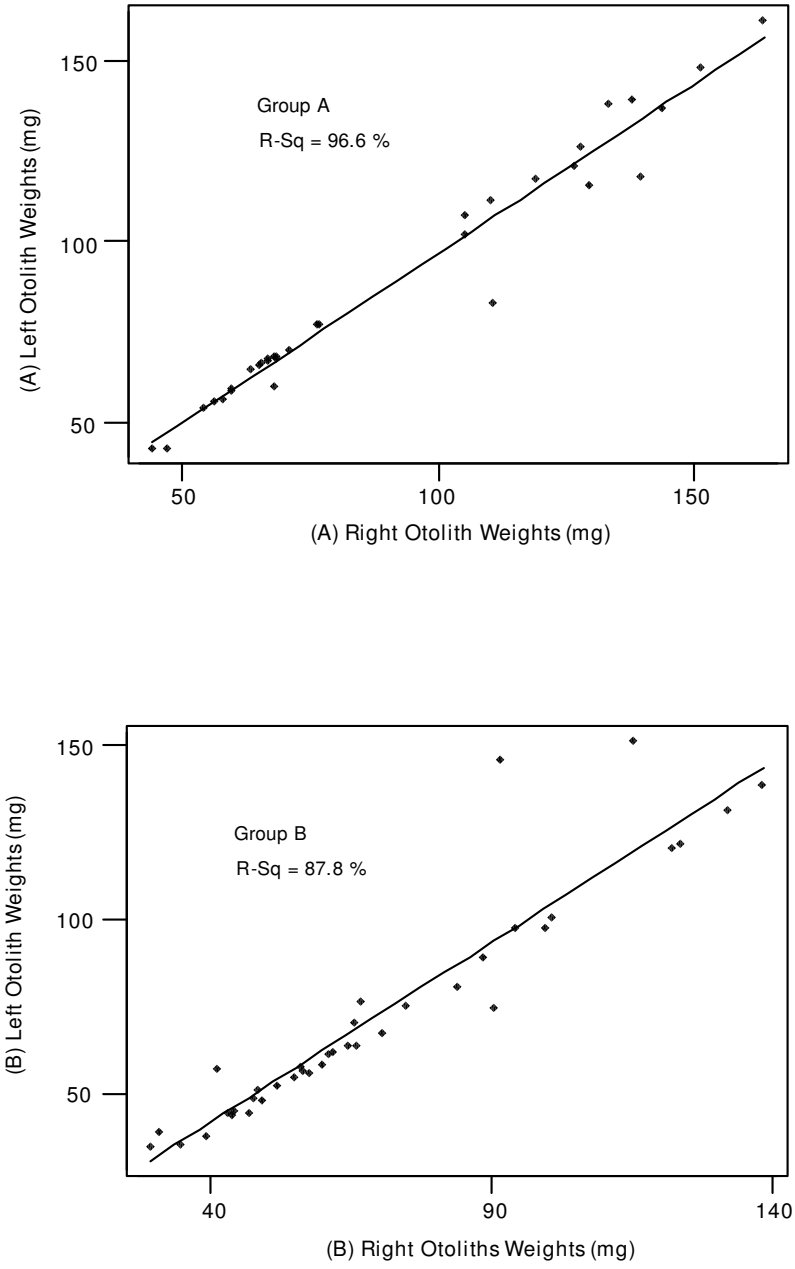


Figure 9. Regression analysis of otolith weights between pairs from (A) red drum on commercial diet ($r^2 = 0.966$, $p < 0.001$) and B) red drum on pinfish diet ($r^2 = 0.878$, $p < 0.001$).

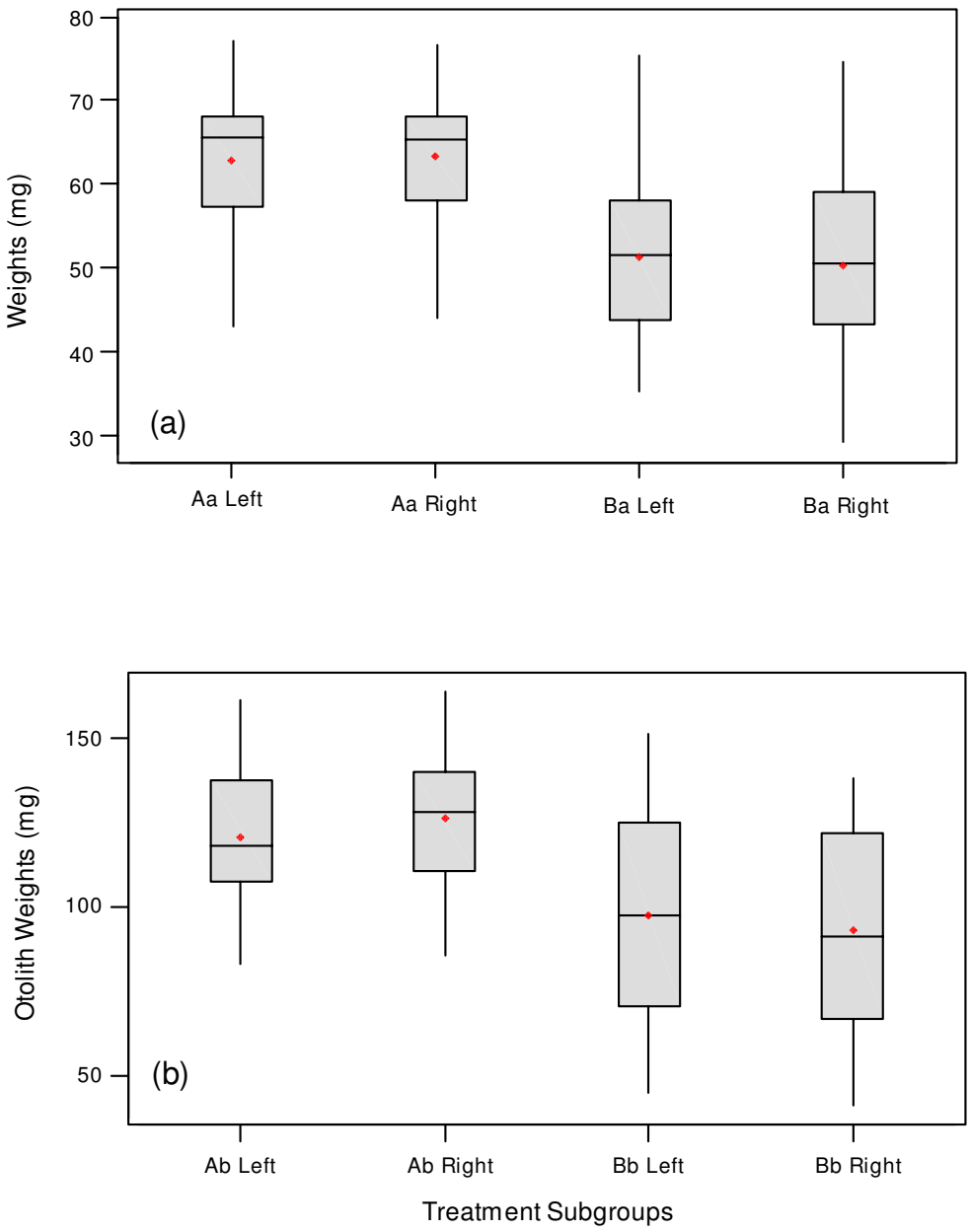


Figure 10. Otolith weights in milligrams (mg) of right and left otoliths from (a) younger age class of fish raised on Aquamax (A) and pinfish (B). Otolith pairs within each diet group were similar (Group A: $p = 0.87$; Group B: $p = 0.78$) while otolith pairs differed significantly between groups ($p < 0.001$) and (b) older age class of fish raised on Aquamax (A) and fish raised on pinfish (B). Otolith pairs within each diet group were similar (Group A: $p = 0.49$; Group B: $p = 0.63$) while otolith pairs differed significantly between groups ($p = 0.001$). ♦ = mean otolith weights.

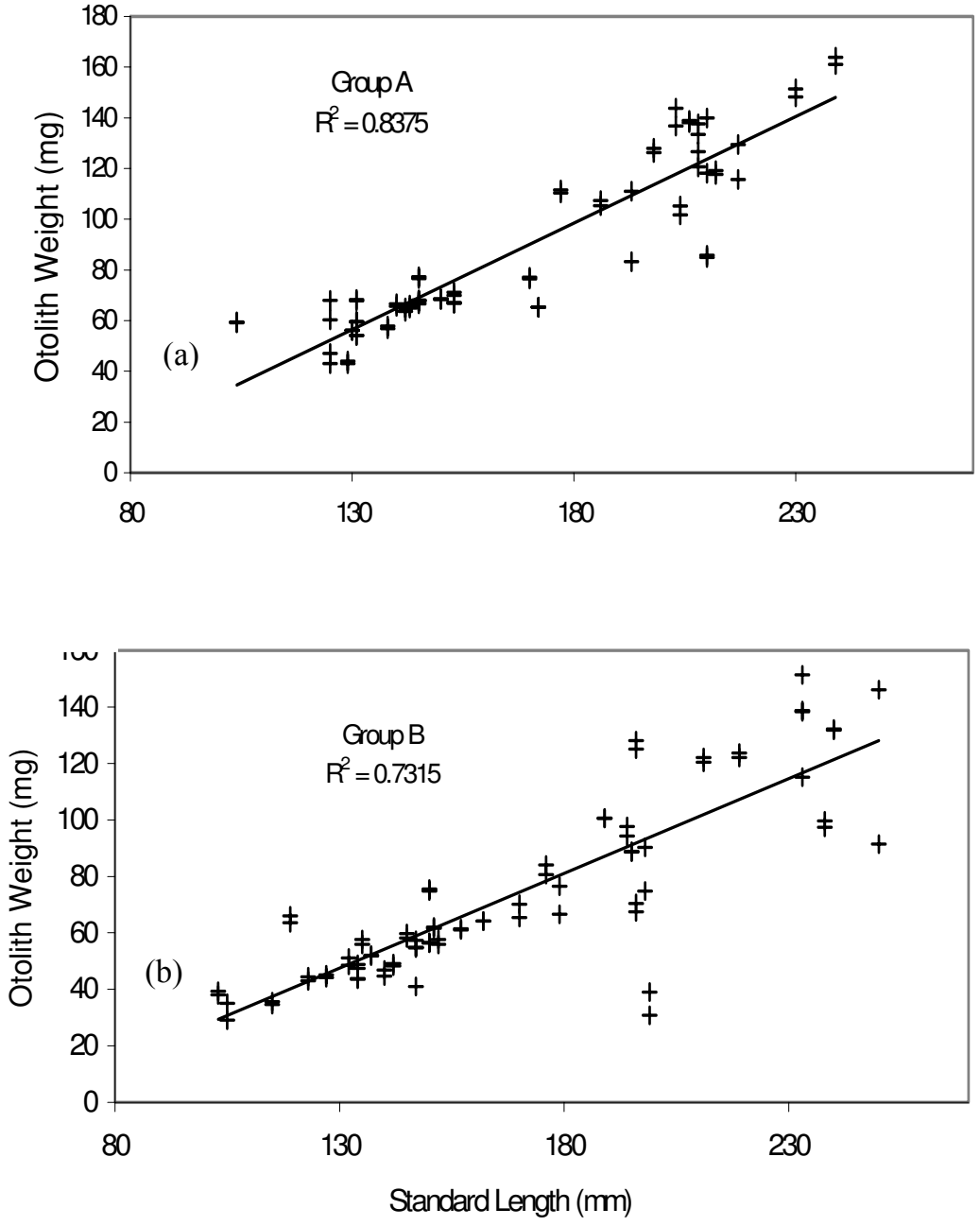


Figure 11. Regression of otolith weights in milligrams (mg) and standard lengths in millimeters (mm) of red drum. a) Group A fish raised on Aquamax had a stronger correlation ($r^2 = 0.84$) than b) Group B fish raised on pinfish ($r = 0.73$).

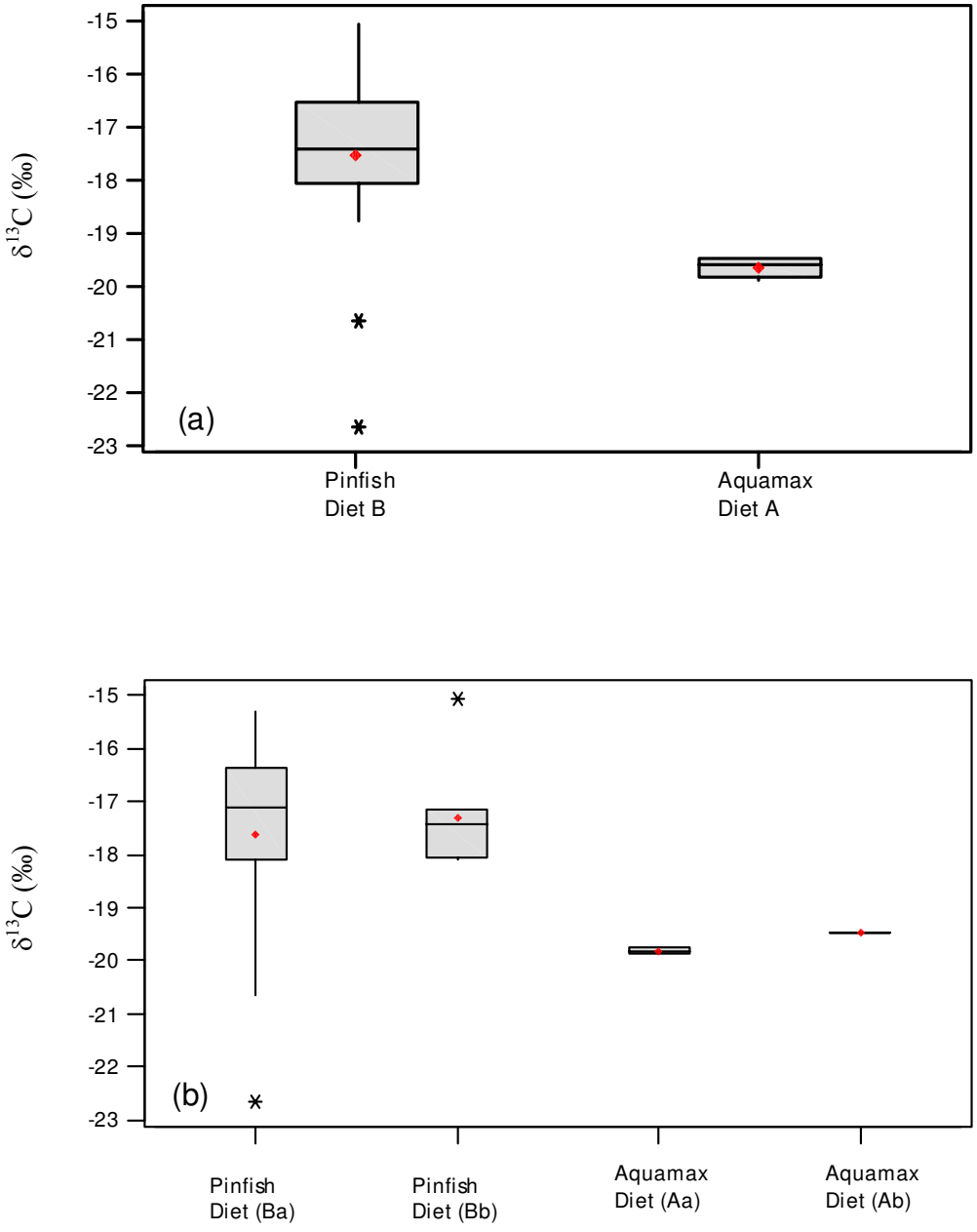


Figure 12. a) Carbon isotopic composition ($\delta^{13}C$) of the two diets fed to red drum during experiment differed by 2.12‰ ($p < 0.001$). b) Carbon isotope composition ($\delta^{13}C$) of diets fed in each part of experiment were not significantly different for the Aquamax diet ($p = 0.10$) or for the pinfish diet ($p = 0.62$). ∗ = data points outside upper or lower quartiles, ♦ = $\delta^{13}C$ means.

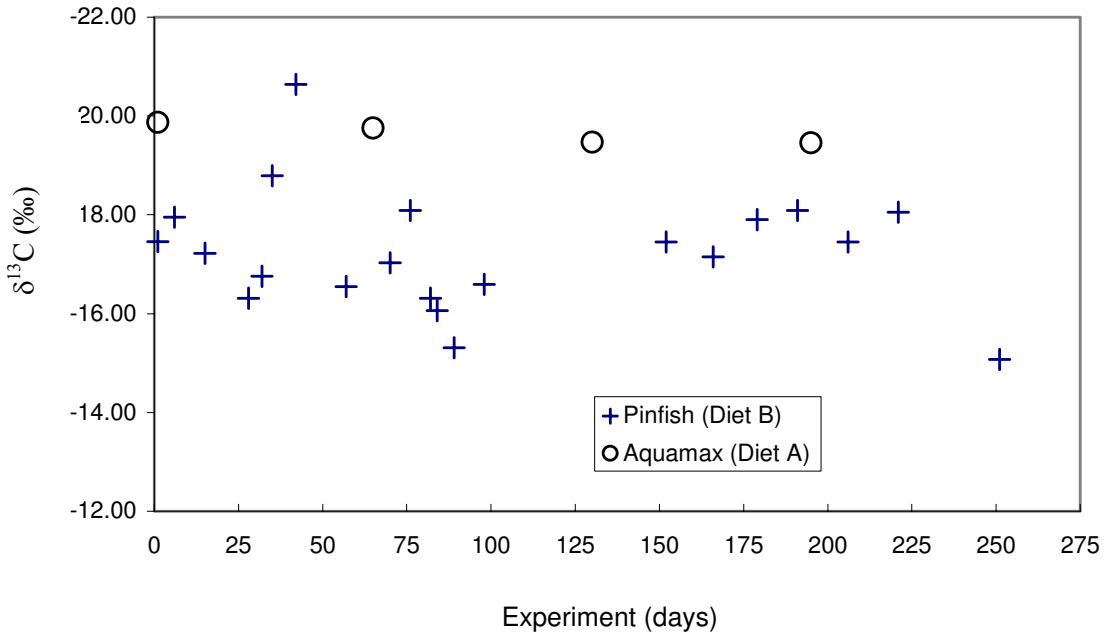


Figure 13. Carbon isotopic composition ($\delta^{13}C$) of two diets fed plotted throughout the course of the experiment (days). The Aquamax diet was much more constant (mean $\delta^{13}C = -19.64 \pm 0.21‰$) than the pinfish diet (mean $\delta^{13}C = -17.52 \pm 1.62‰$). The difference (2.12‰) between diet means was significant ($p < 0.001$). o = Aquamax (diet A) and + = pinfish (diet B).

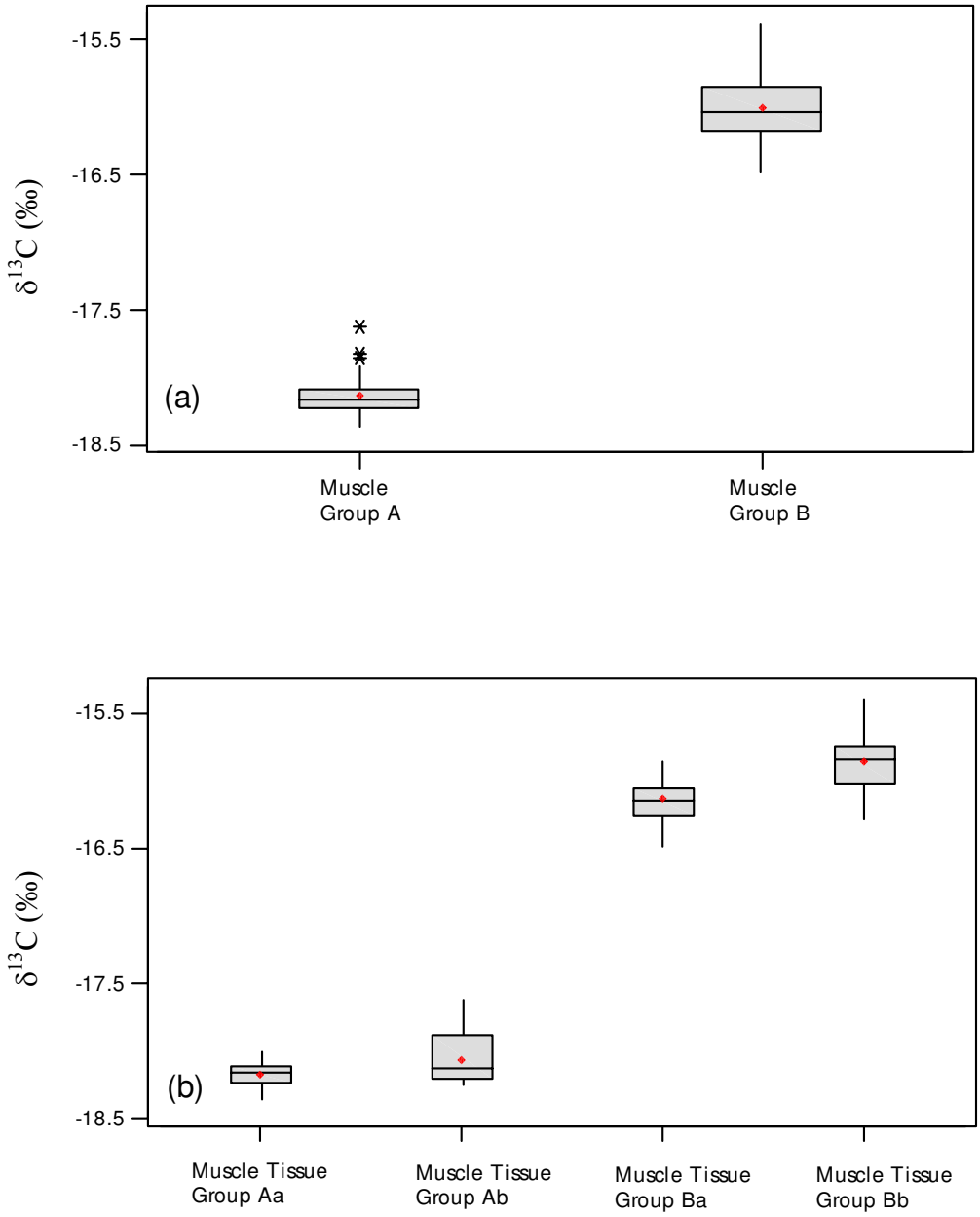


Figure 14. Carbon isotopic composition ($\delta^{13}C$) of muscle tissue from a) Group A fish (mean $\delta^{13}C$ = -18.13 ± 0.15‰) was significantly different $p < 0.0010$) than Group B muscle tissue (mean $\delta^{13}C$ = -16.01 ± 0.24‰). b) Younger fish from Group A ($\delta^{13}C$ = -18.06 ± 0.20‰) differed significantly ($p < 0.0010$) from Group B fish ($\delta^{13}C$ = -15.86 ± 0.21‰). Muscle tissue from older fish in Group A ($\delta^{13}C$ = -18.18 ± 0.10‰) differed significantly ($p < 0.0010$) from Group B fish ($\delta^{13}C$ = -16.14 ± 0.17‰). ♦ = $\delta^{13}C$ means, ∗ = data points outside upper quartiles.

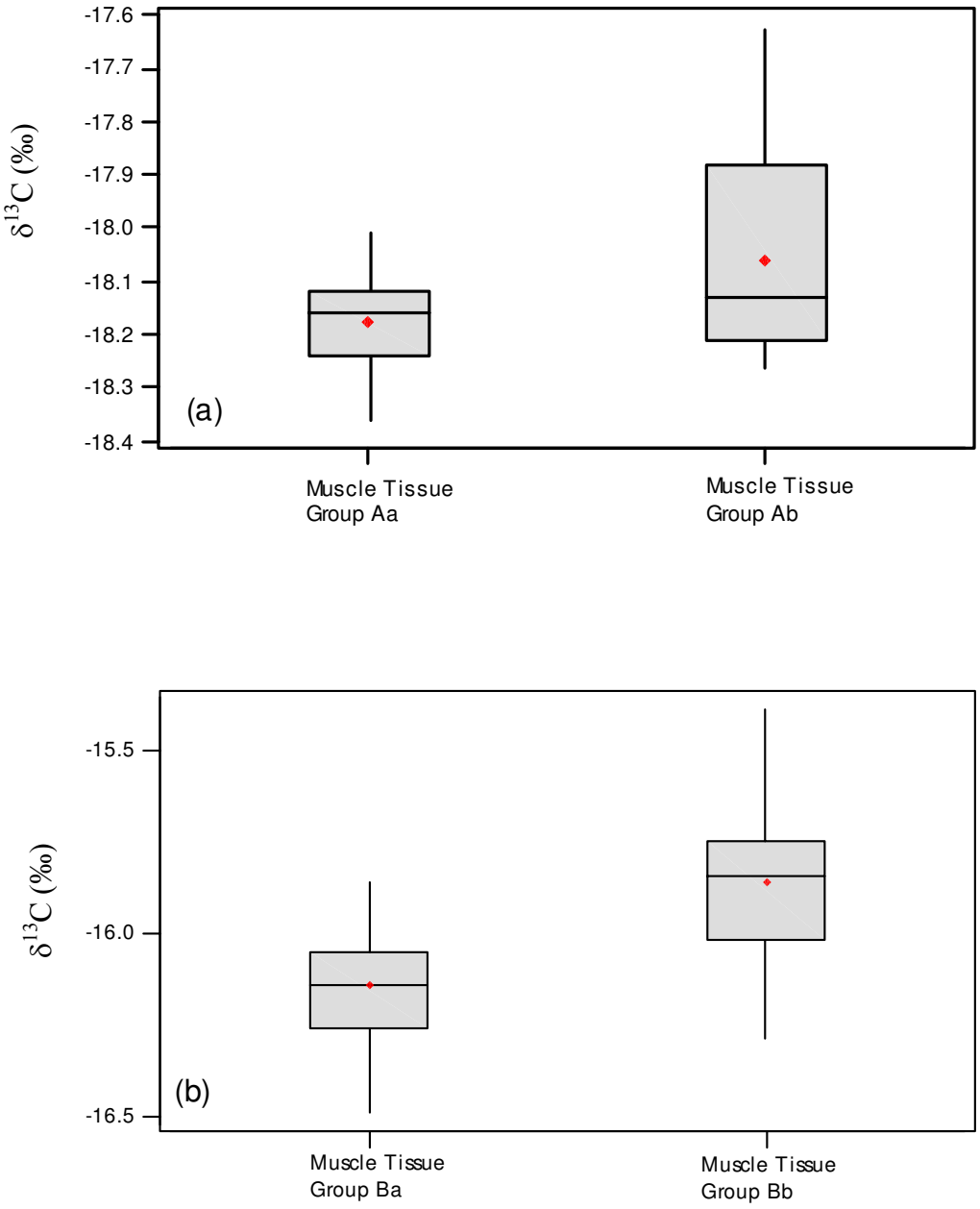


Figure 15. Carbon isotopic composition ($\delta^{13}C$) of muscle tissue a) from younger Group A fish ($\delta^{13}C$ = -18.06 ± 0.20‰) were similar (p = 0.64) to older fish in Group A ($\delta^{13}C$ = -18.18 ± 0.10‰). b) from younger Group B fish ($\delta^{13}C$ = -16.14 ± 0.17‰) were different (p = 0.0001) than older fish in Group B ($\delta^{13}C$ = -15.86 ± 0.21‰). ♦ = $\delta^{13}C$ means.

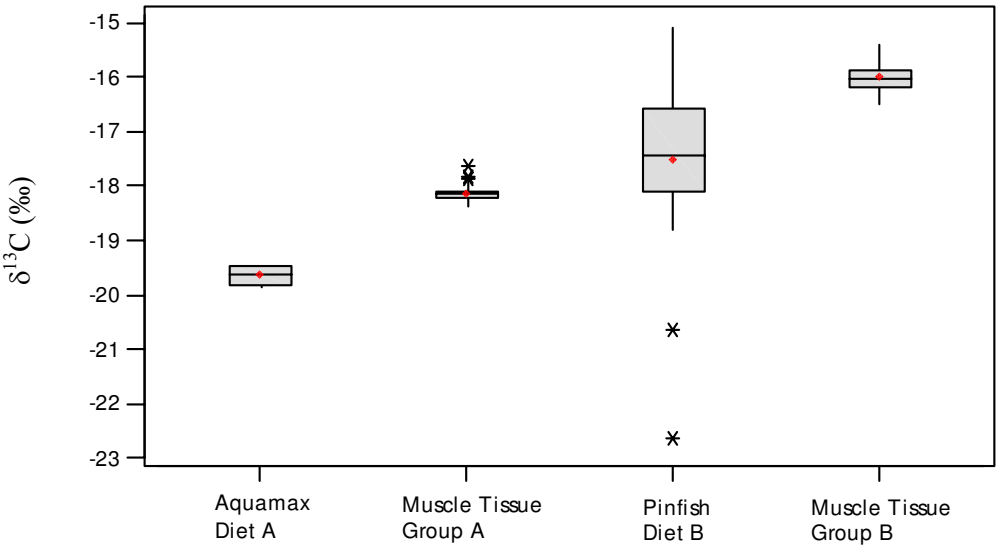


Figure 16. Carbon isotopic composition ($\delta^{13}C$) of the two diets and muscle issue from both treatment groups. Muscle tissue from Group A was enriched 1.51‰ relative to the Aquamax diet. Group B muscle tissue was enriched 1.51‰ relative to the pinfish diet. ♦ = $\delta^{13}C$ means; ∗ = data points outside upper and lower quartiles

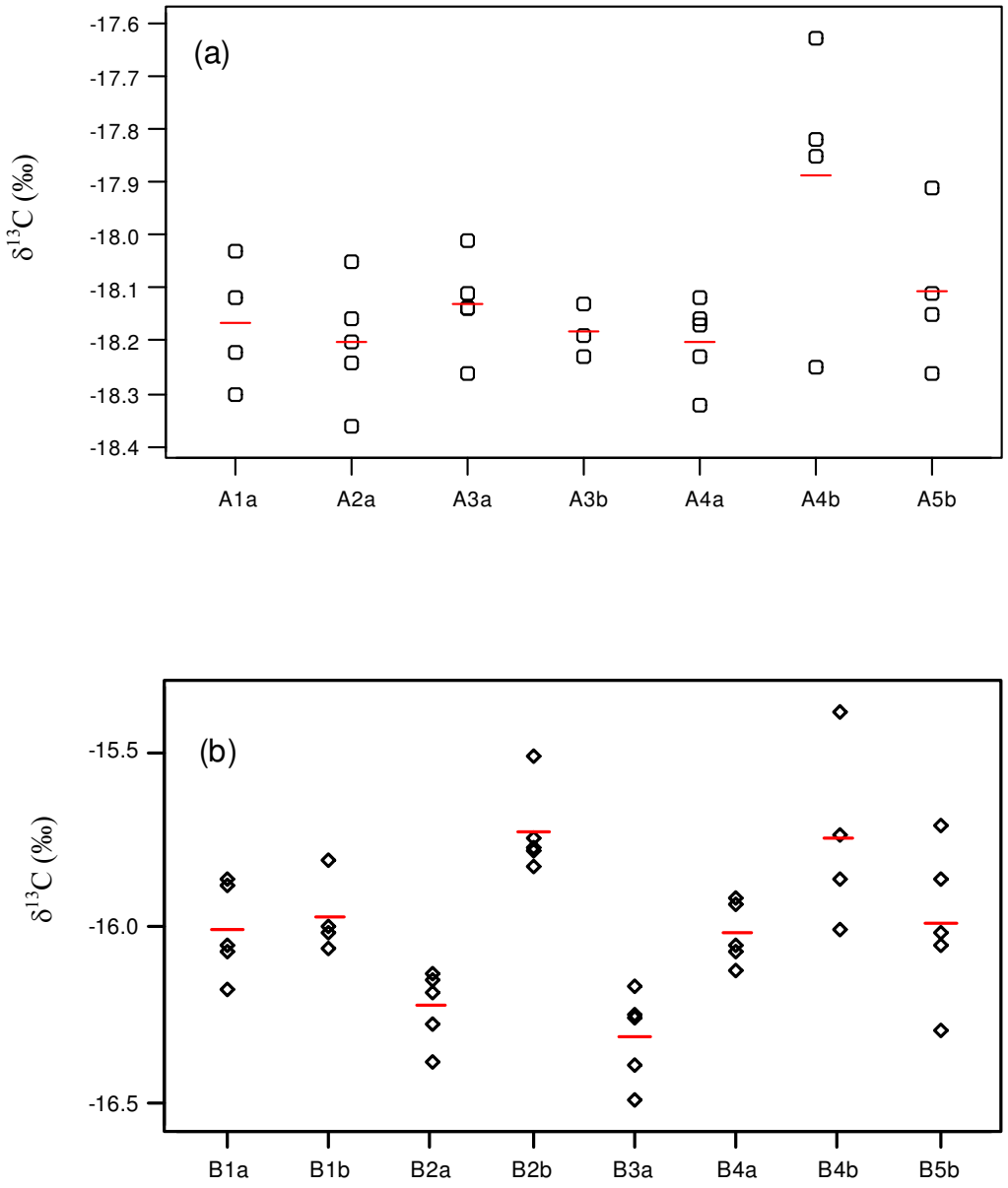


Figure 17. Carbon isotopic composition ($\delta^{13}C$) of red drum muscle tissue from subgroups of the two treatment groups. a) Group A muscle tissue was less variable ($\delta^{13}C$ range = -18.36 to -17.63‰) than b) Group B muscle tissue ($\delta^{13}C$ range = -16.49 to -15.39 ‰). ─ = $\delta^{13}C$ means.

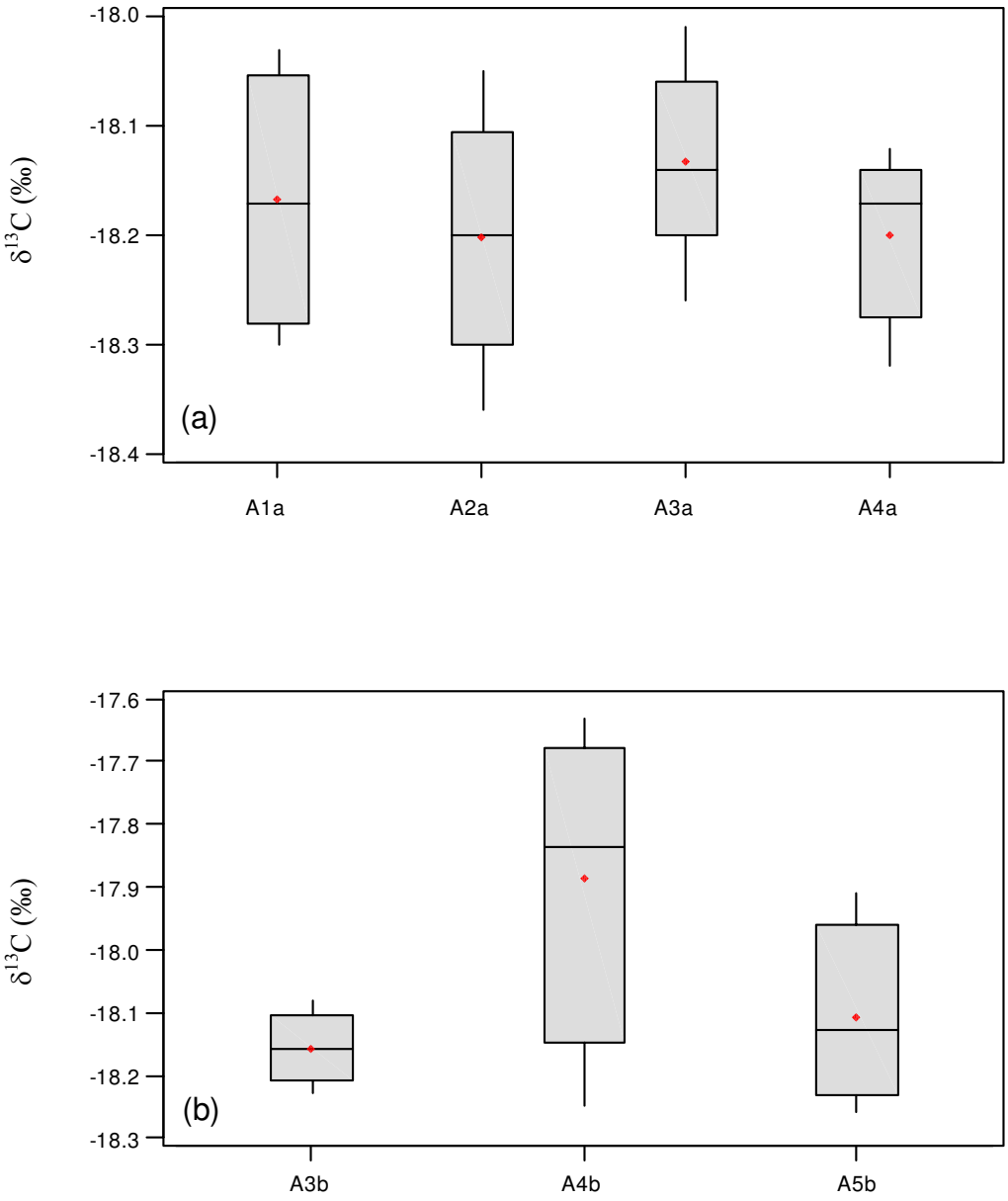


Figure 18. Carbon isotopic composition ($\delta^{13}C$) of red drum muscle tissue from subgroups in the Aquamax diet group. a) The younger fish of Group A had similar $\delta^{13}C$ values ($p = 0.659$) while b) the older fish in Group A were more varied in $\delta^{13}C$ but were not significantly different ($p = 0.088$). ♦ = mean $\delta^{13}C$.

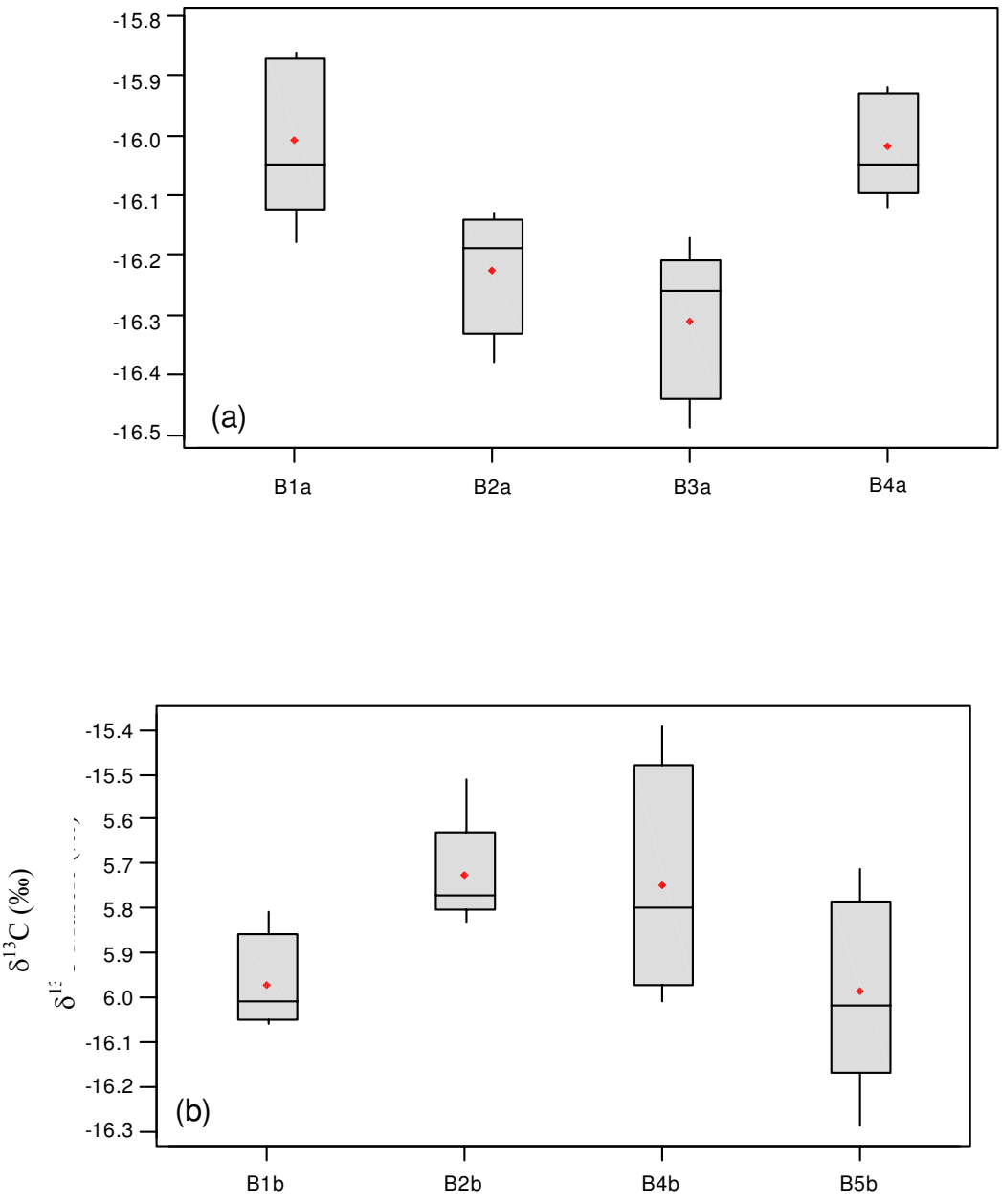


Figure 19. Carbon isotopic composition ($\delta^{13}C$) of red drum muscle tissue from subgroups in the pinfish diet group (B). a) The younger fish of Group B had significantly different $\delta^{13}C$ means ($p = 0.001$) while b) the older fish in Group B were different but not significantly ($p = 0.104$). ♦ = mean $\delta^{13}C$ values.

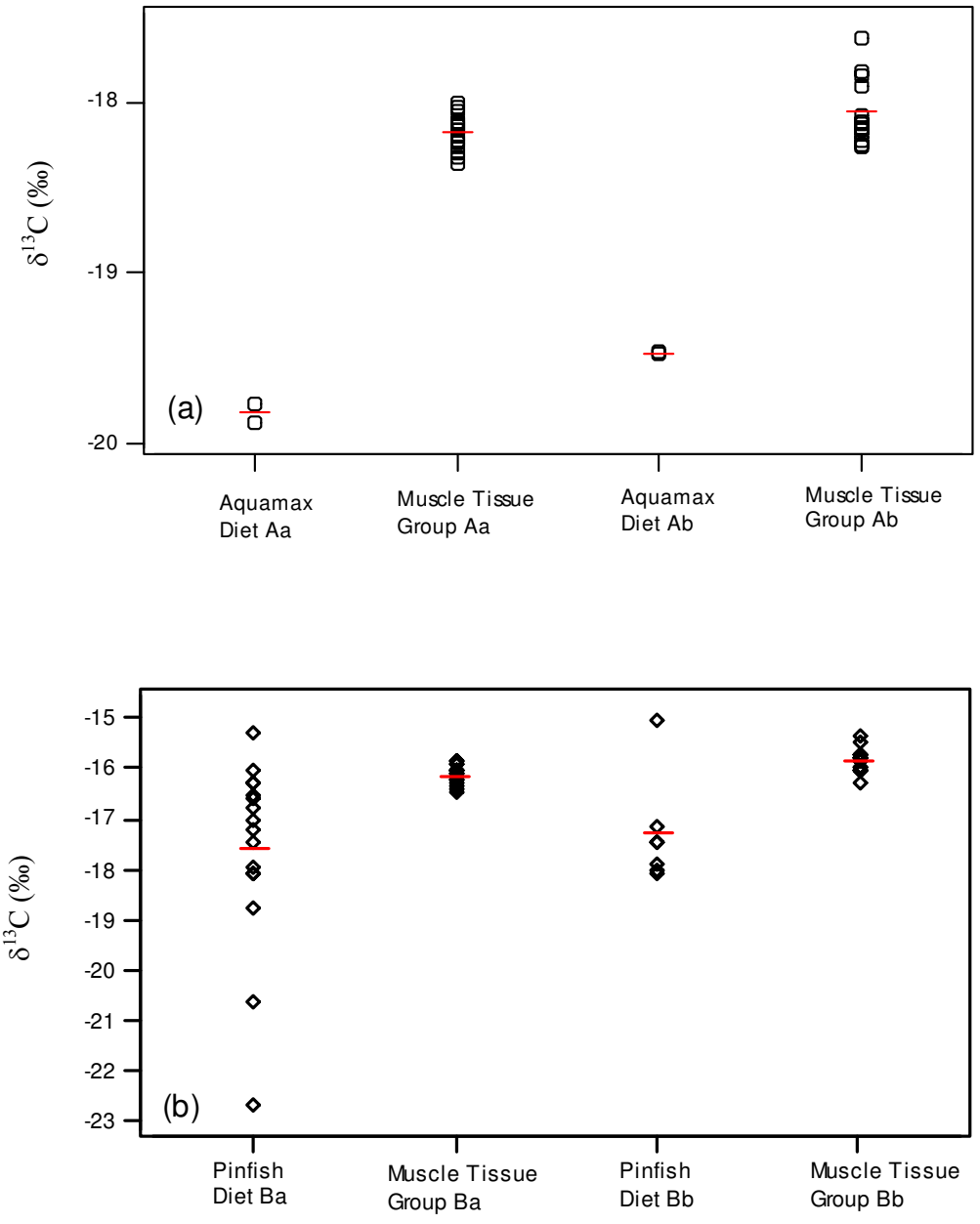


Figure 20. Carbon isotopic composition ($\delta^{13}C$) of muscle tissue and diet from the two age classes in both treatment groups. a) The muscle tissue of the Group A younger fish was enriched 1.64‰ relative to the diet and 1.41‰ in the older fish. b) The muscle tissue from the younger fish in Group B was enriched 1.47‰ relative to the diet and enriched 1.45‰ in the older fish. — = $\delta^{13}C$ means.

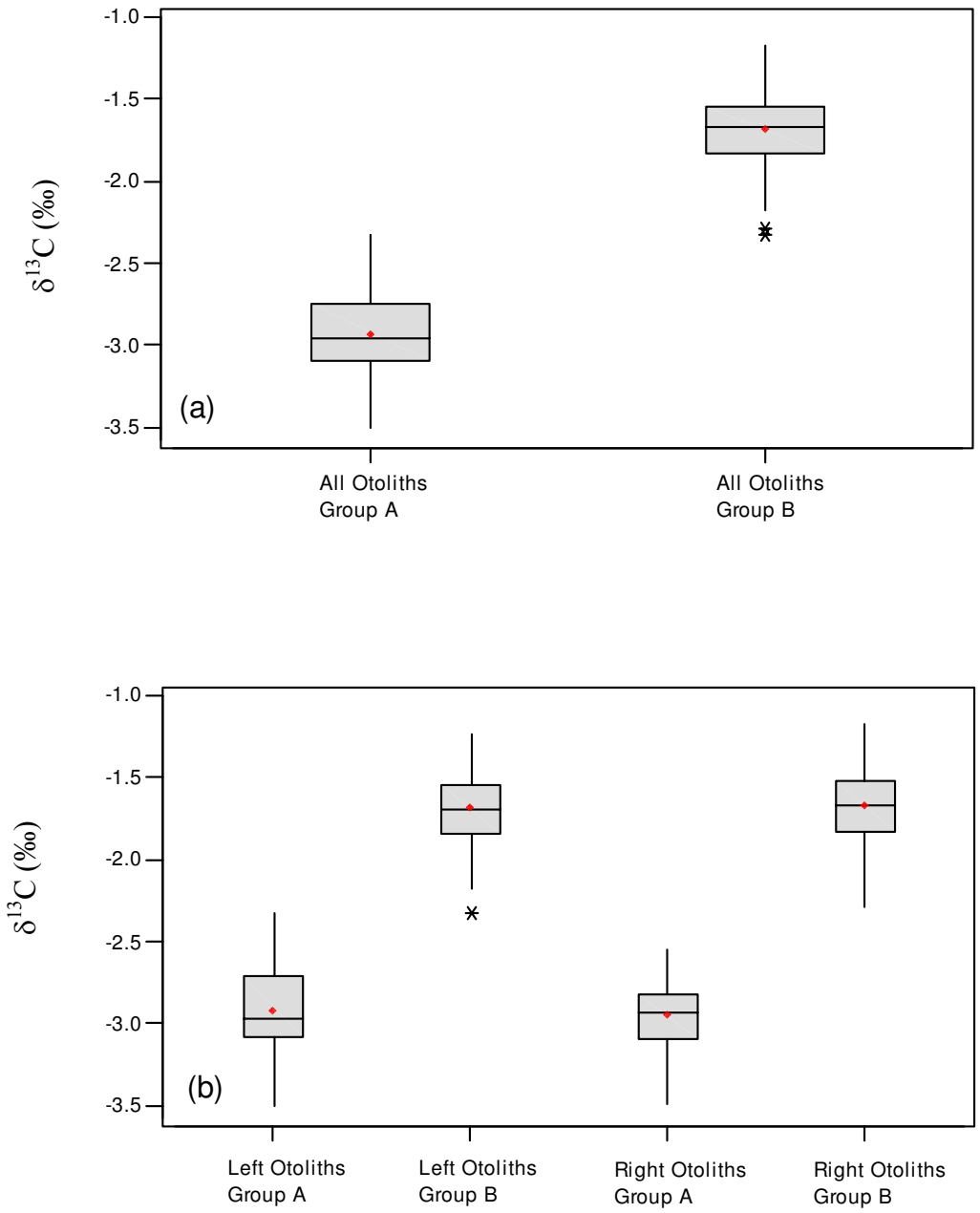


Figure 21. Carbon isotopic composition ($\delta^{13}C$) of red drum otoliths differed between diet groups. a) All otoliths combined in group A (mean $\delta^{13}C$ = -2.94 ± 0.24‰, n = 66) were significantly different ($p < 0.001$) from combined otolith pairs in Group B (mean $\delta^{13}C$ = -1.68 ± 0.25‰, n = 76). b) Otolith pairs from both treatment groups had similar means (Group A: $p = 0.69$; Group B: $p = 0.76$). ♦ = mean $\delta^{13}C$; ∗ = data outside lower quartile.

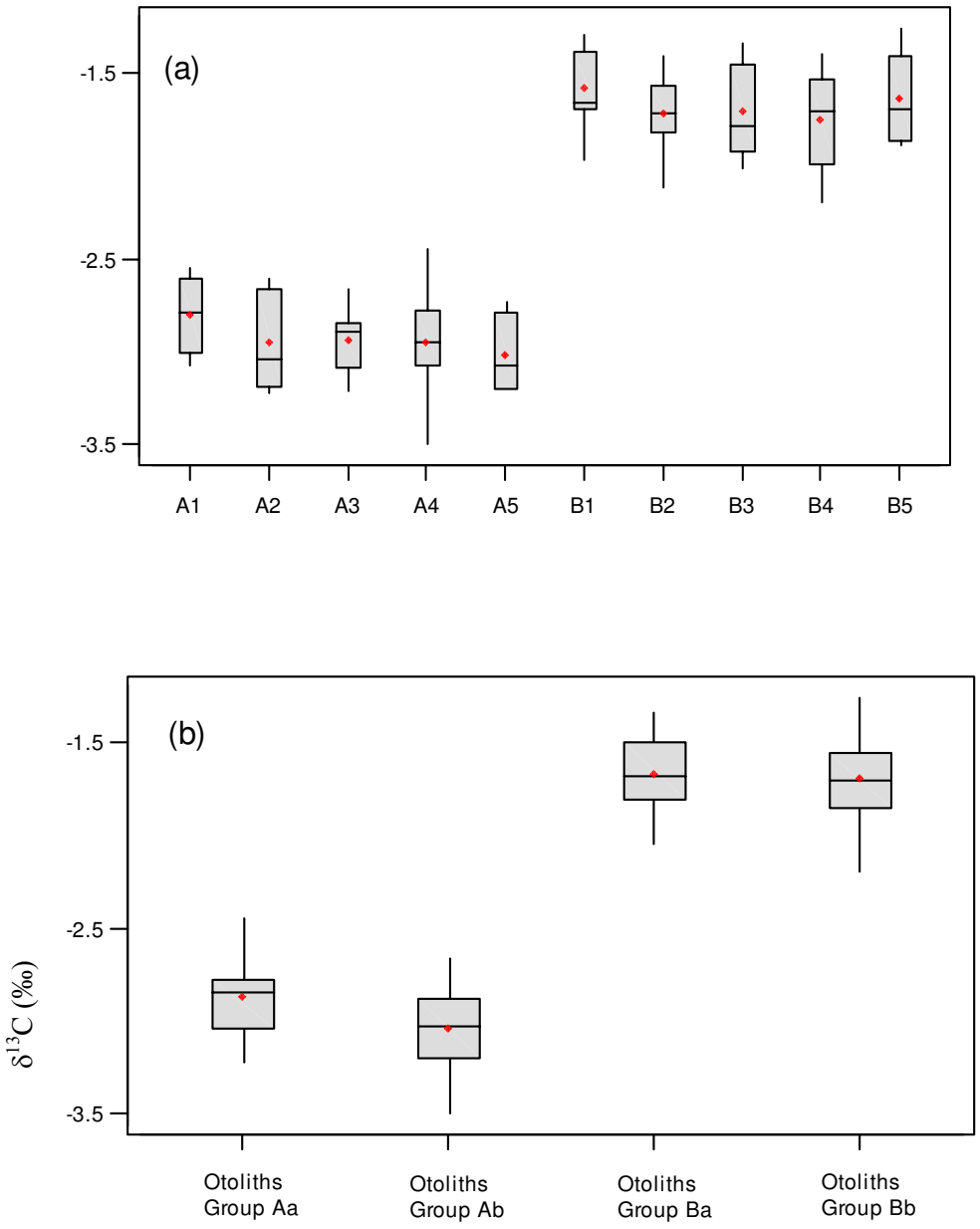


Figure 22. Carbon isotope composition ($\delta^{13}C$) of otoliths pairs. a) The combined age class subgroups were significantly different ($p < 0.001$) between both treatment groups and similar within subgroups (A: $p = 0.747$; B: $p = 0.608$). b) The otoliths from the younger Group A fish (mean $\delta^{13}C = -2.87 \pm 0.21‰$) were significantly lighter ($p = 0.035$) than otoliths from the older fish (mean $\delta^{13}C = -3.03 \pm 0.22‰$). Otoliths from the younger Group B fish (mean $\delta^{13}C = -1.66 \pm 0.21‰$) were lighter than otoliths from the older fish (mean $\delta^{13}C = -1.69 \pm 0.26‰$) but not significantly ($p = 0.71$). ♦ = $\delta^{13}C$ means.

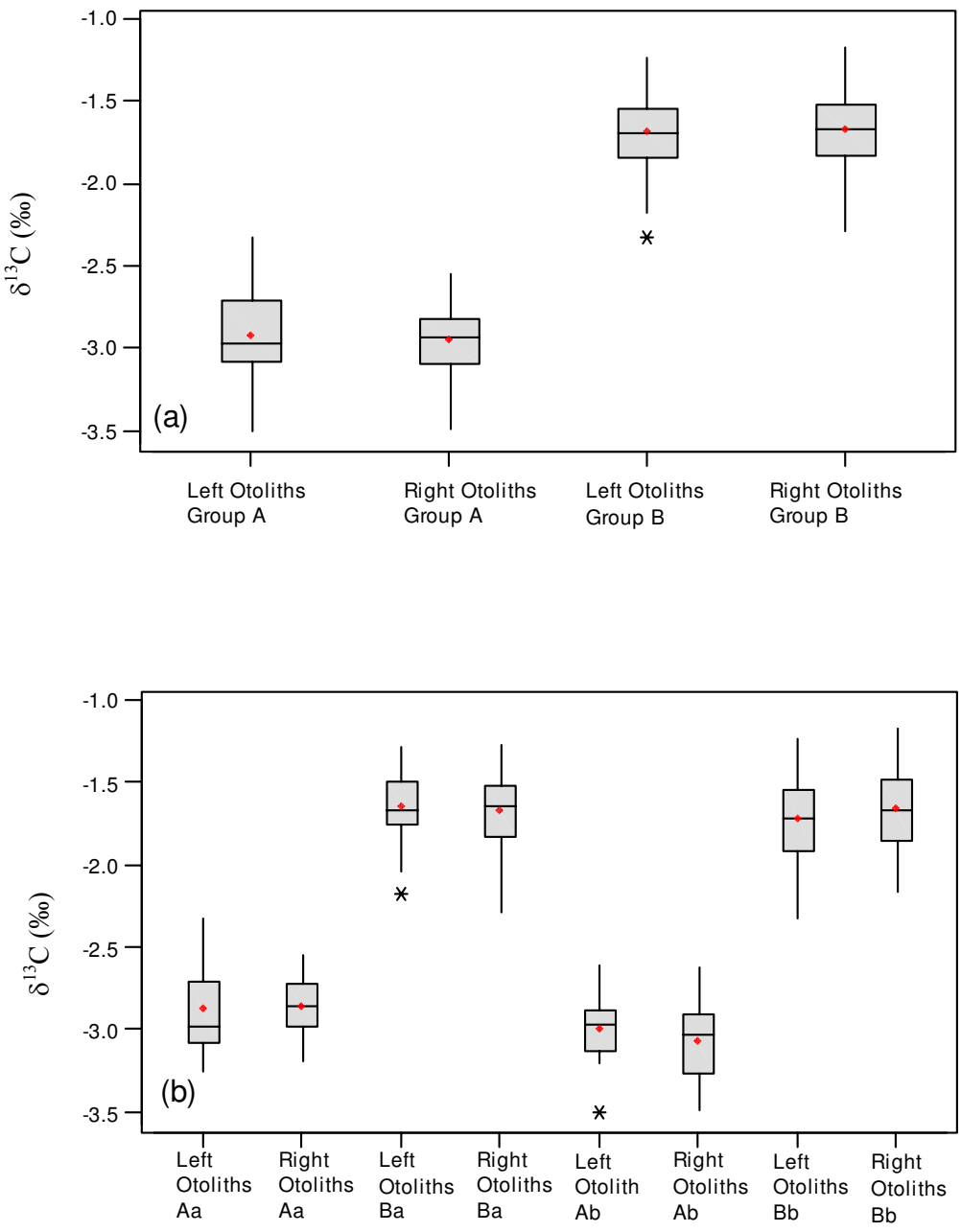


Figure 23. A comparison of carbon s isotope composition ($\delta^{13}C$) between left and right otoliths from both diet groups. a) Otolith pairs did not exhibit significant differences in Group A ($p = 0.69$) or Group B ($p = 0.76$). b) Otolith pairs within the two age classes from both treatment groups had some detectable but not appreciable differences: younger fish Group A ($p = 0.82$); older fish Group A ($p = 0.37$); younger fish Group B ($p = 0.74$); older fish Group B ($p = 0.48$). ♦ = mean $\delta^{13}C$; ∗ = data points outside lower and upper quartiles.

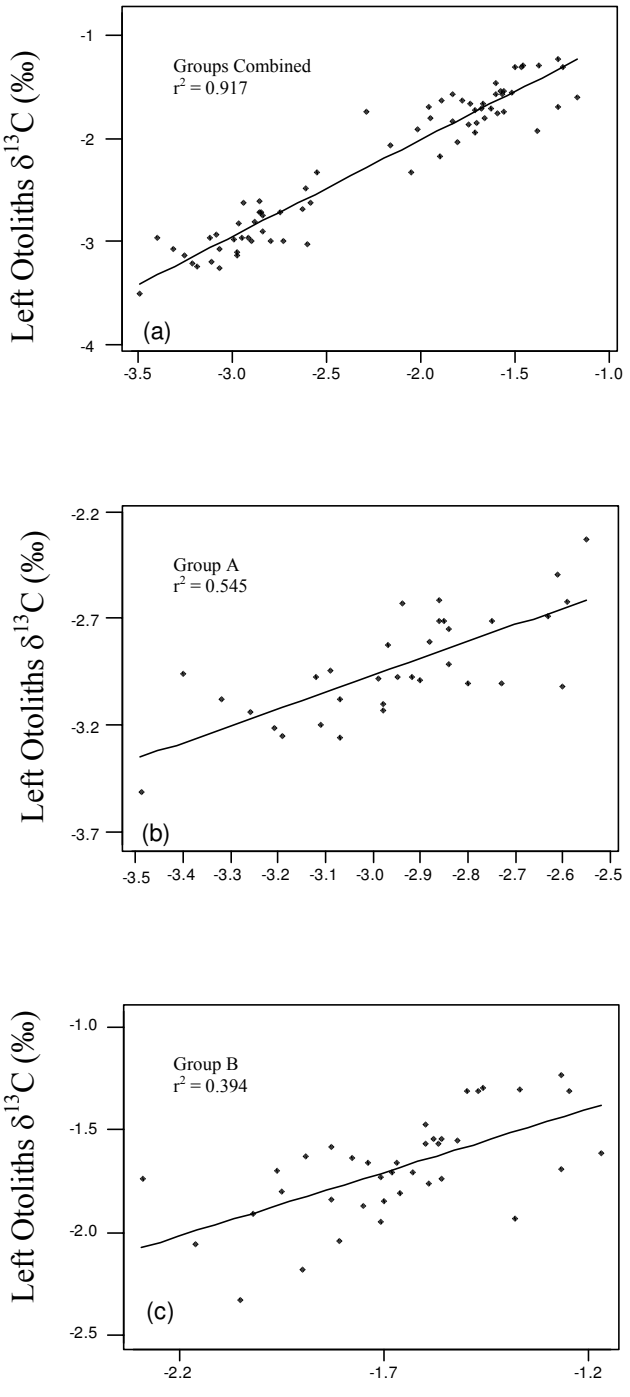


Figure 24. Regression analysis of carbon isotopic composition ($\delta^{13}C$) between otolith pairs for a) two treatment groups combined reveals a strong, significant relationship ($r^2 = 0.92$, $p < 0.001$). b) Otolith pairs from the Aquamax treatment group (A) showed a positive relationship ($r^2 = 0.55$, $p < 0.001$). c) There was a weaker relationship between otolith pairs from the pinfish diet group (B) ($r^2 = 0.39$, $p < 0.001$).

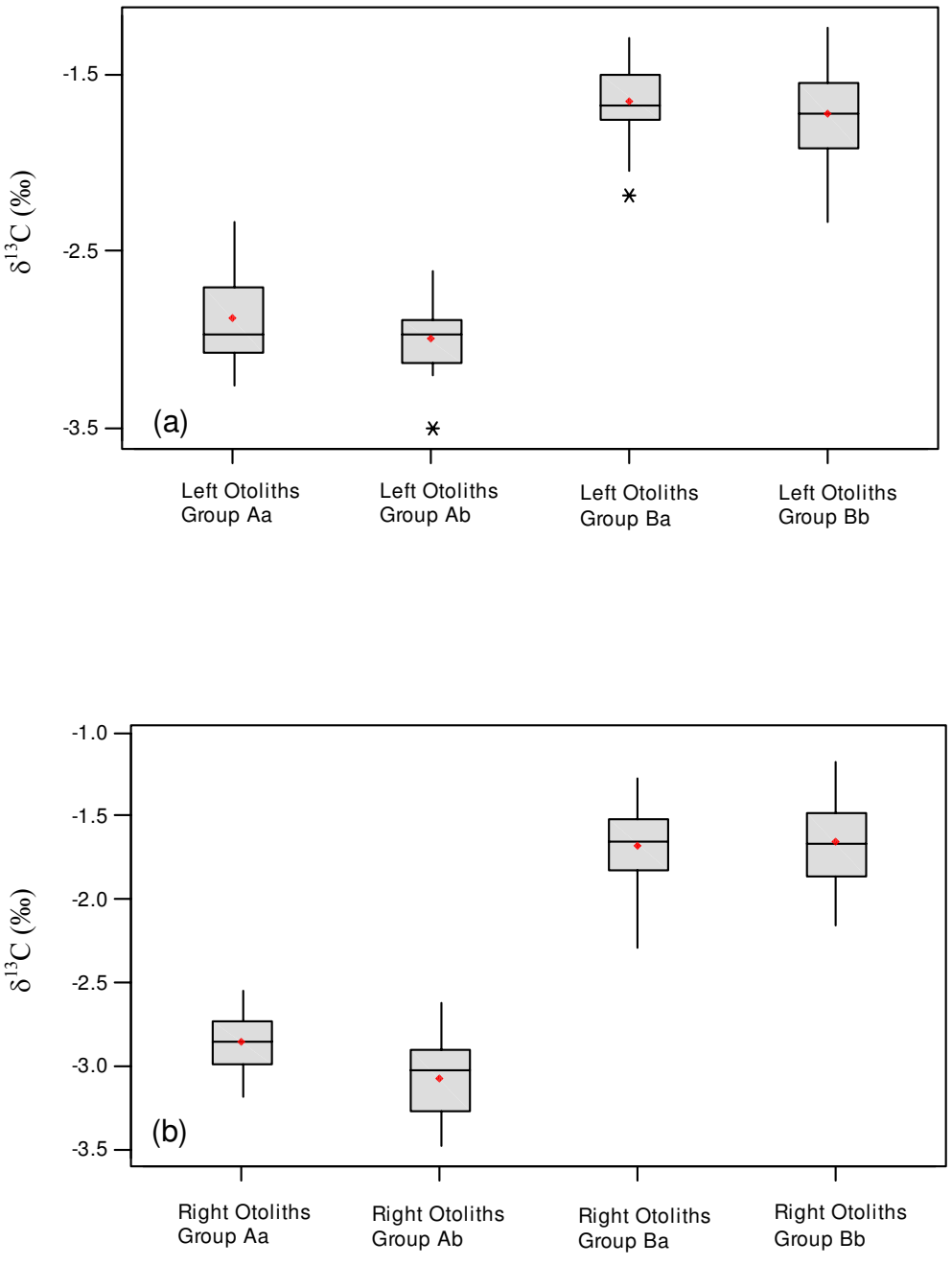


Figure 25. Carbon isotope composition ($\delta^{13}C$) of red drum otoliths from the two age classes of both treatment groups. a) Left otoliths between age classes were similar (Group A: $p = 0.17$; Group B: $p = 0.38$). b) Right otoliths were similar between Group B age classes ($p = 0.83$) but significantly different between Group A age classes ($p = 0.035$). ♦ = $\delta^{13}C$ means; ∗ = data points outside lower quartile.

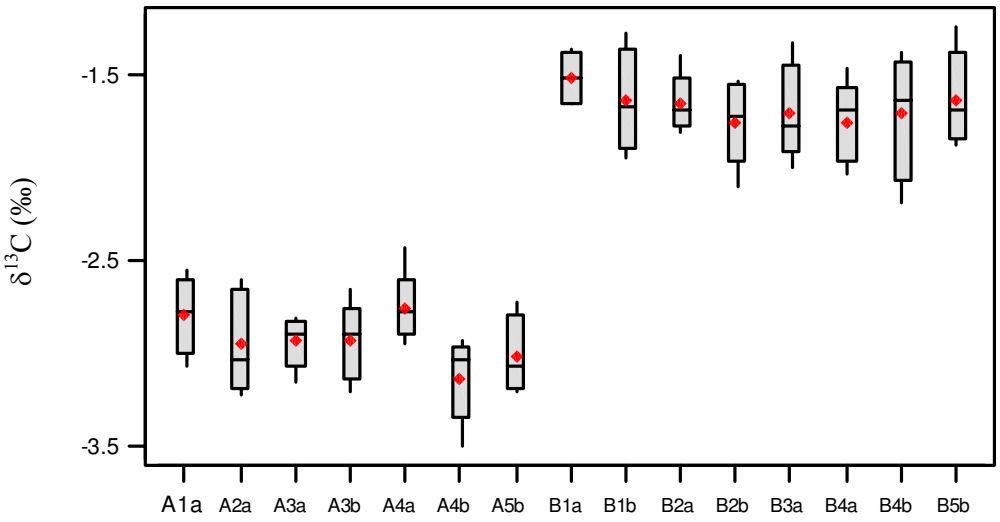


Figure 26. Carbon isotope composition ($\delta^{13}C$) of red drum otolith pairs of all age class subgroups from both treatment groups. The otoliths pairs of all Group A subgroups were significantly different from Group B subgroups ($F_{14,56} = 38.89$, $p < 0.001$). ♦ = $\delta^{13}C$ means of otolith pairs.

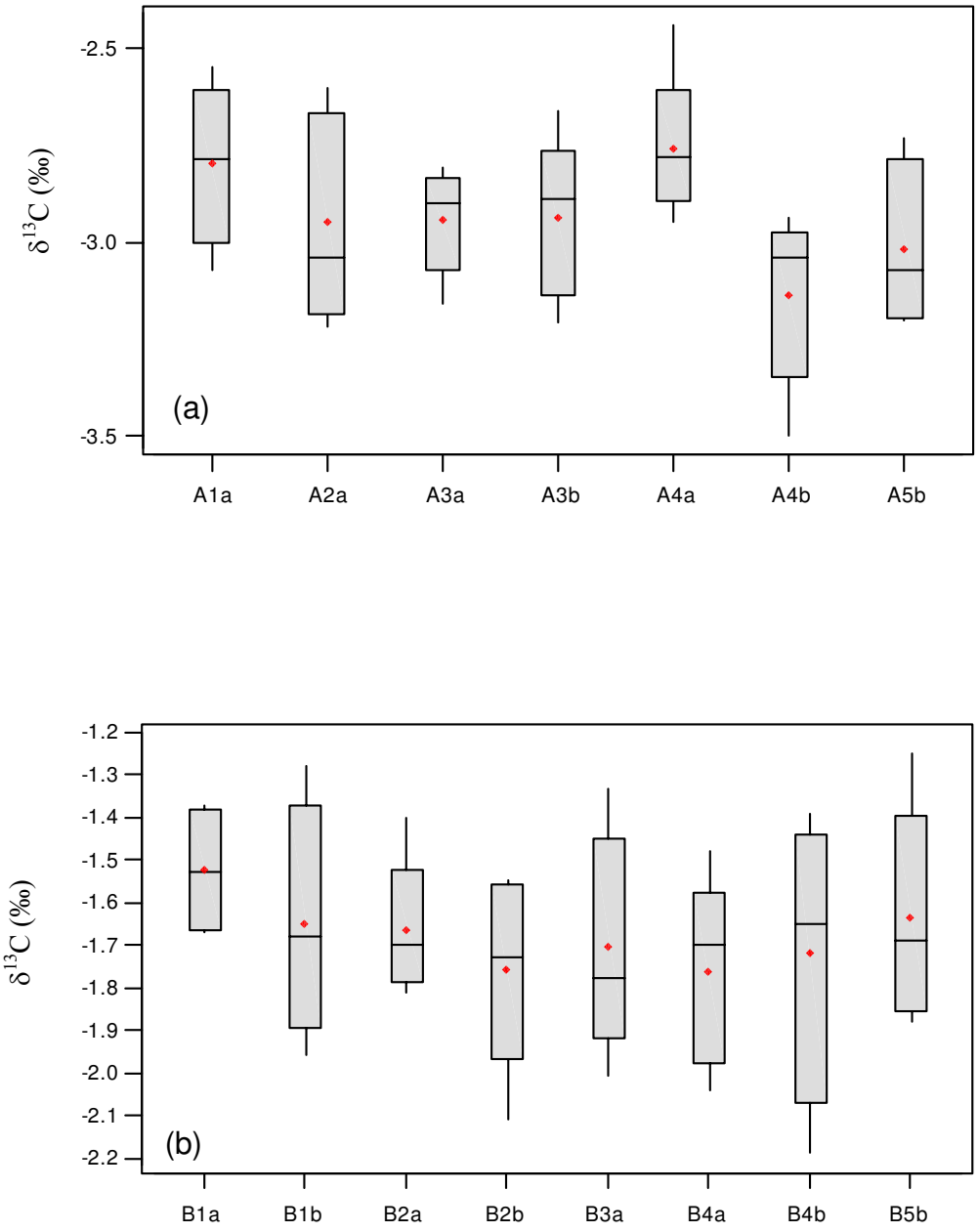


Figure 27. Carbon isotopic composition ($\delta^{13}C$) of red drum otolith pairs from subgroups from both treatment groups. a) Group A subgroups were not significantly different ($F_{13,52} = 1.91$, $p = 0.050$) and b) otolith of Group B subgroups were similar ($F_{15,60} = 0.65$, $p = 0.823$). ♦ = $\delta^{13}C$ means.

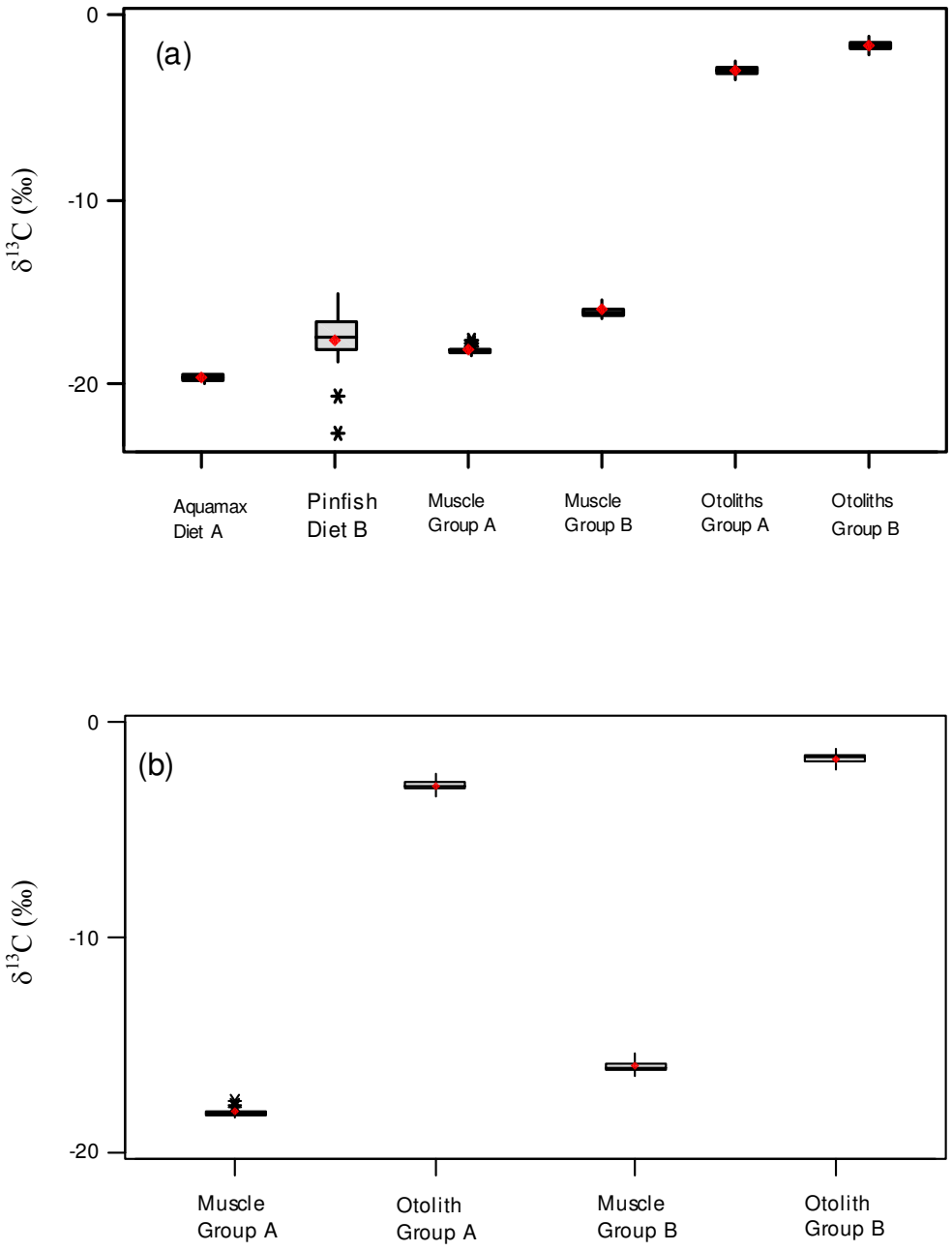


Figure 28. a) The mean $\delta^{13}C$ of muscle tissue and otoliths in both treatment groups displayed similar fractionation from the respective diets. b) Otoliths from Group A fish were enriched 15.19‰ relative to the muscle tissue while Group B otoliths were enriched 14.33 ‰ relative to the muscle tissue. ♦ = $\delta^{13}C$ means; ∗ = data points outside lower or upper quartiles.

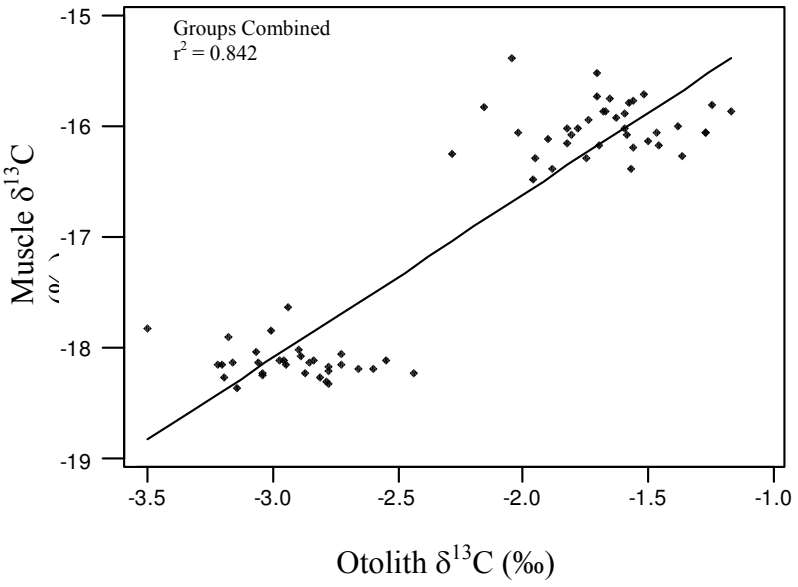


Figure 29. A simpler linear regression reveals a positive correlation ($r^2 = 0.84$, $p < 0.001$) between carbon isotopic composition ($\delta^{13}C$) of red drum muscle tissue and otoliths for diet treatment groups combined. There is distinct isotopic signature between the two groups.

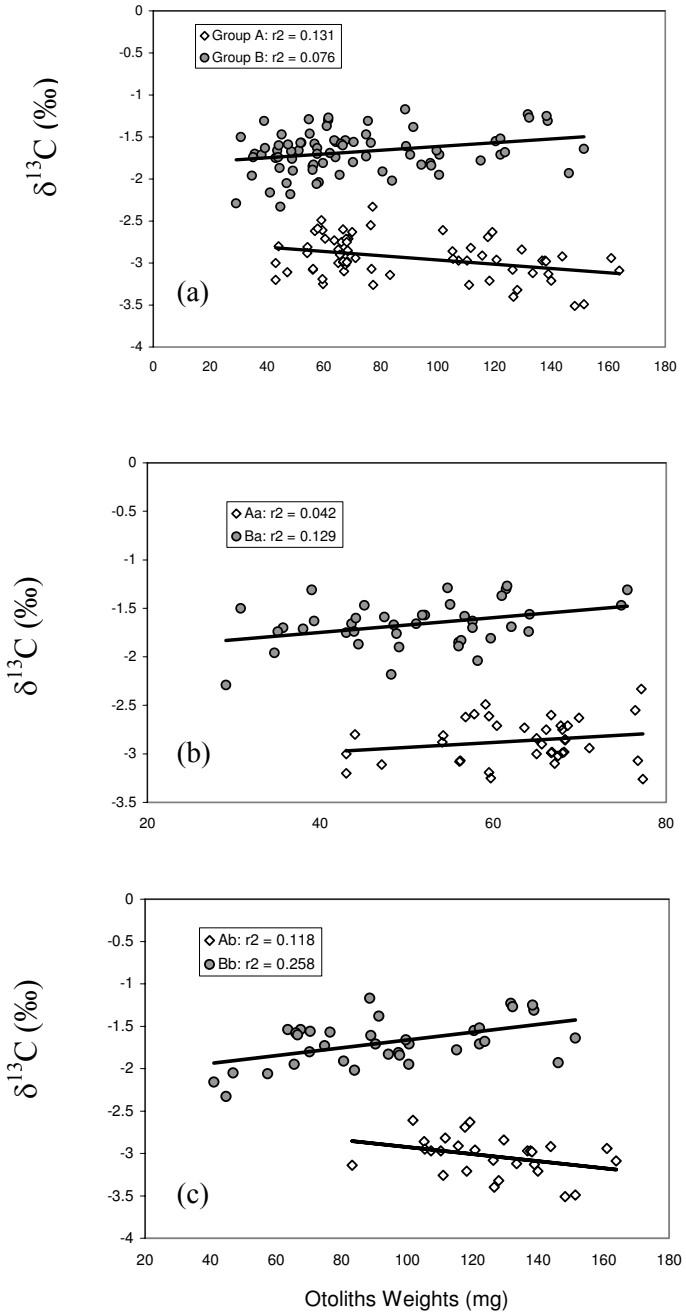


Figure 30. Otolith weights revealed weak correlation with otolith carbon $\delta^{13}C$ but some were significant. a) All otolith pairs in Group A showed a slight ^{13}C depletion with otoliths weight ($r^2 = 0.13$, $p = 0.003$) while Group B became slightly enriched ($r^2 = 0.076$, $p = 0.016$). b) The younger fish from both diet treatment groups exhibited a slight ^{13}C enrichment with increasing otolith weight (Group Aa: $r^2 = 0.04$, $p = 0.218$; Group Ba: $r^2 = 0.13$, $p = 0.023$). c) Older fish in the two groups had differing trends. Group Ab fish had a slight ^{13}C depletion with otolith weight ($r^2 = 0.12$, $p = 0.074$) while otoliths in Group Bb were more ^{13}C enriched with increasing otolith weight ($r^2 = 0.26$, $p = 0.002$).

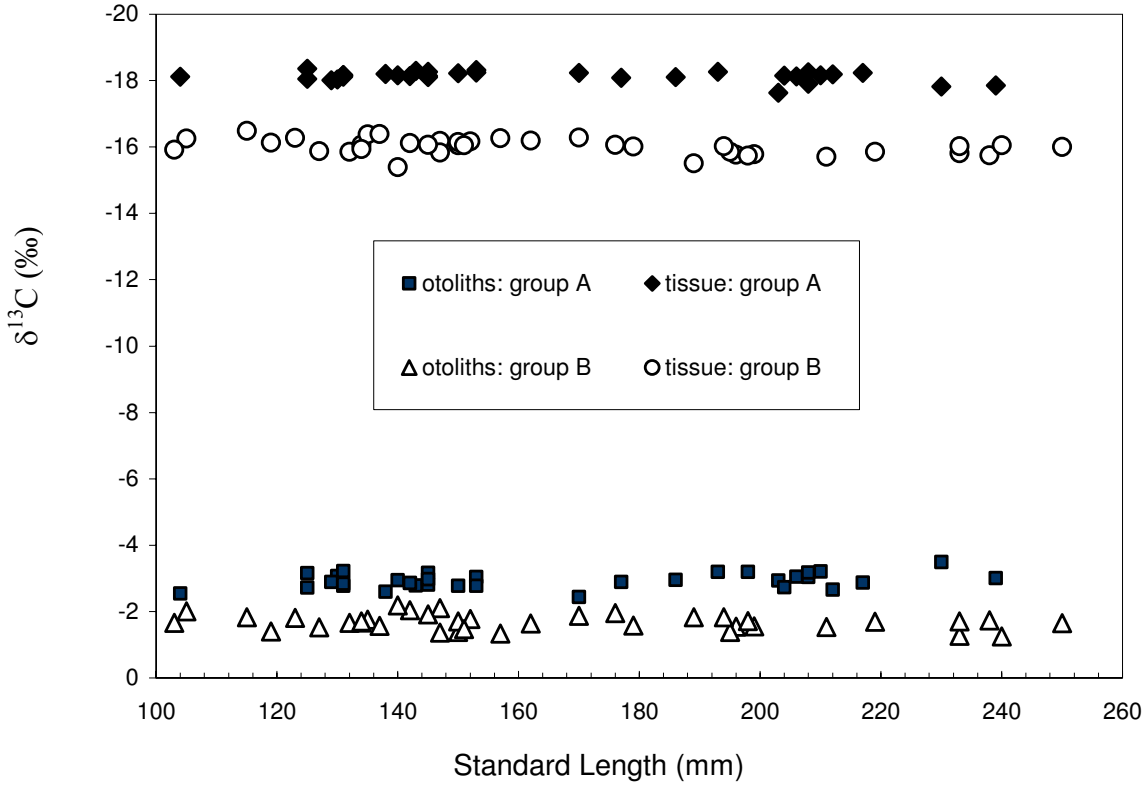
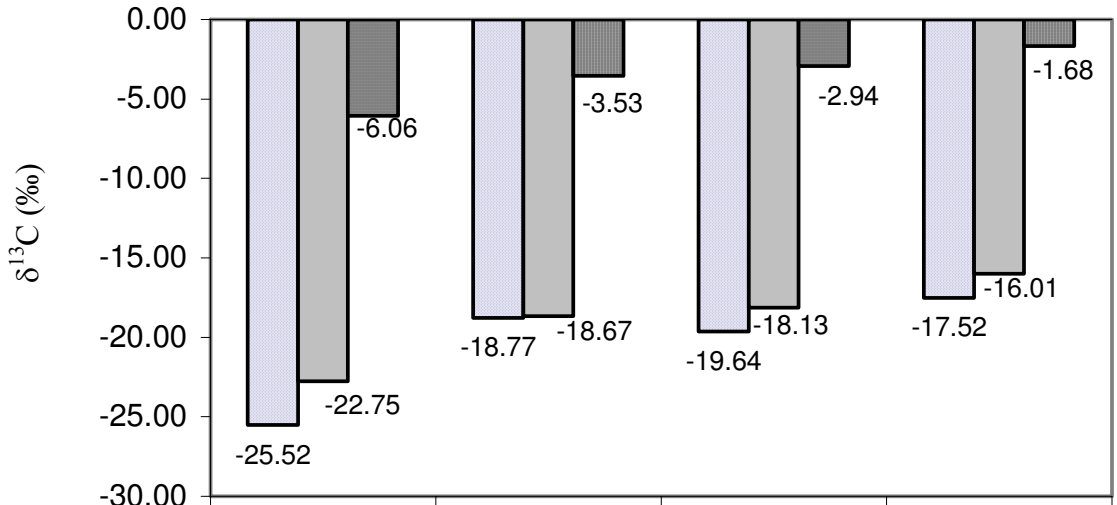


Figure 31. Carbon composition ($\delta^{13}C$) of red drum muscle tissue and otoliths from Aquamax (A) and pinfish (B) diets plotted against ending standard lengths in millimeters (mm) of fish. Otolith and muscle ($\delta^{13}C$) remained relatively constant with increasing length.



	Spirulina	Cod Chow	Aquamax	Pinfish
Diet	-25.52	-18.77	-19.64	-17.52
Muscle	-22.75	-18.67	-18.13	-16.01
Otoliths	-6.06	-3.53	-2.94	-1.68

Figure 32. Comparison of results from two similar feeding studies. Cod raised on Spirulina and Cod Chow (Radtke et al., 1996) had similar ^{13}C enrichment as red drum raised on Aquamax and Pinfish in the present study.

APPENDIX A1

CARBON ISOTOPIC RATIOS OF DISSOLVED INORGANIC CARBON IN SEAWATER DURING EXPERIMENT

Table A1 Complete listing of DIC seawater measurements taken from randomly selected individual tanks during course of the experiment. ∗ = samples that were not included in the analysis due to extremely low values; na = tank sampled unknown due to missing label.

Date	Tank	Sample #	$^{13}C/^{12}C$ DIC 1st reading	$^{13}C/^{12}C$ DIC 2nd reading	Date	Tank	Sample #	$^{13}C/^{12}C$ DIC 1st reading	$^{13}C/^{12}C$ DIC 2nd reading
08/14/01	E2	4A	-3.34		04/02/02	D1	13A	-11.82	-12.11
08/14/01	F1	4B	-6.18	-5.14	04/02/02	F1	13B	-3.51	
08/14/01	C1	4C	-5.13	-5.46	04/02/02	E3	13C	-3.81	-3.61
09/26/01	D1	5A	-5.42	-5.35	04/11/02	D3	14A	-11.46	-10.88
09/26/01	C3	5B	-4.27	-4.24	04/11/02	F1	14B	-2.99	
09/26/01	F2	5C	-3.92		04/11/02	F1	14B	-2.99	
10/18/01	F3	6A	-3.91		04/11/02		14C	-5.91	-5.69
10/18/01	F1	6B	-3.53		05/01/02	F3	15A	-4.79	-4.38
10/18/01	D1	6C	-4.59	-4.28	05/01/02	C3	15B	-4.10	-4.09
11/19/01	E3	7A	-3.59		05/01/02	D2	15C	-4.83	-4.51
11/19/01	D3	7B	-3.32		05/23/02	D3	16A	-2.90	
11/19/01	D1	7C	-3.01	-2.85	05/23/02	F1	16B	-2.57	
12/16/01	E3	8A	-2.83		05/23/02	C3	16C	-2.97	
12/16/01	D3	8B	-3.19		06/04/02	D1	17A	-2.75	
12/16/01	D1	8C	-3.87		06/04/02	D3	17B	-2.32	
01/15/02	F2	9A	-3.75	-3.65	06/04/02	E3	17C	-2.71	
01/15/02	F3	9B	-3.73						
01/15/02	D3	9C	-3.79	-4.49					
01/31/02	D3	10A	-4.31	-4.10					
01/31/02	E3	10B	-4.56						
01/31/02		10C	-4.24						
02/25/02	E3	11A	-3.88						
02/25/02	D2	11B	-4.60						
02/25/02	C3	11C	-2.93						
03/11/02	D1	12A	-3.56						
03/11/02	F1	12B	-2.91						
03/11/02	D3	12C	-3.52						

APPENDIX A2

DAILY TEMPERATURE AND SALINITY MEASUREMENTS OF SEAWATER DURING EXPERIMENT

Table A2 Complete data set for daily temperatures and salinities measured at Florida State University Marine Laboratory.

Date	Time	Temperature º C	Salinity ppt
08/10/01	70000	28.73	27.1
	150000	29.54	27.7
	230000	29.28	27.6
08/11/01	70000	28.24	28.0
	150000	29.10	27.8
	230000	29.40	27.8
08/12/01	70000	28.18	27.6
	150000	29.12	26.8
	230000	29.65	26.5
08/13/01	70000	28.63	26.4
	150000	29.26	39.8
	230000	30.14	38.4
08/14/01	70000	29.14	38.5
	150000	29.20	25.1
	230000	29.06	24.8
08/15/01	70000	28.22	25.1
	150000	26.32	26.9
	230000	29.50	25.7
08/16/01	70000	28.89	26.4
	150000	29.60	27.9
	230000	30.14	26.0
08/17/01	70000	29.43	26.4
	150000	30.30	27.6
	230000	30.86	26.7
08/18/01	70000	30.18	26.9
	150000	30.71	27.2
	230000	31.05	26.8
08/19/01	70000	29.84	27.4
	150000	29.44	27.7
	230000	29.04	27.6
08/20/01	70000	27.68	28.0
	150000	29.92	28.7
	230000	28.55	29.7
08/21/01	70000	27.60	29.7
	150000	29.10	30.5
	230000		
08/22/01	70000		
	150000	30.18	30.3
	230000	30.69	30.3
08/23/01	70000	29.22	30.8
	150000	30.60	30.7
	230000	30.58	31.0
08/24/01	70000	28.73	31.4
	150000	30.64	31.2
	230000	31.46	31.2
08/25/01	70000	29.52	31.1
	150000	30.76	31.0
	230000	31.07	31.2
08/26/01	70000	29.66	31.2
	150000	30.76	31.2
	230000	31.49	31.1
08/27/01	70000	29.80	31.1
	150000	30.74	31.2
	230000	31.11	31.3
08/28/01	70000	29.68	31.4
	150000	30.16	31.4
	230000	30.64	31.5
08/29/01	70000	29.12	31.8
	150000	30.24	31.6
	230000	30.78	31.6
08/30/01	70000	29.52	31.6
	150000	30.46	31.5
	230000	31.56	31.2
08/31/01	70000	30.36	31.3

	150000	31.03	31.3
	230000	31.19	31.1
09/01/01	70000	30.37	31.3
	150000	30.72	31.2
	230000	30.42	30.9
09/02/01	70000	29.34	31.3
	150000	30.20	31.2
	230000	29.82	31.1
09/03/01	70000	29.16	31.3
	150000	28.79	31.3
	230000	28.61	31.1
09/04/01	70000	27.95	31.2
	150000	28.49	31.1
	230000	28.06	31.3
09/05/01	70000	27.28	31.6
	150000	28.81	31.4
	230000	29.06	31.5
09/06/01	70000	28.53	31.4
	150000	29.74	31.4
	230000	29.64	31.4
09/07/01	70000	28.65	31.5
	150000	29.52	31.5
	230000	29.90	31.5
09/08/01	70000	29.01	31.4
	150000	29.60	31.2
	230000	29.46	31.3
09/09/01	70000	28.69	31.1
	150000	29.32	30.8
	230000	30.00	30.7
09/10/01	70000	28.73	30.8
	150000	29.42	30.8
	230000	30.08	30.6
09/11/01	70000	29.18	30.7
	150000	30.29	30.7
	230000	30.30	30.1
09/12/01	70000	29.02	29.7
	150000	29.46	29.2
	230000		
09/13/01	70000		
	150000	28.08	28.1
	230000	27.76	28.2
09/14/01	70000	26.50	28.3
	150000	26.82	28.3
	230000	25.43	25.7
09/15/01	70000	24.89	27.9
	150000	25.61	28.3
	230000		
09/16/01	70000		
	150000		
	230000		
09/17/01	70000		
	150000	25.54	26.0
	230000	26.16	27.7
09/18/01	70000	25.15	28.6
	150000	26.18	28.7
	230000	26.91	28.6
09/19/01	70000	25.74	28.8
	150000	27.03	28.9
	230000	27.97	29.0
09/20/01	70000	27.18	29.1
	150000	28.55	29.1
	230000	28.71	29.2
09/21/01	70000	27.76	29.4
	150000	29.32	29.0
	230000	29.76	29.2
09/22/01	70000	28.34	29.4
	150000	30.12	29.3
	230000	30.97	29.3

09/23/01	70000	29.78	29.4
	150000	30.12	29.6
	230000	29.94	29.8
09/24/01	70000	28.79	29.2
	150000	27.99	29.0
	230000	27.68	29.4
09/25/01	70000	26.67	29.4
	150000	25.86	29.1
	230000	25.86	29.3
09/26/01	70000	24.63	29.5
	150000	24.95	29.7
	230000	24.27	29.6
09/27/01	70000	23.05	29.9
	150000	23.92	29.9
	230000	24.71	29.7
09/28/01	70000	23.14	30.4
	150000	24.23	30.1
	230000	24.91	29.9
09/29/01	70000	23.61	30.3
	150000	23.92	30.3
	230000	23.18	30.5
09/30/01	70000	22.07	30.7
	150000	22.41	30.8
	230000	22.04	31.0
10/01/01	70000	21.05	31.3
	150000	21.81	31.2
	230000	21.72	31.1
10/02/01	70000	20.26	31.2
	150000	21.97	31.0
	230000	22.27	31.2
10/03/01	70000	21.31	31.2
	150000	22.48	31.1
	230000	22.77	31.2
10/04/01	70000	22.05	31.2
	150000	23.61	31.1
	230000	24.45	31.1
10/05/01	70000		
	150000	25.30	30.9
	230000	25.54	30.8
10/06/01	70000	25.43	30.7
	150000	26.14	30.5
	230000	25.71	30.5
10/07/01	70000	23.89	30.7
	150000	24.43	30.6
	230000	24.38	30.7
10/08/01	70000	22.75	30.9
	150000	23.41	30.8
	230000	23.76	31.0
10/09/01	70000	21.97	31.4
	150000	23.72	31.0
	230000	23.65	31.0
10/10/01	70000	22.27	31.2
	150000	23.32	31.2
	230000	24.16	31.1
10/11/01	70000	22.95	30.9
	150000	23.67	30.2
	230000	24.29	29.9
10/12/01	70000	23.99	29.7
	150000	24.05	29.6
	230000	24.25	29.4
10/13/01	70000	23.99	29.4
	150000	24.38	29.4
	230000	24.91	29.3
10/14/01	70000	24.78	29.0
	150000	25.22	28.5
	230000	24.47	28.6
10/15/01	70000	23.40	28.7
	150000	23.92	24.8

	230000	23.58	25.3
10/16/01	70000	22.05	26.7
	150000	23.60	27.4
	230000	22.27	28.3
10/17/01	70000	20.77	29.5
	150000	20.65	29.5
	230000	19.54	29.9
10/18/01	70000	18.05	30.2
	150000	20.19	29.8
	230000	20.86	30.0
10/19/01	70000	19.83	30.1
	150000	21.07	30.1
	230000	21.17	30.3
10/20/01	70000	20.79	30.3
	150000	22.45	30.2
	230000	22.57	30.3
10/21/01	70000	22.29	30.5
	150000	23.36	30.3
	230000	23.78	30.5
10/22/01	70000		
	150000	24.30	30.5
	230000	25.21	30.3
10/23/01	70000	23.90	30.5
	150000	24.85	30.4
	230000	25.47	30.4
10/24/01	70000	24.74	30.4
	150000	25.71	30.4
	230000	26.33	30.3
10/25/01	70000	25.86	30.3
	150000	26.29	30.2
	230000	25.02	30.3
10/26/01	70000		
	150000	22.93	30.6
	230000	21.61	30.6
10/27/01	70000	20.14	30.8
	150000	20.21	31.0
	230000	19.09	31.1
10/28/01	70000	17.44	31.3
	150000	17.43	31.4
	230000	16.73	31.4
10/29/01	70000	15.66	31.6
	150000	16.54	31.4
	230000	16.85	31.4
10/30/01	70000	15.71	31.7
	150000		
	230000		
10/31/01	70000		
	150000	17.39	30.4
	230000	18.73	31.1
11/01/01	70000	18.56	31.5
	150000	19.71	31.4
	230000	20.87	31.3
11/02/01	70000	20.39	31.4
	150000	21.56	31.2
	230000	22.39	31.2
11/03/01	70000	21.86	31.3
	150000	22.86	31.1
	230000	23.04	31.2
11/04/01	70000	22.16	31.3
	150000	23.02	30.8
	230000	23.00	31.2
11/05/01	70000	21.67	31.4
	150000	22.29	30.7
	230000	21.26	30.5
11/06/01	70000	19.73	31.2
	150000	20.68	31.2
	230000	20.47	31.4
11/07/01	70000	18.73	31.7

	150000	20.19	31.6
	230000	20.28	31.6
11/08/01	70000	18.83	31.8
	150000	19.92	31.7
	230000	19.86	31.7
11/09/01	70000	19.02	31.7
	150000	20.23	29.1
	230000	20.56	30.7
11/10/01	70000	19.28	31.4
	150000	20.18	31.5
	230000	20.40	31.6
11/11/01	70000	19.74	31.8
	150000	20.26	31.7
	230000	20.40	31.7
11/12/01	70000	19.35	31.8
	150000	20.12	31.7
	230000		
11/13/01	70000		
	150000	19.48	31.8
	230000	19.50	31.8
11/14/01	70000	18.68	31.8
	150000	18.92	31.5
	230000	19.09	31.6
11/15/01	70000	18.23	31.7
	150000	19.14	30.7
	230000	19.09	31.3
11/16/01	70000	18.01	31.7
	150000	19.19	31.2
	230000	18.80	31.6
11/17/01	70000	17.96	31.8
	150000	18.97	24.8
	230000	18.95	29.0
11/18/01	70000	17.81	30.5
	150000	19.23	30.8
	230000	19.50	31.2
11/19/01	70000	18.47	31.6
	150000	19.57	31.5
	230000	19.85	31.6
11/20/01	70000	19.21	31.7
	150000	19.88	31.7
	230000	19.76	31.7
11/21/01	70000	18.59	31.9
	150000	19.18	31.8
	230000	18.85	31.8
11/22/01	70000	17.67	32.0
	150000	18.75	31.9
	230000	19.47	31.8
11/23/01	70000	19.67	32.0
	150000	19.62	32.1
	230000	19.62	31.4
11/24/01	70000	19.48	31.2
	150000	20.09	31.1
	230000	20.68	31.2
11/25/01	70000	20.79	31.4
	150000	21.37	31.3
	230000	22.05	31.2
11/26/01	70000	21.86	31.1
	150000	22.11	31.0
	230000	22.57	30.9
11/27/01	70000	22.30	30.9
	150000	22.62	30.8
	230000		
11/28/01	70000		
	150000	22.30	23.8
	230000		
11/29/01	70000		
	150000	22.20	29.5
	230000	22.20	30.1

11/30/01	70000	21.95	30.5
	150000	21.51	30.5
	230000	21.51	30.8
12/01/01	70000	20.72	30.9
	150000	20.86	30.6
	230000	20.82	30.9
12/02/01	70000	19.78	31.0
	150000	20.04	30.9
	230000	19.86	30.9
12/03/01	70000	18.58	31.1
	150000	21.65	31.5
	230000		
12/04/01	70000		
	150000	19.59	30.9
	230000	19.83	31.2
12/05/01	70000	19.04	31.3
	150000	20.02	31.2
	230000		
12/06/01	70000		
	150000		
	230000		
12/07/01	70000		
	150000	20.39	20.3
	230000	21.17	21.2
12/08/01	70000	20.44	21.7
	150000	20.63	23.1
	230000	21.37	24.2
12/09/01	70000	21.29	25.5
	150000	21.61	26.9
	230000	21.91	27.8
12/10/01	70000	21.58	28.6
	150000	21.56	29.2
	230000		
12/11/01	70000		
	150000		
	230000		
12/12/01	70000		
	150000		
	230000		
12/13/01	70000		
	150000	21.65	31.5
	230000		
12/14/01	70000		
	150000	22.50	31.3
	230000	23.04	31.3
12/15/01	70000	22.96	31.2
	150000	23.38	31.0
	230000	23.56	31.1
12/16/01	70000	23.11	31.2
	150000	22.66	31.2
	230000	22.71	31.3
12/17/01	70000	22.21	31.3
	150000	22.62	31.2
	230000	23.09	31.1
12/18/01	70000	22.13	31.1
	150000	21.65	30.8
	230000	21.05	31.3
12/19/01	70000	19.61	31.4
	150000	19.86	31.8
	230000	19.73	32.2
12/20/01	70000	18.42	32.3
	150000	18.23	31.7
	230000	17.66	32.5
12/21/01	70000	16.25	32.7
	150000	16.77	32.5
	230000	16.72	32.6
12/22/01	70000	15.11	32.8
	150000	15.64	32.8

	230000	16.08	32.7
12/23/01	70000	16.62	32.7
	150000	17.08	32.6
	230000	17.37	32.2
12/24/01	70000	16.95	32.1
	150000	16.59	32.2
	230000	16.49	32.3
12/25/01	70000	15.43	32.3
	150000	14.86	32.4
	230000	14.28	32.4
12/26/01	70000	12.99	32.4
	150000	13.27	32.6
	230000	13.19	32.7
12/27/01	70000	11.94	32.6
	150000	13.80	32.5
	230000	13.27	32.8
12/28/01	70000	12.43	32.8
	150000	13.22	32.7
	230000	13.90	32.6
12/29/01	70000	13.55	32.6
	150000	14.28	32.5
	230000	15.31	32.5
12/30/01	70000	14.57	32.4
	150000	14.34	32.3
	230000	14.43	32.4
12/31/01	70000	13.40	32.7
	150000	12.96	
	230000	12.91	
01/01/02	70000	12.05	33.0
	150000	11.97	32.8
	230000		
01/02/03	70000		
	150000	10.92	32.5
	230000	10.61	32.7
01/03/03	70000	10.14	33.0
	150000	9.97	32.5
	230000	9.81	32.9
01/04/03	70000	8.70	33.0
	150000	10.69	32.6
	230000	10.91	32.8
01/05/03	70000	9.84	33.0
	150000	11.35	32.9
	230000	11.90	32.8
01/06/03	70000	13.05	33.2
	150000	13.32	32.8
	230000	13.24	32.5
01/07/03	70000	12.61	32.6
	150000	12.46	32.6
	230000	11.92	32.6
01/08/03	70000	11.43	32.6
	150000	11.15	32.5
	230000	11.10	32.8
01/09/03	70000	9.96	32.7
	150000	11.48	32.5
	230000	12.18	32.6
01/10/03	70000	12.31	32.5
	150000		
	230000		
01/11/03	70000		
	150000	14.71	32.2
	230000	15.27	32.3
01/12/03	70000	14.81	32.5
	150000	15.24	32.2
	230000	15.56	32.3
01/13/03	70000	14.33	32.1
	150000	14.97	31.9
	230000	14.99	31.9
01/14/03	70000	14.56	31.9

	150000	14.46	31.7
	230000	14.48	31.5
01/15/03	70000	14.04	31.4
	150000	15.07	31.1
	230000	14.71	31.2
01/16/03	70000	13.60	31.3
	150000	14.61	30.9
	230000	14.57	31.1
01/17/03	70000	13.63	31.1
	150000	15.12	30.9
	230000	15.06	31.0
01/18/03	70000	14.48	31.1
	150000	15.87	31.0
	230000	16.83	31.0
01/19/03	70000	17.01	31.0
	150000	17.40	31.1
	230000	18.03	31.0
01/20/03	70000	16.91	31.0
	150000	16.66	31.3
	230000	17.06	31.2
01/21/03	70000	17.17	31.1
	150000	17.64	30.9
	230000	17.45	30.8
01/22/03	70000	16.86	30.8
	150000	17.01	30.9
	230000	17.32	30.8
01/23/03	70000	17.23	30.6
	150000	17.52	30.9
	230000	18.15	30.9
01/24/03	70000	17.83	30.3
	150000	18.06	29.8
	230000	19.43	29.4
01/25/03	70000	19.93	29.1
	150000	19.28	29.0
	230000	19.74	29.5
01/26/03	70000	19.69	29.5
	150000	19.28	29.5
	230000	19.40	29.8
01/27/03	70000	19.16	29.8
	150000	18.78	29.5
	230000	18.49	29.6
01/28/03	70000	18.37	29.5
	150000	18.78	28.0
	230000	19.26	29.2
01/29/03	70000	18.97	29.6
	150000	19.21	28.2
	230000	19.81	29.5
01/30/03	70000	19.28	29.9
	150000	20.44	29.7
	230000	20.46	29.9
01/31/03	70000	20.23	30.0
	150000	21.12	29.8
	230000	21.08	29.9
02/01/02	70000	20.61	30.0
	150000	21.56	29.8
	230000	21.70	29.9
02/02/03	70000	20.44	30.1
	150000	20.58	29.7
	230000	19.83	30.0
02/03/03	70000	18.18	30.1
	150000	19.09	30.1
	230000	19.12	30.3
02/04/03	70000	18.30	30.2
	150000	17.96	30.4
	230000	17.30	30.6
02/05/03	70000	16.16	30.1
	150000		
	230000		

02/06/03	70000	13.10			150000		
	150000				230000		
	230000			02/18/03	70000	15.00	
02/07/03	70000	14.20	31.4		150000		
	150000				230000		
	230000			02/19/03	70000		
02/08/03	70000	14.00			150000		
	150000				230000		
	230000			02/20/03	70000	17.00	
02/09/03	70000				150000		
	150000				230000		
	230000			02/21/03	70000	17.70	
02/10/03	70000	15.00			150000		
	150000				230000		
	230000			02/22/03	70000	16.20	
02/11/03	70000				150000		
	150000				230000		
	230000			02/23/03	70000		
02/12/03	70000	14.80			150000		
	150000				230000		
	230000			02/24/03	70000	13.50	
02/13/03	70000				150000		
	150000				230000		
	230000			02/25/03	70000	15.40	
02/14/03	70000				150000		
	150000				230000		
	230000			02/26/03	70000		
02/15/03	70000		31.4		150000	17.39	32.0
	150000				230000	17.79	32.0
	230000			02/27/03	70000	15.96	32.3
02/16/03	70000	16.20			150000	14.46	32.3
	150000				230000	14.31	32.5
	230000			02/28/03	70000	12.89	32.7
02/17/03	70000	16.00			150000	13.98	32.4

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03/01/02	70000	13.04	32.8
	150000	14.06	32.5
	230000	14.48	32.7
03/02/03	70000	14.91	32.3
	150000	15.67	31.5
	230000	16.31	30.6
03/03/03	70000	16.93	29.9
	150000	16.95	30.1
	230000	15.92	30.1
03/04/03	70000	20.16	
	150000	13.29	28.9
	230000	12.53	29.7
03/05/03	70000	18.29	
	150000	12.97	29.4
	230000	13.43	30.0
03/06/03	70000	12.17	29.5
	150000	14.09	30.1
	230000	14.97	29.8
03/07/03	70000	15.17	29.0
	150000	15.91	28.7
	230000	17.00	27.1
03/08/03	70000	16.63	26.0
	150000	17.42	25.1
	230000	19.05	24.1
03/09/03	70000	19.00	23.8
	150000	19.76	23.9
	230000	20.87	23.8
03/10/03	70000	19.57	24.1
	150000	17.55	28.7
	230000	16.48	29.4
03/11/03	70000	14.99	29.7
	150000	16.34	24.7
	230000	17.66	24.8
03/12/03	70000	17.79	24.9
	150000	18.32	25.2
	230000	18.56	25.2
03/13/03	70000	17.72	25.7
	150000	18.49	26.1
	230000	18.30	27.1
03/14/03	70000	17.32	28.3
	150000	18.30	28.8
	230000	19.86	28.7
03/15/03	70000	19.57	28.8
	150000	20.75	28.7
	230000	21.49	28.9
03/16/03	70000	20.91	29.3
	150000	21.77	29.1
	230000	22.50	28.9
03/17/03	70000	22.23	28.8
	150000	22.66	28.7
	230000	22.93	28.7
03/18/03	70000	21.84	28.7
	150000	22.89	28.7
	230000		
03/19/03	70000		
	150000	23.54	28.6
	230000	24.19	28.6
03/20/03	70000	23.41	28.6
	150000	23.67	28.7
	230000	24.12	28.7
03/21/03	70000	24.03	28.6
	150000	23.99	28.3
	230000	22.98	28.5
03/22/99	70000	21.49	27.4
	150000	20.52	28.9
	230000	20.14	29.5
03/23/03	70000	17.39	29.4

	150000	18.11	29.7
	230000	18.40	29.8
03/24/03	70000	16.48	30.6
	150000	18.27	30.4
	230000	19.36	29.9
03/25/03	70000	18.99	30.6
	150000	20.32	30.8
	230000	21.19	31.3
03/26/03	70000	21.07	31.5
	150000	22.00	31.2
	230000	22.37	30.8
03/27/03	70000	21.51	31.0
	150000	21.58	31.0
	230000	21.86	30.9
03/28/03	70000	20.09	31.0
	150000	20.82	31.3
	230000	21.29	31.2
03/29/03	70000	19.38	31.5
	150000	21.33	31.2
	230000	21.82	30.7
03/30/03	70000	21.08	31.2
	150000	22.30	31.1
	230000	22.77	30.9
03/31/03	70000	22.45	31.5
	150000	23.40	31.6
	230000	23.31	31.5
04/01/02	70000	22.86	31.3
	150000	23.67	31.3
	230000	23.49	31.3
04/02/03	70000	22.25	31.4
	150000	23.00	31.3
	230000	23.58	31.3
04/03/03	70000	23.27	31.1
	150000	23.34	31.1
	230000	23.90	31.1
04/04/03	70000	23.36	31.0
	150000	23.56	31.3
	230000	24.10	31.2
04/05/03	70000	21.47	31.2
	150000	22.41	31.5
	230000	22.16	31.6
04/06/03	70000	21.63	31.3
	150000	21.26	31.5
	230000	21.38	31.7
04/07/03	70000	20.47	31.9
	150000	20.46	31.8
	230000	21.51	32.2
04/08/03	70000	20.86	32.0
	150000	20.72	31.0
	230000	21.77	30.7
04/09/03	70000	21.52	30.9
	150000	21.65	30.1
	230000	21.58	29.5
04/10/03	70000	21.28	29.3
	150000	21.35	29.3
	230000	21.82	29.1
04/11/03	70000	21.63	29.1
	150000	22.50	28.9
	230000	23.23	28.7
04/12/03	70000	22.43	28.9
	150000	22.77	29.0
	230000	22.59	29.2
04/13/03	70000	22.13	29.6
	150000	22.43	29.6
	230000	23.18	29.6
04/14/03	70000	22.62	29.6
	150000	23.22	29.5
	230000	24.09	29.4

04/15/03	70000	23.52	29.5
	150000	24.49	29.7
	230000	25.13	29.6
04/16/03	70000	24.05	29.6
	150000	25.11	29.5
	230000	25.61	29.4
04/17/03	70000	24.67	29.6
	150000		
	230000		
04/18/03	70000		
	150000	26.31	29.5
	230000	27.12	29.5
04/19/03	70000	26.04	29.4
	150000	27.35	29.4
	230000	27.45	29.5
04/20/03	70000	26.69	29.4
	150000	27.01	29.6
	230000	27.49	29.5
04/21/03	70000	26.19	29.5
	150000	26.74	29.6
	230000	27.01	29.6
04/22/03	70000	25.60	29.7
	150000	27.05	29.7
	230000	27.37	29.8
04/23/03	70000	25.97	29.5
	150000	25.88	29.9
	230000	26.10	29.8
04/24/03	70000	24.71	30.0
	150000	25.06	30.1
	230000	26.01	30.0
04/25/03	70000	25.17	30.1
	150000	26.12	30.1
	230000	27.28	30.1
04/26/03	70000	26.01	30.2
	150000	26.65	30.2
	230000	27.08	30.0
04/27/03	70000	26.01	30.3
	150000	26.95	30.5
	230000	27.35	30.4
04/28/03	70000	26.51	30.6
	150000	27.10	30.6
	230000	27.76	29.8
04/29/03	70000	26.55	30.5
	150000	27.12	30.5
	230000	26.99	30.2
04/30/03	70000	26.16	30.3
	150000	27.05	23.9
	230000	27.77	27.0
05/01/02	70000	26.38	30.0
	150000	26.89	30.7
	230000	27.43	30.5
05/02/03	70000	26.23	30.7
	150000	27.10	30.8
	230000	27.79	30.6
05/03/03	70000	26.89	30.3
	150000	27.64	29.8
	230000	28.40	28.8
05/04/03	70000	27.43	28.3
	150000	28.28	27.7
	230000	28.92	27.4
05/05/03	70000	27.72	27.2
	150000	28.57	26.9
	230000	29.70	26.8
05/06/03	70000	27.85	27.5
	150000	27.68	27.2
	230000		
05/07/03	70000		
	150000	28.16	27.8

	230000	29.08	27.7
05/08/03	70000	28.16	27.9
	150000	29.06	27.8
	230000	30.04	27.6
05/09/03	70000	28.85	27.6
	150000	29.52	27.6
	230000	30.16	27.7
05/10/03	70000	28.88	27.8
	150000	29.84	27.8
	230000	30.50	28.2
05/11/03	70000	29.52	28.6
	150000	29.88	28.7
	230000	29.94	28.6
05/12/03	70000	28.79	29.3
	150000	28.85	29.9
	230000	29.18	29.9
05/13/03	70000	28.24	30.1
	150000	28.85	29.8
	230000	29.10	29.2
05/14/03	70000	27.01	29.6
	150000	26.84	29.7
	230000	26.31	29.7
05/15/03	70000	24.97	30.0
	150000	24.82	30.1
	230000	25.13	30.0
05/16/03	70000	23.89	31.1
	150000	25.00	31.2
	230000	26.31	31.0
05/17/03	70000	25.63	31.0
	150000	26.46	31.1
	230000	27.41	31.0
05/18/03	70000	26.72	31.0
	150000	26.25	30.7
	230000	25.58	30.5
05/19/03	70000	23.11	30.4
	150000	22.89	30.8
	230000	22.43	31.0
05/20/03	70000	20.30	31.0
	150000	22.39	31.0
	230000	22.52	31.0
05/21/03	70000	20.37	31.2
	150000	22.41	31.1
	230000	22.41	31.2
05/22/03	70000	20.26	31.4
	150000	21.81	31.3
	230000	22.32	31.4
05/23/03	70000	20.54	31.7
	150000	21.98	31.6
	230000	24.16	31.4
05/24/03	70000	23.13	31.8
	150000	24.40	31.9
	230000	25.69	31.8
05/25/03	70000	24.29	31.9
	150000	25.56	31.8
	230000	26.48	31.5
05/26/03	70000	25.30	31.9
	150000	26.31	31.8
	230000	26.97	31.5
05/27/03	70000	26.04	31.6
	150000	26.84	32.0
	230000		
05/28/03	70000		
	150000	27.28	32.4
	230000	28.14	32.2
05/29/03	70000	26.36	32.5
	150000	27.43	32.4
	230000	28.03	32.3
05/30/03	70000	27.05	32.4

	150000	27.10	32.4
	230000	26.51	32.2
05/31/03	70000	25.41	32.2
	150000	26.65	32.1
	230000	28.12	32.1
06/01/02	70000	27.01	32.1
	150000	27.87	32.0
	230000	29.18	32.0
06/02/02	70000	27.91	32.1
	150000	28.75	32.1
	230000	29.46	32.0
06/03/02	70000	28.63	32.0
	150000	29.22	32.0
	230000	29.70	32.0
06/04/02	70000	28.81	31.9
	150000	29.56	31.9

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BIOGRAPHICAL SKETCH

Chad W. Hanson

Education

2004: M.S. in Biological Oceanography, Florida State University, Tallahassee, Florida, under direction of Jeff Chanton. Thesis: *The influence of diet on stable carbon isotopic composition in otoliths of juvenile red drum (Sciaenops ocellatus).*

1995: B.S. in Biology at University of Wisconsin-Eau Claire, Eau Claire, Wisconsin.

1995: Gulf Coast Research Laboratory Summer Program, Ocean Springs Mississippi.

1993: National Student Exchange Program at University of South Carolina, Columbia, South Carolina.

Professional Experience

1999 – present: Research Staff, Florida Marine Research Institute (Florida Fish and Wildlife Conservation Commission), Apalachicola Field Lab, Eastpoint, Florida.

Field sampler for Fisheries Dependent Monitoring program. Responsible for collecting data and biological tissue samples for Marine Recreational Fisheries Statistics Survey and Trip Interview Program.

March – September 1999: Fisheries Research Technician, Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, Florida.

Assisted in graduate student projects pertaining to population structure and dynamics of freshwater fisheries. Participated in field sampling, fish-aging techniques, and identification of largemouth bass stomach contents.

September 1998 – March 1999: Marine Fisheries Research Technician, Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, Florida.

Assisted in white grunt age and growth study. Responsible for dissection of tissue samples including otoliths for aging fish. Catalogued tissue samples and aged several thousand specimens.

April – August 1998: Biological Laboratory Technician, Cooperative Fishery Research Unit, Tennessee Technological University, Cookeville, Tennessee.

Assisted in graduate students' and research assistants' freshwater mussel and fisheries projects. Participated in field sampling, experiment design and set of aquatic recirculating systems.

August – October 1997: Natural Area Restoration Crew Member, Arkansas Field Office, The Nature Conservancy, Little Rock, Arkansas.

Participated in activities associated with restoration of upland hardwood forest habitats. Assisted in prescribed burns and habitat/plant monitoring.

April – August 1997: Biological Science Technician, Department of Forestry, Wildlife, and Fisheries, Louisiana State University, Baton Rouge, Louisiana.

Assisted in graduate student's radio telemetry of coyote project. Responsible for collecting field telemetry data in freshwater marshlands of Sabine National Wildlife Refuge.

Research Presentation

Hanson, C.W. and J.P. Chanton. The Influence of diet on stable carbon isotopic composition in otoliths of juvenile red drum (*Sciaenops ocellatus*). Florida Chapter American Fisheries Society 24th Annual Meeting, Withlacoochee State Forest Training Center, Brooksville, Florida, February 23, 2004.

Poster Presentation

Hanson, C.W. and J.P. Chanton. Carbon stable isotope composition of red drum otoliths: A reflection of diet? Florida Chapter American Fisheries Society 23rd Annual Meeting, Withlacoochee State Forest Training Center, Brooksville, Florida, February 25-27, 2003.