2005

Platform recruited reef fish, Phase I: do platforms provide habitat that increase the survival of juvenile reef fishes?

Lauren Kay Nowling
Louisiana State University and Agricultural and Mechanical College

PLATFORM RECRUITED REEF FISH, PHASE I: DO PLATFORMS PROVIDE HABITAT THAT INCREASE THE SURVIVAL OF JUVENILE REEF FISHES?

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Oceanography and Coastal Sciences

by
Lauren Kay Nowling
B.S., Auburn University, 2001
December 2005

To my brother Joseph, who can do anything he puts his mind to if he believes in himself.

ACKNOWLEDGEMENTS

I would like to thank Dr. James H. Cowan, Jr., my major advisor, for supporting me during my time at LSU. Dr. Cowan encouraged me to do my best and believed in me at times when I didn't believe in myself. I am also thankful for the guidance I received from my other committee members: Dr. Richard Shaw, Dr. Sam Bentley, and Dr. Barry Moser. Their help and support were invaluable to the completion of my degree. I would like to acknowledge that the Minerals Management Service funded this research. I sincerely thank my colleagues at the University of Hawaii who put their time and effort into completing this project. They are Dr. Bob Gauldie, Dr. Eric DeCarlo, and Yvonne Perry. I would also like to thank Dr. Khal Spencer for his help originating the otolith digestion procedure for this study. There are many graduate students and research associates in the Coastal Fisheries Institute and elsewhere that were a great help to me. I would like to thank Melissa Woods Jackson, Jered Jackson, Jessica McCawley, Mike McDonough, Megan Westmeyer, and Dave Nieland for their assistance in the field. I am positive that this project would have been lacking without their aid in collecting samples and data. I also owe thanks to Nicole Smith, Thorpe Halloran, Andy Fischer, Pam MacRae, and Dave Wells for their assistance in the laboratory. I am indebted to the recreational charter boats that allowed my collection of red snapper. I would like to extend my thanks to Captain Mike Thierry of Dauphin Island, Alabama, as well as Captain Steve Tomeny of Port Fourchon, Louisiana. I would like to thank Dr. Will Patterson and Dr. Walter Ingram for their help and advice on this project. Lastly, I would like to thank my family and my husband David for their continual support, both emotional and financial. I could not have done any of this without you! Thank you all!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

There are currently over 4000 functioning oil and gas platforms in the northern Gulf of Mexico (Gulf). Platform operations, and their prior drilling operations, produce trace amounts of lead, barium, vanadium, and lanthanum residues that are leached into the surrounding waters and are deposited on the sea floor. These residues have isotopic ratios different from those typical of the Gulf seafloor and can be used as harmless 'fingerprints' if they become incorporated into hard-parts or tissues in fishes associated with oil and gas platforms. From 2002 to 2004, 115 red snapper were collected from oil and gas platforms and artificial reefs off Louisiana and Alabama. Otoliths were removed and analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The objective of this study was to determine if a trace element isotope ratio fingerprint could be detected and described as unique to red snapper inhabiting the platforms. Stepwise and canonical discriminant function analyses were used to compare red snapper otolith fingerprints from on and off platforms, and from east and west of the Mississippi River. Classification accuracies based on the probability of an individual fish being correctly classified into the habitat from which it was sampled were over 90% for each of the two main comparisons. When comparing the elemental composition of red snapper otoliths from Louisiana oil and gas platforms and Louisiana artificial reefs, the classification accuracy was 93.75%. When comparing the elemental composition of red snapper otoliths from Louisiana artificial reefs and Alabama artificial reefs, the classification accuracy was 91.06%. Vanadium 51, Lead 206, Lead 207, and Lead 208 all appear to be linked with oil and gas platforms or their prior drilling operations, as the concentrations of these four elements or isotopes were significantly higher in otoliths sampled on

platforms in Louisiana than in otoliths sampled from artificial reefs in either Louisiana or Alabama. Results from this study indicate that it may be possible in future studies to determine if oil and gas platforms contribute disproportionately to the survival of juvenile and adult red snapper, and as such can be considered viable management tools for stock rebuilding.

INTRODUCTION

Red Snapper (*Lutjanus campechanus*)

The Magnuson-Stevens Fishery Conservation and Management Act, the 1996 revision and reauthorization of the 1976 Public Law 94-265 (Act), requires that fishery management plans include an identification and description of essential fish habitat (EFH), adverse impacts on EFH (including the effects from fishing), and actions to conserve and enhance EFH (Gallaway *et al.*, 1999). One of the most pressing federal fisheries management concerns in the Gulf of Mexico (Gulf) region is the overfished status of red snapper *Lutjanus campechanus* (Schmitten, 1999). National Marine Fisheries Service (NMFS) data collection provides evidence that the primary cause of overfishing on red snapper is bycatch of age-0 and age-1 (juvenile) red snapper by shrimp trawls (Schirripa and Legault, 1999). As such, the conservation and enhancement of red snapper EFH may act to reduce the impacts of shrimp trawl mortality on juvenile red snapper (Schmitten, 1999).

Red snapper support the most economically valuable finfish fishery in the Gulf and are among the most targeted fishes by commercial and recreational fishers (Goodyear, 1995). The red snapper stock in the Gulf has been subjected to heavy fishing pressure since the 1800's and is currently estimated to be severely overfished (Goodyear, 1995; Schirripa and Legault, 1999). Since 1991 both commercial and recreational fisheries have been constrained by size limits, creel or trip limits, and quotas as established by the Gulf of Mexico Fishery Management Council (Wilson and Nieland, 2001).

Red snapper are demersal fish that are distributed along the continental shelf throughout the Gulf. Young red snapper spend most of their first year of life over sand and mud bottoms on the shrimping grounds in the northern Gulf, after which they move offshore to reef environments (Patterson *et al.*, 1998). Adult red snapper are found in deeper offshore waters near coral, rocks, banks, outcrops, and manmade submerged objects such as oil and gas platforms and artificial reefs (Workman and Foster, 1994). They are reef-associated fish and only utilize artificial reefs for certain parts of their life history. It has been noted that red snapper may use reefs for protection from predation but move off the reef to spawn and feed (Gallaway *et al.*, 1981; Bradley and Bryan, 1975). Once red snapper recruit to offshore reefs, such as oil and gas platforms, they have been found to feed primarily on benthic organisms in the surrounding sediment. As these fish mature, they tend to become more piscivorous, even feeding on juvenile red snapper (McCawley, 2003).

The way in which different life stages of red snapper utilize these various habitats as they grow, e.g. for feeding, protection or both, is unknown. Information on age-0 red snapper is sparse, especially at first settlement (Szedlmayer and Conti, 1999). However, it has been demonstrated that as red snapper mature they show strong preferences for natural and artificial habitat with vertical relief (Gallaway *et al.*, 1999; Szedlmayer and Conti, 1999; Workman and Foster, 1994). It is these areas that are considered by many to constitute red snapper EFH.

Red Snapper Site Fidelity

Many studies have been performed to measure red snapper site fidelity and results have been variable. Some studies off of the Alabama coast have indicated that red

snapper have high site fidelity to reefs and remain there for long periods of time. Szedlmayer and Shipp (1994) performed a mark and recapture study off of coastal Alabama in 1994. They concluded that red snapper showed a high degree of residence to artificial reefs, with most fish recaptured within 2 km of their release site, even after extended periods of time. In contrast, a more extensive study by Patterson *et al.* (2001) off of Alabama found that only 55% of recaptures not at liberty during hurricanes had remained at their release sites. They demonstrated that over time, even fish not exposed to hurricanes had a relatively low probability of remaining at their release sites (Patterson *et al.*, 2001).

Opinions of red snapper site fidelity have begun to change. It is now believed by some that red snapper site fidelity is much lower than previously suggested. Peabody (2004) concluded that red snapper site fidelity on Louisiana oil and gas platforms is dependent upon the time scale being examined. Results showed that red snapper displayed high short-term fidelity to oil and gas platforms on the order of days, weeks, to even a month or two (Peabody, 2004). However, Peabody (2004) also concluded that in the longer-term, over a period of a few or more months, red snapper exhibited low fidelity and it is unlikely that they will remain at one location for more than a few consecutive months.

Oil and Gas Platforms in the Gulf of Mexico

In the waters of the northern Gulf there are over 4000 functioning oil and gas platforms (Stanley and Wilson, 1998). Since the first platform was installed, fishers and scientists have been aware of their associated nekton assemblages (Stanley and Wilson, 1997). They act as artificial reefs by: 1) providing habitat that potentially increases the

growth and survival of individuals; 2) affording shelter for protection from predation and spawning substrate; and, 3) acting as a visual attractant for organisms not otherwise dependent upon hard bottom (Gallaway *et al.*, 1981). Oil and gas platforms differ from most natural habitats and from traditional artificial reefs in that their vertical profile extends throughout the entire water column into the photic zone (Stanley and Wilson, 1991). Increased habitat quality on, or immediately around, oil and gas platforms is believed to be derived from increased *in situ* food production associated with encrustation by fouling organisms, but this concept has not been proven. Any amount of fortification of the bottom for support of the structure is also believed to increase habitat quality on, or around, oil and gas platforms.

Due to the noted presence of red snapper around oil and gas platforms, recreational and commercial fishing efforts have continually been concentrated in those areas. Gallaway *et al.*, (1981) reported that of all species caught by bottom fishing in the Buccaneer gas and oil field area off Texas, red snapper comprised 80% of the catch (Gallaway *et al.*, 1981). Later, Stanley and Wilson (1990) showed that a high diversity of fish (including red snapper) existed around oil and gas platforms off coastal Louisiana. They reported that catch rates of private vessel anglers and charter boat operators around oil and gas platforms in the northern Gulf were high, with their effort targeted towards red snapper while offshore bottom fishing (Stanley and Wilson, 1990).

Artificial reefs, such as oil and gas platforms, may be useful tools for fishery managers if they increase fisheries production, but many researchers question whether or not they are a positive influence on reef stock dynamics (Bohnsack, 1989; Grossman *et al.*, 1997). There have been doubts about whether or not they produce or attract fish and

the resolution to this question is essential to the management of reef fish stocks (Seaman, 1997; Lindberg, 1997). If they indeed constitute EFH for reef fish, then they can be considered as viable management tools. If they are simply attracting fish to the area, they may merely promote overfishing. Bohnsack (1989) offered a conceptual model which inferred that increased production is most likely at locations isolated from natural reefs, and for habitat-limited, demersal, philopatric, territorial, and obligatory reef species. Attraction should be more important in locations with abundant natural reef habitat; where exploitation rates are high; and for recruitment-limited, pelagic, highly mobile, partially reef-dependent, and opportunistic reef species (Bohnsack, 1989). The question of habitat limitation lies at the heart of the artificial reef controversy (Grossman *et al.*, 1997). Bohnsack (1989) also stated that artificial reefs are unlikely to benefit heavily exploited or overfished populations without other management actions. Currently, the Gulf Management Council does not include oil and gas platforms, or any other artificial reef habitat, in their treatment of EFH.

Otoliths

The teleost otolith is composed of calcium carbonate, mainly in the form of aragonite, and an organic matrix including proteins, carbohydrates and lipids, as well as trace elements that are deposited during otolith deposition (Takagi *et al.*, 2000). Otoliths are located in the head of fishes in three pairs (sagittae, lapilli, and asteriscii) and function in the acoustico-lateralis system (Patterson, 1997). They grow, or accrete, relative to somatic growth, forming concentric opaque and translucent rings; increments in otoliths can be deposited sub-daily, daily, and annually.

Otoliths are bathed in endolymph within the inner ear sacs and the otolith grows without touching any cells (Takagi, 2002). It is generally believed that the organic matrix is first constructed, followed by aragonite crystallization. The cells of the membranous wall of the otolith organ synthesize components of the otolith matrix. The components are secreted into the endolymph, a framework is constructed and the aragonite crystallization occurs on that framework (Takagi *et al.*, 2000). Therefore, the calcification process of otoliths is heavily dependent upon the composition of the endolymphatic fluid (Campana, 1999). The key regulating factors appear to be pH of the endolymph, which is determined by the concentration of bicarbonate ions in the endolymph (Romanek and Gauldie, 1996; Payan *et al.*, 1997, 1998), and temperature. Calcium carbonate can crystallize as any one of three crystal morphs (calcite, aragonite or vaterite) and the rate and type of calcium carbonate crystals formed in otoliths is regulated by proteins (Campana, 1999). Aragonite is the norm for sagittae and lapilli otoliths, while most asteriscii are made of vaterite. Strontium carbonate is virtually isostructural with aragonite making substitution of Sr ions for Ca in aragonite very likely. Ions similar to Ca and Sr, such as other alkaline earth metals (Mg and Ba), can also be substituted for Ca in the aragonite matrix.

Otolith Microchemistry

In the field of fisheries biology and management, the analysis of otolith microstructure is a quickly expanding field of prime importance (Payan *et al.*, 1997). Fish otoliths have traditionally been used as a hard part to age fish, but recent research indicates that they may also serve as ideal natural markers of individual fish or fish populations (Campana *et al.*, 1994). Some goals of otolith research focus on transport,

movement, and mixing hypotheses, as well as understanding the mechanisms by which minor and trace elements are incorporated into otoliths, and developing tools with which to measure the elements present (Patterson, 1997). Secor *et al.* (1995) stated that concerted efforts at the suborganismal and organismal level are required to determine the effect of the environment on otolith composition.

Early studies using otolith chemistry to differentiate between fish populations or stocks were carried out through the use of proton and electron microprobe analysis. Microprobe analysis can be useful to elucidate the elemental composition of calcified tissues of fish. The nondestructive nature and the multi-elemental sensitivity of these techniques make them useful. With this method samples are irradiated with photons or electrons or even protons, which results in the emission of x-rays. The wavelength and energy dispersion of the resultant x-rays are specific to each element and the amount of that element being analyzed (Radtke and Shafer, 1992). Another advantage of electron microprobe analysis is that the electron probe provides good resolution over small spatial scales (5 μm), as is needed to trace daily and sub-daily chemical histories in larval and juvenile fish otoliths (Patterson, 1997). While this method is widely used, it does have a few drawbacks. This instrumentation is only capable of detecting a small number of elements, one to six. Recent studies indicate that a much broader suite of elements exist in otoliths, but at concentrations well below that detectable with the electron microprobe. There can be a great deal of variation in sample preparation, equipment and experimental design and there is a need to standardize techniques (Radtke and Shafer, 1992).

One of the most recent techniques that is commonly used to assay otoliths is Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). This technique has been

extensively developed as a standard first approach for bulk analysis of micro-composition and has shown that trace elements are present in otoliths down to parts per trillion (ppt) (Thresher, 1999; Patterson, 1997). It involves the use of argon gas plasma produced in a quartz torch at temperatures of greater than 5000^{o} K. According to Thresher (1999), these high temperatures induce intense spectral lines from a wide range of elements, which facilitates multi-element analysis. The advantages of ICP-MS over other approaches include the ability to simultaneously assay numerous elements (and isotopes of elements) quickly and inexpensively, with a sensitivity that matches or exceeds that of x-ray microanalysis, the proton microprobe, neutron activation analysis, atomic absorption spectroscopy, inductively-coupled plasma atomic emission spectroscopy, and x-ray fluorescence (Campana and Gagne, 1995).

Isotope dilution ICP-MS (ID-ICPMS), a variant of ICP-MS, is often used and is thought to be the most accurate of the otolith analytical techniques currently available. ID-ICPMS consists of dissolving whole otoliths into solution and analyzing them using the inductively coupled plasma-mass spectrometer. Advantages of whole-otolith assays include ease of preparation, absence of error associated with sampling or identifying growth increments, and the availability of accurate and precise assay protocols (Campana, 1999). The major disadvantage is associated with the inability to take advantage of the chronological growth sequence recorded in the otolith. Inductively Coupled Plasma-Mass Spectrometry has emerged as the instrument of choice for assays due largely to its capability for rapid and accurate isotopic and elemental assays over a wide range of elements and concentrations (Campana, 1999). The use of ID-ICPMS also minimizes (although not eliminates) the effects of instrumental drift.

The elements useful for site-specific, chemical location markers in otoliths are: lead (Pb), strontium (Sr), barium (Ba), lanthanum (La), vanadium (V) and the elements of the thorium-uranium (Th-U) series. This group of elements forms carbonates whose crystal structure is isostructural with that of aragonite. By having carbonates that are similar in structure to aragonite, these elements are embedded in the crystal matrix of the aragonite itself, and therefore cannot be mobilized by minor pH changes or by the effects of washing and handling (Proctor and Thresher, 1998). Lead is a desirable element to consider as a site-specific marker where there has been industrial activity, such as is the case for oil and gas platforms. Combustion engine operations, electrical motors and some kinds of metal priming paints result in lead residues, although in very small amounts. Another source of potential contamination to otoliths is the corrosion and leaching of submerged platform structures and corrosion of oil and gas platform gratings or decks causing small metal flakes to be released into the water column. In the Buccaneer gas and oil field study, Gallaway *et al.* (1981) stated that observed metal flakes were probably a major source of the trace metal contamination of sediments taken adjacent to the platforms.

A further advantage for this study is that platform drilling operations usually involve the use of drilling muds that contain bentonite (mostly the mineral montmorillionite) that has traces of rare earths and barium. Drilling muds perform several functions integral to the drilling process. The most important of these include transport of drill cuttings to the surface, balance of subsurface and formation pressures thus preventing a blowout, and to cool, lubricate and support part of the weight of the drill bit and drill pipe (Neff *et al*., 1987). The industrial lead compounds mentioned previously,

and montmorillionite, are derived from a single lead mine in Wisconsin and a single montmorillionite mine in Wyoming. Both industrial lead compounds and montmorillionite-containing bentonite will have mine-specific isotope ratios that reflect the unique geochemical histories of the mines involved. It is believed that existing oil and gas platform operations, and their prior history of drilling operations, are likely to produce trace amounts of residues of Pb, Ba, V, and La whose isotope ratio signatures will be different from those typical of the Gulf seafloor away from platforms. We hypothesize that this can be used as a harmless 'tag' in the otoliths of the juvenile fish that have spent time in close association with oil and gas platforms.

Incorporation of Elements into Otoliths

Minor and trace elements may be incorporated into otoliths generally by incorporation into the otoliths' protein structure or by replacing calcium, carbon, or phosphorus in the aragonite crystals of otoliths (Kalish, 1989). Certain elements may be more labile within otoliths than others (Rooker *et al.*, 2001). Those associated with the organic matrix rather than those that substitute for elements within the lattice may be more prone to contamination from, or loss to, surrounding solutions. Postmortem handling procedures can cause changes in elemental concentrations within both the endolymph and otolith (Proctor and Thresher, 1998). Therefore, special care has to be exercised when selecting the elements to be analyzed and when handling, storing, and processing the otoliths to minimize the risk of contamination. Rooker *et al.* (2001) demonstrated that *Thunnus albacares* otoliths easily acquired trace impurities but acid washing was found to successfully remove surface contaminants.

The pathway of a given element or ion from the environment into the otolith is a multi-stage process and is characterized by a sequence of more or less independent barriers (Campana, 1999). The basic pathway of inorganic elements into the otolith is from the water to the blood plasma via the gills or intestine, then into the endolymph, and finally into the crystallizing otolith (Campana, 1999). Elements entering the blood plasma via the gills is the primary method in freshwater fishes, while the continual drinking of seawater by marine fish is the main source of elements being incorporated in the intestine. It is believed that only a minimal amount of elements are gathered from food sources, but the specific portion is unknown.

The intestine-water interface in saltwater fishes is likely one of the biggest barriers to elemental uptake. Osmoregulation regulates movement of water-bourne ions into the fish. The next barrier is the blood plasma-endolymph interface and cellular transport is responsible for moving the water-bourne elements there. Lastly, elements must pass through the endolymph-otolith interface, which occurs during the otolith crystallization process. In general, the composition of the endolymph appears to be closer to the composition of the otolith than is the water or blood plasma (Campana, 1999). Payan *et al.* (1998) indicated that the elemental composition of the endolymph is less affected by starvation than is the plasma.

Otoliths as Natural Tracers

There are two observations of otoliths that make otolith microchemistry successful: (1) otoliths grow throughout the life of the fish and, unlike bone, are metabolically inert; once deposited, otolith material is unlikely to be reabsorbed or altered; and, (2) the calcium carbonate and trace elements that make up 90% of the otolith

appear to be mainly derived from the water (Casselman, 1987; Simkiss, 1974). The majority of elements are present at minor (> 100 ppm) and trace (< 100 ppm) levels, with many more trace elements presumably awaiting detection (Campana, 1999). The minor elements are represented by Na, Sr, K, S, N, Cl, and P, while the bulk of the trace elements are present at concentrations of less than about 10 ppm (Campana, 1999; Rooker *et al.*, 2001). Broad spectrum elemental assays of otoliths have resulted in trace and minor element concentrations of less than 1% of the whole otolith and protein concentrations of about 3 to 4% (range of 1 to 8%) (Edmonds *et al.*, 1992).

The theory behind using otoliths as natural tracers of individual fish or stocks is a fairly simple concept. Since the otolith grows continuously throughout a fish's life, the elemental fingerprint of the whole dissolved otolith integrates across the entire lifetime of the fish, and thus can be used to distinguish among fish which have experienced different overall environmental exposures (Edmonds *et al.*, 1992; Campana and Gagne, 1995). It is thought that the environmental information stored upon the formation of fish otoliths is preserved internally, and that the otoliths contain not only information about age but also a large amount of other ecological data (Kakuta *et al.*, 1997). The elemental composition of otoliths can reflect the elemental composition of the water body in which the fish lives, the elemental composition of the food of the fish, and the short and long term behavior of the pH of the fish inner ear resulting in changes in otolith chemistry in response to food, otolith growth rate, temperature, salinity and even stress induced by chasing (Mugiya and Satoh, 1995; Arai *et al.*, 1996).

The use of otolith elemental fingerprints as natural tags relies upon three central assumptions: (1) There are characteristic and reproducible markers for each group of

interest. If the elemental fingerprints of the groups of interest do not differ significantly, little more can be done; (2) All possible groups contributing to the group mixture have been characterized. Uncharacterized groups of fish present in the mixture could be mistakenly interpreted as one or more of the reference groups; and, (3) The marker remains stable over the temporal interval between characterization and mixing. The potential for drift in elemental concentration of otoliths becomes greater as the interval length is extended (Campana, 1999).

Campana (1999) showed the extent to which otolith trace element concentration reflects (or fails to reflect) that of the ambient water from a comparison of water, blood and otolith composition from two very distinct environments: fresh water and salt water. The concentrations of many of the most common elements (Ca, Na, K, Mg, Cl) differed substantially between fresh and salt water, yet those differences did not appear to be reflected in the otolith when compared. Other elements such as P, Cu, and S that are physiologically important appeared to remain uninfluenced by the relative concentrations in the environment (Campana, 1999). When Campana (1999) examined the corresponding blood concentrations for most of the elements noted above, there was little or no difference in plasma concentrations between freshwater and saltwater. This stability in blood plasma composition is completely consistent with the strict osmoregulatory control required for the fish's survival. Campana (1999) concluded that because the elements deposited in the otolith are derived penultimately from the blood plasma, it is clearly unrealistic to expect the otolith content of physiologically regulated elements to reflect environmental abundance. Through his comparisons Campana (1999) also concluded that the relative concentration of trace elements such as Sr, Zn, Pb, Mn, Ba,

and Fe in freshwater and marine otoliths is consistent with an environmental effect. While it may be possible to use the concentration of any given element to distinguish between groups of fish, it is preferable to use all elements at once, e.g., a multivariate elemental fingerprint (Campana *et al.*, 2000).

Objectives

The main objective of this project (Phase I for ease of notation) is to test the hypothesis that association with oil and gas platforms during early life imparts a detectable 'trace element isotope ratio fingerprint' in the otoliths of juvenile red snapper. If this phase of the project is successful, it will set the stage for future research (Phase II) that will test whether adult fishes containing the 'platform fingerprint' in their otoliths contribute disproportionately to adult stocks on nearby natural and artificial reefs. The rationale for the choice of red snapper in Phase I of the project is due to their site fidelity as well as their recreational and commercial importance. Although there are differing conclusions on red snapper site fidelity, I believe that they exhibit the appropriate model life history to prove the concept of this study. Patterson *et al*. (2001) showed that red snapper have high site fidelity (70%) during their first years as recruits on offshore habitats, with site fidelity decreasing as fish get older. Based on the work by Patterson *et al*. (2001), I believe that red snapper remain at oil and gas platforms long enough to incorporate a signal into their otoliths, if one is available. Determination of oil and gas platforms as red snapper EFH would be an important tool for fisheries managers and may help to restore stock size. The determination of the impacts of artificial reefs (of any type) on the demographics of reef-associated fishes is also a necessary step in moving towards final resolution of the production vs. attraction debate.

The specific objective is as follows:

- To test whether association with oil and gas platforms imparts a detectable "trace element isotope ratio fingerprint" in the otoliths of juvenile red snapper, making Phase II possible.

Hypotheses

H_o1: Association with oil and gas platforms during early life does not impart a detectable 'trace element isotope ratio fingerprint' in the otoliths of juvenile reef fishes, represented here by red snapper.

H_A1: Association with oil and gas platforms during early life imparts a detectable 'trace element isotope ratio fingerprint' in the otoliths of juvenile reef fishes, represented here by red snapper.

MATERIALS AND METHODS

Red Snapper Collection

A total of 115 red snapper were collected from both artificial reefs (anything other than oil and gas platforms - controls) and oil and gas platforms in Alabama and Louisiana over a two-year period from 2002 to 2004. The first sampling trip took place on October 18, 2002; fish were collected on artificial reefs off coastal Alabama. The red snapper sampled (N = 45) were caught aboard the recreational fishing vessel Lady Ann using hook and line. Exact location of capture of each fish is unknown but all were caught approximately 15 to 20 miles south of Dauphin Island in a grid of artificial reefs located within the Hugh Swingle Permit Area (Figure 1). Upon collection, measurements of fork length (mm ± 1), total length (mm ± 1), dorsoventral height (distance from first dorsal spine to pectoral fin in mm ± 1), sex, and gonad development (if possible) were taken. Weight was calculated using a length-weight regression compiled by Patterson (1999) and is discussed in more detail below. Once these measurements were recorded, fish were cleaned and the head of each fish was removed using an electrical fillet knife. The heads were placed in individual plastic storage bags and then stored on ice until they were returned to Louisiana State University (LSU) for immediate otolith removal. For otolith processing at a later date, heads were kept frozen to minimize contamination.

The equations used in this study to convert red snapper length (mm) to weight (kg) were derived by Patterson (1999) based upon fish caught off Alabama. He also fitted weight-length equations for each sex. Since only length measurements were taken as part of this study, the equations formulated by Patterson (1999) were used to calculate weights

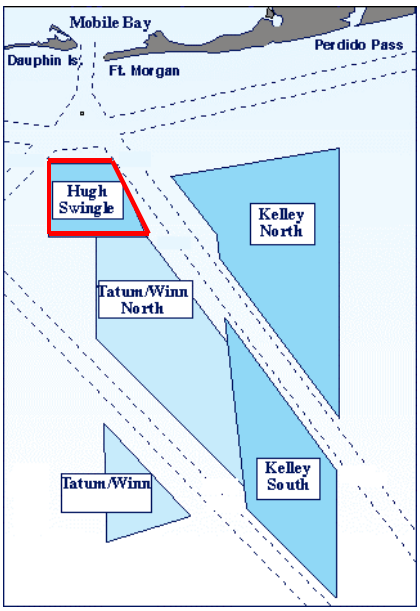

Figure 1. Map of study area where red snapper were collected from artificial reefs in the Hugh Swingle Permit Area off Alabama (Taken from Patterson, 1999).

for my male and female red snapper. The equations used are as follows:

$$\text{Male: } Wt = 5.32 \times 10^{-9} * (TL)^{3.16}$$

$$\text{Female: } Wt = 3.26 \times 10^{-9} * (TL)^{3.23}$$

Although these equations were formulated using red snapper caught off Alabama, I used them to calculate weights for both Louisiana and Alabama samples in my study.

The next sampling trip occurred on May 28, 2003. Thirty-five red snapper were collected on oil and gas platforms aboard a recreational charter boat in the South Timbalier Circle off the Louisiana coast, which is located in Minerals Management Service lease blocks 128, 134, 135, 151 and 152 (Figure 2). These fish were caught using hook and line. Upon collection, measurements of fork length (mm), total length (mm), dorsoventral height (mm), sex, and gonad development (if possible) were taken. Once these measurements were obtained, the heads of the red snapper were then removed and

stored in individual plastic storage bags on ice. The heads were returned to LSU for otolith removal.

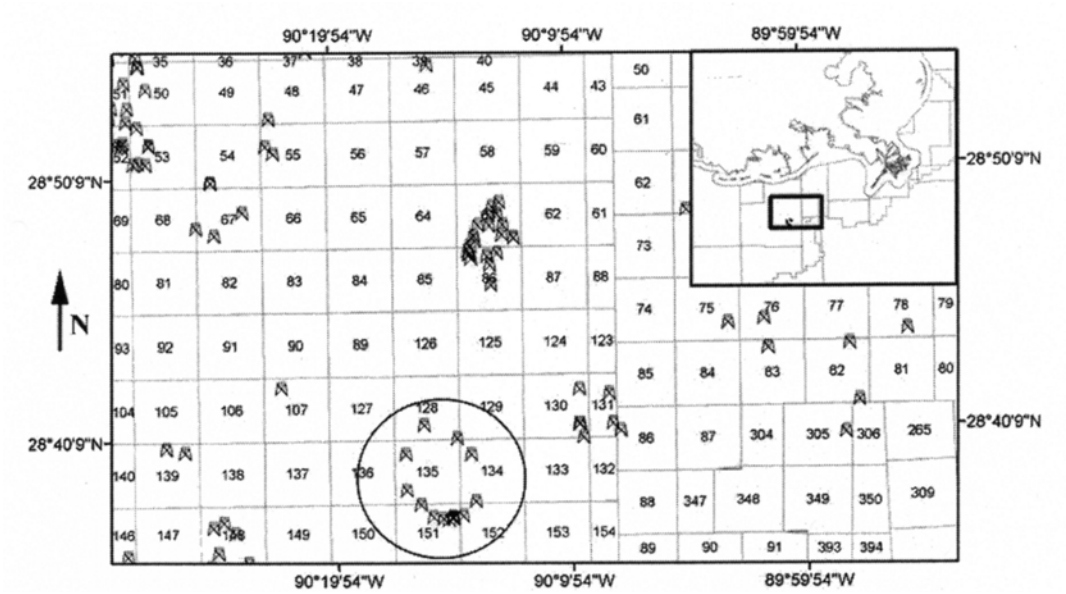

Figure 2. Map of study area where red snapper were collected from oil and gas platforms off Louisiana in the South Timbalier Lease Block (Taken from Peabody, 2004).

In addition, thirty-five red snapper were collected on artificial reefs south of Port Fourchon, Louisiana, during two trips that took place on September 19, 2003, and October 17, 2003 (Figure 3). The first ten red snapper were collected on the September trip using hook and line while fishing on sunken armored personnel carriers (APC's), boats, and barges in the South Timbalier Lease Block. The other twenty-five specimens were collected using hook and line on the October trip while fishing in deeper water within the Grand Isle Lease Block. The same measurements of fork length (mm), total length (mm), dorsoventral height (mm), sex, and gonad development (if possible) were taken prior to cleaning. The heads were then removed using a fillet knife and were stored individually in plastic storage bags for transport to LSU. Although my sampling design called for collection of red snapper on oil and gas platforms east of the Mississippi River

delta, I had difficulty catching them in the spring of 2004. Several attempts were made to collect these fish on recreational and private fishing trips once the red snapper season opened in April but all were unsuccessful. Therefore, that particular sample was excluded from my study.

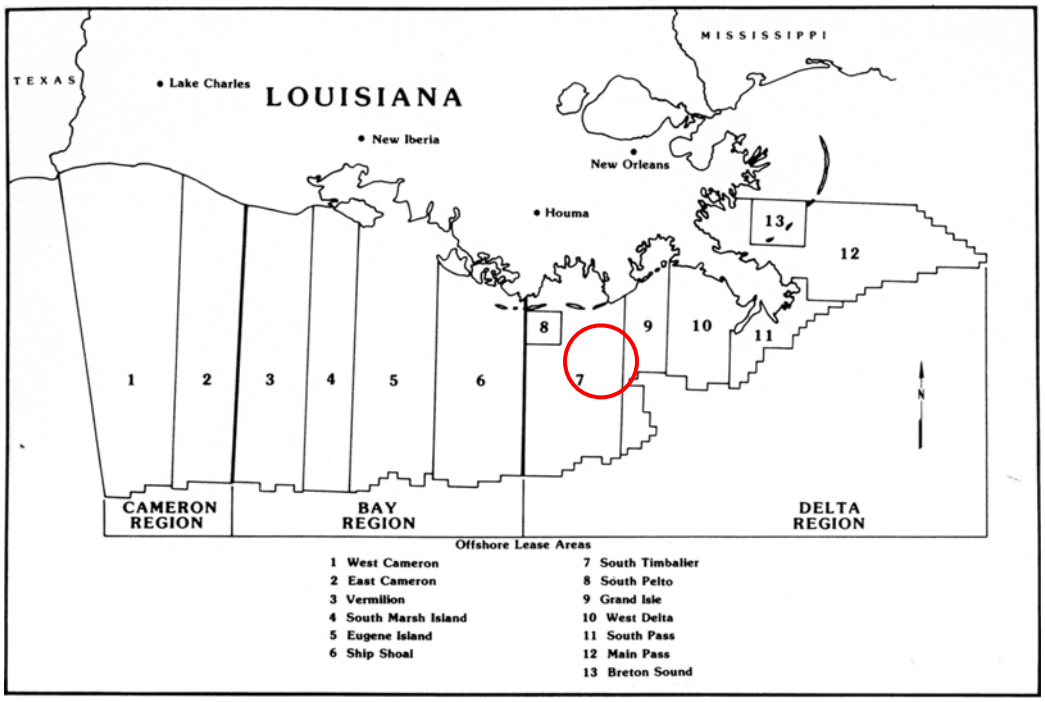

Figure 3. Study area south of Port Fourchon, Louisiana, for both artificial reef and oil and gas platform red snapper samples (Taken from Stanley and Wilson, 1990).

Otolith Removal

For all 115 red snapper collected, the heads were thawed in the laboratory in sealed plastic bags inside collection containers. Dissection and handling tools were cleaned first with 18 megohm water, followed by rinsing with quartz-distilled water (QED). Laboratory surfaces were cleaned with de-ionized water (DI) and covered with clean paper. All samples were dissected in a clean (Class-100) laboratory in a High

Efficiency Particulate Air (HEPA) filtered air module. All tools and specimens were handled with clean-room grade vinyl gloves.

Otoliths were removed in the HEPA filtered air module using a chisel and Teflon forceps. The red snapper head was laid in a collection container within the air module. The operculum was pulled back and the gill rakers were cut and moved to the side using the chisel. The cavity containing the sagittal otolith was then exposed and pried open with the chisel. The otolith was removed using plastic forceps. This procedure was performed on each side of the head to obtain both of the sagittal otoliths. Once an otolith was removed it was wiped clean using sterile laboratory wipes and then placed in a clean, labeled glass vial. Vials were packed in boxes and made ready for shipment. Since all mass spectrometry of the otoliths was performed in laboratories at the University of Hawaii at Manoa, otoliths were immediately shipped to Honolulu.

Otolith Digestion at the University of Hawaii at Manoa

Once otoliths arrived at the University of Hawaii they were prepped for digestion. They were transferred from the glass vials used for shipping to acid washed petri-dishes. Otoliths from artificial reefs in Louisiana (fall 2003 sample) were the first to be digested. The first 15 pairs of otoliths (AR-03-LA to AR-18-LA) from this sample were processed using the digestion procedure described below. This procedure was later modified and those modifications are described in full detail.

Original Otolith Digestion Procedure:

One otolith from each pair was placed into a tared plastic beaker and weighed and the weight (g ± 0.00001) was recorded. A 1-ml volumetric pipette was then used to rinse the otolith with 2.5 ml of DI water to remove surface contaminants (Figure 4). The water

was then transferred out of the plastic beaker. The otolith was again covered with 3 ml of DI water and sonicated for 1 minute. The 1-ml pipette was again used to remove the water from the beaker. For a final rinse, 3 ml of DI water were used to cover the otolith and then removed as before. These same steps were also performed on a 'blank' beaker for comparison.

Figure 4. Addition of DI water to beakers and otoliths for rinsing off surface contaminants (Yvonne Perry – UH at Manoa).

The otolith was then transferred to an acid leached beaker. The tare weight of the beaker was recorded (g ± 0.00001). Four ml of DI water and a small amount of ~8-N HNO_3 were added to the beaker to begin the otolith digestion. The beaker was covered loosely and put aside. Two-hundred and fifty µL of HNO_3 were added every few minutes until the otoliths were completely dissolved. Once the otoliths were completely dissolved, an additional 250 µL of HNO_3 was added to each vial. The same procedure was performed on the 'blank' as well. The otolith samples and blank were dried individually

under heat lamps. Due to the limited number of dry down units and heat lamps (Figure 5), this procedure only allowed for 5 otolith samples and one blank to be processed at a time.

Figure 5. Heat lamps and units used to dry down otolith samples after addition of acid (UH at Manoa).

Once the samples were dry, 1.5 ml of 10.5-N HCl and 1 ml of 11.2-N HNO_3 (a mixture known as aqua regia) were added to the vials. The vials were capped tightly and left over night on low heat under red lamps. The next day, samples were held in front of a light source and checked for residue remaining on the walls of the vials. If no residue was present, vials were again put into dry down units and dried slowly under heat lamps. Two ml of 7.5-N HNO_3 were added to each sample and the blank to remove some of the chloride from the aqua regia stage. Once otolith samples were dried to a 'puffy mass', they were dissolved in approximately 6 ml of 1.5-N HNO_3. The blank was dried to a small drop before adding 6 ml of 1.5-N HNO_3. The samples were then completely digested and ready to be analyzed using ICP-MS.

Modified Otolith Digestion Procedure:

The original otolith digestion procedure using heat lamps to dry down samples was then modified to a procedure using microwave digestion. The new procedure allowed for 11 samples and one blank to be digested at a time and the digestion took less time than the previous method. Therefore, all other otoliths from artificial reefs in Louisiana, as well as all other otolith samples collected, were prepared using the microwave digestion procedure. The new otolith digestion procedure required the use of Advanced Composite Vessels® (ACV) and a CEM Corporation MDS 2100 Microwave Digestion System® (Figure 6).

Otoliths were placed into tared plastic beakers and weighed (g ± 0.00001). Weight was noted before washing. Otoliths were then rinsed with 2.5 ml of DI water. A 1-ml pipette was used to squirt water over the samples to loosen surface contaminants. The pipette was then used to transfer the water out of the beaker. The otolith samples were covered with 3 ml of DI water and sonicated for 1 minute. Water was again removed from the beaker using the pipette. Otoliths were transferred to a nitric cleaned ACV® made for the microwave digestion system. Two ml of quartz-distilled HNO_3 was slowly added to the ACV® to dissolve the otolith. Samples were allowed to sit for at least two hours to allow for complete otolith dissolution. The same methods were also used on a blank ACV® for analysis.

Once otoliths had been completely dissolved, another 2 ml of HNO_3 were added to the samples and the blank. A rupture membrane was placed into the caps of the ACV's®, they were closed tightly, and loaded onto the microwave tray. Two programs were devised for the microwave digestion procedure, and were stored in the microwave. The first program called 'OTOLITH' had two stages. Stage 1 lasted for 10 minutes at

80% power and a pressure of 60 pounds per square inch (PSI). Stage 2 heated the otolith samples for a total of 15 minutes at 80% power. During this stage the pressure was increased from 60 PSI to 90 PSI, but this new pressure was only maintained for 10 of the 15 minutes. Once the OTOLITH program had been performed the samples were allowed to cool. The caps of the ACV's® were removed and the "OTOLITH DRY DOWN' program was begun. This program was used repeatedly until the otolith samples were completely dry. The first time the "OTOLITH DRY DOWN' procedure was performed it lasted for 10 minutes at 10% power. The power was increased from 10 to 15 to 20% with each run to obtain maximum dryness of each sample. Once the samples were completely dry they were transferred into vials using a total of 5 ml of 2% HNO_3. The tare and final weight (g ± 0.00001) of the vials were noted to calculate the weight of the otolith sample. Once samples had been transferred to plastic vials using the HNO_3 they were digested and ready for analysis using ICP-MS.

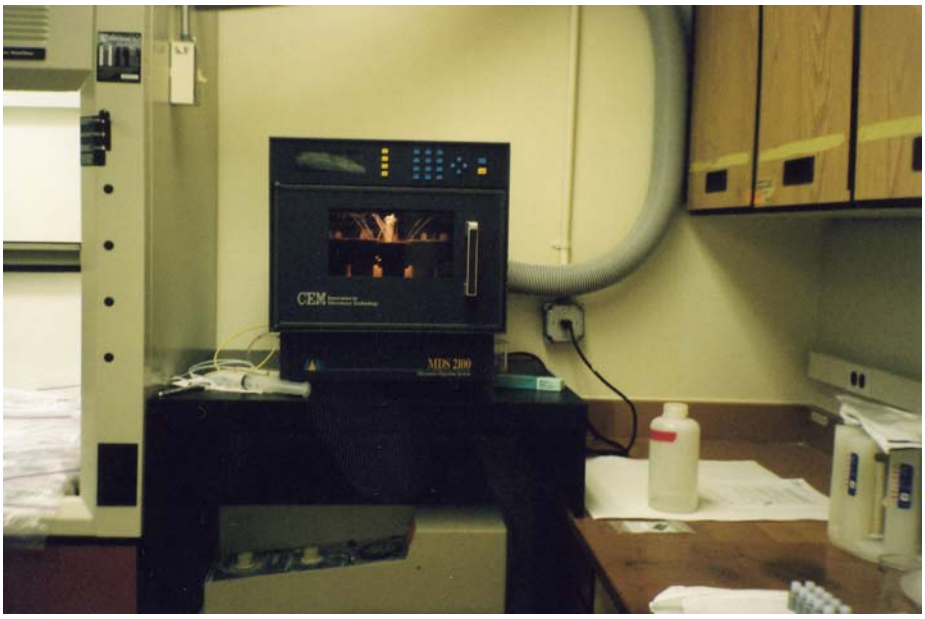

Figure 6. MDS 2100 Microwave Digestion System® used to dissolve otolith samples and blanks (UH at Manoa).

Inductively Coupled Plasma-Mass Spectrometry

There are six major systems within an ICP-MS (Figure 7). The first system is the sample introduction area consisting of a spray chamber and a nebulizer. The job of the sample introduction area is to generate a fine aerosol of the sample so it can be efficiently ionized in the plasma discharge (Thomas, 2001). This system has often been referred to as the Achilles heel of ICP-MS because only 1-2% of the sample actually makes it into the plasma.

The sample is transported from the sample introduction area to the plasma torch. The basic components used to generate the plasma source are a plasma torch, a radio frequency (RF) coil, and a RF power supply (Thomas, 2001). The plasma torch is made up of three tubes, which are usually made from quartz. The process begins with a flow of argon gas through the quartz torch. The RF power supply is then administered to the coil and an electromagnetic field is formed. A high voltage spark produces free electrons, which are accelerated by the RF field, causing collisions and ionization of the argon gas (Thomas, 2001). The inductively coupled plasma is formed at the open end of the quartz torch and the sample is introduced into the plasma.

The sample is then moved to the interface region consisting of two cones, the sampler cone and the skimmer cone. The role of this system is to transport the ions efficiently to the mass analyzer region. After ions are generated in the plasma, they pass through the first cone, the sampler cone, which has an orifice diameter of 0.8-1.2 mm (Thomas, 2001). They travel a short distance to the skimmer cone, which has a much smaller orifice of 0.4-0.8 mm in diameter. The interface region is probably the most critical area of the whole ICP-MS system because the most challenging problems to

overcome are the movement of ions from the plasma to the mass spectrometer (Thomas, 2001).

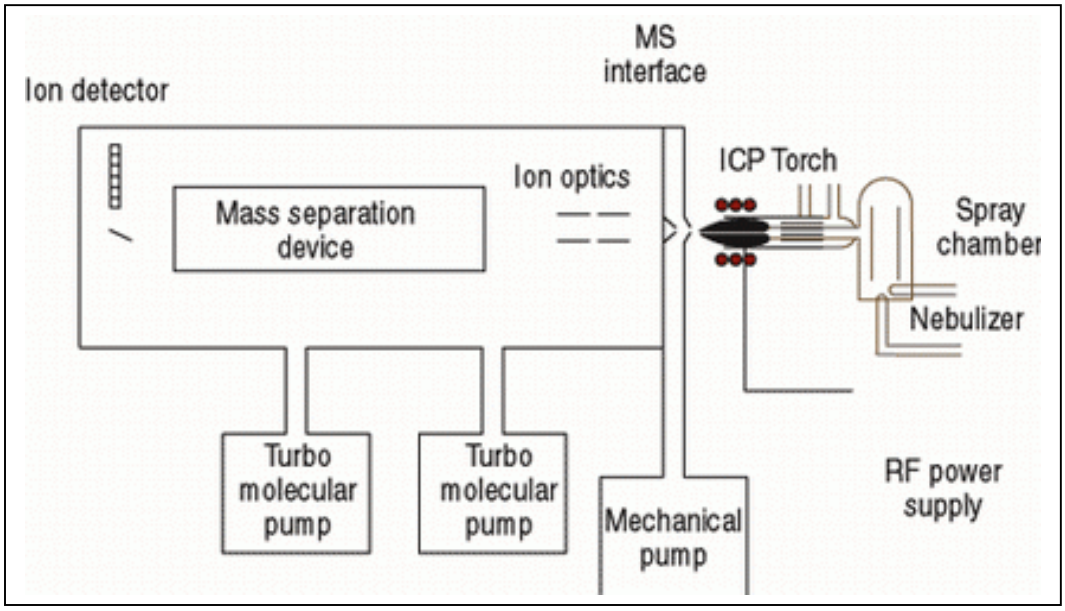

Figure 7. ICP-MS diagram showing 6 main systems (Taken from Thomas, 2001).

Ions emerge from the skimmer cone and are directed through the ion focusing system. This system consists of one or more electrostatically controlled lens components that are made up of a series of metallic plates, barrels, or cylinders. The main role of this system is to transport the maximum number of analyte ions from the interface region to the mass separation device (Thomas, 2001). This system rejects as many of the matrix components and non-analyte-based species as possible. These species cause signal instability and contribute to background levels, which ultimately affect the performance of the machine (Thomas, 2001).

Ions then move from the ion focusing system to the mass separation device where they are separated according to their mass-to-charge ratio (Thomas, 2001). This device is made up of a number of metallic rods of the same length and diameter. A direct current

(DC) field is placed on one pair of rods and a RF field is placed on the opposite pair of rods. By doing this, ions of a selected mass are allowed through the rods to the detector, while the others are ejected from the mass separation device (Thomas, 2001). This scanning process is then repeated for another analyte at a different mass-to-charge ratio until all analytes in a multi-element analysis have been measured (Thomas, 2001).

Ions emerge from the mass separation device and are converted to an electrical pulse by the ion detector (Thomas, 2001). The RF-DC voltage of the mass separation device is repeatedly scanned at different mass-to-charge ratios and the ions as electrical pulses are stored and counted by a multichannel analyzer. The multichannel data acquisition system typically has 20 channels per mass, and as the electrical pulses are counted in each channel, a profile of the mass is built up over the 20 channels, corresponding to the spectral peak of that ion (Thomas, 2001). In a multielement analysis, repeated scans are made over the entire suite of analyte masses.

Red snapper otolith samples were processed in a Fisons/VG PlasmaQuad® inductively coupled plasma-mass spectrometer (Figure 8) using flow injection analysis (FIA) for the presence of 15 different elements or isotopes. The flow injection system pulses a sample through the ICP-MS in a continuous flow of cleaning solution to reduce the amount of sample required and lessen the build up of deposits on the sampler and skimmer cones (Dove *et al.*, 1996). The ICP-MS standard used for comparison of elemental recovery was comprised of red snapper otolith material gathered in the Gulf of Mexico and analyzed at the National Standards Laboratory (James H. Cowan, Jr.[1] – personal communication).

[1] Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803

Figure 8. Fisons/VG PlasmaQuad® inductively coupled plasma-mass spectrometer used at the University of Hawaii at Manoa.

The 15 elements or isotopes analyzed in this study were Vanadium 51, Cobalt 59, Nickel 62, Zinc 64, Copper 65, Zinc 66, Silver 107, Silver 109, Cadmium 110, Cadmium 111, Cadmium 114, Lead 206, Lead 207, Lead 208, and Uranium 238. Although the elements I chose to analyze are not the usual suit of characters used in otolith microchemistry work (Campana, 1999; Campana *et al.*, 1994; Dove *et al.*, 1996;Edmonds *et al.*, 1992; Patterson *et al.*, 1998), I picked the elements and isotopes I felt would be present due to platform operations and in the drilling muds used during drilling of the well (Continental Shelf Associates, Inc., 2004; Neff, 1987; Neff *et al*., 1987). These elements are also heavy and replace calcium in the otoliths' aragonite matrix. In addition, with the exception of zinc, I felt that these elements would not be associated with a food web signal, but would be derived from the surrounding water mass (Campana, 1999; Ni *et al*., 2000).

Statistical Treatment of Data

All data obtained from mass spectrometry was standardized using the following equation:

$$[X]_{sample} = \frac{[X_{solution} - X_{blank}](\text{solution weight})(\text{dilution factor})}{\text{sample weight}}$$

A number of different statistical methods were used to determine the elemental fingerprint of otoliths from each environment tested (platforms and reefs). A simple linear regression was used to determine the relationship between otolith weight (g) and fish weight (g). Differences between environments for individual elements or isotopes were tested using Multivariate Analysis of Variance (MANOVA) to determine which isotopes were most important in inter-environment fingerprint differences. An alpha (α) level of 0.05 was used to judge significance.

A principal components analysis (PCA) was used to describe the variation in the ICP-MS data gathered for all the study sites combined. This analysis is used to describe the variation in a set of multivariate data in terms of a set of new, uncorrelated variables, each of which is a particular linear combination of the original variables (Der and Everitt, 2002). The main objective of this analysis was to determine whether the first few principal components accounted for a large part of the variation in the data, in which case they can be used to provide a suitable summary of the complete data set. The correlation matrix was used in this analysis as opposed to the covariance matrix due to the higher Zinc 64 and Zinc 66 concentrations than those of the other 14 elements analyzed.

Unique signatures of each environment were determined using discriminant function analysis, with all isotopes as dependent variables. Using a set of observations

containing one or more quantitative dependent variables and a classification variable defining groups of observations, discriminant function analysis computes linear or quadratic discriminant functions classifying observations (red snapper otoliths) into two or more groups (platforms vs. other) on the basis of one or more of the quantitative variables (elements and isotopes) (SAS Institute, 1985). The discriminant functions (classification criterion) that are derived will be stored for later use to classify the adult snapper in Phase II of the project.

A stepwise discriminant analysis (SDA) was performed to determine what elements were the most influential in discriminating between oil and gas platforms and artificial reefs in the Gulf of Mexico. Stepwise discriminant analysis finds a set of the original quantitative variables that best discriminate among the two sites or groups. A significance value of 0.05 was used in the analysis to determine when variables were chosen to enter and leave the discriminant function. To exclude variables that are highly correlated, the singular statement was set at a value of 0.2. Singularity is equal to 1 minus the tolerance, so setting the singularity to 0.2 infers an optimum tolerance value of 0.8. Tolerance values range from 0 to 1 and a small value indicates a high correlation between that variable with 1 or more of the other variables included in the discriminant function (Rooker *et al*., 2001).

A canonical discriminant analysis (CDA) was also used to compare the two environments. The canonical discriminant procedure determines the best linear combinations of the quantitative variables in which the means of the groups are most different. The canonical discriminant analysis was performed three times, first to compare the elemental concentration of otoliths from all sites, second to compare otoliths

from both Louisiana sites, and third to compare otoliths from the two artificial reefs sites (Louisiana and Alabama) east and west of the Mississippi River. All statistical analyses were performed using SAS Version 9.0.

RESULTS

Red snapper analyzed (N = 98 of 115 total collected) for this study ranged in size from 318 mm to 776 mm in total length. These were divided into 5 size classes (mm TL): 301 – 400; 401 – 500; 501 – 600; 601 – 700; and 701 – 800. Twenty red snapper were within the first size class, 55 were in the second, 13 were in the third, 8 were in the fourth, and 2 red snapper were in the fifth and largest class (Figure 9). Of those, 45 were males and 53 were females. When compared by site, the sex ratios were not evenly distributed. Of the thirty-two red snapper analyzed from Louisiana oil and gas platforms, 11 were males and 21 were females. Similarly, of red snapper collected on artificial reefs in Louisiana, 12 were males and 19 were females. Of red snapper collected on artificial reefs in Alabama, 22 were males and only 13 were females. By location, 32 otoliths came from Louisiana oil and gas platforms, 31 from Louisiana artificial reefs, and 35 from Alabama artificial reefs.

The discrepancy between the total number of otoliths collected and the number analyzed occurred because there were some failures while running the mass spectrometer due to exceptionally high concentrations of some elements. This caused the instrument detector to trip from pulse counting to analog mode. By the time the detector had settled into analog mode the entire otolith sample had already passed through the machine and was not properly analyzed. Unless there was enough otolith sample left to rerun that analysis, those samples could not be rerun and therefore were not included in the results of this study.

Simple Linear Regression

Otolith weight (g) was related to fish weight (g) using a simple linear regression.

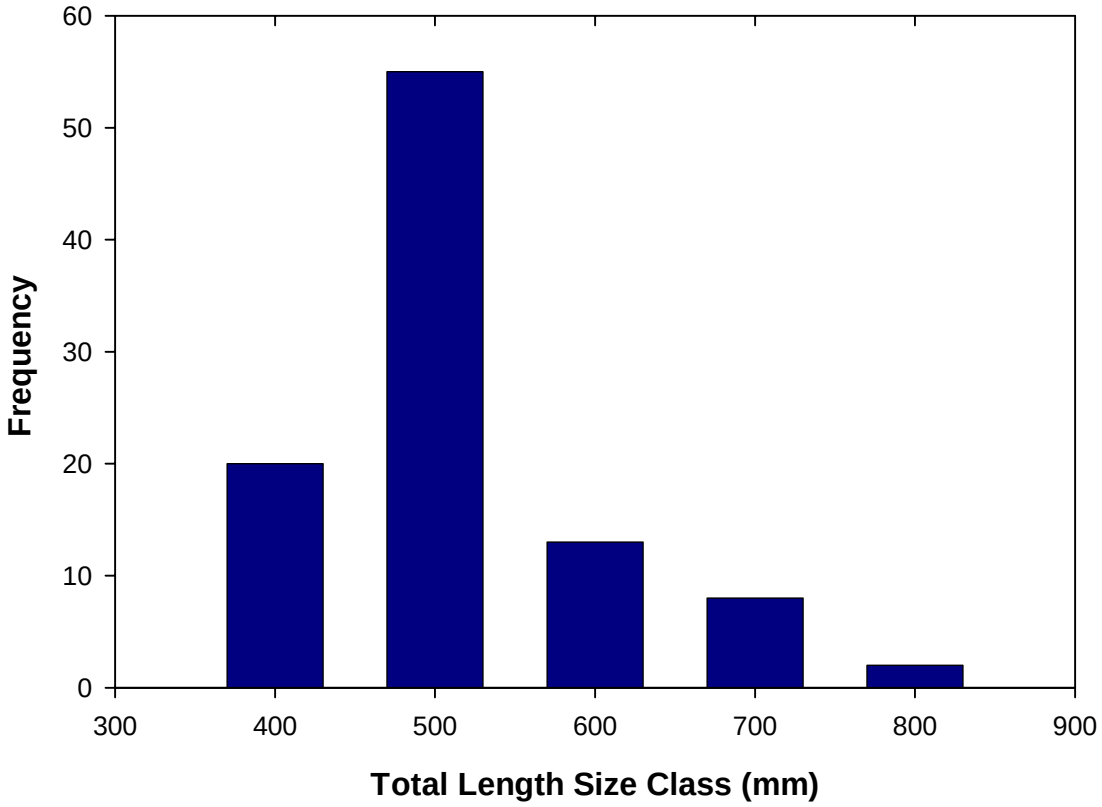

Figure 9. Length frequency distribution for red snapper from all study sites combined (N = 98).

The regression relationship is significant ($\alpha = 0.05$) producing an R^2 value = 0.95 (Figure 10). It is expected that as fish weight increases, otolith weight also increases. While the relationship appears slightly non-linear, log transformation of the data only increases the R^2 by < 1.0%.

Multivariate Analysis of Variance (MANOVA)

A MANOVA was used to compare the means of all 15 elements in otoliths between each of the study sites. For all of the following analyses, site 1 represents Louisiana oil and gas platforms, site 2 represents Louisiana artificial reefs, and site 3 represents Alabama artificial reefs. Ten of the 15 elements analyzed have significantly different means ($\alpha = 0.05$) in otoliths collected from Louisiana oil and gas platforms (site

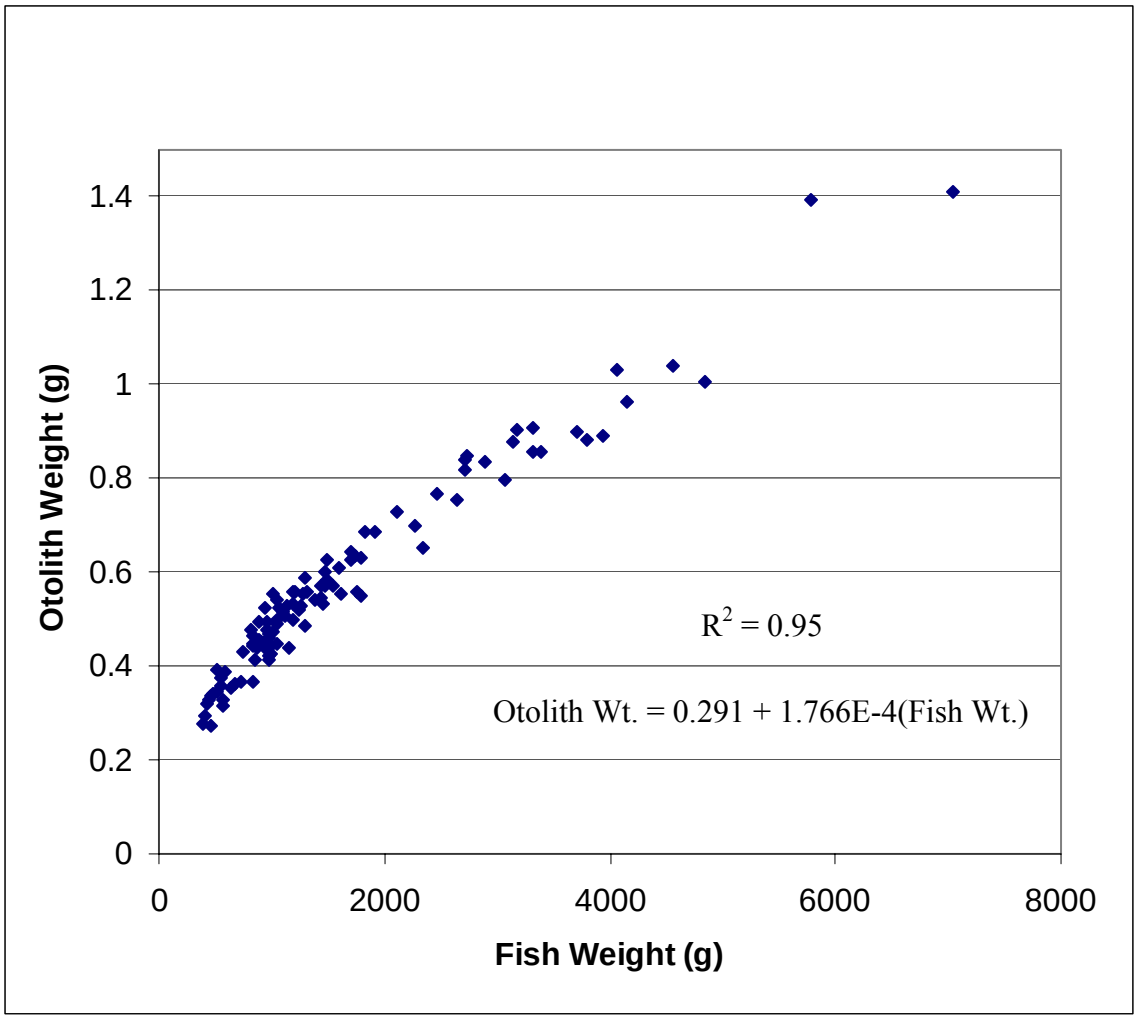

Figure 10. Linear regression relating otolith weight (g) to fish weight (g) (Wt = weight).

1), Louisiana artificial reefs (site 2), and Alabama artificial reefs (site 3) (Figures 11, 12, and 13). These 10 elements, and their corresponding p-values, are as follows: Cobalt 59 ($p=0.0070$), Zinc 64 ($p=0.0339$), Copper 65 ($p=0.0255$), Silver 107 ($p<0.0001$), Silver 109 ($p<0.0001$), Cadmium 110 ($p=0.0091$), Cadmium 111 ($p=0.0229$), Cadmium 114 ($p=0.0012$), Lead 206 ($p=0.0464$), and Lead 207 ($p=0.0331$). Lead 208 ($p=0.0958$) and Uranium 238 ($p=0.0888$) are close to being significantly different between the three sites, with p-values greater than 0.05 but less than 0.10.

When comparing the means of each element in otoliths collected from Louisiana oil and gas platforms and Louisiana artificial reefs, the MANOVA results show that 8 of the 15 elements in question have significantly different means in otoliths from these two areas. The elements are Vanadium 51 ($p=0.0001$), Zinc 64 ($p=0.0035$), Zinc 66 ($p=0.0003$), Silver 107 ($p=0.0015$), Silver 109 ($p=0.0001$), Lead 206 ($p=0.0075$), Lead 207 ($p=0.0092$), and Lead 208 ($p=0.0094$) (Figures 14, 15, and 16).

When comparing otoliths from the two artificial reef sites, one in Louisiana and one in Alabama (sites 2 and 3), 9 of the 15 elements analyzed are significantly different, and are believed to reflect water mass characteristics east and west of the Mississippi River. Those elements that have significantly different means are Vanadium 51 ($p=0.0048$), Copper 65 ($p<0.0001$), Zinc 66 ($p=0.0495$), Silver 107 ($p=0.0001$), Silver 109 ($p=0.0107$), Cadmium 114 ($p=0.0004$), Lead 206 ($p=0.0218$), Lead 208 ($p=0.0103$), and Uranium 238 ($p=0.0374$) (Figures 17, 18, and 19). Cobalt 59 ($p=0.0588$), Cadmium 110 ($p=0.0720$), and Lead 207 ($p=0.0529$) are close to being significant, having p-values larger than 0.05 but less than 0.10.

Principal Components Analysis

Principal components analysis (PCA) was performed using all 98 observations to determine the variation in the data set. The 15 elements analyzed in this study were included in the analysis as variables. Based on the results of the PCA, and when referring to the eigenvalues of the correlation matrix, the first four principal component scores explain 70% of the variance in the ICP-MS data (Table 1). The first two principal component scores alone explain 47% of the variance suggesting that these two scores, for each of the elements analyzed, provide an adequate description of the data. When

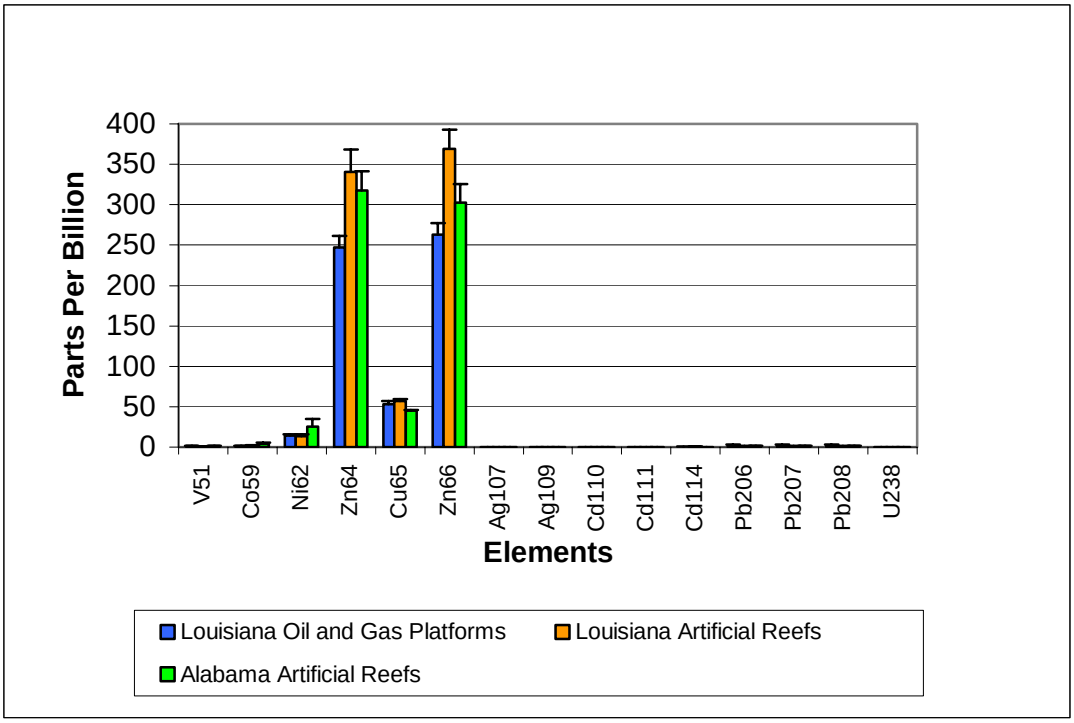

Figure 11. Comparison of means of 15 elements from otoliths analyzed for all three study sites.

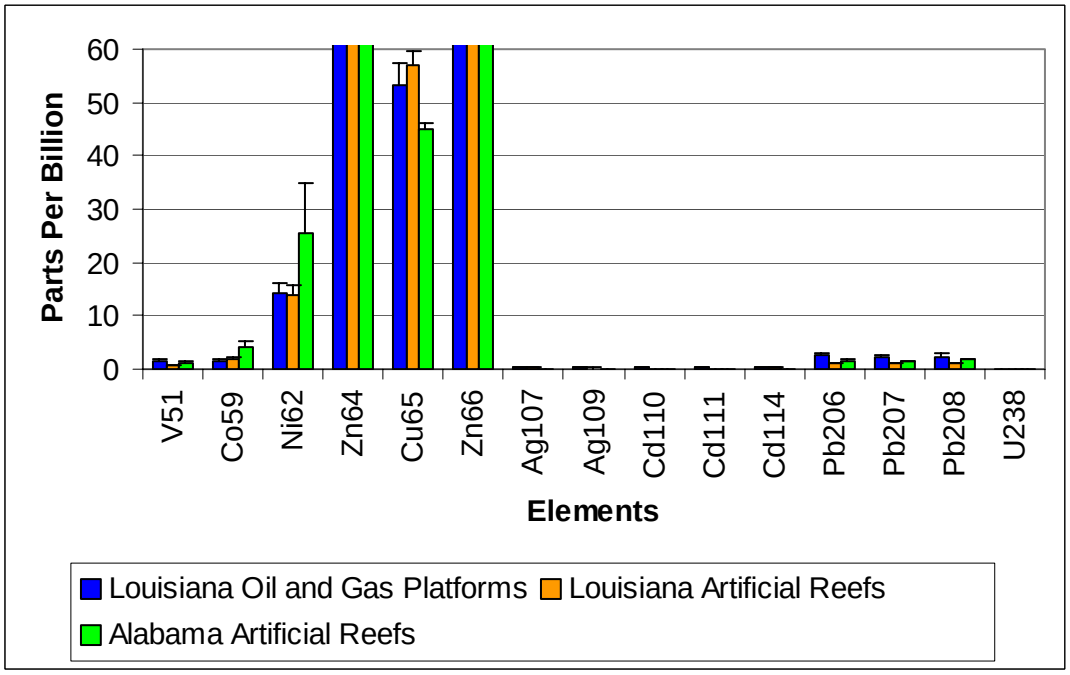

Figure 12. Enlarged view of comparison of means of 15 elements from otoliths analyzed for all three study sites.

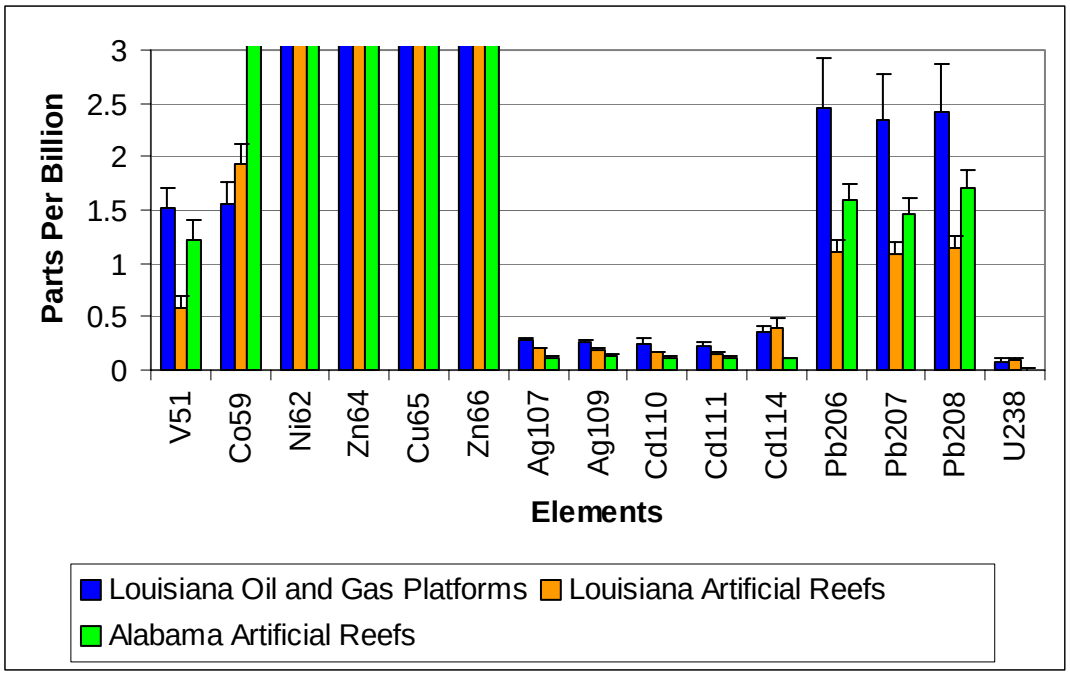

Figure 13. Enlarged view of comparison of means of 15 elements from otoliths analyzed for all three study sites.

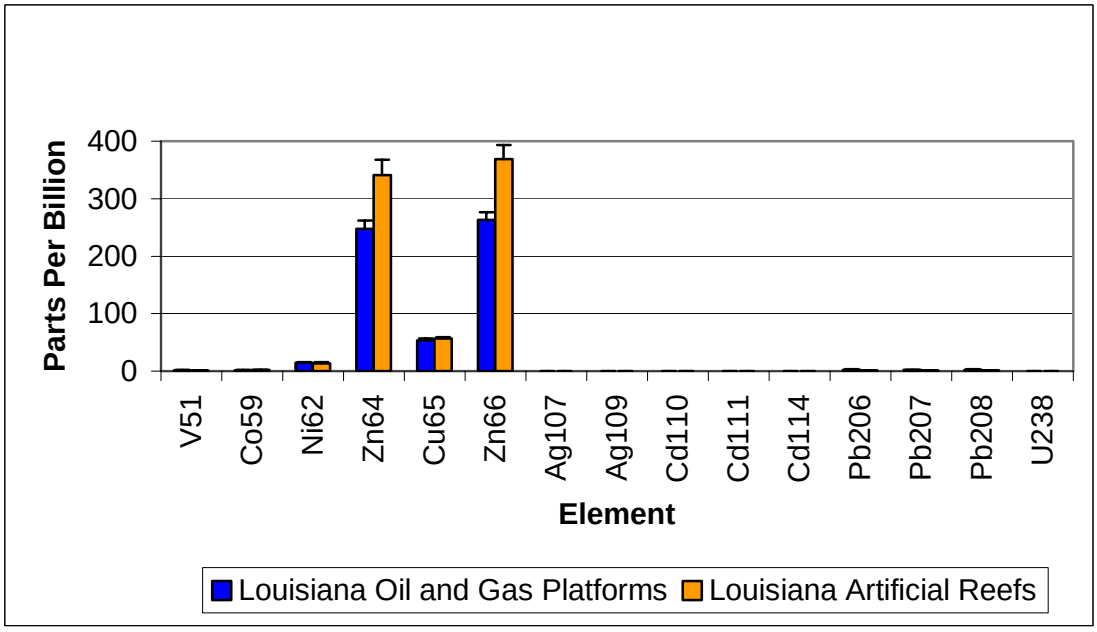

Figure 14. Comparison of means of elemental concentration in otoliths from Louisiana oil and gas platforms and Louisiana artificial reefs.

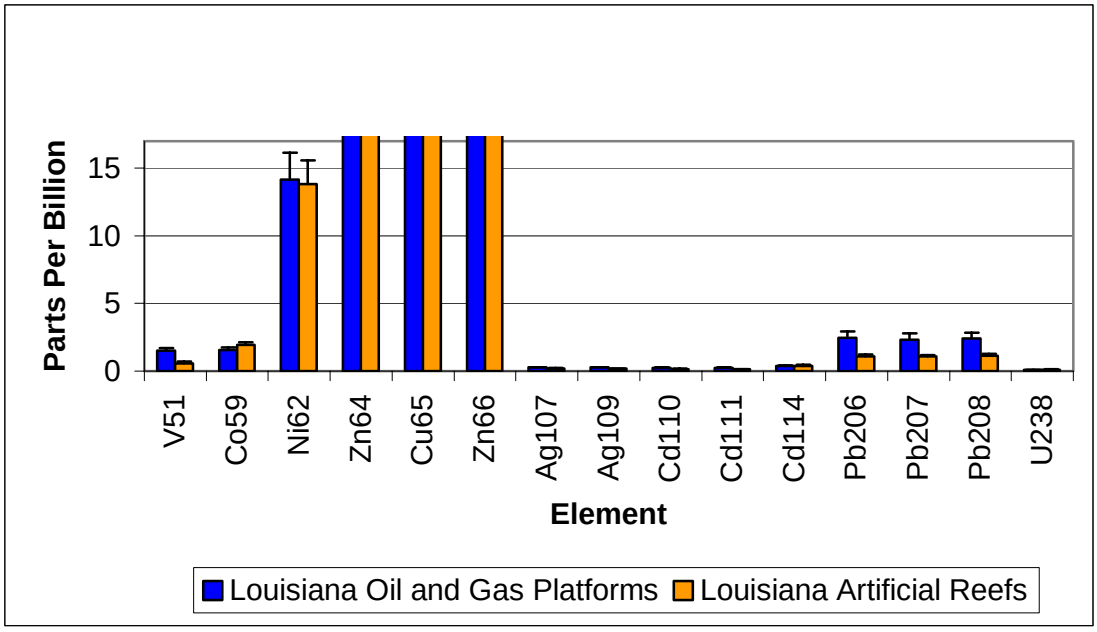

Figure 15. Enlarged view of comparison of means of elemental concentration in otoliths from Louisiana oil and gas platforms and Louisiana artificial reefs.

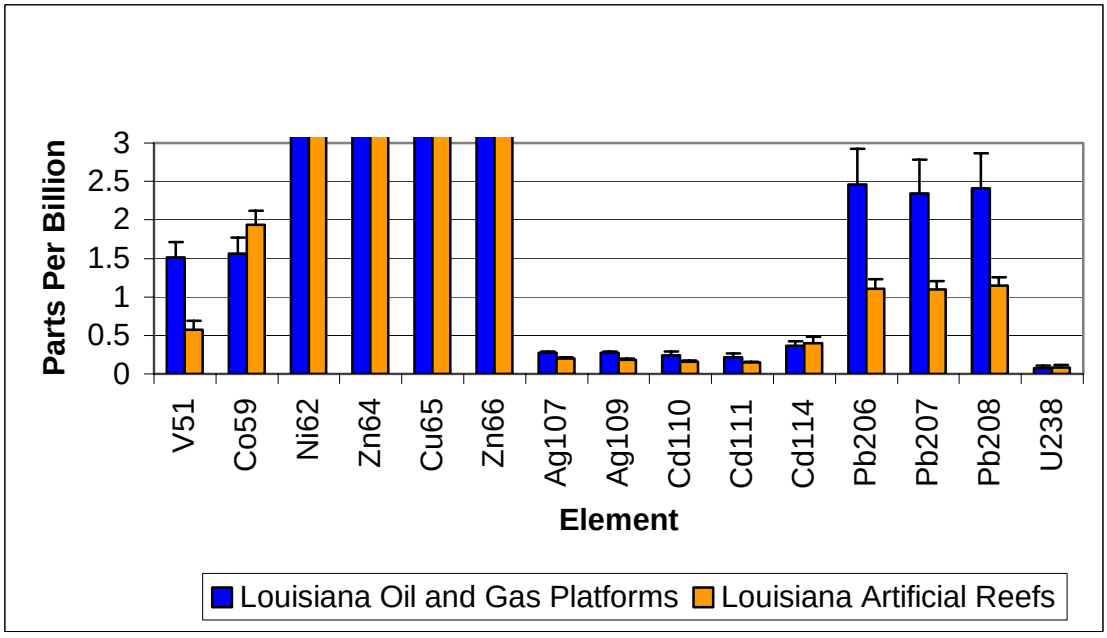

Figure 16. Enlarged view of comparison of means of elemental concentration in otoliths from Louisiana oil and gas platforms and Louisiana artificial reefs.

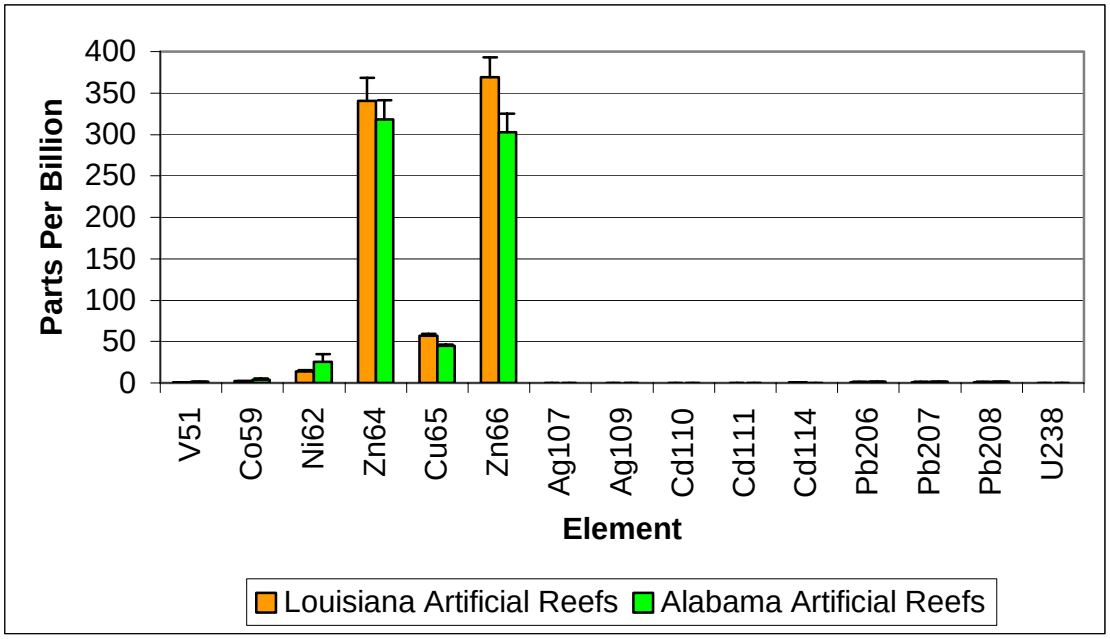

Figure 17. Comparison of means of elemental concentration in otoliths from Louisiana artificial reefs and Alabama artificial reefs.

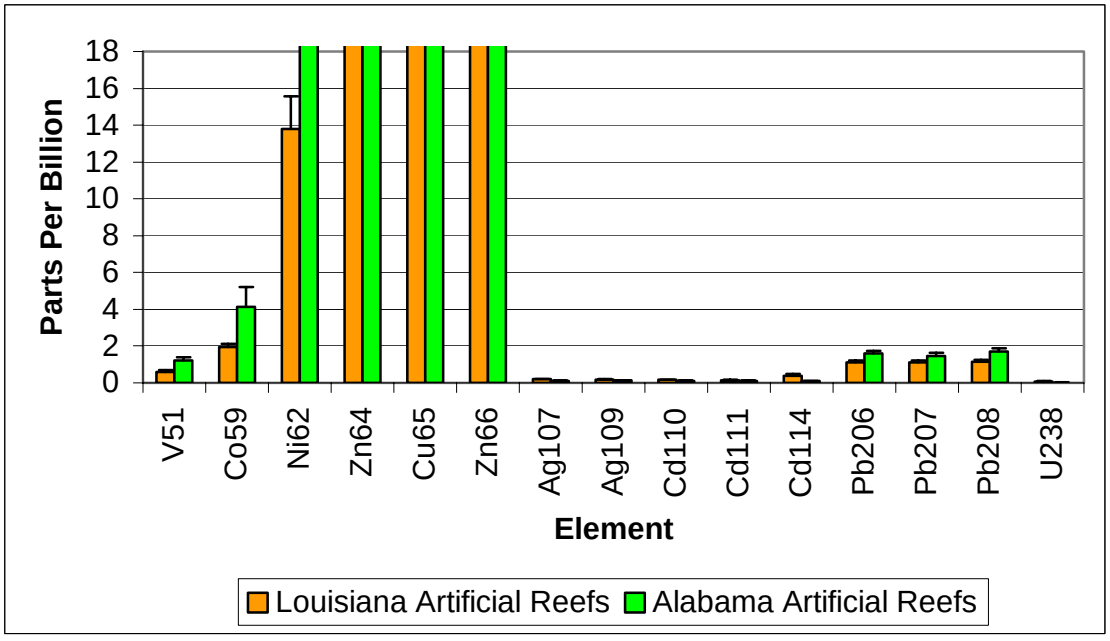

Figure 18. Enlarged view of comparison of means of elemental concentration in otoliths from Louisiana artificial reefs and Alabama artificial reefs.

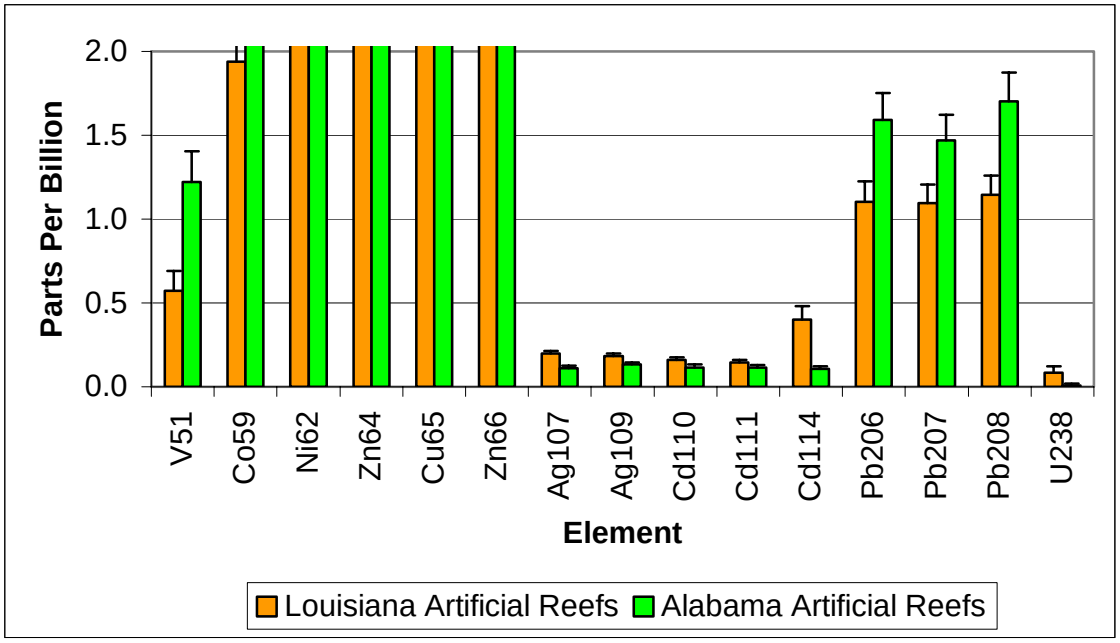

Figure 19. Enlarged view of comparison of means of elemental concentration in otoliths from Louisiana artificial reefs and Alabama artificial reefs.

referring to the eigenvectors of principal components 1 and 2, a number of different elements account for the variation in the ICP-MS data. Silver 107, Silver 109, Cadmium 110, Cadmium 111, Cadmium 114, Lead 206, Lead 207, and Lead 208 have some of the largest eigenvectors and appear to be explaining the greatest portion of the variance when looking at principal component 1(Table 2). When referring to principal component 2, Zinc 64, Zinc 66, Vanadium 51, Cobalt 59, and Nickel 62 have the largest eigenvectors (Table 2). Figure 20 shows a scatter plot of principal component 1 versus principal component 2 with observations labeled by site. Both principal component scores appear to be ranking observations collected from the same location closely together.

Table 1. Eigenvalues of the correlation matrix from the PCA showing cumulative percent of variation explained by each principal component score.

	Eigenvalue	Cumulative %
1	5.02168453	0.3348
2	2.02398796	0.4697
3	1.74382553	0.5860
4	1.67318932	0.6975

Table 2. Eigenvectors of principal components 1 and 2 for all 15 elements analyzed.

	Principal Component 1	Principal Component 2
V 51	0.023242	0.276143
Co 59	-.092396	0.289524
Ni 62	-.018790	0.296602
Zn 64	0.184254	-.501276
Cu 65	0.208920	-.115625
Zn 66	0.194150	-.523766
Ag 107	0.253426	-.104995
Ag 109	0.250578	-.112389
Cd 110	0.339336	0.139852
Cd 111	0.342344	0.134835
Cd 114	0.286382	-.107563
Pb 206	0.380085	0.214434
Pb 207	0.383314	0.200276
Pb 208	0.375109	0.216806
U 238	0.042430	-.083117

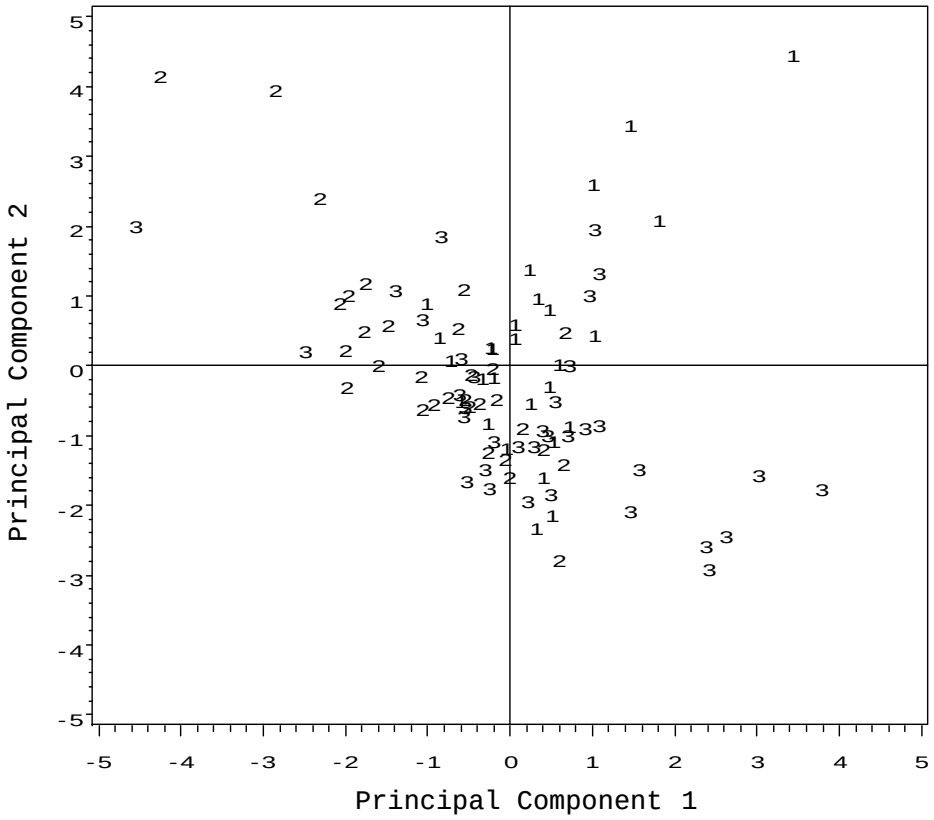

Figure 20. Scatter plot of principal component score 1 versus principal component score 2 with observations labeled by site (site 1 = LA plat.; site 2 = LA reefs; site 3 = AL reefs).

Linear and Stepwise Discriminant Analysis

Comparison Among All Study Sites:

Linear discriminant analysis (LDA) was performed using all 98 observations to determine if study sites could be discriminated based upon the elemental composition of otoliths. The variables used include the 15 elements analyzed, and the classes consist of the 3 sites previously designated. The F approximations for all of the multivariate statistics in this analysis are significant with p-values < 0.0001 (Table 3) indicating that it is possible to discriminate between the 3 sites based upon red snapper otolith elemental composition. It is also apparent that the two artificial reef sites (site 2 and site 3) cannot be combined as one large sample due to differences in the elemental composition of otoliths collected in these areas. Overall, the LDA resulted in a cross-validation misclassification rate of only 19.51%, and the number of observations and percent classified into each of the 3 sites are shown in Table 4.

Table 3. Multivariate statistics and F approximations for linear discriminant analysis comparing the elemental concentration of otoliths among all study sites.

Statistic	F-Value	Pr > F
Wilks' Lambda	8.80	<0.0001
Pillai's Trace	8.81	<0.0001
Hotelling-Lawley Trace	8.80	<0.0001
Roy's Greatest Root	10.66	<0.0001

Table 4. Number of observations and percent of otoliths classified into each of 3 sites using linear discriminant analysis and cross-validation.

From Site	1	2	3	Total
1	23 71.88	5 15.63	4 12.50	32 100.00
2	3 9.68	26 83.87	2 6.45	31 100.00
3	3 8.57	2 5.71	30 85.71	35 100.00
Total	29 29.59	33 33.67	36 36.73	98 100.00

A stepwise discriminant analysis (SDA) was then used to determine which elements were most important in discriminating otoliths from fish collected among the sites. All 98 observations were included in the analysis (PROC STEPDISC; SAS V 9.0), the 15 elements analyzed in the study were used as variables, and the 3 sites remained as class levels. Results of the SDA indicate that 9 of the 15 elements are useful to discriminate among otoliths collected from the 3 sites. These 9 elements are Silver 107, Zinc 66, Vanadium 51, Lead 208, Cadmium 114, Cobalt 59, Copper 65, Uranium 238, and Nickel 62. The LDA was then rerun comparing otoliths from all 3 sites, but using only the 9 elements identified above; the number of observations and the classes remained the same. Rerunning the LDA using these 9 elements lowers the cross-validation misclassification rate from 19.51% to 15.03%. The number of observations and the percent of otoliths classified into each site are shown in Table 5.

Table 5. Number of observations and percent of otoliths classified into each of 3 sites after rerunning the linear discriminant analysis using only the 9 most important elements.

From Site	1	2	3	Total
1	25 78.13	4 12.50	3 9.38	32 100.00
2	0 0.00	30 96.77	1 3.23	31 100.00
3	4 11.43	3 8.57	28 80.00	35 100.00
Total	29 29.59	37 37.76	32 32.65	98 100.00

Because it was determined that otoliths collected from all of the study sites are significantly different in elemental composition and I could not combine the artificial reef sites, two other discriminant analyses were performed. The first analysis compared the Louisiana sites to determine if I could successfully discriminate between red snapper

otoliths from Louisiana oil and gas platforms (site 1) and Louisiana artificial reefs (site 2). Since no red snapper were collected on oil and gas platforms in Alabama, the Louisiana and Alabama artificial reef samples (sites 2 and 3) were compared to determine if I could discriminate between otoliths taken from east and west of the Mississippi River.

Comparison of Louisiana Sites:

The elemental composition of otoliths from Louisiana oil and gas platforms (site 1) and Louisiana artificial reefs (site 2) were compared using LDA to determine if otoliths from the two sites could be successfully discriminated. The analysis included a total of 63 red snapper otoliths (32 from site 1 and 31 from site 2), 15 variables or elements, and 2 classes (site 1 and site 2). All four of the multivariate statistics and their exact F statistics are significant, with p-values < 0.0001 (Table 6). Otoliths from site 1 and 2 could successfully be discriminated using this procedure, and the analysis resulted in a cross-validation misclassification rate of 15.83%. The number of observations and percent of otoliths classified into each of the two sites are shown in Table 7.

Table 6. Multivariate statistics and exact F statistics for linear discriminant analysis comparing the elemental concentration of otoliths from Louisiana oil and gas platforms (site 1) and Louisiana artificial reefs (site 2).

Statistic	F-Value	Pr > F
Wilks' Lambda	8.23	<0.0001
Pillai's Trace	8.23	<0.0001
Hotelling-Lawley Trace	8.23	<0.0001
Roy's Greatest Root	8.23	<0.0001

An SDA was then performed to determine the most important elements in discriminating otoliths from site 1 and 2. This procedure identifies 6 elements as being important in differentiating between elemental composition of otoliths; these are Vanadium 51, Zinc 66, Silver 109, Lead 206, Uranium 238, and Nickel 62. Using only

Table 7. Number of observations and percent of otoliths classified into Louisiana oil and gas platforms (site 1) and Louisiana artificial reefs (site 2) using linear discriminant analysis and cross-validation.

From Site	1	2	Total
1	26 81.25	6 18.75	32 100.00
2	4 12.90	27 87.10	31 100.00
Total	30 47.62	33 52.38	63 100.00

these 6 elements to rerun the LDA lowers the original cross-validation misclassification rate from 15.83% to 6.25%. The number of observations and percent of otoliths classified into site 1 and site 2 after rerunning the LDA are shown in Table 8.

Table 8. Number of observations and percent of otoliths classified into Louisiana oil and gas platforms (site 1) and Louisiana artificial reefs (site 2) after rerunning the linear discriminant analysis using only the 6 most important elements.

From Site	1	2	Total
1	28 87.50	4 12.50	32 100.00
2	0 0.00	31 100.00	31 100.00
Total	28 44.44	35 55.56	63 100.00

Comparison Between Artificial Reef Sites:

Because no red snapper were collected on oil and gas platforms in Alabama (east of the Mississippi River), otoliths from fishes collected at the two artificial reef sites were compared to determine if otoliths collected east and west of the Mississippi River could be successfully discriminated. The LDA consisted of 66 red snapper otoliths (31 from site 2 in Louisiana and 35 from site 3 in Alabama), the 15 elements analyzed, and 2 classes

(site 2 and site 3). All of the multivariate statistics and exact F statistics from this analysis are significant with p-values < 0.0001 (Table 9). Otoliths collected on Louisiana and Alabama artificial reefs can successfully be discriminated, with a cross-validation misclassification rate of 12.35%. The number of observations and percent of otoliths classified into site 2 and site 3 are shown in Table 10.

Table 9. Multivariate statistics and exact F statistics for linear discriminant analysis comparing the elemental concentration of otoliths from Louisiana (site 2) and Alabama artificial reefs (site 3).

Statistic	F-Value	Pr > F
Wilks' Lambda	9.90	<0.0001
Pillai's Trace	9.90	<0.0001
Hotelling-Lawley Trace	9.90	<0.0001
Roy's Greatest Root	9.90	<0.0001

Table 10. Number of observations and percent of otoliths classified into Louisiana artificial reefs (site 2) and Alabama artificial reefs (site 3) using linear discriminant analysis and cross-validation.

From Site	2	3	Total
2	26 83.87	5 16.13	31 100.00
3	3 8.57	32 91.43	35 100.00
Total	29 43.94	37 56.06	66 100.00

An SDA was performed to determine which elements are the most influential in discriminating otoliths from Louisiana and Alabama artificial reefs (sites 2 and 3). This analysis identifies 6 of the 15 elements as being the most important for discrimination. The 6 elements are Copper 65, Silver 107, Lead 208, Cobalt 59, Vanadium 51, and Uranium 238. The LDA was then rerun using only these 6 elements. Rerunning the analysis in this manner lowers the original cross-validation misclassification rate of

12.35% to 8.94%. The number of observations and the percent of otoliths classified into site 2 (Louisiana) and site 3 (Alabama) after rerunning the LDA are shown in Table 11.

Table 11. Number of observations and percent of otoliths classified into Louisiana artificial reefs (site 2) and Alabama artificial reefs (site 3) after rerunning the original linear discriminant analysis using only the 6 most important elements.

From Site	2	3	Total
2	29 93.55	2 6.45	31 100.00
3	4 11.43	31 88.57	35 100.00
Total	33 50.00	33 50.00	66 100.00

Canonical Discriminant Analysis (CDA)

Comparison Among All Three Study Sites:

Although results from LDA and SDA are commonly reported in otolith microchemistry research, a CDA was also used to compare the red snapper otolith samples. Although these statistical techniques are similar to one another, the LDA will later allow an otolith of unknown origin to be classified into one of the three habitats mentioned above. The use of CDA allows for better graphical visualization of the discrimination of otolith elemental concentrations from the three study areas.

The first CDA included all study sites to determine what quantitative variables were contributing most to the discrimination of red snapper otoliths from among the sites. This procedure included all 98 otolith samples, the 15 elements used in the previous analyses, and 3 classes (site 1, site 2, and site 3) (PROC CANDISC; SAS V 9.0). Based upon results from the CDA, and referring to the pooled with-in class standardized canonical coefficients, a number of different elements are identified as important for

discriminating otoliths from all sites (i. e., have high coefficients). The CDA identifies Vanadium 51, Zinc 64, Zinc 66, Lead 207 and Lead 208 as the most important variables in discriminating between otolith composition among these 3 areas (Table 12). Nickel 62, Silver 107, and Cadmium 114 may also play a role in the discrimination between red snapper otoliths from the three sites, but these have smaller coefficients than the elements mentioned above. A vertical bar chart of canonical variable 1, with observations separated by site, shows a clear distinction between site 1 (LA oil and gas platforms), site 2 (LA artificial reefs), and site 3 (AL artificial reefs) (Figure 21). A scatter plot of canonical variable 1 versus canonical variable 2, with observations separated by site, also shows the discrimination of these three study areas (Figure 22).

Table 12. Pooled with-in class standardized canonical coefficients for canonical discriminant analysis comparing elemental concentration of otoliths from all 3 sites.

Variable	Can 1	Can 2
Van 51	-0.326287286	**1.271772749**
Co 59	-0.364269086	-0.111424522
Ni 62	0.164067374	**-0.830804671**
Zi 64	**-2.614762035**	0.455984967
Cu 65	0.226111971	-0.051381164
Zi 66	**2.124772164**	**-1.061174832**
Ag 107	**0.727485480**	0.043888748
Ag 109	-0.232535143	0.423190943
Cd 110	0.165261904	0.004690588
Cd 111	-0.550891827	0.138192167
Cd 114	**0.711935361**	-0.182457948
Pb 206	-0.261392235	0.668427423
Pb 207	**3.391593583**	**0.770820882**
Pb 208	**-3.253403361**	**-0.991748720**
U 238	0.395437128	-0.665813787

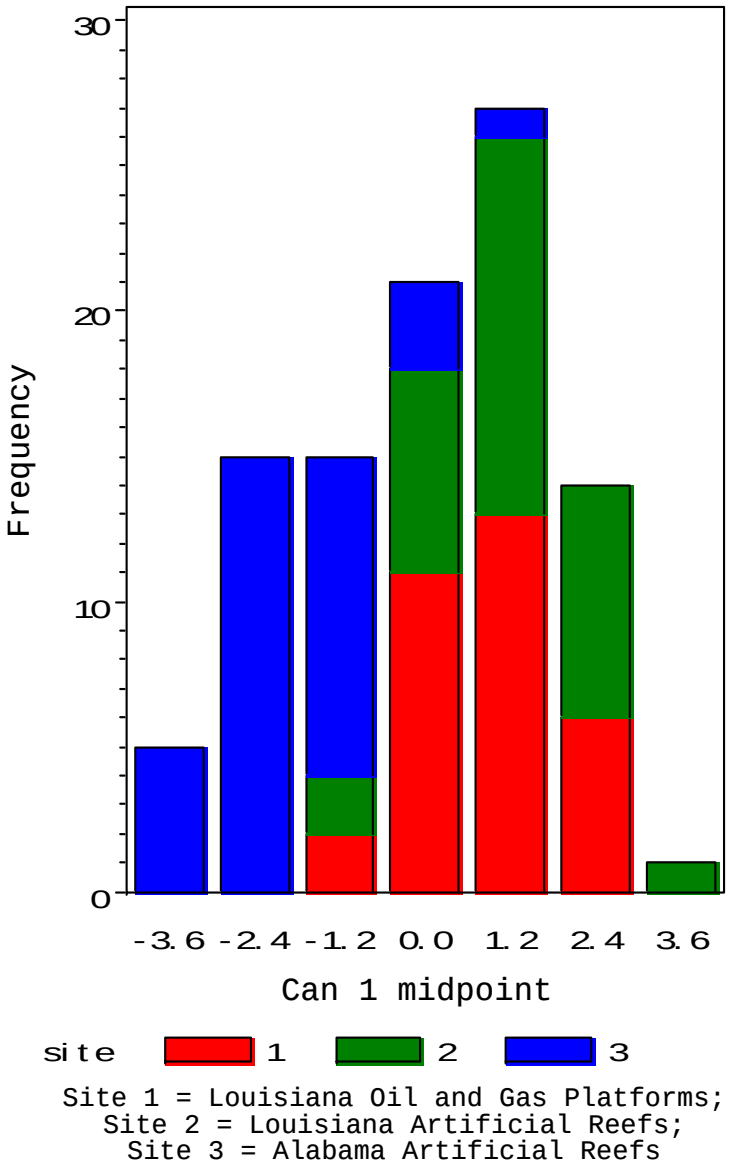

Figure 21. Vertical bar graph of canonical variable 1, with observations separated by site, for a comparison of elemental concentration of otoliths collected from all three study sites.

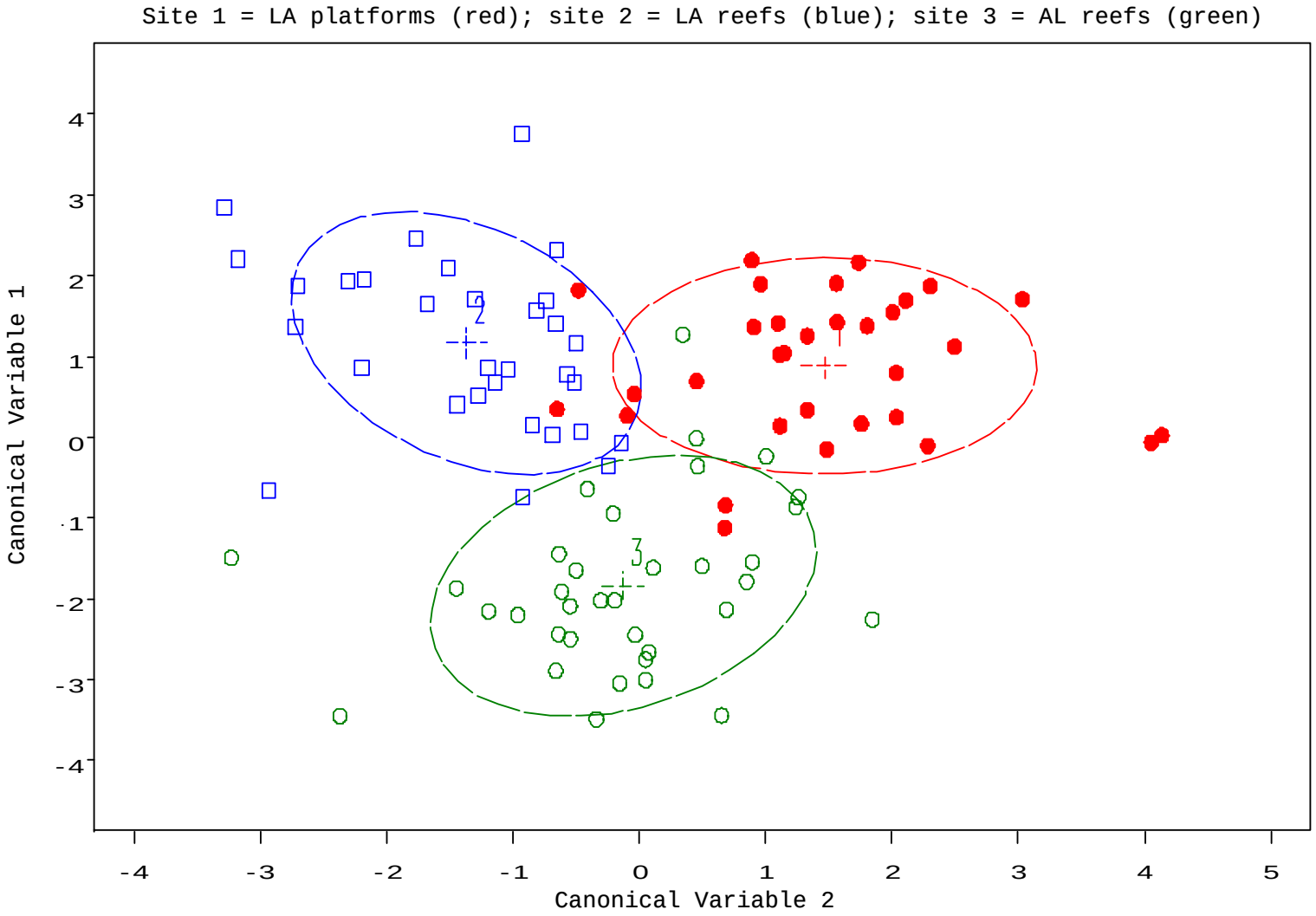

Figure 22. A scatter plot of canonical variable 1 versus canonical variable 2, with observations separated by site, for a comparison of elemental concentration of otoliths collected from all three study sites.

Comparison Between Louisiana Sites:

A CDA was also performed comparing the elemental composition of otoliths from Louisiana oil and gas platforms (site 1) and Louisiana artificial reefs (site 2). This procedure contained a total of 63 red snapper otoliths, all 15 elements, and 2 classes (site 1 and site 2). Based upon results from the CDA, and referring to the pooled with-in class standardized canonical coefficients, 7 elements are identified to be most important (i. e., have high coefficients); these are Vanadium 51, Silver 109, Lead 206, and Lead 207 (Table 13). Silver 107, Lead 208, and Uranium 238 may also play a minor role in the discrimination of site 1 from 2, but their coefficients are smaller. A vertical bar graph of canonical variable 1, with the 63 otolith samples separated up by site, shows a clear separation of elemental composition of otoliths from Louisiana oil and gas platforms (site 1) and Louisiana artificial reefs (site 2) (Figure 23).

Table 13. Pooled with-in class standardized canonical coefficients for canonical discriminant analysis comparing elemental concentration of otoliths from Louisiana oil and gas platforms and Louisiana artificial reefs.

Variable	**Can 1**
Van 51	**1.150511481**
Co 59	-0.315485812
Ni 62	-0.386722369
Zi 64	-0.510542370
Cu 65	0.075796429
Zi 66	-0.407428082
Ag 107	**-0.828019113**
Ag 109	**1.288229683**
Cd 110	0.266889436
Cd 111	-0.200465328
Cd 114	0.067606075
Pb 206	**1.319984482**
Pb 207	**-1.428899832**
Pb 208	**0.741607422**
U 238	**-0.758786655**

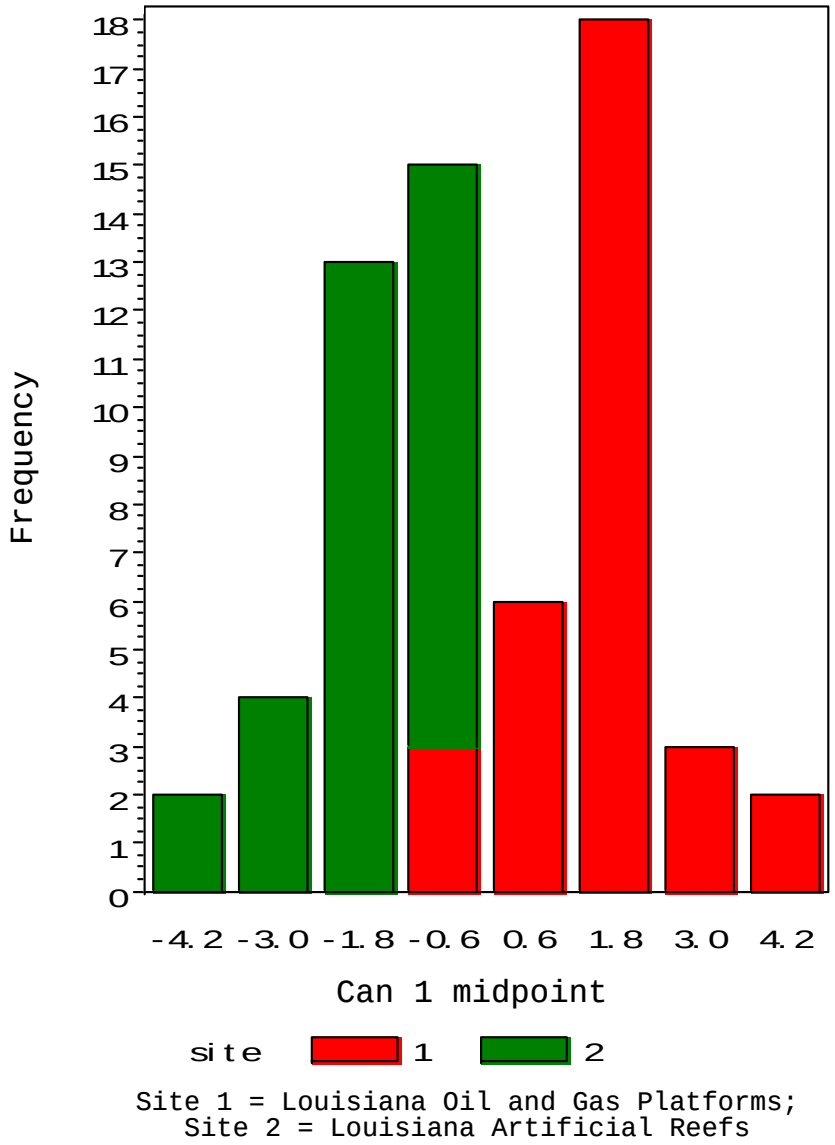

Figure 23. Vertical bar graph of canonical variable 1, with observations separated by site, for a comparison of elemental concentration of otoliths collected from Louisiana oil and gas platforms (site 1) and Louisiana artificial reefs (site 2).

Comparison Between Artificial Reef Sites:

Elemental composition of otoliths collected from Louisiana artificial reefs (site 2) and Alabama artificial reefs (site 3) were compared using CDA. This comparison contained 66 red snapper otoliths, 15 elements, and 2 classes (site 2 and site 3). Based upon results from the CDA, and referring to the pooled with-in class standardized canonical coefficients, 7 of the 15 elements included are identified as being important in discriminating between otoliths from sites 2 and 3 (i. e., have high coefficients); these elements are Vanadium 51, Nickel 62, Zinc 64, Zinc 66, Lead 207, Lead 208, and Uranium 238 (Table 14). A vertical bar graph of canonical variable 1, with all 66 otoliths separated by site, shows a clear distinction between the composition of otoliths from Louisiana and Alabama artificial reefs (Figure 24).

Table 14. Pooled with-in class standardized canonical coefficients for canonical discriminant analysis comparing elemental concentration of otoliths from Louisiana artificial reefs and Alabama artificial reefs.

Variable	**Can 1**
Van 51	**-1.599721728**
Co 59	-0.219237785
Ni 62	**1.344612829**
Zi 64	**-2.600825746**
Cu 65	0.123169822
Zi 66	**2.264223297**
Ag 107	0.307630655
Ag 109	0.104474403
Cd 110	0.224670174
Cd 111	-0.306991225
Cd 114	0.575792743
Pb 206	-0.326919757
Pb 207	**1.042553437**
Pb 208	**-0.910109476**
U 238	**0.942455608**

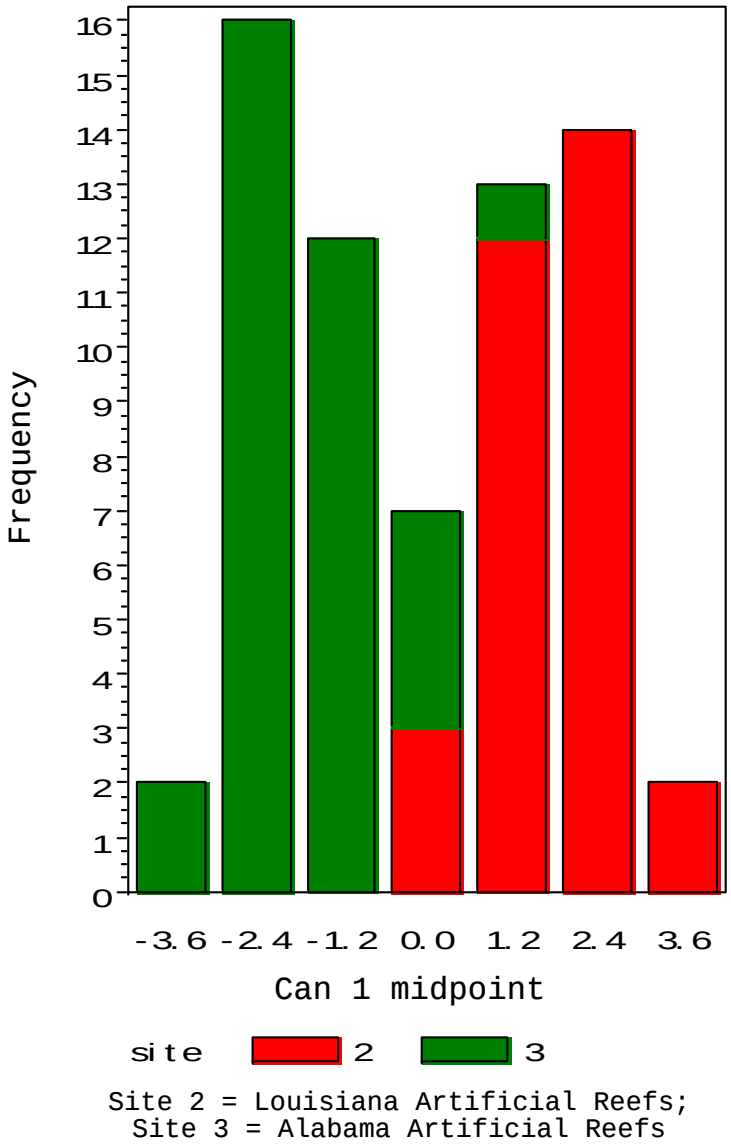

Figure 24. Vertical bar graph of canonical variable 1, with observations separated by site, for a comparison of elemental concentration of otoliths collected from Louisiana artificial reefs (site 2) and Alabama artificial reefs (site 3).

Possible Influence of the Mississippi River

A number of the same elements are identified as being important in both the stepwise and canonical discriminant analyses. Of the 15 elements analyzed, 13 reoccur frequently in the results. The 13 most important elements identified in discrimination of red snapper otoliths using the two statistical techniques are Vanadium 51, Cobalt 59, Nickel 62, Copper 65, Zinc 64, Zinc 66, Silver 107, Silver 109, Cadmium 114, Lead 206, Lead 207, Lead 208, and Uranium 238. These elements are essential in differentiating between red snapper otoliths collected from Louisiana oil and gas platforms and artificial reefs, as well as between otoliths from artificial reefs east and west of the Mississippi River. To determine if the concentrations of these important elements are being influenced by the discharge of the Mississippi River, I plotted and compared concentrations of the elements in red snapper otoliths collected at all three study sites.

I reasoned that those elements attributable to Mississippi River discharge should have mean concentrations in otoliths from both Louisiana sites that were significantly higher than in otoliths collected at site 3 in Alabama. The elements that meet this criteria are Cadmium 114 (Figure 25), Copper 65 (Figure 26), Uranium 238 (Figure 27), Silver 107 (Figure 28), and Silver 109 (Figure 28), all having means significantly higher in red snapper otoliths from coastal Louisiana, west of the Mississippi River. Conversely, Cobalt 59 (Figure 29) and Nickel 62 (Figure 30) have significantly higher means in otoliths from site 3, east of the Mississippi River and perhaps reflect discharge from Mobile Bay. However, otolith concentrations of Lead 206 (Figure 31), Lead 207 (Figure 32), Lead 208 (Figure 33), and Vanadium 51 (Figure 34) do not vary geographically, yet remain significantly higher in otoliths from Louisiana oil and gas platforms than from

either artificial reef site. Otolith Zinc 64 and Zinc 66 concentrations (Figure 35) also show no significant geographical distribution, but are higher in otoliths collected on Louisiana and Alabama artificial reefs than in those from Louisiana oil and gas platforms.

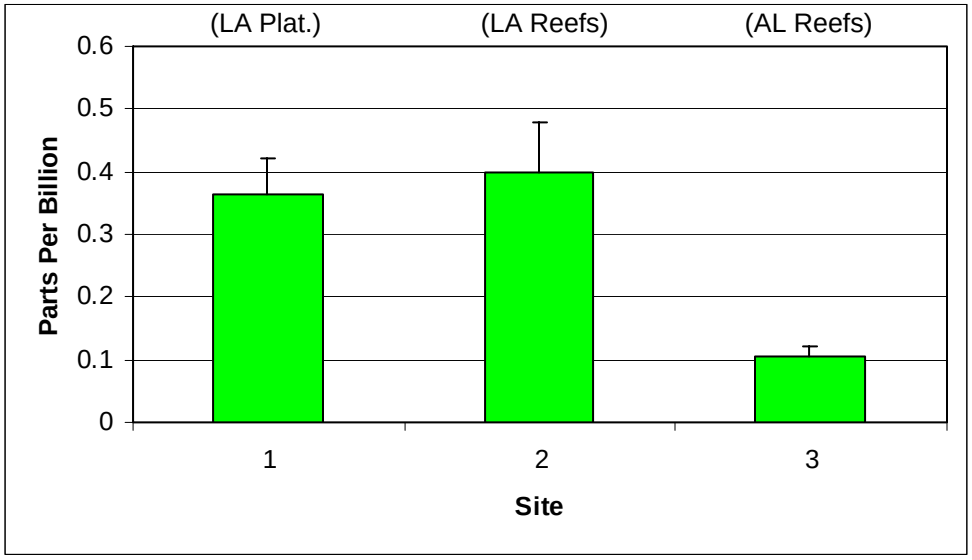

Figure 25. Cadmium 114 concentration in otoliths all three study sites showing a geographical distribution with higher concentrations west of the Mississippi River.

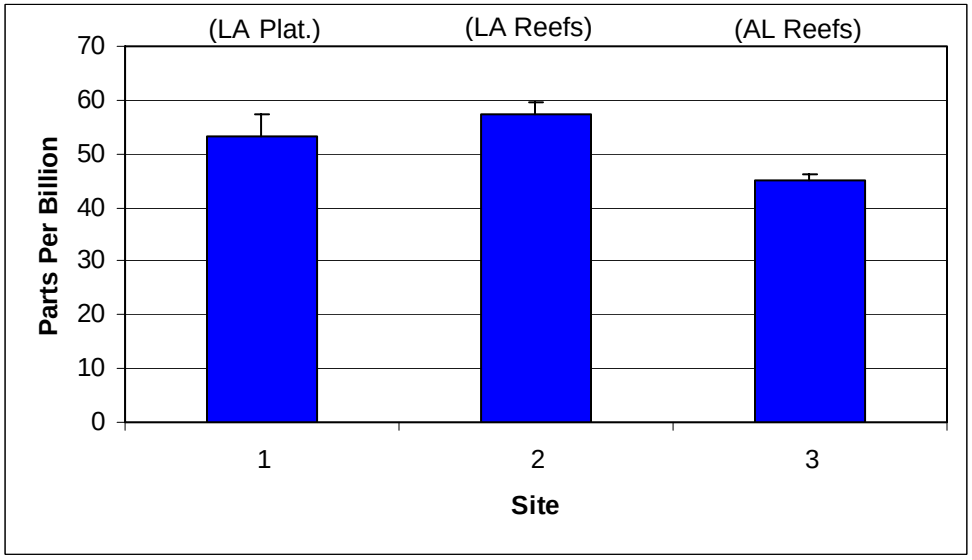

Figure 26. Copper 65 concentration in otoliths at all three study sites showing a geographical distribution with higher concentrations west of the Mississippi River.

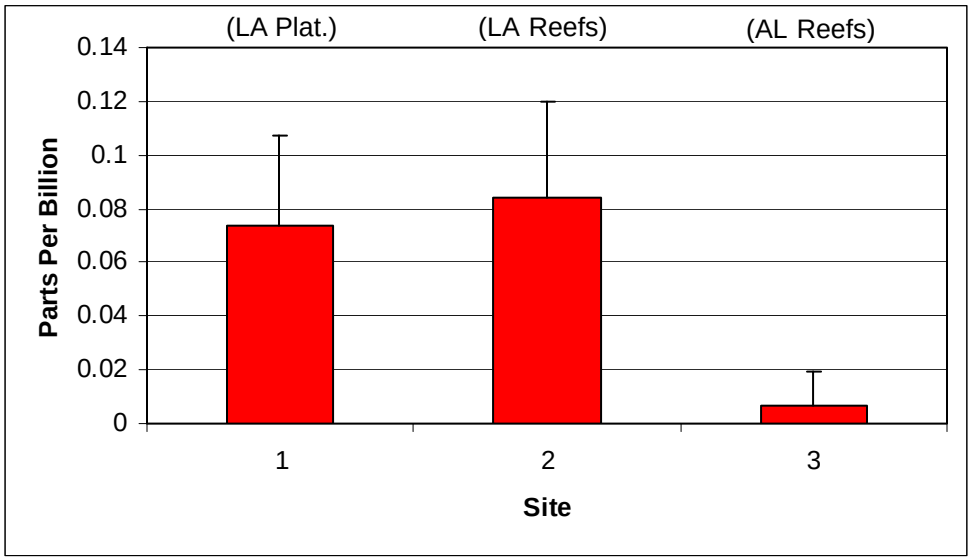

Figure 27. Uranium 238 concentration in otoliths at all three study sites showing a geographical distribution with higher concentrations west of the Mississippi River.

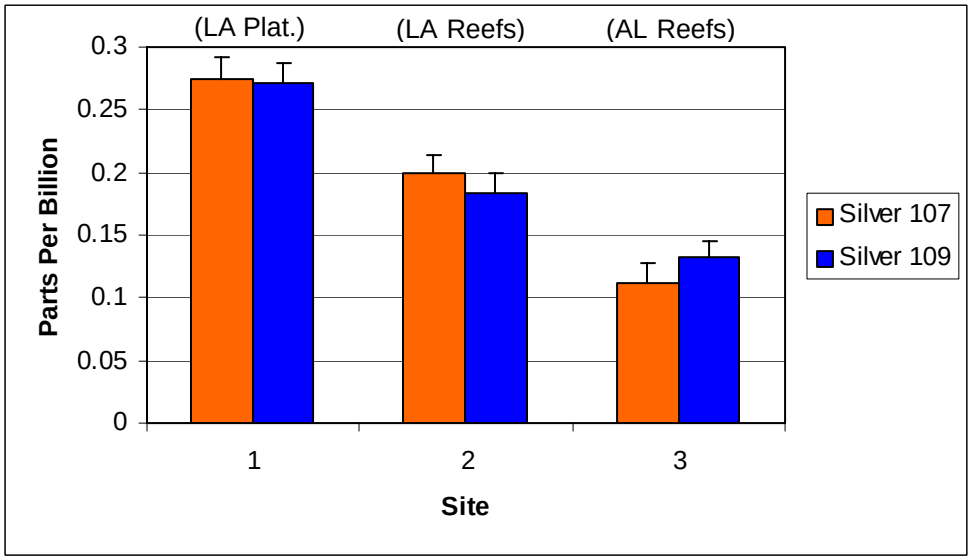

Figure 28. Silver 107 and 109 concentrations in otoliths at all three study sites showing a geographical distribution with higher concentrations west of the Mississippi River.

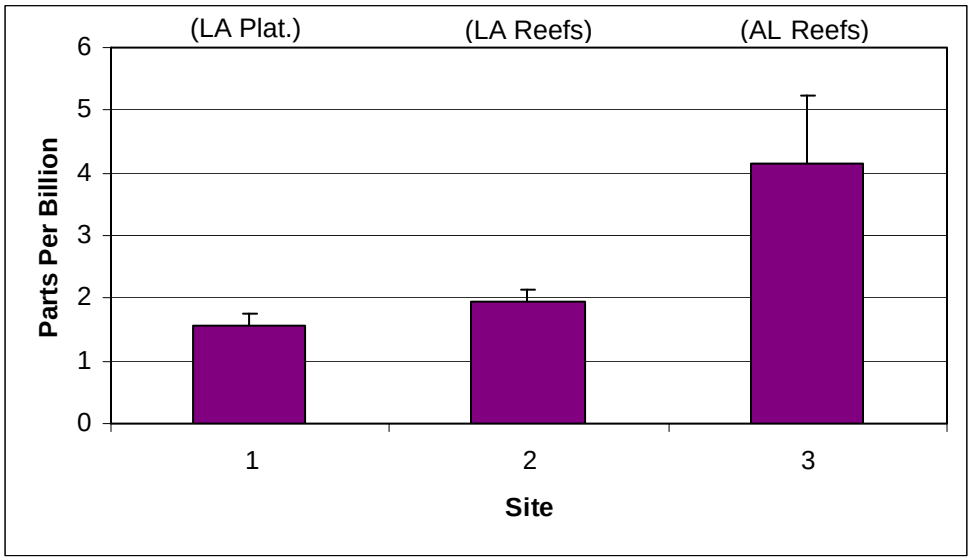

Figure 29. Cobalt 59 concentration in otoliths at all three study sites showing a geographical distribution with higher concentrations east of the Mississippi River.

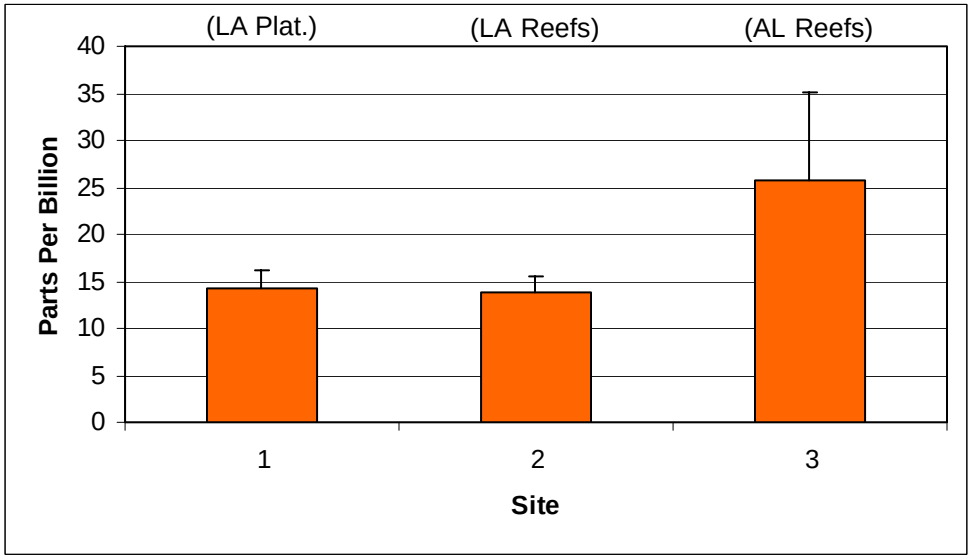

Figure 30. Nickel 62 concentration in otoliths at all three study sites showing a geographical distribution with higher concentrations east of the Mississippi River.

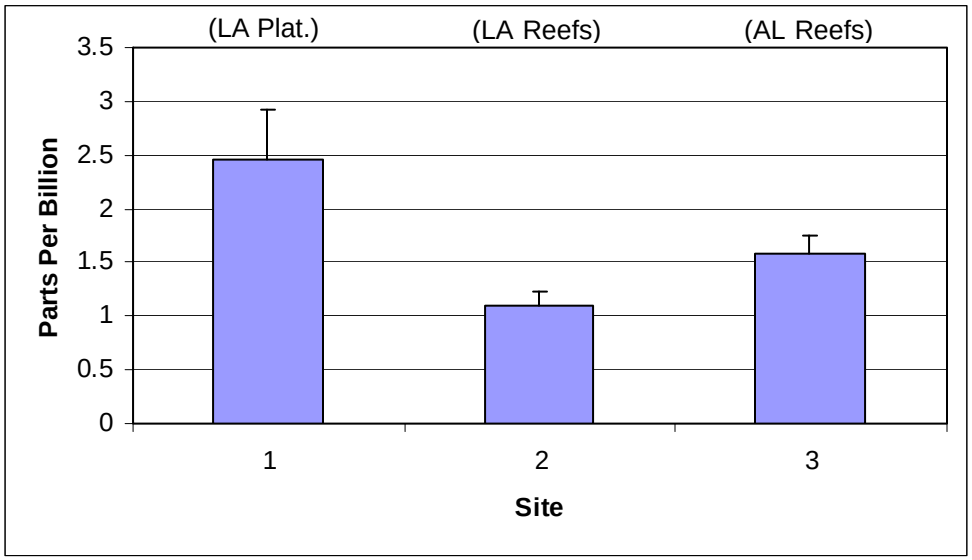

Figure 31. Lead 206 concentration in otoliths at all three study sites showing no significant geographical distribution but having higher concentrations on Louisiana oil and gas platforms.

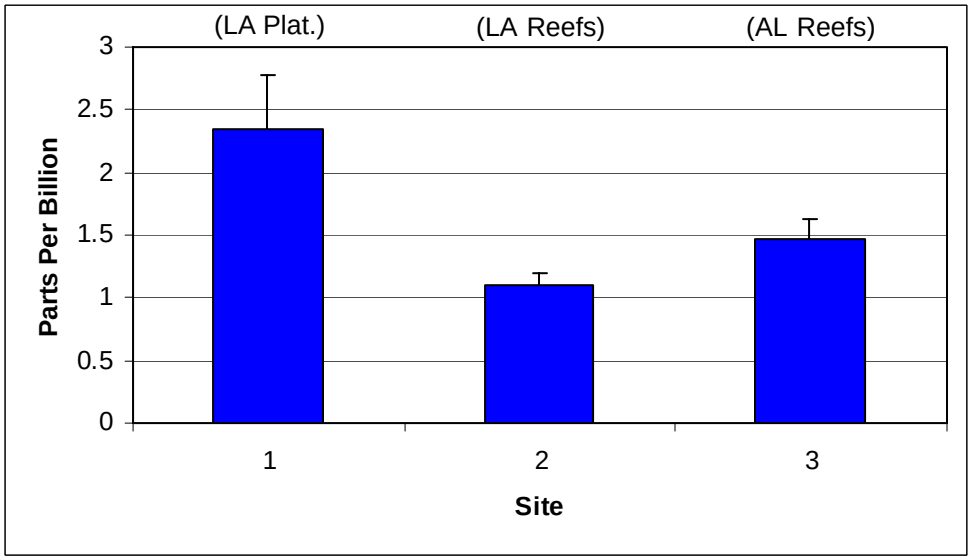

Figure 32. Lead 207 concentration in otoliths at all three study sites showing no significant geographical distribution but having higher concentrations on Louisiana oil and gas platforms.

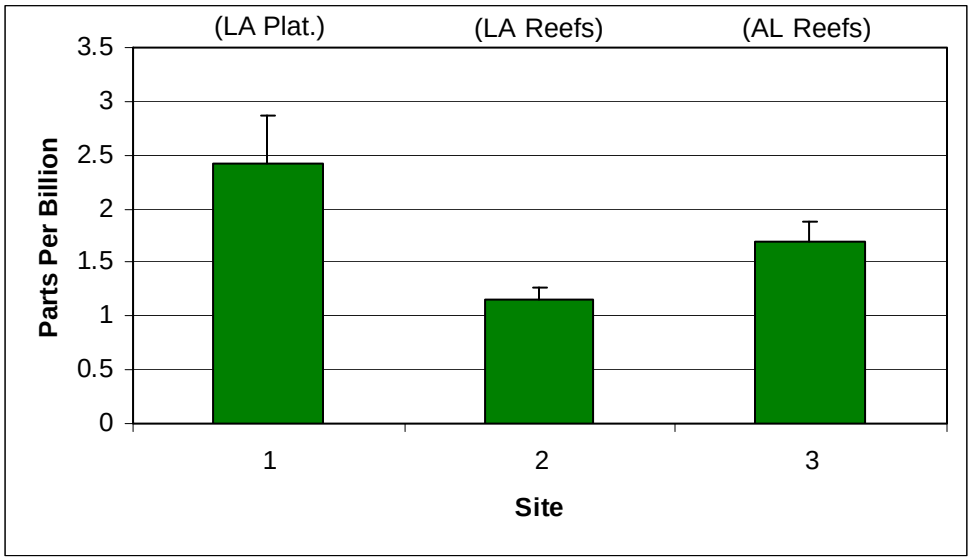

Figure 33. Lead 208 concentration in otoliths at all three study sites showing no significant geographical distribution but having higher concentrations on Louisiana oil and gas platforms.

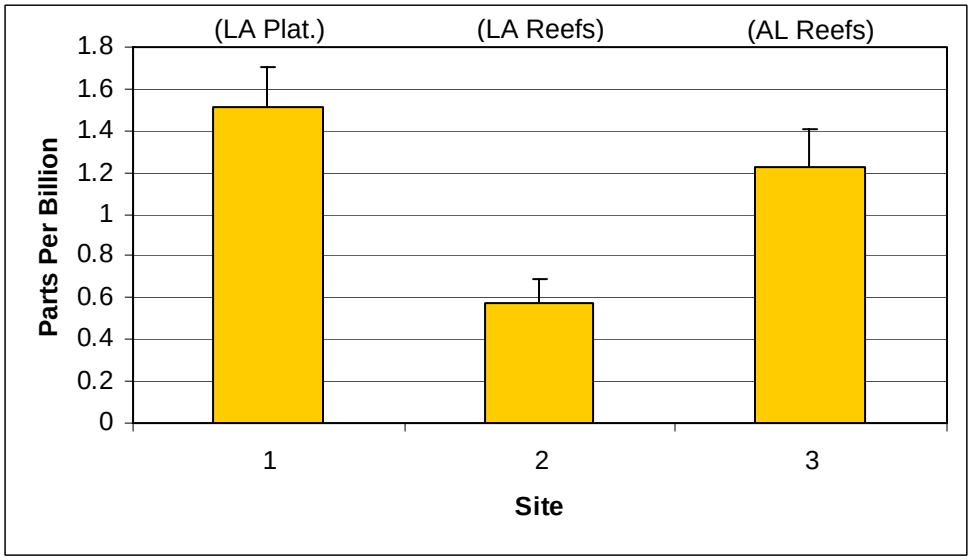

Figure 34. Vanadium 51 concentration in otoliths at all three study sites showing no significant geographical distribution but having higher concentrations on Louisiana oil and gas platforms.

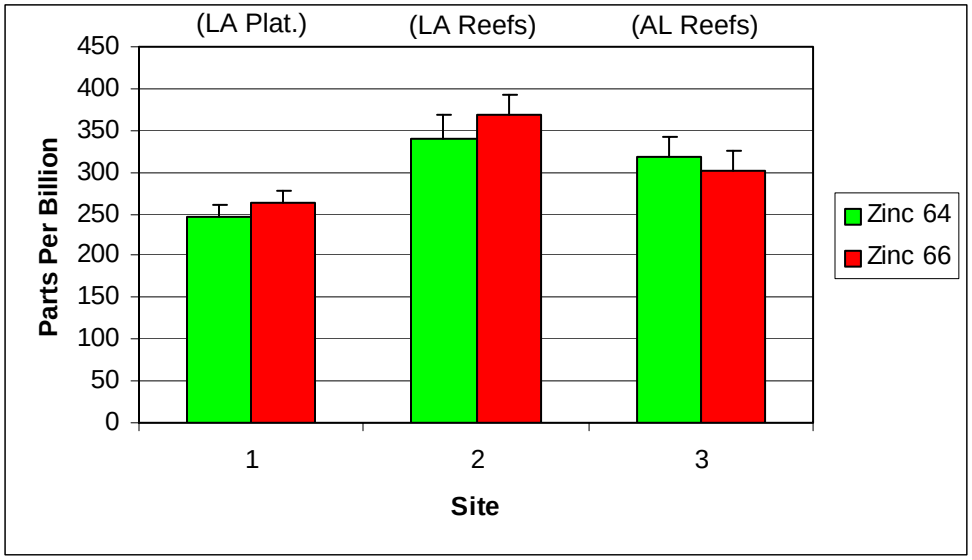

Figure 35. Zinc 64 and Zinc 66 concentrations in otoliths at all three study sites showing no clear geographical distribution but having higher concentrations on both artificial reef sites.

Trends of Important Elements with Red Snapper Sex and Total Length

An important potential influence on otolith elemental site-specific markers detected in this study are the sex and total length of red snapper collected for analysis. To investigate these trends, the concentrations of the 13 most important elements identified in both of the discriminant techniques for all sites combined were plotted against sex and total length. The 13 elements are Vanadium 51, Cobalt 59, Nickel 62, Copper 65, Zinc 64, Zinc 66, Silver 107, Silver 109, Cadmium 114, Lead 206, Lead 207, Lead 208, and Uranium 238. Any significant relationship between otolith concentration and red snapper sex or total length could reduce confidence in the concept of an 'oil and gas platform' site-specific marker.

When compared with red snapper sex, only 2 of the 13 elements show a significant relationship. Vanadium 51 (Figure 36) and Uranium 238 (Figure 37) both have higher mean concentrations in otoliths of male red snapper than females at all three

study areas. None of the elements show any significant trends when compared to red snapper total length. Figures 38, 39, and 40 are a few examples of the graphic results obtained when comparing otolith elemental concentration at all three study sites to red snapper total length. Although a number of elements appear to have higher concentrations in otoliths of smaller (younger) red snapper, i.e., those less than 500 mm in total length, I believe this to be a spurious correlation attributable to the high number of individuals collected in the smaller size classes relative to fish greater than 500 mm TL.

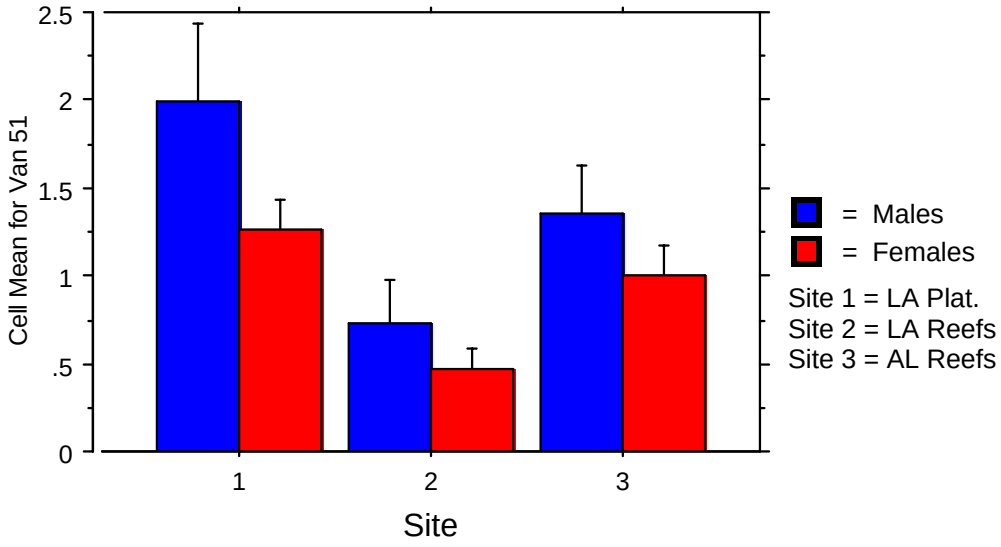

Figure 36. Vanadium 51 concentration in otoliths at all three study sites showing higher concentrations in male red snapper.

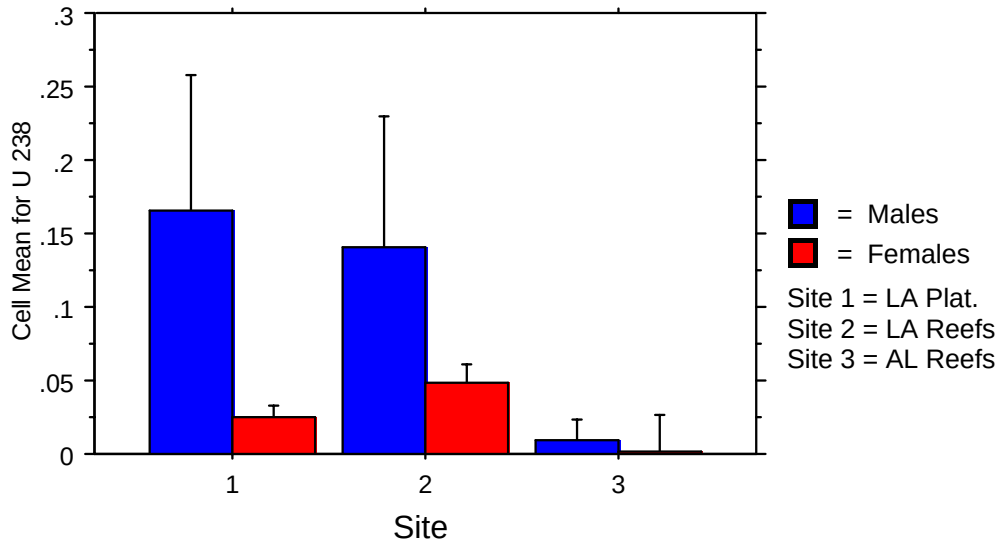

Figure 37. Uranium 238 concentration in otoliths at all three study sites showing higher concentrations in male red snapper.

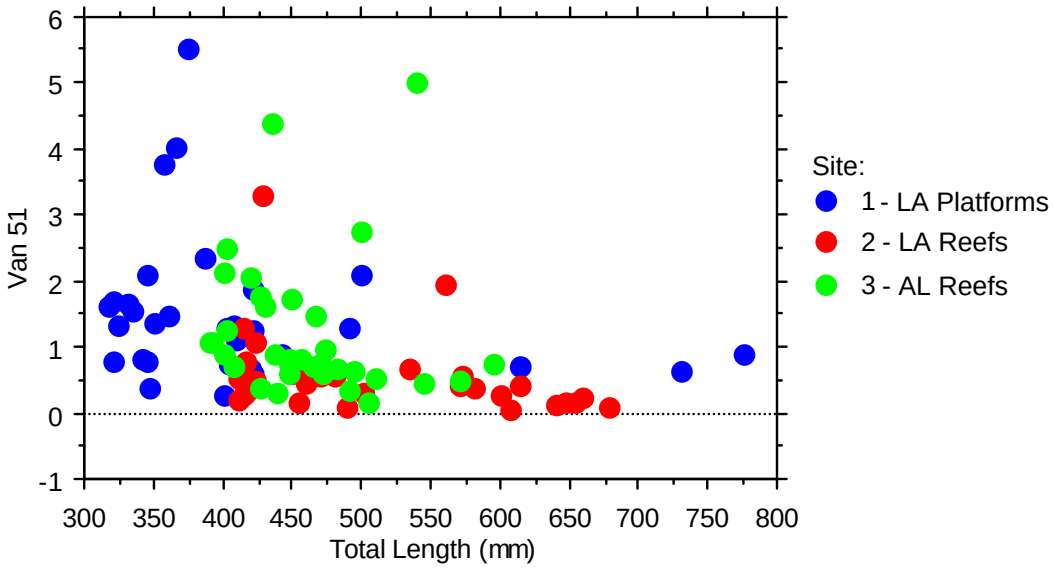

Figure 38. Vanadium 51 concentration in otoliths at all three study sites showing no significant trend with red snapper total length.

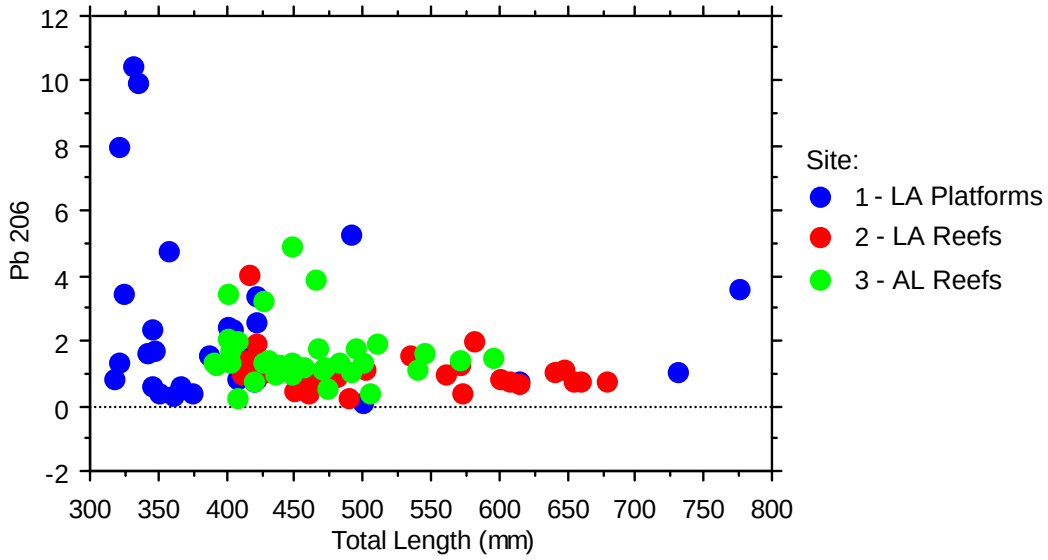

Figure 39. Lead 206 concentration in otoliths at all three study sites showing no significant trend with red snapper total length.

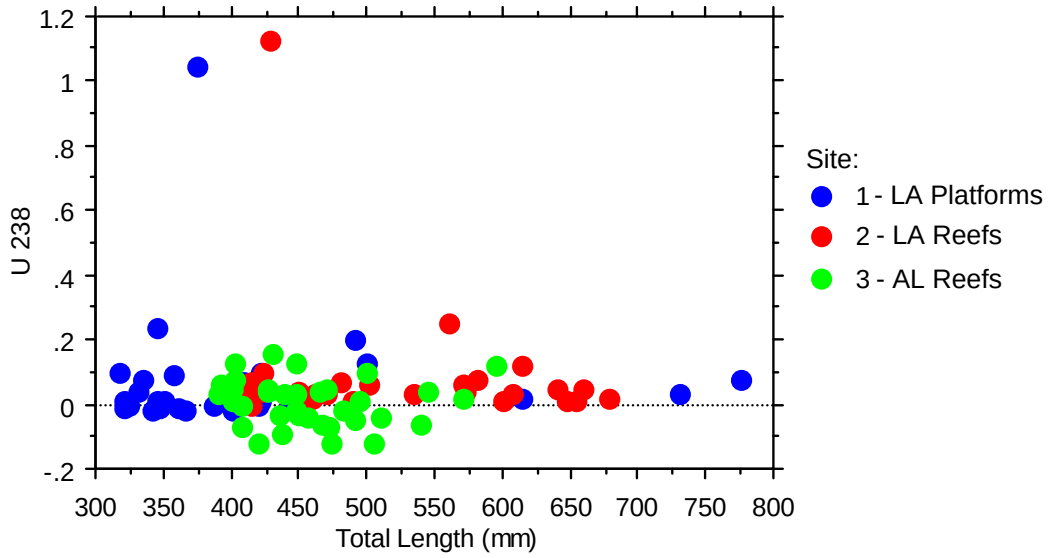

Figure 40. Uranium 238 concentration in otoliths at all three study sites showing no significant trend with red snapper total length.

DISCUSSION

Results of chemical and statistical analyses show that red snapper collected in the northern Gulf of Mexico had otolith microchemical 'fingerprints' unique to oil and gas platforms off coastal Louisiana. The results also show that red snapper collected had 'fingerprints' unique to artificial reefs located east and west of the Mississippi River. Once I discovered these differences, further analyses proved much more revealing and interesting. The discriminant function classification accuracies based on the probability of an individual fish being correctly classified into the habitat from which it was sampled are over 90% for each of the two main comparisons. If these results prove to be temporally stable, it may be possible to take any red snapper, analyze the otoliths using ICP-MS, and determine if that individual spent any portion of its life associated with oil and gas platforms. This will, therefore, help to determine if red snapper that have the 'oil and gas platform fingerprint' have disproportionately high survival rates when compared to the survival of red snapper without the fingerprint if red snapper with the fingerprint are present in high numbers on other habitat types or in different locations. For example, if large numbers of red snapper collected in the eastern Gulf of Mexico, where oil and gas platforms are relatively few, exhibit the 'oil and gas platform signature', this may indicate that platforms act as sources that supply recruits to other locations, in this case from Louisiana to Florida. This hypothesis is as of yet untested, but a proof of concept was necessary to determine if the oil and gas platform signature could be detected.

If my findings hold true, the core of red snapper otoliths collected from any location could be analyzed to determine if the red snapper had at one time been resident on a platform, and single annual increments could be isolated to determine temporal patterns

of residency. Such knowledge would be valuable for fisheries managers. If platforms are indeed refugia that are acting as source locations, they may be acting in much the way that marine protected areas (MPA's) are thought to behave, and perhaps should be considered for designation as MPA's. The determination of the impacts of artificial reefs (of any type) on the demographics of reef-associated fishes such as red snapper is also a necessary step in moving towards final resolution of the production vs. attraction debate.

Vanadium 51, Lead 206, Lead 207, and Lead 208 may be dissolution products linked with oil and gas platform operations or their prior drilling operations (Continental Shelf Associates, Inc., 2004; Neff, 1987; Neff *et al*., 1987) and the presence of these elements or isotopes in the otoliths of reef-associated fishes could indicate their association with these structures. The concentrations of these four isotopes are significantly higher in otoliths sampled on oil and gas platforms in Louisiana than in otoliths sampled from artificial reefs in either Louisiana or Alabama. The feeding of red snapper on benthic organisms in the sediment surrounding oil and gas platforms may also be a method of incorporating these elements into otoliths, although the specific portion of elements gathered from food sources is thought to be minimal.

In contrast, concentrations of Copper 65, Silver 107, Silver 109, Cadmium 114, and Uranium 238 in otoliths of red snapper may be markers attributable to Mississippi River discharge. These five isotopes have significantly higher concentrations in otoliths collected west of the Mississippi River both on Louisiana oil and gas platforms and Louisiana artificial reefs. Cobalt 59 and Nickel 62 concentrations are both higher in otoliths sampled east of the Mississippi River off Alabama. Due to the close proximity of the sampling area to Mobile Bay, this fingerprint may be derived from Mobile Bay

discharge, or from materials used to construct artificial reefs from which red snapper were sampled in Alabama. It must be noted, however, that the inferences above are speculative and will require more study before firm conclusions can be drawn.

When comparing otolith elemental concentration with red snapper sex, Vanadium 51 and Uranium 238 both have higher mean concentrations in otoliths of male red snapper than females at all three study areas. One possible explanation of these results may be that these elements are lipophilic. Female red snapper may be distributing these elements into egg yolk and 'dumping' them during spawning, where as, male red snapper do not have this opportunity. These findings suggest the need for more research into how these particular elements and reproduction interact. The ideas mentioned are speculative and require more research before firm conclusions can be drawn.

The main statistical techniques utilized to discriminate red snapper otolith elemental concentration in those fish sampled on oil and gas platforms and on artificial reefs were LDA, SDA, and CDA. These analyses were chosen based upon their wide use in otolith microchemistry research. For example, Campana *et al.* (1994), Campana and Gagne (1995), and Patterson *et al*. (1998) all used SDA while Edmonds *et al*. (1992), Edmonds *et al*. (1995), and Milton *et al*. (1997) utilized CDA instead to interpret their otolith microchemical data.

Although SDA and CDA are very similar techniques and both successfully discriminated between otoliths collected on oil and gas platforms and artificial reefs, elements or isotopes identified by these methods were not entirely consistent. Canonical discriminant analysis employed a larger number of isotopes than did SDA. For example, when discriminating between Louisiana oil and gas platforms and Louisiana artificial

reefs, both techniques identify Vanadium 51, Silver 109, Lead 206, and Uranium 238 as key isotopes. However, SDA also identifies Zinc 66 and Nickel 62 as important for discrimination between the two habitats. In contrast, CDA did not indicate Zinc or Nickel as being important, but included Lead 207, Lead 208, and Silver 107. Although differences exist, both statistical techniques identify the main elements I now believe to be indicative of association with oil and gas platforms in coastal Louisiana (Continental Shelf Associates, Inc., 2004; Neff, 1987; Neff *et al*., 1987), namely Vanadium and the 3 isotopes of Lead previously mentioned.

When comparing the results of the SDA and CDA for the discrimination of otoliths collected east and west of the Mississippi River, Lead 208, Vanadium 51, and Uranium 238 are chosen as important for discrimination by both techniques. SDA identifies Copper 65, Silver 107, and Cobalt 59 as important isotopes for discrimination as well. The CDA did not identify any of these isotopes as important, and instead recognizes Nickel 62, Zinc 64, Zinc 66, and Lead 207. Most importantly, while the two techniques use different elements to discriminate between otoliths collected on artificial reefs east and west of the Mississippi River, neither employs elements associated with the platform signature. All of the elements identified by SDA and CDA as being important for discrimination in any of the three comparisons were also identified by the principal components analysis as explaining a large portion of the variance within the ICP-MS data.

Although the results of this study are promising, there are some identifiable weaknesses that should be mentioned. My inability to collect red snapper on oil and gas platforms east of the Mississippi River limited the ability to determine if the

microchemical signature in otoliths collected on Louisiana oil and gas platforms are evident and similar to those in fishes collected from platforms east of the River. Future studies should address this issue by sampling red snapper east of the Mississippi River and rerunning the analyses described here. Also, Zinc anodes that are attached to boat hulls and oil and gas platforms to prevent electrolysis may have contributed to the high Zinc 64 and Zinc 66 concentrations that were seen in otoliths collected in all three of the study areas.

The red snapper collected on Louisiana oil and gas platforms were smaller in total length (mm) than the specimens collected both on Louisiana artificial reefs (by 100 mm on average) and Alabama artificial reefs (by 50 mm on average). Therefore, the otoliths from the Louisiana sample were smaller also. Because it has been determined that otolith growth rates of larval and juvenile fish may affect otolith elemental microchemistry (Thorrold *et* al., 1997; Patterson *et* al., 1998), I assumed that otolith growth rates were similar despite differences in size of red snapper collected. This assumption was based on the results of Fischer *et al*. (2004) who examined growth rates, size-at-age, and length and weight information of red snapper collected from the recreational harvests off Alabama, Louisiana, and Texas from 1999 to 2001. Fischer *et al*. (2004) collected red snapper from Dauphin Island, Alabama, Port Fourchon, Louisiana, and Port Aransas, Texas and recorded pertinent morphometric measurements. Red snapper growth was modeled from weighted mean fork length at age and mean total weight at age by using the von Bertalanffy growth equation (Fischer *et al*., 2004). Results found that models of mean red snapper fork length at age for Alabama and Louisiana were similar, with

likelihood ratio tests showing no significant differences between growth rates of fish collected in Louisiana and Alabama (Fischer *et al*., 2004).

It is important to reemphasize that the 15 elements analyzed in this study are not the usual suite of elements analyzed in most otolith microchemistry research (Campana, 1999; Campana *et al.*, 1994; Dove *et al.*, 1996; Edmonds *et al.*, 1992; Patterson *et al.*, 1998). The elements chosen for analysis were those that other data suggested would be present as a unique 'oil and gas platform signature'. I expected this signature to be attributable to both drilling muds used during the initial drilling operations of the oil and gas platforms, as well as from the natural weathering processes that occur on these structures over time (Continental Shelf Associates, Inc., 2004; Neff, 1987; Neff *et al*., 1987; Gallaway *et al*., 1981). Some examples of these weathering processes include natural corrosion and degradation of the platform gratings, decks, and 'legs' into the surrounding water column. The elements chosen for analysis are also heavy metals and replace calcium in the aragonite crystal matrix. In addition, with the exception of zinc, I felt that these elements would not be associated with a food web signal, but would be incorporated from the surrounding water mass and sediments (Campana, 1999; Ni *et al*., 2000). Elements that are associated with a food web signal tend to be light and get incorporated into the protein matrix rather than the aragonite crystal matrix (James H. Cowan, Jr. – personal communication).

Otolith microchemistry research has been a widely expanding field, yet such work involving red snapper in the Gulf of Mexico has been limited. Most otolith microchemistry studies, particularly in the early years, were aimed primarily at questions of stock discrimination. Now there are more studies that are attempting to use this

technique to answer questions about habitat value and differential sources of recruitment to adult habitats, especially for fishes like red snapper where early life history stages occupy different habitats than adults.

For example, a previous study by Patterson *et al*. (1998) aimed to differentiate between three important nursery areas of age-0 red snapper in the Gulf of Mexico using otolith microchemical fingerprints. Patterson *et al*. (1998) collected age-0 red snapper from the near-shore waters of south Texas, western Louisiana, and Alabama/Mississippi, which are thought to be three of the main nursery areas for red snapper in the Northern Gulf. Otoliths collected were dissolved and analyzed using inductively coupled plasma-atomic emissions spectrometry (ICP-AES). The presence of twelve elements was detected in the red snapper otolith solutions with the ICP-AES and those elements were Al, As, Ba, Ca, Fe, K, Mg, Mn, Na, Se, Sr, and Zn. Otolith elemental data was analyzed statistically to determine the microchemical fingerprints from each nursery area (Patterson *et al*., 1998). Statistical analyses included an analysis of covariance (ANCOVA), an analysis of variance (ANOVA), and a multivariate analysis of variance (MANOVA).

The results of the study by Patterson *et al*. (1998) found 10 of the 12 elements analyzed to be significantly different among the three nursery areas; Al, As, Ca, Fe, Mg, Mn, Se, Zn, K, and Na. However, only Al, As, Ca, Fe, Mg, Mn, Se, and Zn were used in subsequent multivariate analyses to determine otolith microchemical fingerprints of the three nursery areas (Patterson *et al*., 1998). K and Na were excluded because incorporation of these elements into otoliths is most likely under physiological control and thus may not result form true differences in water chemistry (Campana and Gagne,

1995; Kalish, 1989). Those eight elements were then entered into a stepwise discriminant function analysis that was significant, with the best model including Mg, Se, As, Fe, and Al entered in that order (Patterson *et al*., 1998). Classification accuracies based upon the probability of an individual fish being correctly classified into the nursery area from which it came were over 90% for each nursery area (Patterson *et al*., 1998). The results of the chemical and statistical analyses showed that 1995 age-0 red snapper in the northern Gulf of Mexico had otolith microchemical fingerprints unique to their nursery area (Patterson *et al*., 1998). Once Patterson *et al*. (1998) determined that they could successfully use otolith microchemistry to determine the nursery area signature of juvenile red snapper in the Gulf of Mexico, they inferred that they could extend this hypothesis to adult red snapper, allowing them to sample adults on offshore habitats and determine whether fish were recruiting from nearby or distant nursery areas. Their results showed that most recruits to offshore reefs were derived from nearby nursery grounds, but the movement between reefs was more frequent as fish got older. More importantly, Patterson *et al*. (1998) clearly demonstrated that otolith microchemical fingerprints were useful for estimating patterns of long-term movement of reef-associated species.

Despite some obvious differences, my experiment is an extension of the 'nursery area hypothesis' explored by Patterson *et al*. (1998). Instead of determining the signatures of different red snapper nursery areas, or natural habitats, my goal was to determine if it was possible to identify the signature of oil and gas platforms, or man-made red snapper habitat, which are much smaller in spatial extent despite the large number of individual platforms.

Similar to the work by Patterson *et al*. (1998), a number of other studies have attempted to use otolith microchemistry to link fishes to specific habitats. Gillanders and Kingsford (1996), on the east coast of Australia, aimed to determine if populations of blue grouper, *Achoerodus viridis*, on rocky reefs were sustained by (1) recruitment to estuarine seagrass habitat followed by movement to rocky reefs, (2) direct recruitment to rocky reefs, or (3) a combination of the two. Recruits were collected from estuarine seagrass and rocky reef habitats and elements in their otoliths were analyzed by ICP-MS to determine if different habitat specific elemental fingerprints could be found (Gillanders and Kingsford, 1996). With further analysis, the larger goal was to determine the possible source (i.e., seagrass or rocky reef) of adult reef fish by analyzing the core of otoliths from adults (i.e., deposited during the juvenile recruitment phase) and relating this to the composition of otoliths from recruits from each environment.

Significant differences in the elemental composition of otoliths were found between recruits collected from seagrass and rocky reef environments (Gillanders and Kingsford, 1996). Strontium occurred in significantly higher concentrations in otoliths from fish collected on rocky reefs, whereas Ba, Mn, and Co occurred in significantly greater concentrations in otoliths of fish collected from seagrass habitats. Strontium, Mn, and Ba were used as variables in a 2 group (seagrass and rocky reef) LDA that resulted in a significant separation of recruits between rocky reef and seagrass environments. There was a misclassification error rate of 5.5% when classifying recruits using the cross-validation method. The algorithms generated from recruits classified 59% of adult fish as having settled directly on rocky reefs while 41% of the adults had first recruited to seagrass environments before moving to the reefs (Gillanders and Kingsford, 1996).

In another example, Thorrold *et al*. (1997) used laser ablation ICP-MS to assay sectioned otoliths from juvenile Atlantic croaker (*Micropogonias undulates*) collected from the Neuse River, North Carolina, and the Elizabeth River, Virginia. They hoped to identify factors that potentially influenced larval survival and transport from spawning sites to juvenile nursery areas and to gain insight into the geographic extent of self-recruiting populations of Atlantic croaker in the Mid-Atlantic and South Atlantic Bights. Yet, they found little evidence for differences in the trace element composition between the cores of otoliths from croakers collected in the Neuse and Elizabeth Rivers. More variation between locations was seen in assays from the otolith edge with Mg:Ca and Ba:Ca being significantly different. Both elements were found to be significantly higher in otoliths from the Neuse River than in those from the Elizabeth River (Thorrold *et al*., 1997). Despite this variability, Thorrold *et al*. (1997) were unable to reject the hypothesis that croaker larvae form north and south of Cape Hatteras originated from different spawning sites, which might indicate that the larvae were spawned in close geographic proximity.

Finally, Gillanders and Kingsford (2000) collected juvenile trumpeter *Pelates sexlineatus* over a 2-year period from two sites in seven estuaries along the east coast of Australia. The main objective of the study was to determine variation in elemental fingerprints among and within estuaries. Otoliths were removed and analyzed by solution-based ICP-MS; preliminary analyses suggested that seven elements (Mg, Mn, Cu, Zn, Sr, Ba, and Pb) were detectable in the otoliths (Gillanders and Kingsford, 2000). However, their results showed that significant differences were found in the otolith chemistry of juvenile trumpeter from different estuaries, but that success rates of

classification to recruited estuaries ranged from 50 to 100% (Gillanders and Kingsford, 2000). While their results were promising, Gillanders and Kingsford (2000) suggested that the addition of further elements to the discriminant function and the use of stable isotopes might improve their classification accuracies.

There are several such examples in the literature similar to the studies described above (Secor *et al.*, 1995; Jessop *et al*., 2002; Sanchez-Jerez *et al*., 2002; Swan *et al*., 2003; Arai *et al*., 2004; Brazner *et al*., 2004; Chittaro *et al*., 2004; and Arslan and Secor, 2005, to name but a few). Although the study by Patterson *et al*. (1998) was the only other from the Gulf of Mexico using red snapper, it is plausible to compare my study to those discussed above, as they represent direct comparisons to my research and others in the current literature, with one notable exception. My study is unique because it deals with an elemental signature of oil and gas platforms, i.e., a fingerprint derived from a man-made habitat.

A study by Spencer *et al*. (2000) that utilized distinct anthropogenic sources of lead in fish otoliths as a potential nursery ground stock marker in Hawaii is the 'bridge' that closes the gap between my study and those in the previous literature. Spencer *et al*. (2000) collected three species of juvenile tropical reef fish (parrotfish, sergeant major, and domino damselfish) at 5 locations in Kaneohe Bay, Oahu, and used ICP-MS for otolith analysis. Variations measured in the lead stable isotope ratios in the otoliths reflected mixing of anthropogenic lead from the Kaneohe Bay watershed and 'background' lead characteristic of the adjacent ocean (Spencer *et al*., 2000). They found that the lead isotopic composition of the watershed has a low $^{206}Pb/^{204}Pb$ signature primarily reflecting past combustion of tetra-ethyl Pb additives in fuels, while the ocean

water has a high $^{206}Pb/^{204}Pb$ isotopic composition (Spencer *et al*., 2000). The key issue was that the characteristic anthropogenic Pb isotope ratios are a qualitative rather than quantitative marker, so that the reliable detection of the presence of distinct Pb isotopes is all that is required for nursery ground discrimination (Spencer *et al*., 2000). The use of an anthropogenic otolith signature instead of naturally occurring markers inspired me to expand examination of the 'nursery area hypothesis' to man-made rather than natural nursery habitats.

The overall objectives of all of the studies mentioned involve determination of the origin of adult recruits in coastal and offshore environments. This is very similar to the main goal of the next phase of this research; namely to determine if adult red snapper now recruiting to habitats in the eastern Gulf and elsewhere have spent any portion of their lives on oil and gas platforms. The real distinction between my research and the studies performed earlier in the literature is that it deals with determining the otolith 'elemental fingerprint' of reef fishes attributable to their association with man-made habitats rather than natural habitats, and I believe this to be the first of its kind. If my findings hold true, it may provide a new direction in which this type of research may expand.

Despite the fact that the main goal of this study was to prove the concept that otolith microchemistry could be used to determine association of red snapper with oil and gas platforms in the Gulf of Mexico, a number of other important conclusions can be drawn. They are as follows:

- This method was successful; otolith microchemistry can be used to determine the trace element signature of oil and gas platforms in otoliths of red snapper.

- Vanadium 51, Lead 206, Lead 207, and Lead 208 may be dissolution products incorporated into red snapper otoliths from oil and gas platform operations and their prior drilling operations.
- It is plausible to move forward with sub-sampling otoliths of adult fish to determine age-specific habitat affinity, and to determine if the new recruits that are now expanding into the eastern Gulf were associated with oil and gas platforms during some portion of their early life.
- It may be possible to determine if there are a disproportionate number of adult red snapper in the eastern Gulf and elsewhere that have this 'oil and gas platform signature' in their otoliths.
- If the above statement is true, oil and gas platforms may constitute red snapper essential fish habitat and, as such, should be considered as viable tools in management of red snapper.

REFERENCES

Arai, N., W. Sakamoto, and K. Maeda. 1996. Correlation between ambient seawater temperature and strontium calcium concentration ratios in otoliths of the Red Sea Bream *Pagrus major*. *Fisheries Science* 62: 652-653.

Arai, T., A. Kotake, P. M. Lokman, M. J. Miller, and K. Tsukamoto. 2004. Evidence of different habitat use by New Zealand freshwater eels *Anguilla austalis* and *A. dieffenbachia*, as revealed by otolith microchemistry. *Marine Ecology Progress Series* 266: 213-225.

Arslan, Z. and D. H. Secor. 2005. Analysis of trace transition elements and heavy metals in fish otoliths as tracers of habitat use by American eels in the Hudson River Estuary. *Estuaries* 28(3): 382-393.

Bohnsack, J. A. 1989. Are high densities of fishes at artificial reefs the result of habitat limitation or behavioral preference? *Bulletin of Marine Science* 44(2): 631-645.

Bradley, E. and C. E. Bryan. 1975. Life History and fishery of the red snapper (*Lutjanus campechanus*) in the northwestern Gulf of Mexico: 1970-1974. *Gulf and Caribbean Fisheries Institute* 27: 77-106.

Brazner, J. C., S. E. Campana, and D. K. Tanner. 2004. Habitat fingerprints for Lake Superior coastal wetlands derived from elemental analysis of yellow perch otoliths. *Transactions of the American Fisheries Society* 133: 692-704.

Campana, S. E., A. J. Fowler, and C. M. Jones. 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. *Canadian Journal of Fisheries and Aquatic Sciences* 51: 1942-1950.

Campana, S. E. and J. A. Gagne. 1995. Cod stock discrimination using ICPMS elemental analysis assays of otoliths. P. 671-692 In D. H. Secor, J. M. Dean and S. E. Campana (eds.), *Recent Developments in Fish Otolith Research*. University of South Carolina Press. Columbia, SC.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series* 188: 263-297.

Campana, S. E., G. A. Chouinard, J. M. Hanson, A. Frechet, and J. Brattey. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. *Fisheries Research* 46: 343-357.

Casselman, J. M. 1987. Determination of age and growth. p. 209-242 In A.H. Weatherley and H.S. Gill (eds.), *The Biology of Fish Growth*. Academic Press, New York.

Chittaro, P. M., B. J. Fryer, and P. f. Sale. 2004. Discrimination of French grunts (*Haemulon flavolineatum*, Desmarest, 1823) from mangrove and coral reef habitats using otolith microchemistry. *Journal of Experimental Marine Biology and Ecology* 308: 169-183.

Continental Shelf Associates, Inc. 2004. Gulf of Mexico comprehensive synthetic based muds monitoring program volume I: technical final report for the SBM Research Group, Shell Global Solutions U. S., Houston, TX.

Der, Geoff and B. S. Everitt. 2002. Principal components analysis and factor analysis: The Olympic decathlon and statements about pain. In Chapman and Hall/CRC, eds. *A handbook of statistical analyses using SAS, Second Edition*. CRC Press LLC, Florida. pp. 237-262.

Dove, S. G., B. M. Gillanders, and M. J. Kingsford. 1996. An investigation of chronological differences in the deposition of trace metals in the otoliths of two temperate reef fishes. *Journal of Experimental Biology and Ecology* 205: 15-33.

Edmonds, J. S., R. C. J. Lenanton, N. Caputi, and M. Morita. 1992. Trace elements in the otoliths of yellow-eye mullet (*Aldrichetta forsteri*) as an aid to stock identification. *Fisheries Research* 13: 39-51.

Edmonds, J. S., N. Caputi, M. J. Moran, W. J. Fletcher, and M. Morita. 1995. Population discrimination by variation in concentrations of minor and trace elements in sagittae of two western Australian teleosts. P. 655-670 In D. H. Secor, J. M. Dean and S. E. Campana (eds.), *Recent Developments in Fish Otolith Research.* University of South Carolina Press. Columbia, SC.

Fischer, A. J., M. S. Baker, Jr., and C. A. Wilson. 2004. Red snapper (*Lutjanus campechanus*) demographic structure in the northern Gulf of Mexico based on spatial patterns in growth rates and morphometrics. *Fishery Bulletin* 102: 593-603.

Gallaway, B. J., L. R. Martin, R. L. Howard, G. S. Boland, and G. D. Dennis. 1981. Effects on artificial reef and demersal fish and macro-crustacean communities. In *Environmental effects of offshore oil production: the Buccaneer gas and oil field study*. *Edited by* B. S. Middleditch. Mar. Sci. Vol. 14. Plenum Press, New York. Pp. 237-299.

Gallaway, B. J., J. G. Cole, R. Meyer, and J. P. Roscigno. 1999. Delineation of essential habitat for juvenile red snapper in the northwestern Gulf of Mexico. *Transactions of the American Fisheries Society* 128: 713-726.

Gillanders, B. M. and M. J. Kingsford. 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. *Marine Ecology Progress Series* 141: 13-20.

Gillanders, B. M. and M. J. Kingsford. 2000. Elemental fingerprints of otoliths of fish may distinguish estuarine 'nursery' habitats. *Marine Ecology Progress Series* 201: 273-286.

Goodyear, C. P. 1995. Red snapper in U. S. waters of the Gulf of Mexico. National Marine Fisheries Service, Southeast Fisheries Science Center, Miami Laboratory, Miami MIA-95/96-05.

Grossman, G. D., G. P. Jones, and W. J. Seaman, Jr. 1997. Do artificial reefs increase regional fish production? A review of existing data. *Fisheries* 22(4): 17-27.

Jessop, B. M., J.–C. Shiao, Y. Iizuka, and W.–N. Tzeng. 2002. Migratory behavior and habitat use by American eels *Anguilla rostrata* as revealed by otolith microchemistry. *Marine Ecology Progress Series* 233: 217-229.

Kakuta, I., M. Yukawa, and Y. Nishimura. 1997. Effect of water salinity on the elemental composition of otoliths from *Tridentiger obscurus* obscurus. *Bulletin of the Society of Sea Water Science of Japan* 51: 388-394.

Kalish, J. M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *Journal of Experimental Marine Biology and Ecology* 132: 151-178.

Lindberg, W. J. 1997. Can science resolve the attraction-production issue? Fisheries 22(4): 10-13.

McCawley, J. R. 2003. Diet and prey demand of red snapper, *Lutjanus campechanus*, on Alabama artificial reefs. Thesis, Department of Marine Sciences, University of South Alabama.

Milton, D. A., S. R. Chenery, M. J. Farmer, and S. J. M. Blaber. 1997. Identifying the spawning estuaries of the tropical shad, terubok *Tenualosa toli*, using otolith microchemistry. *Marine Ecology Progress Series* 153: 283-291.

Mugiya, Y. and C. Satoh. 1995. Strontium-Calcium ratios corresponding to microincrements in otoliths of the goldfish *Carassius auratus*. *Fisheries Science* 61: 361-362.

Neff, J. M. 1987. Biological effects of drilling fluids, drill cuttings, and produced waters. In D. F. Boesch and N. N. Rabalais, eds. *Long-Term Environmental Effects of Offshore Oil and Gas Development*. Elsevier Applied Science Publishers, London. pp. 469-538.

Neff, J. M., N. N. Rabalais, and D. F. Boesch. 1987. Offshore oil and gas development activities potentially causing long-term environmental effects. In D. F. Boesch and N. N. Rabalais, eds. *Long-Term Environmental Effects of Offshore Oil and Gas Development*. Elsevier Applied Science Publishers, London. pp. 149-173.

Ni, I. H., W. X. Wang, and Y. K. Tam. 2000. Transfer of Cd, Cr, and Zn from zooplankton prey to mudskipper *Periophthalmus cantonensis* and glassy *Ambassis urotaenia* fishes. *Marine Ecology Progress Series* 194: 203-210.

Patterson, W. F., III. 1997. Applications of Otolith Microstructure to problems in Fisheries Biology: Examining Recruitment Processes and Stock/Recruitment Relationships.

Patterson, W. F., III. 1999. Aspects of the population ecology of red snapper, *Lutjanus capechanus*, in an artificial reef area off Alabama. Dissertation, Department of Marine Sciences, University of South Alabama, pg. 98-99.

Patterson, W. F., III, J. H. Cowan, Jr., E. Y. Graham, and W. B. Lyons. 1998. Otolith microchemical fingerprints of age-0 red snapper, *Lutjanus campechanus* for the northern Gulf of Mexico. *Gulf of Mexico Science* 16: 83-91.

Patterson, W. F., III, J. C. Watterson, R. L. Shipp, and J. H. Cowan, Jr. 2001. Movement of tagged red snapper in the Northern Gulf of Mexico. *Transactions of the American Fisheries Society* 130: 533-545.

Payan, P., H. Kossman, A. Watrin, N. Mayer-Gostan, and G. Boeuf. 1997. Ionic composition of endolymph in teleosts; origin and importance of endolymph alkalinity. *Journal of Experimental Biology* 200: 1905-1912.

Payan, P., G. Borelli, G. Boeuf, and N. Mayer-Gostan. 1998. Relationship between otolith and somatic growth: consequence of starvation on acid-base balance in plasma and endolymph in the rainbow trout *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry* 19: 35-41.

Peabody, M. B. 2004. The fidelity of red snapper (*Lutjanus campechanus*) to petroleum platforms and artificial reefs in the northern Gulf of Mexico. Thesis, Department of Oceanography and Coastal Sciences, Louisiana State University, pp. 51-73.

Proctor, C. H. and R. E. Thresher. 1998. Effects of specimen handling and otolith preparation on concentrations of elements in fish otoliths. *Marine Biology* 131: 681-694.

Radtke, R. and D. Shafer. 1992. Environmental sensitivity of fish otolith microchemistry. *Australian Journal of Marine and Freshwater Research* 43: 935-951.

Romanek, C. S. and R. W. Gauldie. 1996. A predictive model of otolith growth in fish based on the chemistry of the endolymph. *Comp. Biochemistry and Physiology* 114A: 71-79.

Rooker, J. R., D. H. Secor, V. S. Zdanowicz, and T. Itoh. 2001. Discrimination of northern bluefin tuna from nursery areas in the Pacific Ocean using otolith chemistry. *Marine Ecology Progress Series* 218: 275-282.

Rooker, J. R., V. S. Zdanowicz, and D. H. Secor. 2001. Chemistry of tuna otoliths: assessment of base composition and postmortem handling effects. *Marine Biology* 139: 35-43.

Sanchez-Jerez, P., B. M. Gillanders, and M. J. Kingsford. 2002. Spatial variability of trace elements in fish otoliths: comparison with dietary items and habitat constituents in seagrass meadows. *Journal of Fish Biology* 61: 801-821.

SAS Institute, Inc. 1985. SAS Users Guide: Statistics, Version 5 Edition. Box 8000, Cary, North Carolina 27511-8000.

Schirripa, M. J., and C. M. Legault. 1999. Status of red snapper in U.S. waters of the Gulf of Mexico: updated through 1998. NMFS, SFD, Miami. SFD-99/00-75

Schmitten, R. A. 1999. Essential fish habitat: Opportunities and challenges for the next millennium. *American Fisheries Society Symposium* 22: 3-10.

Seaman, W. J., Jr. 1997. What if everyone thought about reefs? *Fisheries* 22: 5.

Secor, D. H., J. M. Dean, and S. E. Campana. (eds.). 1995. *Recent Developments in Fish Otolith Research*. University of South Carolina Press. Columbia, SC.

Secor, D. H., A. Henderson-Arzapalo, and P. M. Piccoli. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? *Journal of Experimental Marine Biology and Ecology* 192: 15-33.

Simkiss, K. 1974. Calcium metabolism of fish in relation to ageing, p. 1-12. *In* T. B. Bagenal [ed.] *The ageing of fish*. Unwin Brothers Ltd., Surrey, England.

Spencer, K., D. J. Shafer, R. W. Gauldie, and E. H. DeCarlo. 2000. Stable lead isotope ratios from distinct anthropogenic sources in fish otoliths: a potential nursery ground stock marker. *Comparative Biochemistry and Physiology Part A* 127: 273-284.

Stanley, D. R. and C. A. Wilson. 1990. A fishery-dependent based study of fish species composition and associated catch rates around oil and gas structures off Louisiana. *Fishery Bulletin* 88: 719-730.

Stanley, D. R. and C. A. Wilson. 1991. Factors affecting the abundance of selected fishes near oil and gas platforms in the Northern Gulf of Mexico. *Fishery Bulletin* 89: 149-159.

Stanley, D. R. and C. A. Wilson. 1997. Seasonal and spatial variation in the abundance and size distribution of fishes associated with a petroleum platform in the northern Gulf of Mexico. *Canadian Journal of Fisheries and Aquatic Science* 54: 1166-1176.

Stanley, D. R. and C. A. Wilson. 1998. Spatial variation in fish density at three petroleum platforms as measured with Dual-Beam hydroacoustics. *Gulf of Mexico Science* 16: 73-82.

Swan, S. C., J. D. M. Gordon, B. Morales-Nin, T. Shimmield, T. Sawyer, and A. J. Geffen. 2003. Otolith microchemistry of *Nezumia aequalis* (Pisces: Macrouridae) from widely different habitats in the Atlantic and Mediterranean. *Journal of the Marine Biological Association of the United Kingdom* 83: 883-886.

Szedlmayer, S. T. and R. L. Shipp. 1994. Movement and growth of red snapper, *Lutjanus campechanus*, from an artificial reef area in the northeastern Gulf of Mexico. *Bulletin of Marine Science* 55(2-3): 887-896.

Szedlmayer, S. T. and J. Conti. 1999. Nursery habitats, growth rates, and seasonality of age-0 red snapper, *Lutjanus campechanus*, in the northeast Gulf of Mexico. U.S. *Fishery Bulletin* 97: 626-635.

Takagi, Y., K. Ishida, and Y. Mugiya. 2000. Carbohydrates of the otolith organ of the rainbow trout *Oncorhynchus mykiss* detected by lectins. *Fisheries Science* 66: 933-939.

Takagi, Y. 2002. Otolith formation and endolymph chemistry: a strong correlation between the aragonite saturation state and pH in the endolymph of the trout otolith organ. *Marine Ecology Progress Series* 231: 237-245.

Thomas, R. 2001. A beginner's guide to ICP-MS Part I. *Spectroscopy* 16(4): 38-42.

Thomas, R. 2001. A beginner's guide to ICP-MS Part II: The sample-introduction system. *Spectroscopy* 16(5): 56-60.

Thomas, R. 2001. A beginner's guide to ICP-MS Part III: The plasma source. *Spectroscopy* 16(6): 26-30.

Thomas, R. 2001. A beginner's guide to ICP-MS Part IV: The interface region. *Spectroscopy* 16(7): 26-34.

Thomas, R. 2001. A beginner's guide to ICP-MS Part V: The ion focusing system. *Spectroscopy* 16(9): 38-44.

Thomas, R. 2001. A beginner's guide to ICP-MS Part VI: The mass analyzer. *Spectroscopy* 16(10): 44-48.

Thorrold, S. R., C. M. Jones, and S. E. Campana. 1997. Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic Croaker (*Micropogonias undulates*). *Limnology and Oceanography* 42(1): 102-111.

Thresher, R. E. 1999. Elemental composition of otoliths as a stock delineator in fishes. *Fisheries Research* 43: 165-204.

Wilson, C. A., and D. L. Nieland. 2001. Age and growth of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico off Louisiana. *Fishery Bulletin* 99: 653-664.

Workman, I. K., and D. G. Foster. 1994. Occurrence and behavior of juvenile red snapper, *Lutjanus campechanus*, on commercial shrimp fishing grounds in the northeastern Gulf of Mexico. *Marine Fisheries Review* 56: 9-11.

APPENDIX: MORPHOMETRIC AND ICP-MS SUMMARY TABLES

Morphometric data for the 32 red snapper collected on oil and gas platforms off of Louisiana.

ID #	Site #	Total Length (mm)	Otolith Wt (g)	Fish Wt (g)	Sex
GP01-LA	1	375	0.36634	724.19	M
GP02-LA	1	500	0.63084	1797.45	M
GP03-LA	1	351	0.37334	542.7	F
GP04-LA	1	360	0.35163	636.54	M
GP05-LA	1	366	0.36236	670.67	M
GPO6-LA	1	318	0.27575	394.51	F
GPO7-LA	1	346	0.3904	518.12	F
GPO9-LA	1	386	0.43072	737.73	F
GP10-LA	1	321	0.29512	406.66	F
GP11-LA	1	342	0.35701	541.3	M
GP12-LA	1	346	0.3163	561.55	M
GP13-LA	1	410	0.44523	960.08	M
GP14-LA	1	332	0.27466	453.41	F
GP15-LA	1	335	0.33814	466.78	F
GP16-LA	1	324	0.31931	419.06	F
GP17-LA	1	357	0.32612	573.24	F
GP18-LA	1	321	0.3267	443.07	M
GP20-LA	1	347	0.34578	522.98	F
GP21-LA	1	400	0.46436	827.7	F
GP23-LA	1	491	0.642	1697.18	M
GP24-LA	1	408	0.4376	945.36	M
GP25-LA	1	615	0.9057	3321.12	F
GP26-LA	1	402	0.442	841.14	F
GP27-LA	1	442	0.53	1142.7	F
GP28-LA	1	422	0.4918	1051.72	M
GP29-LA	1	405	0.4392	861.58	F
GP30-LA	1	419	0.4941	961.54	F
GP31-LA	1	422	0.4693	983.96	F
GP32-LA	1	421	0.4601	976.45	F
GP33-LA	1	404	0.4131	854.73	F
GP34-LA	1	776	1.4123	7038.35	F
GP35-LA	1	730	1.39556	5777.64	F

Morphometric data for the 31 red snapper collected on artificial reefs off of Louisiana.

ID #	Site #	Total Length (mm)	Otolith Wt (g)	Fish Wt (g)	Sex
ARO3-LA	2	416	0.44801	939.48	F
ARO4-LA	2	415	0.44238	997.57	M
ARO5-LA	2	411	0.41423	967.5	M
ARO7-LA	2	454	0.51818	1245.97	F
AR08-LA	2	428	0.51677	1099.7	M
AR10-LA	2	423	0.52221	1059.61	M
AR11-LA	2	415	0.4452	932.21	F
AR13-LA	2	416	0.52625	939.48	F
AR14-LA	2	573	0.75479	2642.76	F
AR17-LA	2	461	0.54027	1390.61	M
AR18-LA	2	460	0.59004	1299.95	F
AR19-LA	2	535	0.7277	2117.39	F
AR21-LA	2	411	0.42337	967.502	M
AR15-LA	2	470	0.57196	1478.23	M
AR16-LA	2	450	0.4869	1288.44	M
AR23-LA	2	615	0.85701	3321.12	F
AR24-LA	2	659	0.96513	4151.62	F
AR25-LA	2	648	0.89098	3931.92	F
AR26-LA	2	600	0.79487	3066.52	F
AR27-LA	2	502	0.68821	1820.27	M
AR28-LA	2	424	0.42704	999.1	F
AR29-LA	2	607	0.90285	3183.59	F
AR30-LA	2	421	0.44649	1043.86	M
AR31-LA	2	641	0.88207	3796.37	F
AR32-LA	2	655	1.03172	4070.77	F
AR33-LA	2	490	0.61123	1594.23	F
AR34-LA	2	678	1.03883	4550.82	F
AR35-LA	2	561	0.76898	2468.13	F
AR36-LA	2	480	0.62642	1491.51	F
AR37-LA	2	570	0.84107	2719.42	M
AR38-LA	2	581	0.83424	2888.74	M

Morphometric data for the 35 red snapper collected on artificial reefs off of Alabama.

ID #	Site #	Total Length (mm)	Otolith Wt (g)	Fish Wt (g)	Sex
AR01-AL	3	500	0.5507	1797.45	M
AR02-AL	3	470	0.6021	1478.23	M
AR03-AL	3	435	0.4406	1157.54	M
AR04-AL	3	438	0.5579	1182.96	M
AR05-AL	3	450	0.5582	1210.86	F
AR06-AL	3	483	0.5557	1611.33	M
AR07-AL	3	457	0.5546	1272.76	F
AR08-AL	3	447	0.5291	1261.49	M
AR09-AL	3	491	0.6259	1697.18	M
AR10-AL	3	539	0.6972	2278.94	M
AR11-AL	3	467	0.5313	1448.61	M
AR12-AL	3	510	0.688	1913.52	M
AR13-AL	3	472	0.5819	1498.2	M
AR14-AL	3	408	0.4527	945.36	M
AR15-AL	3	544	0.6514	2346.41	M
AR16-AL	3	439	0.5072	1117.84	F
AR17-AL	3	430	0.4999	1045.49	F
AR18-AL	3	402	0.4501	902.12	M
AR19-AL	3	426	0.4738	1014.4	F
AR20-AL	3	392	0.3647	833.1	M
AR21-AL	3	448	0.5001	1193.56	F
AR22-AL	3	402	0.4518	902.12	M
AR23-AL	3	390	0.4786	819.74	M
AR24-AL	3	426	0.5526	1014.4	F
AR25-AL	3	596	0.8786	3131.05	M
AR26-AL	3	447	0.5305	1184.98	F
AR27-AL	3	495	0.636	1741.26	M
AR28-AL	3	571	0.8468	2734.52	M
AR29-AL	3	400	0.4541	888.02	M
AR30-AL	3	465	0.5437	1429.1	M
AR31-AL	3	401	0.4459	834.4	F
AR32-AL	3	408	0.4961	882.37	F
AR33-AL	3	505	0.558	1757.31	F
AR34-AL	3	474	0.57	1432.13	F
AR35-AL	3	419	0.4783	961.54	F

Summary table of the ICP-MS data (concentration in ppb) for all three study sites.

SAMPLE:		Gas Platform LA samples (n = 32)				Artificial Reef LA samples (n = 31)				Artificial Reef AL samples (n = 35)			
		AVERAGE	STD DEV	MAX	MIN	AVERAGE	STD DEV	MAX	MIN	AVERAGE	STD DEV	MAX	MIN
Vanadium	V 51	1.51	1.11	5.52	0.28	0.57	0.64	3.31	0.06	1.19	1.07	5.03	0.15
Cobalt	Co 59	1.56	1.17	6.86	0.53	1.94	1.02	3.78	0.51	4.08	6.25	26.32	0.74
Nickel	Ni 62	14.2	11.1	42.1	3.6	13.8	9.9	39.1	4.0	25.1	54.8	269.2	1.2
Zinc	Zn 64	247	84	532	120	341	153	783	129	317	139	844	136
Copper	Cu 65	53.4	21.8	157.5	30.1	57.2	13.0	87.8	18.3	44.9	7.6	68.5	33.9
Zinc	Zn 66	263	78	539	145	369	135	681	151	302	132	833	115
Silver	Ag 107	0.27	0.10	0.48	0.08	0.20	0.08	0.39	0.05	0.11	0.09	0.32	-0.05
Silver	Ag 109	0.27	0.09	0.44	0.10	0.18	0.08	0.41	0.05	0.13	0.08	0.30	0.01
Cadmium	Cd 110	0.24	0.30	1.15	-0.03	0.16	0.08	0.41	0.05	0.12	0.11	0.28	-0.10
Cadmium	Cd 111	0.22	0.29	1.20	-0.04	0.15	0.08	0.35	0.03	0.11	0.10	0.41	-0.02
Cadmium	Cd 114	0.37	0.31	1.37	0.07	0.40	0.45	2.47	0.02	0.11	0.09	0.31	-0.06
Lead	Pb 206	2.46	2.63	10.47	0.15	1.10	0.68	4.07	0.28	1.58	0.95	4.92	0.29
Lead	Pb 207	2.34	2.51	10.01	0.14	1.10	0.61	3.81	0.34	1.46	0.88	4.36	0.10
Lead	Pb 208	2.41	2.55	10.03	0.07	1.15	0.63	3.85	0.37	1.69	1.00	5.10	0.35
Uranium	U 238	0.07	0.19	1.05	-0.02	0.08	0.20	1.13	0.00	0.01	0.07	0.16	-0.12

Summary table of the elements identified as important by each of the statistical analyses performed on the ICP-MS data. (site 1 = LA oil and gas platforms; site 2 = LA artificial reefs; site 3 = AL artificial reefs)

Analysis:	MANOVA			PCA	LDA & SDA			CDA		
Site:	All Sites	1 vs 2	2 vs 3	All Sites	All Sites	1 vs 2	2 vs 3	All Sites	1 vs 2	2 vs 3
V 51		X	X	X	X	X	X	X	X	X
Co 59	X			X	X		X			
Ni 62				X	X	X		X		X
Zn 64	X	X		X				X		X
Cu 65	X		X		X		X			
Zn 66		X	X	X	X	X		X		X
Ag 107	X	X	X	X	X		X	X	X	
Ag 109	X	X	X	X		X			X	
Cd 110	X			X						
Cd 111	X			X						
Cd 114	X		X	X	X			X		
Pb 206	X	X	X	X		X			X	
Pb 207	X	X		X				X	X	X
Pb 208		X	X	X	X		X	X	X	X
U 238			X		X	X	X		X	X

VITA

Lauren Kay Nowling, born to Sandra McLin- and Dan Mione on July 5, 1979, was raised in Daleville, Alabama, until the age of 4. Her father then joined the military and was immediately sent overseas. Lauren grew up living in a number of different places including Alabama, Germany, Alaska, Oklahoma, Washington, and Georgia. She graduated high school in 1997 from Ramstein American High School in Ramstein, Germany. She then pursued an education in marine biology at Auburn University in Auburn, Alabama. During her time at Auburn, she spent two summers taking classes at the Dauphin Island Sea Lab. Once she graduated with a Bachelor of Science in May of 2001, she took a summer internship at the Dauphin Island Sea Lab with Dr. Jim Cowan. After Lauren's internship, she returned to Auburn and began work in the Department of Fisheries and Allied Aquacultures as a temporary research technician. In the summer of 2002, Lauren decided to continue her education at Louisiana State University with the goal of a master's degree in oceanography and coastal sciences. While at school at Louisiana State University, she married David Nowling in December of 2004. After graduate school Lauren plans to pursue a career in fisheries research.