

REGIONAL STOCK STRUCTURE OF GREATER AMBERJACK IN THE  
SOUTHEASTERN UNITED STATES USING OTOLITH SHAPE ANALYSIS

By

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To my parents, who always encouraged me to pursue my passions

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## TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS.....	4
LIST OF TABLES.....	7
LIST OF FIGURES .....	9
ABSTRACT.....	10
CHAPTER	
1    INTRODUCTION.....	12
Fisheries Background.....	12
Stock Identification .....	13
Introduction to the Stock Concept .....	13
Otolith Shape Analysis .....	14
Greater Amberjack .....	15
Background.....	15
GAJ Fishery .....	16
GAJ Stock Assessment.....	18
GAJ Stock Structure.....	19
Study Objectives.....	21
2    METHODS .....	23
Sample Collection.....	23
Otolith Morphology .....	23
Rostrum Exclusion .....	23
Shape Quantification.....	24
Shape indices .....	24
Fourier analysis .....	25
Data Analysis .....	26
Comparison of Left and Right Otoliths .....	27
Comparison of Male and Female Otoliths .....	28
Regional Comparisons .....	28
3    RESULTS.....	34
Sample Collection.....	34
Data Analysis .....	34
Comparison of Left and Right Otoliths .....	34
Comparison of Male and Female Otoliths .....	35
Regional Comparisons .....	35
Data Exploration.....	35

Analysis of Variance.....	35
Discriminant Analysis .....	36
<b>4 DISCUSSION .....</b>	<b>48</b>
<b>LIST OF REFERENCES .....</b>	<b>53</b>
<b>BIOGRAPHICAL SKETCH.....</b>	<b>59</b>

## LIST OF TABLES

<u>Table</u>		<u>page</u>
2-1	Shape indices of greater amberjack ( <i>Seriola dumerili</i> ) sagittae calculated following Tuset et al. (2003), with ML corresponding to maximum length and MH to maximum height of the greatest enclosing rectangle.....	31
3-1	Otolith sample sizes of greater amberjack ( <i>Seriola dumerili</i> ) sagittae by region used in the present study.....	37
3-2	Analysis of Covariance (ANCOVA) of shape indices of greater amberjack ( <i>Seriola dumerili</i> ) sagittae, with forklength as the covariate and region as a factor.....	37
3-3	Pearson's product-moment correlation coefficients resulting from analysis of shape indices of greater amberjack ( <i>Seriola dumerili</i> ) sagittae.....	37
3-4	Paired t-test comparing shape indices of greater amberjack ( <i>Seriola dumerili</i> ) sagittae between left and right otoliths from the same individual. ....	37
3-5	Comparison of male and female greater amberjack ( <i>Seriola dumerili</i> ) otolith shape indices in the Gulf of Mexico stock using Analysis of Variance (ANOVA). .....	38
3-6	Comparison of male and female greater amberjack ( <i>Seriola dumerili</i> ) otolith shape indices in the Atlantic stock using Analysis of Variance (ANOVA).....	38
3-7	Eigenvalues from principal component analysis (PCA) comparing otolith shape between Gulf and Atlantic stocks of greater amberjack ( <i>Seriola dumerili</i> ) .....	39
3-8	Eigenvalues from principal component analysis (PCA) comparing otolith shape among central Florida, north Florida, and Louisiana samples of greater amberjack ( <i>Seriola dumerili</i> ) .....	40
3-9	Comparison of Atlantic and Gulf of Mexico stock greater amberjack ( <i>Seriola dumerili</i> ) otolith shape indices using Analysis of Variance (ANOVA) .....	41
3-10	Comparison of central Florida, north Florida, and Louisiana greater amberjack ( <i>Seriola dumerili</i> ) otolith shape indices using Analysis of Variance (ANOVA). .....	41
3-11	Comparison of central Florida, north Florida, and Louisiana age 3 greater amberjack ( <i>Seriola dumerili</i> ) otolith shape indices using Analysis of Variance (ANOVA). .....	41

- 3-12 Discriminant analysis comparing otolith shape indices between Gulf and Atlantic samples of greater amberjack (*Seriola dumerili*). ..... 42
- 3-13 Discriminant analysis comparing greater amberjack (*Seriola dumerili*) otolith shape among three regions in the Gulf of Mexico, with CF= Central Florida, NF=North Florida, and LA=Louisiana. ..... 42

## LIST OF FIGURES

<u>Figure</u>	<u>page</u>
1-1. Trends in commercial catch of greater amberjack, <i>Seriola dumerili</i> over time in round (whole) weight by state in the Gulf of Mexico (data from SEDAR 2011).....	22
1-2. Commercial and recreational landings by weight for Gulf of Mexico and Atlantic greater amberjack, <i>Seriola dumerili</i> , fisheries (data from SEDAR 2008, 2011), with inclusion of Gulf of Mexico management regimes. ....	22
2-1. Gulf of Mexico regions compared in this study, as delineated by the dotted lines.....	32
2-2. Whole left sagitta otolith of a greater amberjack, <i>Seriola dumerili</i> .....	32
2-3. The maximum height (MH) and maximum length (ML) of the greatest enclosing rectangle, excluding the rostrum, were measured in each greater amberjack ( <i>Seriola dumerili</i> ) otolith; area and perimeter of the otolith posterior to the rostrum were also calculated. .....	33
2-4. Maximum ventral length (MVL) of greater amberjack ( <i>Seriola dumerili</i> ) otoliths across ages and forklengths. .....	33
3-1. Forklength (mm) of greater amberjack ( <i>Seriola dumerili</i> ) samples organized by collection region.....	43
3-2. Age in years of Gulf of Mexico greater amberjack ( <i>Seriola dumerili</i> ) samples organized by collection region. ....	44
3-3. Power analysis on elliptical Fourier descriptors showed that 13 harmonics described 99% of the shape of greater amberjack ( <i>Seriola dumerili</i> ) otoliths....	44
3-4. Values of shape indices of greater amberjack ( <i>Seriola dumerili</i> ) otoliths plotted across forklength show no relationship between size of fish and shape index value. .....	45
3-5. Two-dimensional Principal Component Analysis projection comparing otolith shape between males from the Gulf and Atlantic stocks of greater amberjack ( <i>Seriola dumerili</i> ), with maximum convex polygons enclosing the regions. ....	46
3-6. Two-dimensional Principal Component Analysis projection comparing otolith shape of greater amberjack ( <i>Seriola dumerili</i> ) among regions in the Gulf of Mexico, with maximum convex polygons enclosing the regions. ....	47

Abstract of Thesis Presented to the Graduate School  
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Otolith shape analysis was used to examine the current management hypothesis that greater amberjack (*Seriola dumerilii*) in the Gulf of Mexico belong to a single stock.

Shape of the sagittae was quantified using a combination of the shape indices rectangularity, ellipticity, roundness, and form factor, along with elliptical Fourier analysis for 379 otoliths collected from Louisiana, North Florida, and Central Florida. A smaller quantity (n=69) of Atlantic stock otoliths from fish collected north of the Florida/Georgia border was included to test the validity of the technique for distinguishing Atlantic stock fish from Gulf of Mexico greater amberjack.

No significant differences were detected between left and right otoliths or between male and female otoliths from greater amberjack in the Gulf of Mexico or the Atlantic. Principal Component Analysis showed evidence of grouping in otolith shape between the Gulf and Atlantic stocks, although there was overlap in shape between the two regions; the Gulf showed less clear signs of regional grouping, with no differences in shape visible between North and Central Florida. Discriminant analysis had a 70% classification success rate between otoliths from the Gulf of Mexico and Atlantic stock. In addition, the shape indices form factor and circularity were found to differ significantly

between the stocks. Similarly, a 75% classification rate was attained for fish collected in Louisiana, while only a 25% and 40% classification success rate was attained for otoliths from North and Central Florida respectively. No significant differences were seen in shape indices amongst the three regions; however, when a subset of age-3 fish was tested, significant differences in the shape index rectangularity were present between the Louisiana and Florida samples. This suggests that some differentiation between Florida and Louisiana is present, but overall the analysis supports the current one-stock management of greater amberjack within the Gulf of Mexico.

## CHAPTER 1

### INTRODUCTION

#### **Fisheries Background**

The demand for marine fish is growing, with the annual global catch of fish more than tripling in the past 50 years (Pauly et al. 2002; Walters and Ahrens 2009). The consequence of this global rise in demand and consumption is that many of the world's fisheries are either fully or over-exploited (Pauly et al. 2002; Hilborn et al. 2003; Worm et al. 2006) and an estimated 14-29% of fish stocks had collapsed by the early 2000s (Mullon et al. 2005; Worm et al. 2006; Worm et al. 2009). Management rebuilding efforts have yet to reverse the overall trend of depletion in many stocks, and fisheries management has often been unsuccessful (Cunningham and Bostock 2005; Beddington et al. 2007; Worm et al. 2009). This results from challenges faced by management, including the difficulties associated with balancing a variety of stakeholder interests (Lackey 2005; Beddington et al. 2007; Hilborn 2007) with the biological limitations intrinsic to a species. Notably, insufficient biological knowledge (e.g., fecundity, sex ratio, age at first reproduction, and frequency of reproduction) has made it difficult for management to determine appropriate regulations for some species (Beddington et al. 2007, Smith 2011). Management of a fishery requires a good scientific understanding of the biology and behavior of a species (Beddington et al. 2007); therefore it is important to future management to improve our scientific knowledge. One area fundamental to proper management of fisheries is the appropriate delineation of fish stocks (MacLean and Evans 1981; Begg and Waldman 1999).

## **Stock Identification**

### **Introduction to the Stock Concept**

The concept of stock, which originated in the field of animal husbandry, was first solidified as a basis for fisheries management at the end of the 19<sup>th</sup> century, when A.C. Anderson and later John Peace Babcock noted that the salmon stocks in British Columbia rivers were discrete and varied in abundance from river to river (McDonald 1981). Anderson and Babcock recognized the relationship between abundance of spawners and the number of recruits produced in each stock, and concluded that catch should be regulated differently among rivers (McDonald 1981; Ebbin 1996). In this way, the stock concept became established very early as a basis for managing salmon fisheries (McDonald 1981).

Over time, numerous definitions have arisen to capture the essence of the stock concept, varying from delineations based on harvest to genetically-based distinctions (Ihsen et al. 1981; MacLean and Evans 1981; Carvalho and Hauser 1994; Ebbin 1996; Coyle 1997; Booke 1999). Essentially, the term ‘stock’ refers to units of fish that have temporal, spatial, genetic, and/or morphological differences separating them from each other (Ebbin 1996). Stocks are typically reproductively isolated, often with differing patterns of growth and recruitment, and respond independently to exploitation (McDonald 1981; Carvalho and Hauser 1994; Coyle 1997). This can lead to differential status of stocks within a species, with some stocks sustainably fished and others considered overfished or even collapsed. For example, the Atlantic stock of greater amberjack, *Seriola dumerili*, has recovered from overfishing and is currently considered sustainable, while the Gulf of Mexico stock has been classified as overfished and undergoing overfishing despite an ongoing rebuilding effort (SEDAR 2008, 2011).

Numerous techniques have been employed to identify fish stocks, ranging from simple qualitative observations (such as the differential timing of spawning runs) to highly technical quantitative techniques (Ihsen et al. 1981; MacLean and Evans 1981; Ebbin 1996; Coyle 1997; Begg and Waldman 1999). In a review of methodologies, Begg and Waldman (1999) list catch data, tag recoveries, meristics, morphometrics, scale morphology, parasites, cytogenetics, protein electrophoresis, immunogenetics, mitochondrial (mtDNA) and nuclear DNA, heart tissue fatty acids, otolith elemental composition, osteological interdigitation features, stable isotope measurements, and thermal marking as some of the techniques employed to define stock structure in fisheries species. An additional tool recently found useful in stock delineation is otolith shape analysis (Campana and Casselman 1993).

### **Otolith Shape Analysis**

Otoliths, or “earstones”, are calcium carbonate structures found in the otic capsules of bony fishes that are involved in hearing and balance. Most fish have three sets of paired otoliths: the sagittae, lapilli and asterisci. Otoliths are thought to grow continuously throughout the life of a fish, in proportion to the fish’s somatic growth, and are likely not subject to resorption (Ricker 1975; Campana and Thorrold 2001).

Otolith shape is typically species-specific and is influenced by both genetic and environmental parameters (Campana and Casselman 1993; Cardinale et al. 2004; Vignon and Morat 2010). Because of these influences, otolith shape has been observed to differ between stocks of the same species, including king mackerel, *Scomberomorus cavalla* (DeVries et al. 2002; Shepard et al. 2010), Pacific sardine, *Sardinops sagax* (Felix-Uraga et al. 2005), haddock, *Melanogrammus aeglefinus* (Begg et al. 2001), sole, *Solea solea* (Merigot et al. 2007), Atlantic saury, *Scomberesox saurus saurus* (Aguera

and Brophy 2011), common coral trout, *Plectropomus leopardus* (Bergenius et al. 2006), yellowstripe goatfish, *Mullioidichthys flavolineatus* (Pothin et al. 2006), monkfish, *Lophius piscatorius* (Canas et al. 2012), mulloway, *Argyrosomus japonicus* (Ferguson et al. 2011), and Atlantic cod, *Gadus morhua* (Campana and Casselman 1993; Jonsdottir et al. 2006; Petursdottir et al. 2006). Otolith shape is less variable than fish growth, and the otolith, typical of sensory structures, remains unaffected by short-term changes in fish condition such as starvation that might confound body morphometrics (Campana and Casselman 1993; Pankhurst and Montgomery 1994). While studies of otolith shape cannot distinguish between environmental and genetic influences, the contributing differences in these factors are likely to influence otolith shape among populations that remain at least partially segregated (Campana and Casselman 1993).

### **Greater Amberjack**

#### **Background**

Greater amberjack are widely distributed in marine tropical and subtropical waters. Individuals can attain lengths greater than 1500 mm forklength, making them the largest members of the jack family (Carangidae). In the Western Atlantic the species ranges along the coast from Massachusetts to southeastern Brazil, including the Gulf of Mexico (Robins et al. 1986; McEachern and Fechhelm 2005). Greater amberjack (GAJ) are predatory, consuming invertebrates (including shrimp, crabs, and squid) as well as ray-finned fishes of various species including members of the Clupeidae, Bothidae, and Sparidae (Burch 1979; Manooch and Haimovici 1983).

In the Gulf of Mexico, GAJ are currently managed as two distinct stocks, with one stock along the Southeast Atlantic coast, including the Florida Keys, and the other residing in the Gulf of Mexico. Variation in mtDNA and microsatellite DNA is consistent

with the two-stock hypothesis (Gold and Richardson 1998; Murie et al. 2011). Tagging studies also support the current two-stock management, with McClellan and Cummings (1997) reporting an exchange rate from the Atlantic to the Gulf of Mexico of only 1.3%, with a 1.6% exchange rate from the Gulf of Mexico to the Atlantic.

In the Gulf of Mexico, GAJ are assumed to spawn offshore, and pelagic *Sargassum* mats have been observed to provide nursery habitat for young of year GAJ (Wells and Rooker 2004). As adults, GAJ are typically found schooling around reefs or other structure (such as wrecks and oil rigs) in 10-100 m of water. Because of this, GAJ are considered part of the Reef Fish Management Unit, which includes species such as red snapper *Lutjanus campechanus* and gag grouper *Mycteroperca microlepis*.

Currently, little is known about GAJ movement in the Gulf, although it has been noted that they are often only seasonally abundant in certain parts of their range and may associate with a variety of different habitats or areas each year (SEDAR 2011). From a fisheries perspective, these seasonal movements likely affect local abundance and access to the resource, especially by recreational fishers due to added costs of fuel and distance.

### **GAJ Fishery**

Greater amberjack are fished both commercially and recreationally; they are a popular sport fish (likely due to their aggressive fighting behavior) and important recreational fisheries for this species have existed since the 1950s (Cummings and McClellan 2000). However, it wasn't until the 1980s that the species became commercially popular, possibly spurred by the decline of other commercial species such as red snapper and red drum *Sciaenops ocellatus* (Cummings and McClellan 2000).

Gulf of Mexico greater amberjack are currently assessed as both overfished and undergoing overfishing (SEDAR 2011).

The commercial fishery for greater amberjack utilizes vertical handlines (bandit gear or electric reels), with a small proportion of catch coming from long-lining and spearfishing (SEDAR 2006). Commercial landings for this species in the Gulf are highest in Florida, followed by Louisiana and Texas (SEDAR 2006) (Figure 1-1). Commercial fishery data for GAJ in the Gulf begins in the 1960s for Florida and in the 1980s for all other states (SEDAR 2011). Catch of GAJ in the Gulf exceeds that of the Atlantic (Figure 1-2). Commercial GAJ landings in the Gulf grew from between five to eight thousand pounds (whole weight) in the early 1960s to a peak of 2,055,639 pounds in 1988 (SEDAR 2011). After the 1988 peak, landings fell, stabilizing around 1.2 million pounds between 1994 and 1997 (SEDAR 2011). The decline in landings after 1991 corresponds to the implementation of management regulations (Cummings and McClellan 2000, Fig. 1-2). Following 1997, landings declined again and have remained below one million pounds since, reaching a low of 504,114 pounds in 2008 (SEDAR 2011).

Recreational angling methods for GAJ include hook-and-line and spearfishing. The recreational fishery for GAJ (and other reef species) has high economic value in the Gulf of Mexico. For example, in 2008 charter boats in the reef fish fishery earned approximately \$88,000/vessel and headboats approximately \$461,000/vessel (Rauch 2011); while there are no data on how many of these vessels target GAJ specifically, overall the largest proportion of recreational GAJ catch comes equally from charterboats and private anglers (SEDAR 2011).

## **GAJ Stock Assessment**

The underlying principle in modern fisheries management is that every fish population produces a surplus, and that the largest surplus that can be harvested annually from that population (known as Maximum Sustainable Yield, or MSY) can be estimated through scientific analysis (i.e. stock assessments) (Ricker 1975; Lackey 2005). With this in mind, managers can set target “reference points”, such as fishing pressure and population biomass ( $F_{msy}$  and  $B_{msy}$  respectively), which will maintain MSY (Beddington et al. 2007; Gabriel and Mace 1999). When fishing pressure ( $F$ ) exceeds  $F_{msy}$  ( $F_{current}/F_{msy} > 1$ ) the stock is considered to be undergoing overfishing, and is assessed as overfished when the biomass ( $B$ ) is less than  $B_{msy}$  ( $B_{current}/B_{msy} < 1$ ).

In the 1990s, the Gulf of Mexico Fishery Management Council received anecdotal information from eastern Gulf of Mexico fishermen that the GAJ stock was in decline, notable due to a decrease average size and abundance. In response, NOAA’s Southeast Fisheries Science Center (SEFSC) conducted a stock assessment in 1996 (Hood 2006). Though this assessment was deemed too imprecise to specify allowable catch limits, there was concern that the stock was in decline and so more stringent management regulations were implemented (Hood 2006). In 2000, the stock was reassessed, and the resulting stock assessment models showed an overfished condition (as of 1998) and suggested that overfishing had also occurred (Hood 2006). In 2001, NOAA’s National Marine Fisheries Service (NMFS) officially notified the Gulf of Mexico Fishery Management Council that the stock had been overfished and, in response, the Council developed a 7-year rebuilding plan beginning in 2003 (SEDAR 2011). In 2006, a stock assessment was conducted through the Southeast Data Assessment and Review (SEDAR) process which found the stock to continue to be both

overfished and undergoing overfishing as of 2004, estimating  $F_{2004}/F_{\text{msy}}$  at 1.017 and  $B_{2004}/B_{\text{msy}}$  at 0.706 (SEDAR 2006).

The most recent assessment update (SEDAR 2011) estimated  $F_{2009}/F_{\text{msy}}$  at 1.83, with  $B_{2009}/B_{\text{msy}}$  at 0.31, indicating continued overexploitation. In addition, backwards projections using the updated models estimated conditions to have been worse in 2004 than estimated by the assessment, with  $F_{2004}/F_{\text{msy}}$  at 2.4 and  $B_{2004}/B_{\text{msy}}$  at 0.38. The models estimated that the stock has been overfished continuously, reaching a low in 1997 with a  $B_{1997}/B_{\text{msy}}$  of 0.28. In addition, it was estimated that overfishing has occurred since 1986, with  $F_{1986}/F_{\text{msy}}$  of 1.77, with the exception of 1990 ( $F_{1990}/F_{\text{msy}} = 0.89$ ). Furthermore, models based on current rates of exploitation predict that the stock will not recover, nor will overfishing end, within the time frame of the rebuilding plan (SEDAR 2011).

### **GAJ Stock Structure**

GAJ within the Gulf of Mexico are currently managed as one continuous stock, with presumed mixing across the entire region. Genetic analysis has yielded evidence of continuous gene flow in the northern Gulf, with no signs of regional differentiation (Gold and Richardson 1998; Murie et al. 2011). However, tagging studies in the Gulf have found that many individual GAJ exhibit little net movement between release and recapture, suggesting that mixing across the region may not be continuous. For example, amberjack tagged off of a Louisiana oil platform for age validation in an age and growth study were observed to remain on site for at least 9 months after tagging (Beasley 1993). On a larger scale, McClellan and Cummings (1997) found 30% of fish tagged and recaptured in the Gulf showed zero net movement, with 58% of recaptures made within 25 nautical miles of the release site. Of the fish that moved, 90.6% were

recaptured within 100 nautical miles of the release site. Recapture times ranged from 1-6 years (1.2 on average), with 98% recaptured within 2 years. McClellan and Cummings (1997) also saw a negative relationship between movement and time-at-large, with the highest movement exhibited by fish at large for a short period of time. In addition, Ingram and Patterson (2001) found that 97% of GAJ tagged off of Pensacola were recaptured within the original release area (though no information was given on time at large), and found that fish tagged off of Panama City traveled an average distance of 10.8 km (with a mean time at large of 200 days). Furthermore, a study currently underway by Murie et al. (2011) has found an average distance traveled of 69.54 km for tagged GAJ, with a median distance of 8.0 km, and no relationship between distance traveled, time at large, or size of fish.

Tagging studies have found that some individual GAJ do undergo large-scale movements in the Gulf. For example, Murie et al. (2011) found one fish tagged in Apalachicola, FL was recaptured 11 months later in Tampico, Mexico, with another tagged near Madeira Beach, FL recaptured 10 months later near Port Maria, Jamaica. In addition, Ingram and Patterson (2001) saw one fish tagged off of Panama City Beach, FL recaptured 396 days later off of Port Fourchain, LA. This suggests at least some degree of mixing in the region, which may make it difficult to elucidate regional structure using genetic data. Studies have shown that only a few migrants per generation are sufficient to prevent development of genetic differentiation (Allendorf 1983; Carvalho and Hauser 1994; Coyle 1997). Therefore, a failure to detect genetic differentiation does not mean that functionally no separation exists (Coyle 1997).

## **Study Objectives**

GAJ in the Gulf are both overfished and undergoing overfishing, and have failed to recover despite a rebuilding plan and increasingly stringent management regulations. A failure to accurately grasp the stock structure of GAJ may contribute to the ongoing decline in this species in the Gulf. If the Gulf of Mexico stock is not completely mixed, the disproportionate fishing effort could lead to localized overfishing of the species, particularly off the coast of Florida.

In delineating stock structure, it is important to use a holistic approach that incorporates results from a variety of methodologies (Begg and Waldman 1999). The present study examines the utility of otolith shape analysis in regional comparisons of GAJ in the Gulf of Mexico, and between the Gulf and the Atlantic stocks of greater amberjack. In conjunction with tagging and genetic data, this stock identification method may help form a holistic picture of the stock structure of GAJ in the southeastern United States. This should aid in future management of the species in this region and assist in rebuilding efforts for the Gulf of Mexico stock by allowing managers to accurately understand the structure of the stock.

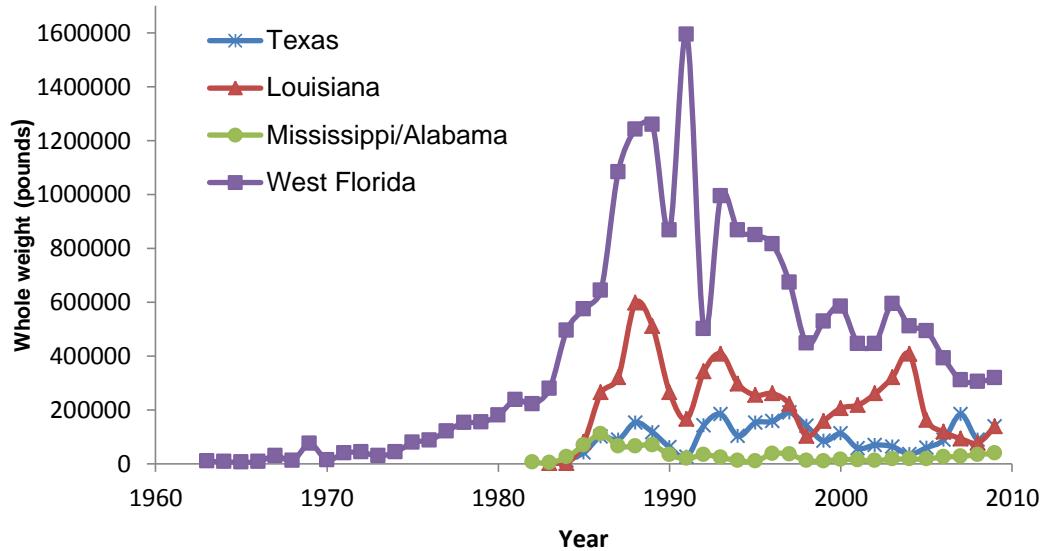


Figure 1-1. Trends in commercial catch of greater amberjack, *Seriola dumerili* over time in round (whole) weight by state in the Gulf of Mexico (data from SEDAR 2011).

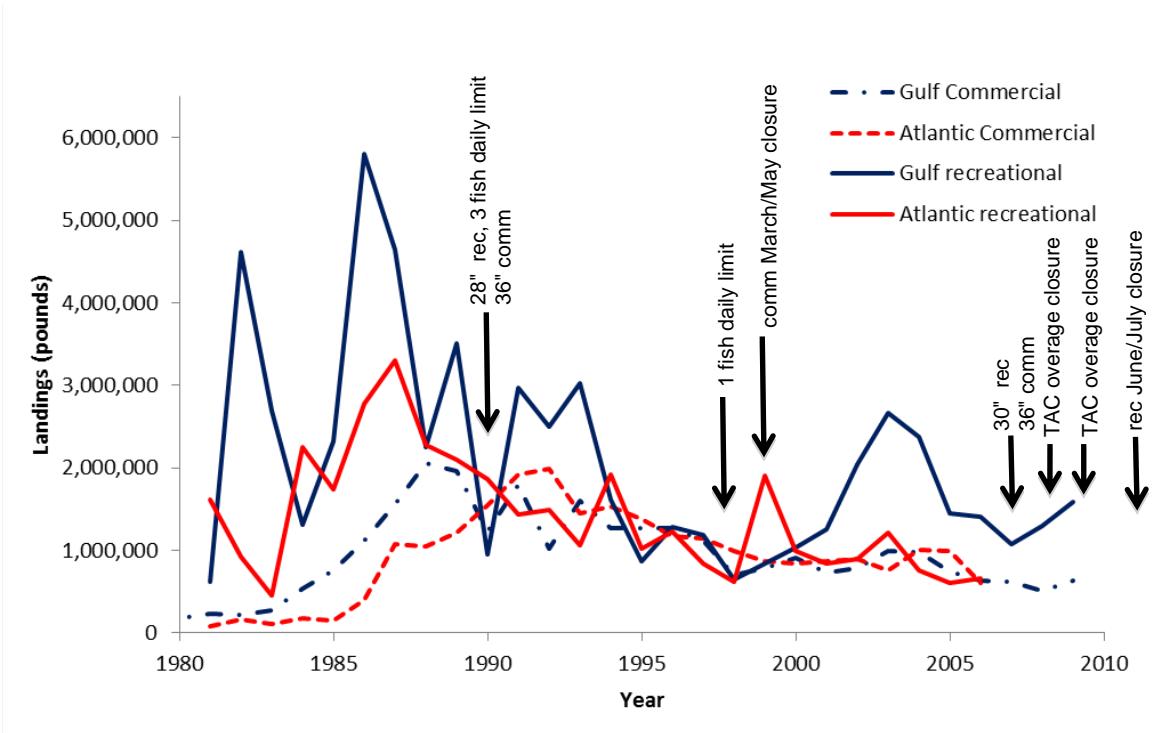


Figure 1-2. Commercial and recreational landings by weight for Gulf of Mexico and Atlantic greater amberjack, *Seriola dumerili*, fisheries (data from SEDAR 2008, 2011), with inclusion of Gulf of Mexico management regimes.

## CHAPTER 2 METHODS

### **Sample Collection**

The present study used otoliths collected from port sampling and scientific sampling by the University of Florida, NOAA Fisheries, and regional state agencies including the Gulf States Marine Fisheries Council (GSMFC). Otoliths utilized were limited to those collected between 2002 and 2008. Samples were restricted to fish between 700-1050 mm forklength (FL) to limit possible size-based variation and to standardize fish samples across regions. Sample regions included central Florida (Madeira Beach to Sarasota), North Florida (Apalachicola to Suwannee), and central and western Louisiana (Figure 2-1). In addition, as otolith shape comparisons have never been conducted for GAJ, otoliths from the Atlantic stock were included for a separate shape comparison between stocks. Atlantic stock otoliths were provided by NOAA Fisheries, and were restricted to otoliths collected north of the Florida/Georgia border to limit the chance that otoliths came from Gulf migrants. The study focused on saggittae, which have been seen to be the most informative for morphological studies (Campana and Casselman 1993) and have been utilized in other studies of this nature (Begg et al. 2001; DeVries et al. 2002; Felix-Uraga et al. 2005; Bergenius et al. 2006; Jonsdottir et al. 2006; Petursdottir et al. 2006; Pothin et al. 2006; Merigot et al. 2007; Shepard et al. 2010; Aguera and Brophy 2011; Ferguson et al. 2011; Canas et al. 2012).

### **Otolith Morphology**

### **Rostrum Exclusion**

Greater amberjack otoliths are fragile and easily broken during removal, in addition to being small in size relative to the body size of the fish (Figure 2-2). As a result of this, a

large proportion of otoliths available to be used in the present study were broken or had chipped rostra. Otolith shape was therefore standardized to exclude the rostral portion. Following DeVries et al. (2002), a straight line was drawn from the dorsal surface of the anterostrum to the ventral edge of the otolith (Figure 2-3), and the rostral portion anterior to this line was excluded from further analysis. Without this methodology, sample size would have been greatly reduced.

### **Shape Quantification**

Although there are many ways to quantify otolith shape, a combination of shape indices developed by Tuset et al. (2003) and elliptical Fourier analysis was chosen for this study, as recommended by Tracey et al. (2006). Otolith images were captured and digitized using Motic Images (v 3.0) software (Motic Group North America, Vancouver, Canada) on a Leica MZ50 dissecting scope using a Panasonic WV-CP224 CCD Microscope Camera. Linear measurements were taken using the Motic software and included maximum height (MH) and maximum length (ML) of the greatest enclosing rectangle of the posterior portion of the otolith (Figure 2-3), as well as area and perimeter of the posterior portion of the otolith; these measures allowed for the calculation of five shape indices.

### **Shape indices**

Shape indices were calculated following Tuset et al. (2003). Shape indices serve to quantify general shape characteristics and have been useful in other studies of otolith shape (Ferguson et al. 2011; Jonsdottir et al. 2006; Pothin et al. 2006; Merigot et al. 2007; Shepard et al. 2010). The shape indices used in the present study include form factor, roundness, circularity, rectangularity and ellipticity (Table 2-1). The index form factor estimates edge irregularity, with a value of 1 representing a perfectly smooth

edge and values < 1 when the edge is irregular. Roundness and circularity describe the similarity of certain features to a perfect circle, with respective minimum values of 1 and  $4\pi$ . Rectangularity quantifies the variations in length and width with respect to area, with a value of 1 representing a perfect square. Finally, ellipticity examines whether the changes in the axes lengths are proportional.

### Fourier analysis

Among the many classes of Fourier analysis, Elliptical Fourier Analysis (EFA) is considered the most powerful for otolith shape analysis (Tracey et al. 2006; Merigot et al. 2007). EFA describes the outline of a shape using a number of representative variables, termed harmonics. These harmonics are each characterized by four coefficients, or elliptical Fourier descriptors,  $a$ ,  $b$ ,  $c$ , and  $d$ , which correspond to the projection of the outline on the  $x$  ( $a$  and  $b$ ) and  $y$  ( $c$  and  $d$ ) axes (Merigot et al. 2007; Canas et al. 2012). Elliptical Fourier analysis was conducted using the Shape program (Iwata and Ukai 2002). Shape inputs the digitized image and calculates the Fourier coefficients, then normalizes them based on Kuhl and Giardina (1982) to correct for differences in size and orientation.

A greater number of harmonics increases the accuracy of shape outline; however, too large a number can overcomplicate analyses. Fourier power analysis was therefore calculated to determine the appropriate number of harmonics using Equation 2-1

$$PF_n = 0.5(a_n^2 + b_n^2 + c_n^2 + d_n^2) \quad (2-1)$$

where  $PF_n$  = power of the Fourier harmonic, with  $a_n$ ,  $b_n$ ,  $c_n$  and  $d_n$  referring to the  $a$ ,  $b$ ,  $c$  and  $d$  coefficients of the  $n^{th}$  harmonic (Pothin et al. 2006; Merigot et al. 2007). The

cumulative power percentage was then calculated using the sum of the previous  $PF_n$ 's. The goal was to reach a threshold cumulative power percentage of 99%; after this, little information would be added by additional harmonics (Pothin et al. 2006; Merigot et al. 2007). Power analysis was run on a randomly selected subsample of 30 otoliths, to determine a cumulative power of 99%.

### Data Analysis

Statistical analyses were run using the SAS® and JMP® software (SAS Institute Inc. 2008). All statistical tests were conducted at the  $\alpha = 0.05$  criterion level unless otherwise stated. Prior to analyses, the shape indices and Fourier coefficients were examined for agreement with statistical assumptions of normality and homoscedasticity using the Kolmogorov-Smirnov test and Levene's test (Zar 1999), respectively. Initial data exploration indicated a number of outliers, which caused the data to vary significantly from normal. Each outlier was checked against its sample image, and it was determined that the outliers corresponded to deformed otoliths (i.e. otoliths with jagged, irregular posterior portions). A total of 18 outliers were therefore removed from the dataset for subsequent analyses. Following outlier removal, parameters still found to vary from normal included the shape index circularity and the harmonics A5, A6, A8, B2, and D8. Circularity was normalized using the square-root transformation, and the harmonics were normalized using the  $\log_e$  transformation (Zar 1999). Following transformation, all parameters conformed to assumptions of normality and homogeneity of variance.

Otoliths grow over the life of a fish, and it is possible that shape varies with fish size. Therefore, Analysis of Covariance (ANCOVA) was used to examine the effect of fish

size (forklength) on each shape index, with sampling region included as a factor and fish length the covariate (Bergenius et al. 2006; Jonsdottir et al. 2006; Petursdottir et al. 2006). Fish length was chosen over age as a covariate because initial data exploration determined that otolith growth has a stronger relationship with fish length than fish age (Figure 2-4). An Analysis of covariance (ANCOVA) was used to examine if the shape indices varied with length

Many of the shape indices were constructed using different combinations of the same parameters, and correlation was suspected amongst the shape indices; therefore Pearson's product-moment correlation coefficients were calculated as a precursor to analysis. All indices were retained for further analyses, but correlations were taken into account when choosing analysis methodology.

### **Comparison of Left and Right Otoliths**

Often, only a single left or right otolith was available in the otolith collection. Consequently, a paired t-test was used to compare left and right otoliths when available from the same individual to look for differences that might bias analyses. Most species previously examined including Atlantic cod, *Gadus morhua*, haddock, *Melanogrammus aeglefinus*, saithe, *Pollachius virens*, golden redfish, *Sebastes marinus*, Atlantic herring, *Clupea harengus*, and Atlantic mackerel, *Scomber scombrus*, appeared to show no significant statistical differences between left and right otoliths (Hunt 1992; Petursdottir et al. 2006). In contrast, common sole, *Solea solea*, (Merigot et al. 2007) have left and right otoliths that differ significantly within an individual, likely a consequence of their side-oriented benthic existence. A paired t-test was used to compare shape indices between left and right otoliths of 25 male GAJ individuals from the same region (Louisiana) to explore this possible source for error in shape analysis.

### **Comparison of Male and Female Otoliths**

Sex-based differences in growth rates between male and female GAJ have been documented in the Atlantic (Harris et al. 2007); differences in growth rates of GAJ in the Gulf of Mexico are less pronounced (Murie and Parkyn 2008) but may still exist. Studies have found correlations between differences in growth rate and differences in otolith shape, and sexual dimorphism in otolith shape has been observed in other species (e.g., cod and haddock) (Campana and Casselman 1993; Begg et al. 2001). Similarly it is possible that male and female GAJ otoliths exhibit morphological differences. Shape indices of male and female GAJ sagittae collected from the same region (Central Florida) were compared using analysis of variance (ANOVA), with a Bonferroni correction for repeated testing, to look for sex-specific differences in GAJ otolith shape. Individual ANOVAs were chosen over multiple analysis of variance (MANOVA) as MANOVA is known to work best with moderately correlated data (Salkind 2010), and most of the indices were found to have low or high correlations. The Bonferroni adjustment gave a significance criterion of  $\alpha = 0.01$ .

### **Regional Comparisons**

Comparisons of otolith shape, as quantified by shape indices and elliptical Fourier analysis, between the Gulf and Atlantic stocks and among three regions in the Gulf of Mexico (Central Florida, Northern Florida and Louisiana) were first explored descriptively using Principal Component Analysis (PCA) (PC-ORD v.6.0). PCA does not require *a priori* assumptions about group membership, and presents an unbiased indication of separation between regions (Begg et al. 2001; Merigot et al. 2007). The

cross-products matrix for the PCA was calculated using the variance/covariance method.

Next, shape indices were compared between the Gulf and Atlantic stocks of GAJ and among the three Gulf regions using multiple individual analyses of variance (ANOVAs) to test for specific shape differences. ANOVA was corrected for multiple testing using Bonferroni's adjustment, giving a significance criterion of  $\alpha = 0.01$ . Though samples were restricted to a size range, they still contained fish of variable ages. Therefore, a complimentary analysis was run on a subset of the data. Shape indices of age 3 fish were compared among the three Gulf of Mexico regions using ANOVA, again corrected using Bonferroni's adjustment for a significance criterion of  $\alpha = 0.01$ . Analysis on the data subset was restricted to ANOVA and excluded from the other multivariate analyses due to the small sample size of age 3 fish.

Linear Discriminant Analysis (DA) was then used as an *a posteriori* test to examine if otolith shape could distinguish among regions. Discriminant analysis investigates the integrity of pre-defined groups (Pothin et al. 2006; Merigot et al. 2007), and has been employed in several recent studies of otolith morphology (Begg et al. 2001; DeVries et al. 2002; Felix-Uraga et al. 2005; Merigot et al. 2007; Petursdottir et al. 2006; Pothin et al. 2006). Like PCA, discriminant analysis allows for the comparison of multiple parameters across groups simultaneously; however, DA requires the sample size of the smallest group to exceed the number of predictor variables by a value of at least  $n - 2$  (with  $n$  = the sample size of the smallest group), though a greater discrepancy is thought to be preferable (Meyers et al. 2006). Shape indices and Fourier analysis combined give a total of 53 parameters to describe GAJ otolith shape. This is

sufficiently smaller than the sample size for each of the Gulf regions; however, it is too large to allow appropriate testing of the Atlantic stock samples. Therefore, DA was run on shape indices alone when comparing the Gulf and Atlantic stock otoliths, but all parameters (shape indices and Fourier descriptors combined) were included for comparison among the three Gulf regions.

Prior to DA, samples were randomly split into a model data set and a test data set. Discriminant analysis was then run on the model data sets, and the resulting discriminant functions were used to test the ability of otolith shape to predict a sample's region of origin. Performance of the DA was evaluated using the Cohen's  $\kappa$  statistic (Fleiss 1981), which compares the discriminatory power of the analysis to what might be expected by random chance alone.

Table 2-1. Shape indices of greater amberjack (*Seriola dumerilii*) sagittae calculated following Tuset et al. (2003), with ML corresponding to maximum length and MH to maximum height of the greatest enclosing rectangle.

Shape index	Equation
Form factor	$4 * \pi * \text{Area}/\text{Perimeter}^2$
Roundness	$4 * \text{Area}/\pi * \text{ML}^2$
Circularity	$\text{Perimeter}^2/\text{Area}$
Rectangularity	$\text{Area}/(\text{ML} * \text{MH})$
Ellipticity	$(\text{ML} - \text{MH})/(\text{ML} + \text{MH})$

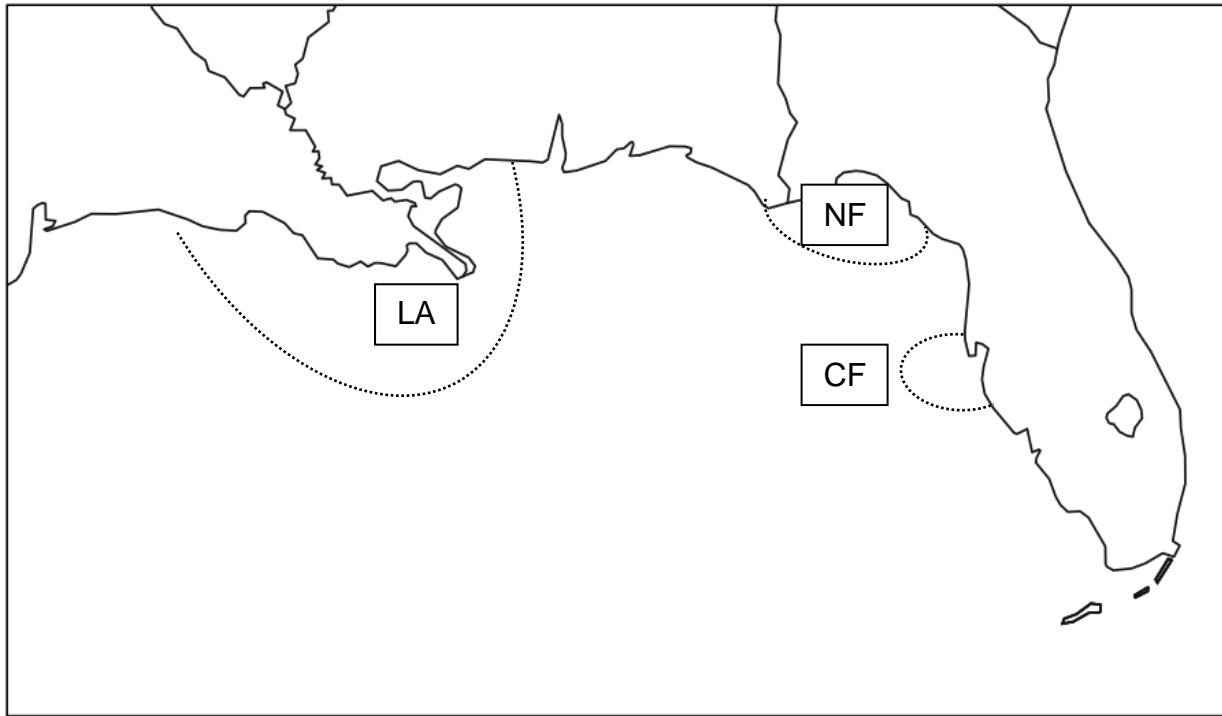


Figure 2-1. Gulf of Mexico regions compared in this study, as delineated by the dotted lines. Region abbreviations correspond to CF: Central Florida (Madeira Beach to Sarasota), NF: North Florida (Apalachicola to Cedar Key, FL) and LA: Louisiana.

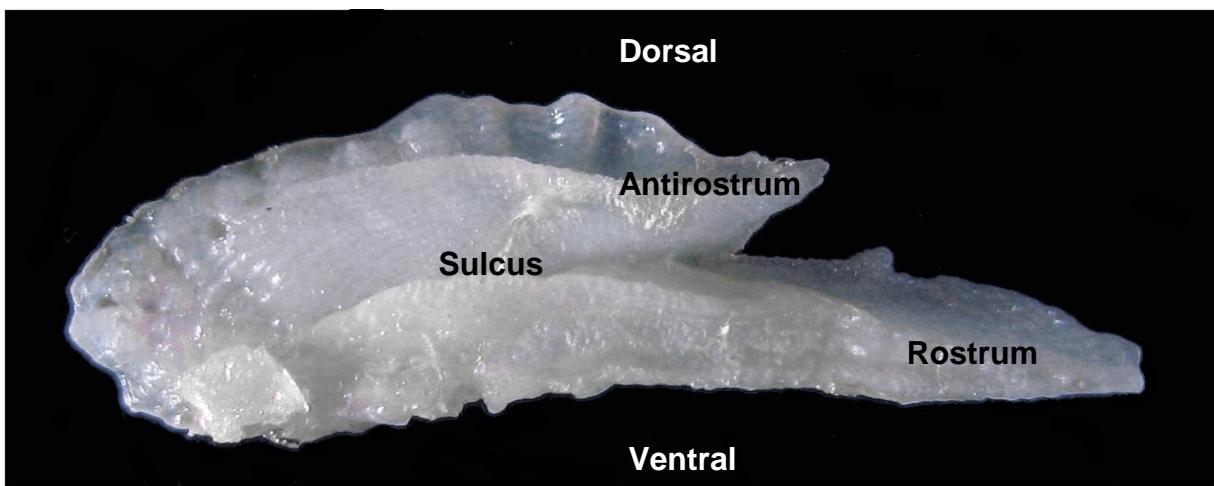


Figure 2-2. Whole left sagitta otolith of a greater amberjack, *Seriola dumerili*.

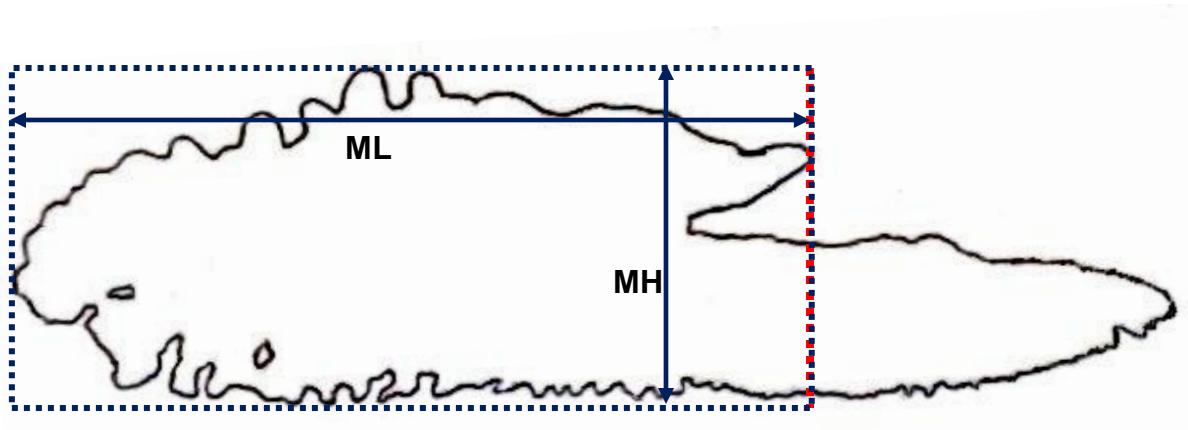


Figure 2-3. The maximum height (MH) and maximum length (ML) of the greatest enclosing rectangle, excluding the rostrum, were measured in each greater amberjack (*Seriola dumerilli*) otolith; area and perimeter of the otolith posterior to the rostrum were also calculated.

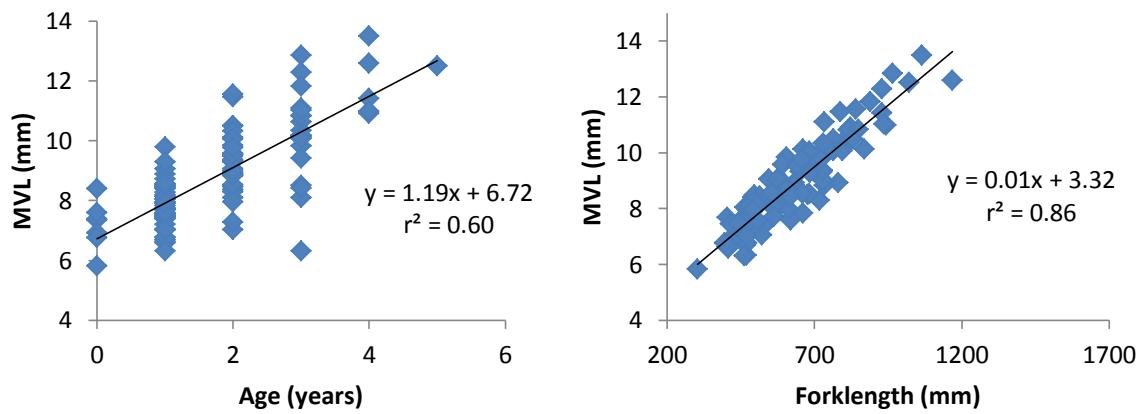


Figure 2-4. Maximum ventral length (MVL) of greater amberjack (*Seriola dumerili*) otoliths across ages and forklengths.

## CHAPTER 3 RESULTS

### **Sample Collection**

In total, 455 samples were analyzed using shape analysis (Table 3-1). All Gulf regions had greater than 100 samples; the Atlantic stock had fewer samples, with a total of 69 analyzed for the region. Final size and age frequencies across regions are depicted in Figures 3-1 and 3-2; most fish were around 3 years of age.

### **Data Analysis**

Power analysis determined that the *a priori* criterion level of 99% was achieved within 13 harmonic calculations (Figure 3-3), indicating that GAJ otolith shape could be summarized by 13 harmonics (or 52 Fourier coefficients). However, the first harmonic was excluded from the analyses because the outline reconstructed by the first coefficients is a simple ellipse (Merigot et al. 2007). Therefore, a final of 12 harmonics, and thus a total of 48 Fourier coefficients, were retained for each otolith.

The ANCOVA demonstrated that none of the shape indices varied significantly with fish length (Table 3-2). In addition, shape indices as a function of fish length showed no visible trends (Figure 3-4), therefore all indices were retained for further analyses. All shape indices were correlated, with most correlations low; however, circularity and form factor were highly correlated (0.99), as were ellipticity and roundness (0.89) (Table 3-3).

### **Comparison of Left and Right Otoliths**

The paired t-test found no significant differences in shape indices between left and right otoliths (Table 3-4). Therefore, when the right otolith was absent, the mirror image of the left (a digital manipulation using the Motic imaging software) was used in the analysis.

## **Comparison of Male and Female Otoliths**

The ANOVAs showed no significant differences in any shape index between male and female otoliths in the Gulf of Mexico (Table 3-5) or Atlantic (Table 3-6) stocks of GAJ. Therefore, the sexes were pooled for subsequent analyses.

## **Regional Comparisons**

### **Data Exploration**

Principal component analysis (PCA) showed that 98% of the variance could be explained by the first two principal component axes (Tables 3-7 and 3-8), therefore the results were examined on a two-dimensional plane. Principal components analysis showed evidence of regional grouping of otolith shape between the Gulf and Atlantic otoliths (Figure 3-5), indicating differences in otolith shape between these two stocks, although there was overlap in otolith shape between the two regions. PCA projections comparing otolith shape among Gulf regions showed less clear signs of grouping (Figure 3-6), with north and central Florida displaying a high degree of overlap. Some differences in otolith shape were evident between Louisiana and the two Florida regions, although grouping was less clear than between the Atlantic and Gulf stocks.

### **Analysis of Variance**

Analysis of Variance (ANOVA) found significant differences in the shape indices form factor and circularity between the Gulf and Atlantic stocks of GAJ (p-values of 0.007 and 0.0012 respectively) (Table 3-9). No significant differences were seen among the three Gulf regions (Table 3-10). However, when limited to the age 3 fish data subset, significant differences were seen in the shape index rectangularity between the two Florida regions and the Louisiana region (Table 3-11).

## **Discriminant Analysis**

The Gulf regions model data set consisted of 270 randomly selected samples (90 per region), with a test data set of 60 randomly selected samples (20 from each region); for comparison between the Atlantic and Gulf, the model data set consisted of 98 randomly selected samples (49 from each stock), with a test data set of 54 randomly selected samples (27 per stock). Discriminant analysis (DA) between the Gulf and Atlantic stocks of GAJ conducted on shape indices alone showed a 70% classification success, suggesting that shape differences are present in otoliths from GAJ in these two regions (Table 3-12). In addition, the Cohen's  $\kappa$  value of  $\kappa = 0.40$  indicated a 40% improvement over random chance. Within the Gulf, however, otolith shape was less able to correctly predicted region, with only a 47% classification success among the three regions overall and Cohen's  $\kappa$  predicting only a 20% improvement over random chance (Table 3-13). DA had the highest success assigning otoliths from Louisiana (75% classification success) but did a poor job assigning otoliths from North Florida (40% classification success) and Central Florida (25% classification success).

Table 3-1. Otolith sample sizes of greater amberjack (*Seriola dumerili*) sagittae by region used in the present study.

Region	n
Central Florida	143
North Florida	115
Louisiana	121
Atlantic	69

Table 3-2. Analysis of Covariance (ANCOVA) of shape indices of greater amberjack (*Seriola dumerili*) sagittae, with forklength as the covariate and region as a factor.

Parameter	df	p-value	F	n
Form factor	1	0.4691	0.53	442
Roundness	1	0.7944	0.07	442
Circularity	1	0.4471	0.58	442
Rectangularity	1	0.2294	1.46	442
Ellipticity	1	0.6719	0.18	442

Table 3-3. Pearson's product-moment correlation coefficients resulting from analysis of shape indices of greater amberjack (*Seriola dumerili*) sagittae.

Index	Roundness	Circularity	Rectangularity	Ellipticity
Form factor	0.32	-0.99	0.20	-0.24
Roundness		-0.32	0.40	-0.89
Circularity			-0.21	0.24
Rectangularity				0.06

Table 3-4. Paired t-test comparing shape indices of greater amberjack (*Seriola dumerili*) sagittae between left and right otoliths from the same individual.

Index	df	t statistic	p-value	n
Form factor	24	2.064	0.617	25
Circularity	24	2.064	0.525	25
Roundness	24	2.064	0.986	25
Rectangularity	24	2.069	0.227	25
Ellipticity	24	2.055	0.393	25

Table 3-5. Comparison of male and female greater amberjack (*Seriola dumerili*) otolith shape indices in the Gulf of Mexico stock using Analysis of Variance (ANOVA).

Index	df	F	p-value	n
Form factor	1	0.0325	0.8573	80
Roundness	1	5.1945	0.0255	80
Circularity	1	0.0188	0.8914	80
Rectangularity	1	0.7295	0.3957	80
Ellipticity	1	3.9309	0.051	80

Table 3-6. Comparison of male and female greater amberjack (*Seriola dumerili*) otolith shape indices in the Atlantic stock using Analysis of Variance (ANOVA).

Index	df	F	p-value	n
Form factor	1	2.9686	0.0911	52
Roundness	1	0.1868	0.6674	52
Circularity	1	3.4152	0.0705	52
Rectangularity	1	1.4023	0.2419	52
Ellipticity	1	0.0027	0.9588	52

Table 3-7. Eigenvalues from principal component analysis (PCA) comparing otolith shape between Gulf and Atlantic stocks of greater amberjack (*Seriola dumerili*)

Number	Eigenvalue	Percent	Cumulative Percent
1	12406886.1624	92.0623	92.0623
2	744966.7353	5.5278	97.5901
3	304486.5123	2.2594	99.8495
4	6447.4660	0.0478	99.8974
5	3679.5648	0.0273	99.9247
6	3091.2776	0.0229	99.9476
7	2313.6054	0.0172	99.9648
8	983.8244	0.0073	99.9721
9	927.5728	0.0069	99.9789
10	598.0848	0.0044	99.9834
11	457.2442	0.0034	99.9868
12	342.2807	0.0025	99.9893
13	246.8228	0.0018	99.9911
14	186.0984	0.0014	99.9925
15	135.0716	0.0010	99.9935
16	123.9071	0.0009	99.9945
17	110.1558	0.0008	99.9953
18	106.3880	0.0008	99.9961
19	99.3859	0.0007	99.9968
20	62.6120	0.0005	99.9973
21	57.8768	0.0004	99.9977
22	49.2333	0.0004	99.9981
23	47.0896	0.0003	99.9984
24	37.9093	0.0003	99.9987
25	30.5891	0.0002	99.9989
26	25.0799	0.0002	99.9991
27	20.9755	0.0002	99.9993
28	19.5557	0.0001	99.9994
29	16.5765	0.0001	99.9995
30	11.9904	0.0001	99.9996

Table 3-8. Eigenvalues from principal component analysis (PCA) comparing otolith shape among central Florida, north Florida, and Louisiana samples of greater amberjack (*Seriola dumerili*)

PCA axis	Eigenvalue	Percent	Cumulative Percent
1	1467.6461	92.5836	92.5836
2	80.6105	5.0852	97.6687
3	34.4548	2.1735	99.8422
4	0.7838	0.0494	99.8917
5	0.5184	0.0327	99.9244
6	0.3714	0.0234	99.9478
7	0.1652	0.0104	99.9582
8	0.1338	0.0084	99.9667
9	0.1090	0.0069	99.9736
10	0.0881	0.0056	99.9791
11	0.0549	0.0035	99.9826
12	0.0482	0.0030	99.9856
13	0.0340	0.0021	99.9878
14	0.0265	0.0017	99.9894
15	0.0220	0.0014	99.9908
16	0.0197	0.0012	99.9921
17	0.0171	0.0011	99.9931
18	0.0154	0.0010	99.9941
19	0.0130	0.0008	99.9949
20	0.0119	0.0007	99.9957
21	0.0087	0.0005	99.9962
22	0.0085	0.0005	99.9968
23	0.0072	0.0005	99.9972
24	0.0053	0.0003	99.9976
25	0.0051	0.0003	99.9979
26	0.0047	0.0003	99.9982
27	0.0038	0.0002	99.9984
28	0.0035	0.0002	99.9986
29	0.0028	0.0002	99.9988
30	0.0027	0.0002	99.9990

Table 3-9. Comparison of Atlantic and Gulf of Mexico stock greater amberjack (*Seriola dumerili*) otolith shape indices using Analysis of Variance (ANOVA).

Index	df	F	p-value	n
Form factor	1	12.153	0.0007	98
Roundness	1	0.2831	0.5959	98
Circularity	1	11.1997	0.0012	98
Rectangularity	1	6.6579	0.0114	98
Ellipticity	1	0.4276	0.5197	98

Table 3-10. Comparison of central Florida, north Florida, and Louisiana greater amberjack (*Seriola dumerili*) otolith shape indices using Analysis of Variance (ANOVA).

Index	df	F	p-value	n
Form factor	2	2.3232	0.0999	270
Roundness	2	0.2649	0.7675	270
Circularity	2	2.4169	0.0911	270
Rectangularity	2	1.7278	0.1797	270
Ellipticity	2	0.0838	0.9196	270

Table 3-11. Comparison of central Florida, north Florida, and Louisiana age 3 greater amberjack (*Seriola dumerili*) otolith shape indices using Analysis of Variance (ANOVA).

Index	df	F	p-value	n
Form factor	2	3.7449	0.0267	114
Roundness	2	2.3634	0.0989	114
Circularity	2	3.983	0.0214	114
Rectangularity	2	7.1636	0.0012	114
Ellipticity	2	0.5467	0.5804	114

Table 3-12. Discriminant analysis comparing otolith shape indices between Gulf and Atlantic samples of greater amberjack (*Seriola dumerili*).

Region	Model n	Test n	Gulf	Atlantic	correctly classified (%)	Cohen's $\kappa$
Gulf	49	20	14	6	70	-
Atlantic	49	20	6	14	70	-
Total	98	40	20	20	70	0.400

Table 3-13. Discriminant analysis comparing greater amberjack (*Seriola dumerili*) otolith shape among three regions in the Gulf of Mexico, with CF= Central Florida, NF=North Florida, and LA=Louisiana.

Region	Model n	Test n	CF	NF	LA	correctly classified (%)	Cohen's $\kappa$
CF	90	20	5	7	8	25	-
NF	90	20	7	8	5	40	-
LA	90	20	3	2	15	75	-
Total	270	60	15	17	28	47	0.199

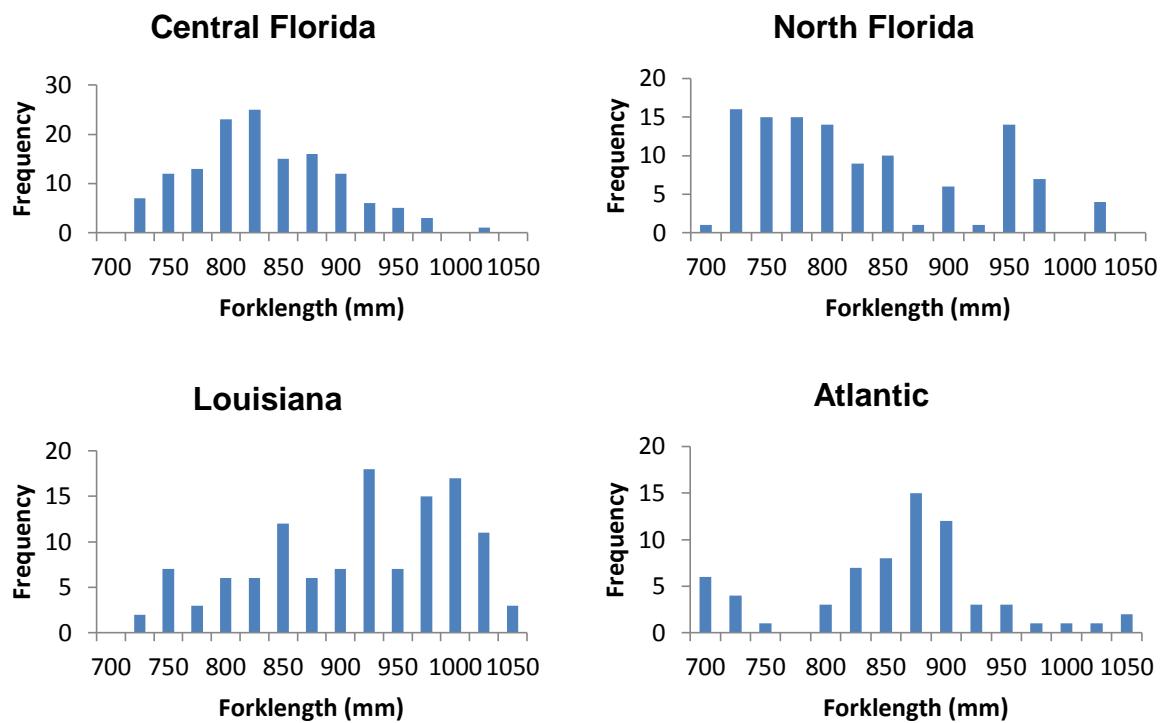


Figure 3-1. Forklength (mm) of greater amberjack (*Seriola dumerilii*) samples organized by collection region.

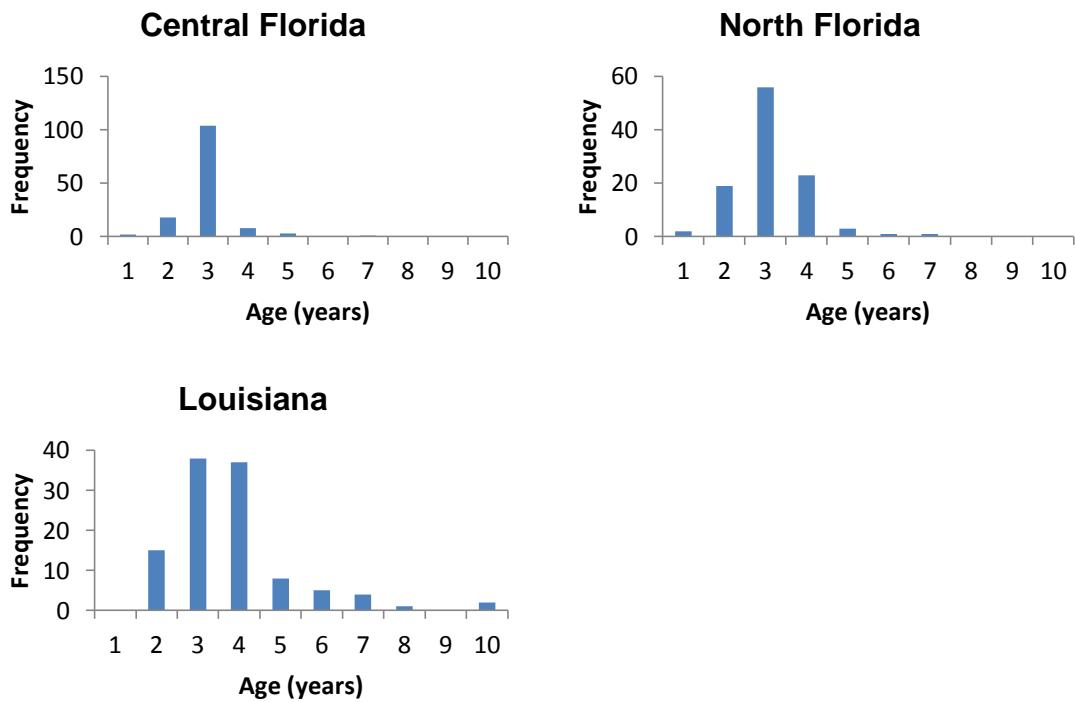


Figure 3-2. Age in years of Gulf of Mexico greater amberjack (*Seriola dumerili*) samples organized by collection region.

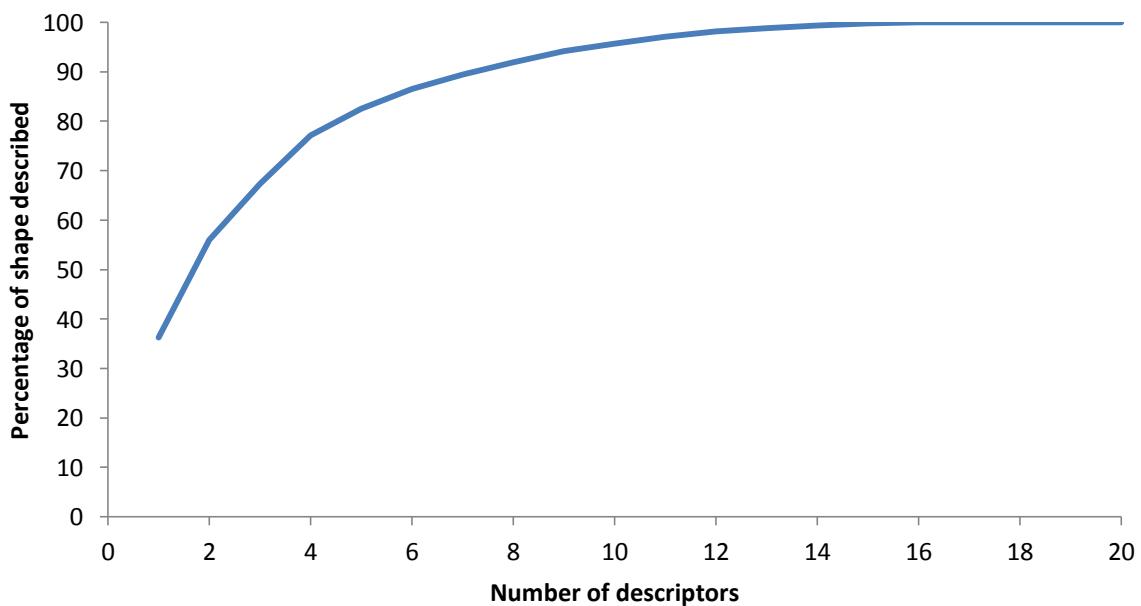


Figure 3-3. Power analysis on elliptical Fourier descriptors showed that 13 harmonics described 99% of the shape of greater amberjack (*Seriola dumerili*) otoliths.

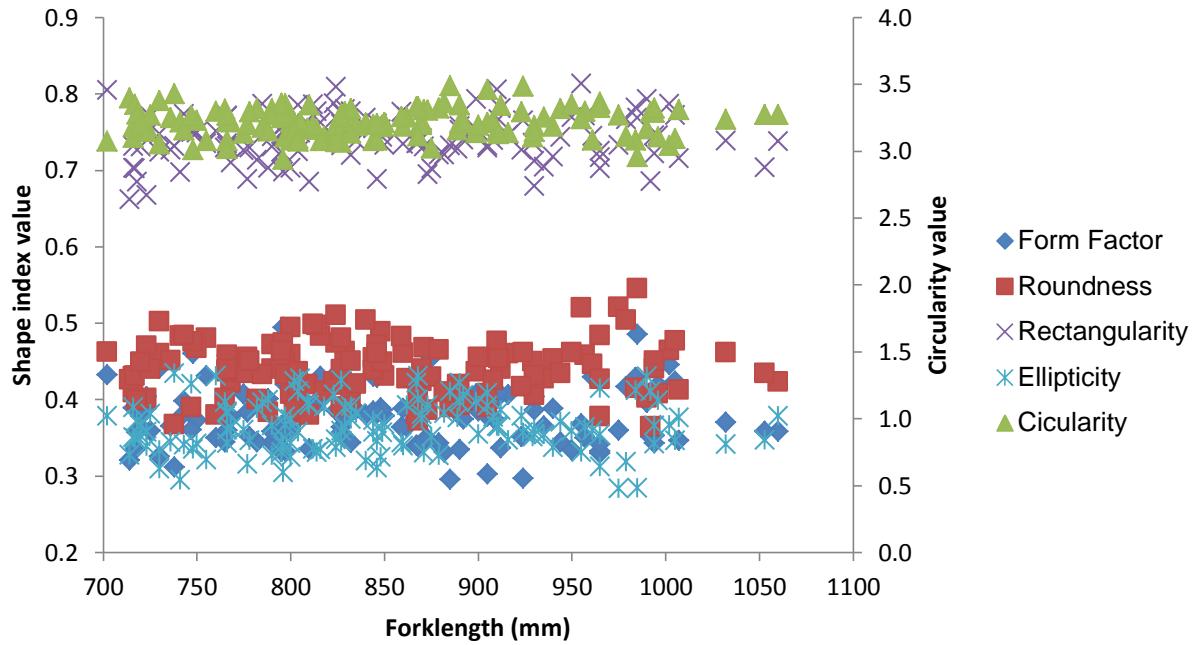


Figure 3-4. Values of shape indices of greater amberjack (*Seriola dumerili*) otoliths plotted across forklength show no relationship between size of fish and shape index value.

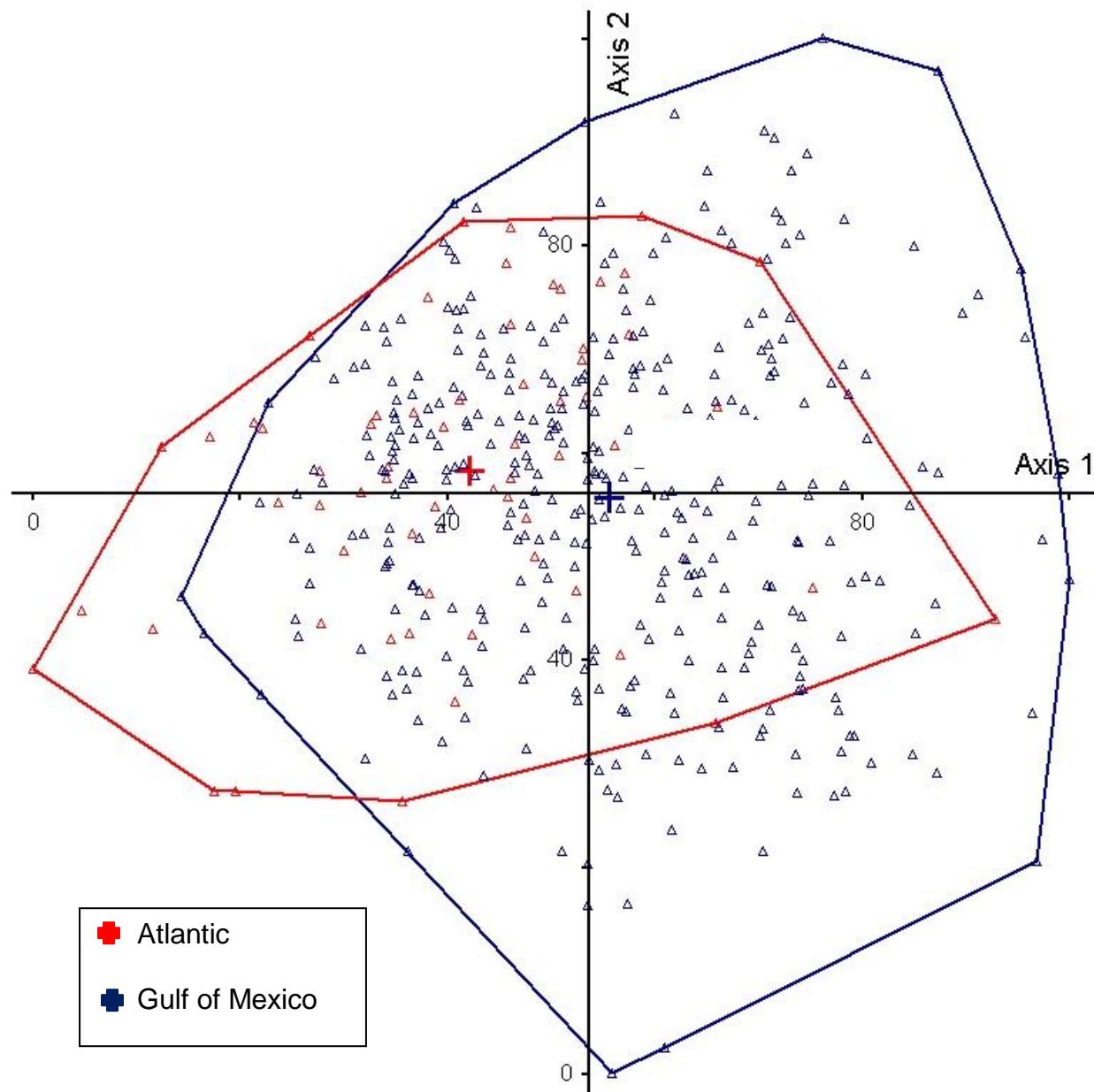


Figure 3-5. Two-dimensional Principal Component Analysis projection comparing otolith shape between males from the Gulf and Atlantic stocks of greater amberjack (*Seriola dumerili*), with maximum convex polygons enclosing the regions.

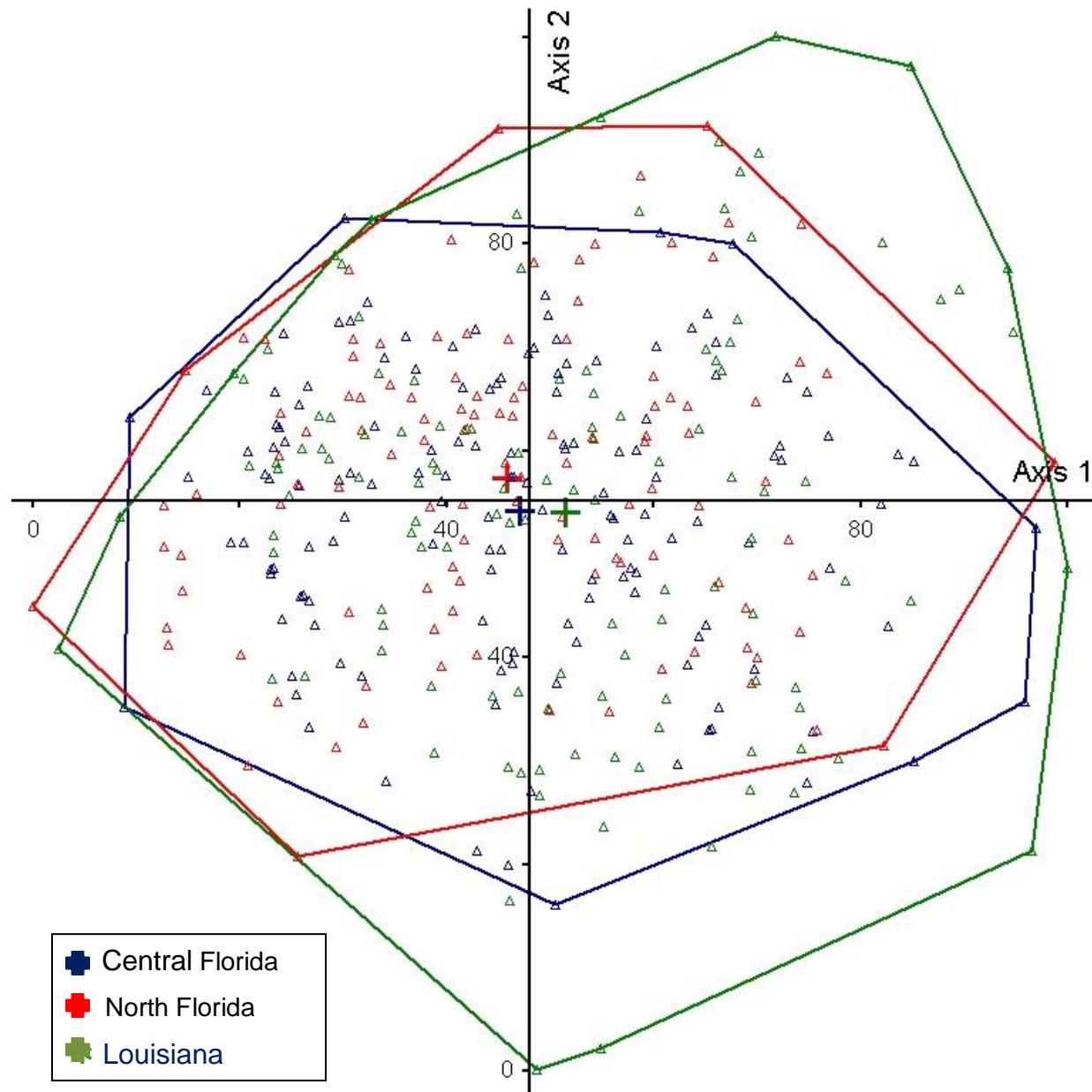


Figure 3-6. Two-dimensional Principal Component Analysis projection comparing otolith shape of greater amberjack (*Seriola dumerili*) among regions in the Gulf of Mexico, with maximum convex polygons enclosing the regions.

## CHAPTER 4 DISCUSSION

There is no consensus in otolith shape analysis as to what constitutes a classification success informative to management, and studies have reported variable levels of success. Jonsdottir et al. (2006) compared cod otoliths from locations in northern and southern Iceland, and found that only 0-44% classified correctly to region based on otolith shape. However, misclassified otoliths were most often classified to adjacent locations, and a high percentage of cod south of Iceland were classified to other southern locations (66-72%) and north of Iceland to other northern locations (61-67%). The authors considered the results to successfully discriminate a northern and southern spawning group for Icelandic cod, which previously had been managed as a single management unit, and suggested that the current single-stock management of Icelandic cod be reconsidered. DeVries et al. (2002) compared otolith shape among Gulf of Mexico and Atlantic king mackerel and found shape correctly classified 80% of Atlantic and 86% of Gulf king mackerel, which was considered high enough to use otolith shape to discern between the two stocks in mixing zones. A later study on king mackerel (Shepard et al. 2011) found classification success rates from 60-73%. Tuset et al. (2003) reported 68.8% classification accuracy in otolith shape between Atlantic and Mediterranean comber; though differences were slight between the two stocks, the authors considered it better than would be expected by chance and therefore reported otolith shape capable of separating the two regions. Campana and Casselman (1993) compared otolith shape among northwestern Atlantic cod and found classification success ranged from 20-80% depending upon location and scale of classification (i.e.

classification was more accurate to region than to specific location), and interpreted this to mean that otolith shape can “sometimes” be a useful tool to discriminate cod stocks.

In the present study, otolith shape was found to correctly classify 70% of Atlantic and Gulf of Mexico otoliths, which is interpreted as a high classification success based upon previous studies. In addition, analysis of variance showed significant differences in the shape indices form factor and circularity between the two stocks, and PCA projections showed evidence of grouping. Overall this confirms otolith shape’s ability to distinguish two stocks of GAJ, validating the tool for this species providing further support for the uniqueness of the Gulf and Atlantic stocks and their separate management.

Using otolith shape to discriminate among Louisiana, north Florida and central Florida GAJ showed variable success. Overall, discriminant analysis had only a 47% classification success, with  $\kappa = 0.199$  indicating little improvement over random chance alone, while PCA projections also revealed a high degree of overlap in otolith shape among regions. Similarly, analysis of variance showed no significant differences in otolith shape overall among the three regions. This supports both the current management assumption of one continuous Gulf of Mexico stock as well as the current genetic data indicating mixing within the Gulf of Mexico stock (Gold and Richardson 1998; Murie et al. 2011). However, when restricted by age, analysis of variance did show significant differences in rectangularity between Florida and Louisiana samples, and despite an overall low classification success, discriminant analysis was able to correctly classify 75% of Louisiana samples, which suggests some otolith shape characteristics local to the subregion. Despite this success, many Florida samples (40% for central Florida and 25% for northern Florida) were incorrectly classified to Louisiana,

which supported the PCA results that suggested a high degree of overlap among the three regions.

Though samples were restricted to a size range of 700-1050 mm in an effort to standardize amongst regions, Louisiana fish were larger and older on average than fish from the two Florida regions. It is possible that this size and age discrepancy could have contributed to the differences seen between Florida and Louisiana otoliths. However, analysis of covariance showed no relationships between size and otolith shape, and differences were still apparent in the age-restricted subset of the data. Therefore it is assumed that the differences seen were due to regional distinctions in otolith shape and not to differences in size or age among the regions.

Otoliths used in this study had been previously collected for age and growth analyses, and not specifically for regional shape comparisons, and otolith collections ranged across years and seasons. However, preliminary data exploration showed no differences in shape between breeding and non-breeding seasons, and so time-of-collection was not taken into account for regional comparisons. However, it is possible that regional differences may be more discernible in a future targeted study of otoliths collected from a single year.

It is also possible that movement may restrict otolith shape's ability to accurately reflect the structure of the Gulf of Mexico stock of GAJ. While tagging data show most individuals recaptured close to the original release site, some tags have been recovered over greater distances (Beasley 1993; McClellan and Cummings 1997; Ingram and Patterson 2001; Murie et al. 2011). This could reflect a stock consisting of both migratory and resident individuals, which has been shown in other species. Tagging

data of Gulf of Mexico cobia, *Rachycentron canadum*, for example, have found that while most individuals migrate from the northern Gulf to south Florida to overwinter, some individuals have been found to remain in the northern Gulf year-round, suggesting separate migratory and non-migratory groups (Hendon and Franks 2010). A stock structure consisting of migratory and resident sub-populations would confound morphological differences in otolith shape, making regional differences among resident individuals difficult to discern. It may therefore be worthwhile to examine this stock using isotope analysis, which could be able to distinguish between migratory and resident individuals and thereby supplement the current genetic and tagging data.

In summary, GAJ otoliths in the Gulf of Mexico did not exhibit clear differences in shape among regions sampled. While there is evidence that Louisiana samples differ, with age three individuals significantly differing in rectangularity and an overall high classification success, there was overlap among the regions, and northern and central Florida regions were indistinguishable. However, this does not necessarily mean that the stock is completely mixed. Otolith shape analysis is a novel approach for exploring stock structure in this species, and while it was able to distinguish between the Gulf and Atlantic stocks, it showed a high degree of variation. Therefore, this form of analysis may not accurately reflect stock structure in the Gulf, and studies should continue to elucidate the structure of this species. If the stock is not continuously mixed, as is strongly suggested by the low movement rates of GAJ in the Gulf of Mexico observed in the tagging data, the disproportionately high fishing effort off of Florida could lead to localized overfishing of the species. Stock delineation is vital to the appropriate

management of fisheries. Therefore, it is important to determine with certainty as the Gulf of Mexico GAJ stock enters the next phase in its rebuilding efforts.

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## BIOGRAPHICAL SKETCH

Chelsey was born in Tampa, Florida, where she attended St. Mary's Episcopal Day School and then H.B. Plant High School. While in Tampa, Chelsey taught Tae Kwon Do for several years, attained the rank of 3<sup>rd</sup> degree blackbelt and competed on the national level. In 2004 she graduated from high school and attended the University of Florida, where she majored in Zoology.

While an undergraduate at UF, Chelsey volunteered on a variety of research projects. In 2005 she worked for Dr. Iske Larkin conducting manatee surveys in Kings Bay, Florida. The next year she was hired to assist graduate student Elise Hoover and Dr. Shirley Baker on a study of triploid clam survival. That fall, she began working in Dr. Harvey Lillywhite's lab with marine and terrestrial snakes. In addition, she assisted on work with cottonmouths on the island of Seahorse Key, FL, and also worked with then graduate student Leslie Babonis researching marine snake osmoregulation. In the summer of 2006, Chelsey worked in the Florida Keys as an intern for the Seakeys program; while there, she also volunteered for the Sea Turtle Hospital on Marathon Key. In 2006, she was awarded a University Scholars grant to study the relative abundance of invasive rats on the island of Seahorse Key. Chelsey also began volunteering with the Florida Program for Shark Research that year, where she worked on shark age and growth and reproduction studies.

In 2007 her work with the Lillywhite lab allowed her to join a research expedition to Orchid Island, Taiwan, where they collected and studied several species of sea krait. That same summer she assisted the Florida Program for Shark Research in the field, tagging cownose rays in the Mosquito Lagoon, FL. Later that year, Chelsey began

volunteering with the Invertebrate Division of the Florida Museum of Natural History, where she was soon hired as a collections assistant. Chelsey also assisted the Paulay lab with their research on species diversity of various sea cucumber groups. During her time at UF, Chelsey also worked with numerous elementary and middle school groups. In addition, she led the students on field trips to Cedar Key, Florida, where the collected and identified marine organisms. Chelsey greatly enjoyed this outreach experience.

Chelsey graduated from the University of Florida with a Bachelor in Science degree in May, 2008. She was then hired as an intern by Dr. Kenneth Emberton on a study of biodiversity in Madagascar's land snail fauna. She was able to travel to Madagascar to conduct field research, and remained there a total of 6 weeks; the rest of my internship was spent analyzing the specimens collected in Gainesville, FL, at the Florida Museum of Natural History. In the fall of 2008, she attained my Science Diver certification, and was invited to assist then graduate student Dr. Mary Hart in field collections of chalk bass in Carrie Bowe Cay, Belize.

In 2009, Chelsey was hired as the field technician for the Florida Program for Shark Research, where she tagged numerous shark species as well as conducted abundance surveys for the Sawfish Recovery Program. Chelsey also joined the Murie/Parkyn lab in 2009 and 2010 as a field assistant in a reef fish habitat use study in St. John's, USVI.

In 2010, Chelsey began work as a M.S. graduate assistant in the University of Florida's Program for Fisheries and Aquatic Sciences with Dr. Daryl Parkyn. Her work embodied this thesis on otolith shape as a tool to examine stock structure in Gulf of Mexico greater amberjack. Her research was recognized with a best poster award at the Florida Chapter of the American Fisheries Society, and the Roger Rottmann

Scholarship as the top Master of Science student in the Florida Chapter of the American Fisheries Society for 2012. Upon completion of her Masters of Science degree, Chelsey will begin a Ph.D. in Interdisciplinary Ecology at the University of Florida.