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Regional stock structure of greater amberjack in the southeastern United States using otolith shape analysis

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Abstract

Otolith shape analysis was used to examine the current management hypothesis that greater amberjack *Seriola dumerili* in the Gulf of Mexico belong to a single stock. Shape of the sagittae was quantified using a combination of the shape indices circularity, rectangularity, ellipticity, roundness, and form factor, along with elliptical Fourier analysis for 379 otoliths collected from Louisiana, North Florida, and Central Florida. An additional 107 Atlantic stock otoliths were 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 overlap in otolith shape between the Gulf and Atlantic stocks and among the three Gulf of Mexico regions. Discriminant analysis of a region-wide sample of amberjack otoliths ranging from North Carolina to Key West, FL, had a 56% classification success rate between otoliths from the Gulf of Mexico and Atlantic stock. However, the shape indices rectangularity and circularity were found to differ significantly between the stocks. A sub-sample of South Atlantic Region otoliths that was restricted to fish collected north of the Florida-Georgia border showed evidence of regional grouping and an overall classification success of 70% when compared to Gulf amberjack. This was indicative of underlying morphological differences between the two stocks. Within the Gulf, 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 of amberjack between Florida and Louisiana is present, but overall the analysis supports the current one-stock management of greater amberjack within the Gulf of Mexico.

Introduction

The stock concept forms the basis for modern fisheries management. A stock is a unit of fish which has been differentiated based upon genetic, phenotypic, environmental, and/or harvest differences (Ihssen et al. 1981; Carvalho and Hauser 1994; Ebbin 1996; Coyle 1997; Booke 1999). 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). Therefore, it is important to appropriately delineate stocks so that management can apply suitable harvest regulations. This is particularly pertinent to overfished fisheries.

Greater amberjack *Seriola dumerili* in the southeastern United States are currently managed as two distinct stocks, with one stock along the southeastern Atlantic coast, including the Florida Keys, and the other stock residing in the Gulf of Mexico. While the Atlantic stock of greater amberjack is considered a recovered, sustainable fishery, the Gulf of Mexico stock is both overfished and undergoing overfishing, and has failed to recover despite a rebuilding plan and increasingly stringent management regulations (SEDAR 2008, 2011).

Greater amberjack in the Gulf of Mexico are currently managed as one continuous stock, with presumed mixing across the entire Gulf of Mexico region. Greater amberjack are capable of traveling large distances (Ingram et al. 2001; Murie et al. 2011) and so it is feasible to predict that the Gulf represents one well-mixed population. Furthermore, genetic analyses 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 data indicate that many individuals exhibit little net movement. Beasley (1993) observed that amberjack tagged off a Louisiana oil platform remained on site for at least nine months after tagging. Compiling a decade of tagging data, McClellan and Cummings (1997) found 30% of fish showed zero net movement between tag and recapture, with 58% recaptured within 25 miles and 90.6% within 100 miles of the original release site, and a negative relationship between movement and time-at-large. Ingram and Patterson (2001) saw 97% of greater amberjack tagged off of Pensacola recaptured within the original release area, and observed fish tagged off of Panama City traveled an average distance of 10.8 km. Furthermore, Murie et al. (2011) have found an average distance traveled of 69.54 km for tagged greater amberjack, with a median distance of 8.0 km, and no relationship between distance traveled and time at large or size of fish for a significant number of fish. These results imply that the region is not continuously mixed, but may have regional sub-structuring.

Tagging studies have found some individual greater amberjack undergo large-scale movements in the Gulf of Mexico. One fish tagged by Murie et al. (2011) near Madeira Beach, Florida, was recaptured 10 months later near Port Maria, Jamaica, with another tagged in Apalachicola, Florida recaptured 11 months later in Tampico, Mexico. In addition, Ingram and Patterson (2001) observed one fish tagged off of Panama City, Florida, recaptured 396 days later off Port Fourchain, Louisiana. These data suggest at least some degree of mixing within the region, which may make it difficult to elucidate regional structure using genetic data, as a small amount of mixing can mask genetic differentiation among populations (Allendorf 1983; Carvalho and Hauser 1994; Coyle 1997). It is possible therefore that genetic data do not accurately reflect greater amberjack stock structure in the Gulf of Mexico.

It is important to use a holistic approach in delineating stock structure, one that incorporates results from a variety of methodologies (Begg and Waldman 1999). As one component of this approach, otolith shape analysis has been demonstrated to be useful. Otolith shape is influenced by both genetic and environmental parameters (Campana and Casselman 1993; Cardinale et al. 2004; Vignon and Morat 2010) and therefore can differ among populations of the same species of fish. Otolith shape analysis has been successfully employed to delineate stock structure in numerous fish species (Campana and Casselman 1993; 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; Shephard et al. 2010; Aguera and Brophy 2011; Ferguson et al. 2011; Canas et al. 2012). 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).

This study examined otolith shape as a potential tool for identification of regional differences in Gulf of Mexico greater amberjack. The results of this study, in conjunction with tagging and genetic data, will help form a holistic picture of the stock structure of greater amberjack in the Southeastern United States. This should aid in management of the species in this region, and assist in future rebuilding efforts for the Gulf of Mexico stock.

Methods

A total of 379 Gulf of Mexico 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) were included in the study (Table 1). Otoliths were collected between 2002 and 2008, and samples were restricted to fish between 700-1100 mm forklength (FL) to limit possible size-based variation and to standardize fish samples across regions, as in general sampled Louisiana fish skewed larger in size than sampled Florida fish. Sample regions included central Florida (Madeira Beach to Sarasota), North Florida (Apalachicola to Suwannee), and central and western Louisiana (Figure 1). In addition, as otolith shape comparisons have never been conducted for greater amberjack, 107 otoliths from the Atlantic stock were included for a separate shape comparison between stocks to confirm the technique's validity for the species. Atlantic stock otoliths were provided by NOAA Fisheries.

The study focused on sagittae, shown to be the most informative otolith for morphological studies (Campana and Casselman 1993) and used 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). Greater amberjack otoliths are fragile and easily broken during removal; as a result, a large proportion of otoliths available had broken or chipped rostra. Otolith shape was therefore standardized to exclude the rostral portion following DeVries et al. (2002) (Figure 2).

A combination of shape indices and elliptical Fourier analysis was chosen to quantify otolith shape, 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), as well as area and perimeter of the posterior portion of the otolith; these measurements allowed for the calculation of five shape indices.

Shape indices were calculated following Tuset et al. (2003). Shape indices 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). 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 4π. 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.

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). 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, but too large a number can overcomplicate analyses. Fourier power analysis was therefore calculated to determine the appropriate number of harmonics using the equation $PF_n = 0.5(a^2_n + b^2_n + c^2_n + d^2_n)$ 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, achieving a cumulative power of 99% within 13 harmonic calculations. The first harmonic was excluded from analyses as the outline constructed by the first coefficients represents a simple ellipse (Merigot et al. 2007); therefore a final of 12 harmonics, and thus 48 Fourier coefficients, were retained for each otolith.

Statistical analyses were run using the SAS® and JMP® software (SAS Institute Inc. 2008). All statistical tests were conducted at the $a = 0.05$ criterion level unless otherwise stated. Prior to analysis, 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 initial 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 cube-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 had a stronger relationship with fish length than fish age (Figure 3). Results found that none of the shape indices varied significantly with fish length (Table 3), therefore all indices were retained for further analyses.

Many of the shape indices were constructed using different combinations of the same parameters and so correlation was suspected amongst the shape indices, therefore Pearson's product-moment correlation coefficients were calculated. 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 4). All indices were retained for further analyses, but correlations were taken into account when choosing further statistical methodology.

Often, only a single left or right otolith was available in the otolith collection. To test if the handedness of an otolith has an influence,, 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,* showed no significant statistical differences between left and right otoliths (Hunt 1992; Petursduttir 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.

Sex-based differences in growth rates between male and female greater amberjack have been documented in the Atlantic (Harris et al. 2007); differences in growth rates are less pronounced in the Gulf of Mexico stock (Murie and Parkyn 2008) but may still exist. Studies have found correlations between differences in growth rate and differences in otolith shape. As well, 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 greater amberjack otoliths exhibit morphological differences. Shape indices of male and female 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 greater amberjack 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 $a = 0.01$.

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). 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 greater amberjack and among the three Gulf regions using multiple individual analyses of variance (ANOVAs) to test for specific shape differences. ANOVA was again corrected for multiple testing using Bonferroni's adjustment, giving a significance criterion of $a = 0.01$. 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 $a = 0.01$. 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 otolith shape's ability to 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). 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 κ statistic (Fleiss 1981), which compares the discriminatory power of the analysis to what might be expected by random chance alone.

Results

The paired t-test found no significant differences in shape indices between left and right otoliths (Table 5); therefore, when the right otolith was absent, the mirror image of the left (a digital manipulation using the Motic software) was used in the analysis. In addition, ANOVA showed no significant differences in any shape index between male and female otoliths in the Gulf of Mexico (Table 6) or Atlantic (Table 7) stocks of greater amberjack; therefore, sexes were pooled for subsequent analyses.

Principal component analysis (PCA) showed overlap in otolith shape both between the Gulf and Atlantic stocks (Figure 4) and among the three Gulf of Mexico regions (Figure 5). Analysis of variance (ANOVA), however, found significant differences in the shape indices circularity and rectangularity between the Gulf and Atlantic stocks of greater amberjack (p-values of < 0.001 and 0.0014 respectively) (Table 8). No significant differences were seen in shape indices among the three Gulf regions using ANOVA (Table 9). However, when restricted to the age 3 fish data subset, significant differences were seen in the shape index rectangularity between the two Florida regions and the Louisiana regions (Table 10).

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 per region); for comparison between the Gulf and Atlantic, the model data set consisted of 160 randomly selected samples (80 from each stock), with a test data set of 50 randomly selected samples (25 per stock). Discriminant analysis (DA) between the Gulf and Atlantic stocks of greater amberjack showed a 58% classification success (Table 11), with a Cohen's $\kappa = 0.16$, indicating a 16% improvement over random chance. Within the Gulf, otolith shape showed a 47% classification success among the three regions overall with $\kappa = 0.20$, predicting a 20% improvement over random chance (Table 12). Discriminant analysis had the highest success assigning otoliths from Louisiana (75% classification success) but did a poor job assigning otoliths from North Florida (40% success) and Central Florida (25% success).

As the Atlantic stock otoliths used had been collected from across the South Atlantic Management Region, from North Carolina to Key West, FL , it was possible that the analysis included some Gulf of Mexico migrants ; therefore, a subsequent comparison was run between Gulf of Mexico otoliths and a subset of Atlantic stock otoliths collected north of the Florida/Georgia border. Due to limitations in the number of otoliths available, the sample size for these northern Atlantic otoliths was only n=69. Discriminant analysis requires distance between the sample size and the number of parameters (which in this case was 53), therefore the discriminant analysis was restricted to comparison of shape indices between stocks; PCA was still run on all parameters. Principal component results indicate more evidence of regional grouping than in the prior, larger range comparison (Figure 6). Analysis of variance again found significant differences in otolith shape, although in this comparison, it was form factor and circularity that differed significantly (p-values of 0.007 and 0.0012 respectively). Similarly, rectangularity was significantly different($p = 0.0114$) (Table 13). Notably, discriminant analysis showed a 70% classification success, with a Cohen's $\kappa = 0.40$ indicating a 40% improvement over random chance, further suggesting shape differences between these two stocks (Table 14).

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.

The comparison of Atlantic and Gulf of Mexico otoliths was a little more complex. For Atlantic otoliths collected from Key West to North Caroline combined, shape was correctly classified only 56% of Atlantic and Gulf of Mexico otoliths, which is relatively low when compared with previous studies. In addition, PCA projections showed a high degree of overlap in otolith shape between the two stocks. However, analysis of variance showed significant differences in the shape indices circularity and rectangularity between Gulf and Atlantic stock otoliths, suggesting some differences in otolith shape. Interestingly, classification was much more effective, under a more restricted scenario, where the northern subset of Atlantic stock otoliths was compared with Gulf of Mexico samples. Greater shape differences were observed, with evidence of regional grouping in the PCA, and an overall 70% classification success. This suggests that the Florida Keys is a zone where mixing from the two stocks may be occurring. This concurs with the observation of movements of amberjack between the keys and Central Gulf regions. Despite the low differences seen in the general DA and PCA results, these findings suggest otolith shape is a valid tool for this species and provides further support for the continued separation of the Gulf and Atlantic stocks for the purposes of management.

Using otolith shape to discriminate among Louisiana, north Florida and central Florida, greater amberjack showed variable success. Overall, discriminant analysis had only a 47% classification success, with $\kappa = 0.199$ indicating little improvement over random chance alone. Similarly, PCA projections also revealed a high degree of overlap in otolith shape among regions. In support of these findings, analysis of variance showed no significant differences in otolith shape overall among the three regions vindicating 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). Interestingly, when restricted by age, ANOVA 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. This suggests some otolith shape characteristics are unique to this sub-region. Despite this success, many Florida samples (40% for central Florida and 25% for northern Florida) were incorrectly classified to Louisiana, which supports the PCA results suggesting a high degree of overlap among the three regions.

The inability of DA and PCA to clearly distinguish the Gulf and Atlantic stocks suggests the possibility that either consistent differences in otolith morphology were absent or that elliptical Fourier analysis was not able to capture shape differences for this species. Greater amberjack otolith outlines are highly variable relative to other species, and this variability may be too high within a region to allow differentiation of stocks using the metrics employed. This would explain why an ANOVA conducted on shape indices alone was able to distinguish Gulf and Atlantic stock otoliths, while PCA and DA conducted including the Fourier coefficients were not. If this is true, it would lend credence to the ability of ANOVA to distinguish between Florida and Louisiana age 3 fish based upon rectangularity, further suggesting that there may be structuring in the Gulf of Mexico stock.

Despite the small sample size in the northern-only Atlantic subset, the observed increase in the ability for otolith shape to distinguish between this region and Gulf of Mexico stock otoliths, suggests stronger differences in otolith shape between the two regions. . Since tagging data have demonstrated some mixing between the Gulf and Atlantic stocks, (Murie et al. 2011) it would explain the limited ability of otolith shape to distinguish the regions in the larger sample including fishes collected in the Florida Keys region of the South Atlantic Management zone. However, the ANOVA results did demonstrate significant differences in shape indices; therefore it is possible that these results are also a product of problems with elliptical Fourier analysis in this species, as the discriminant analysis for the limited sample was run on shape indices alone.

Although samples were restricted to a size range of 700-1050 mm in an effort to standardize amongst regions, Louisiana fish were in general, 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 greater amberjack. 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, greater amberjack 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, overlap was evident 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 greater amberjack 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 greater amberjack stock enters the next phase in its rebuilding efforts.

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Table 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	107

Table 2. Shape indices of greater amberjack (*Seriola dumerili*) 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	$4 * \pi * Area/Perimeter^2$
Roundness	$4 * Area/ \pi * ML^2$
Circularity	$Perimeter^2/Area$
Rectangularity	$Area/(ML*MH)$
Ellipticity	$(ML - MH)/(ML + MH)$

Table 3. Analysis of Covariance (ANCOVA) of shape indices of greater amberjack (*Seriola dumerili*) sagittae, with fork length 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 4. 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 5. 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 6. 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 7. 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 8. 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	3.2245	0.0742	180
Roundness	1	0.4297	0.5130	180
Circularity	1	24.2044	<0.001	180
Rectangularity	1	10.5378	.0014	180
Ellipticity	1	0.7313	0.3936	180

Table 9. 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 10. 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 11. Discriminant analysis comparing otolith shape indices between Gulf and Atlantic samples of greater amberjack (*Seriola dumerili*).

Region	Model n	Test n	Gulf	Atlantic	Correct (%)	Cohen's κ
Gulf	80	25	16	9	64	-
Atlantic	80	25	12	13	52	-
Total	160	50	56	44	56	0.16

Table 12. 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	Correct (%)	Cohen's κ
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

Table 13. Comparison of northern 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 14. Discriminant analysis comparing otolith shape indices between Gulf and northern Atlantic samples of greater amberjack (*Seriola dumerili*).

Region	Model n	Test n	Gulf	Atlantic	Correct (%)	Cohen's κ
Gulf	80	25	16	9	64	-
Atlantic	80	25	12	13	52	-
Total	160	50	56	44	56	0.16

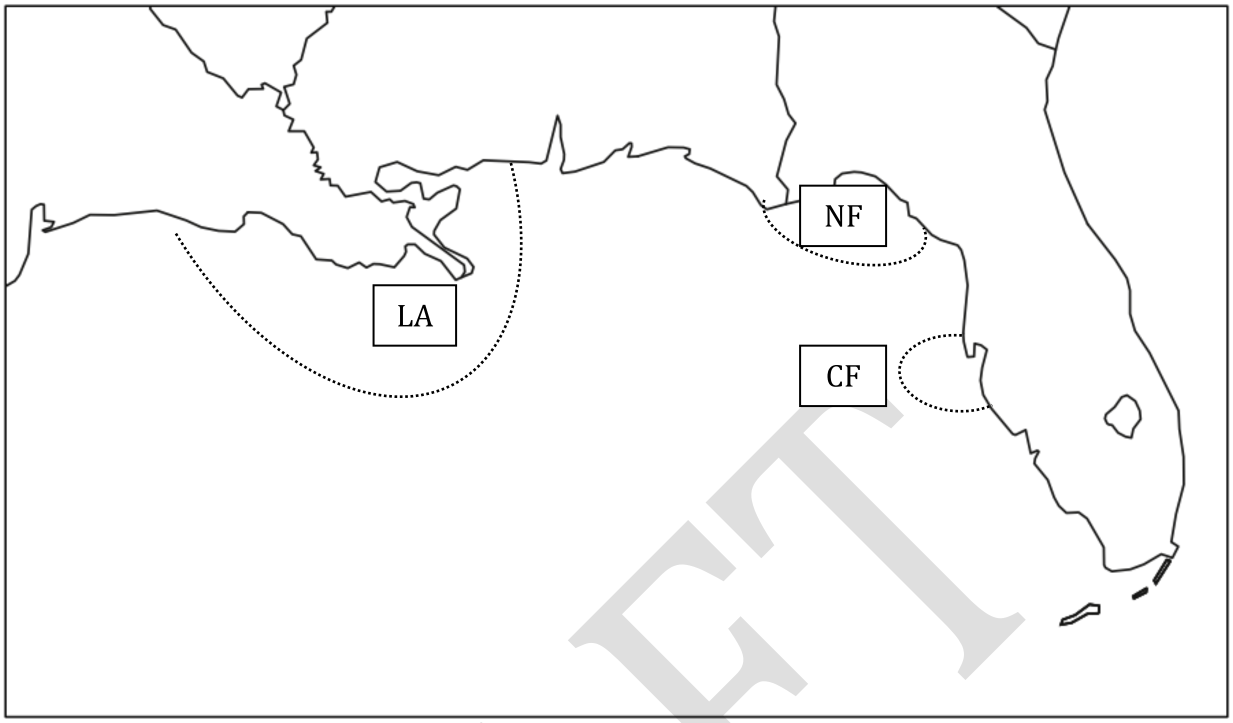

Figure 1. Gulf of Mexico regions compared in this study, as delineated by the dotted lines. Region abbreviations correspond to CF: Central Florida (Madeira Beach to Sarasota), NF: North Florida (Apalachicola to Cedar Key, FL) and LA: Louisiana.

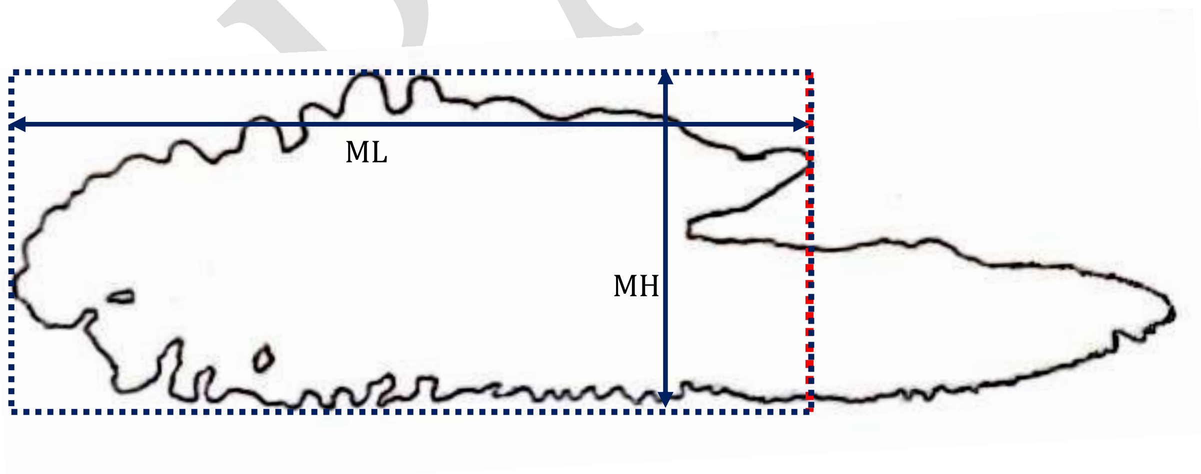

Figure 2. The maximum height (MH) and maximum length (ML) of the greatest enclosing rectangle, excluding the rostrum, were measured in each greater amberjack (*Seriola dumerili*) otolith; area and perimeter of the otolith posterior to the rostrum were also calculated.

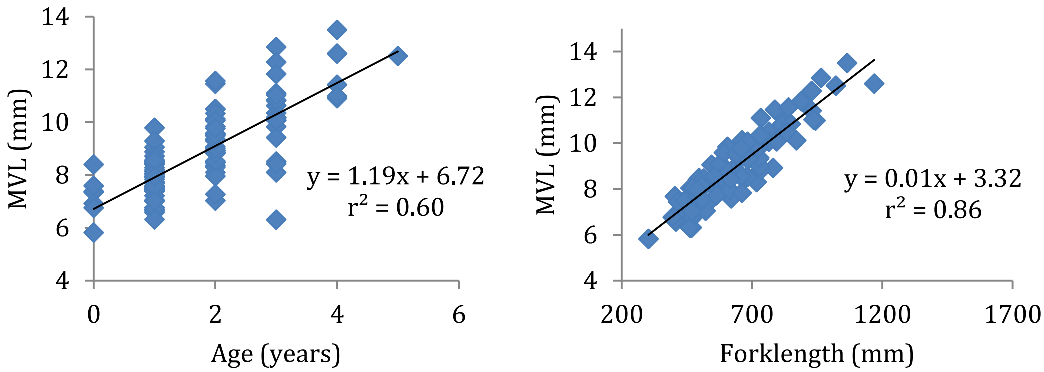

Figure 3. Maximum ventral length (MVL) of greater amberjack (*Seriola dumerili)* otoliths across ages and fork lengths.

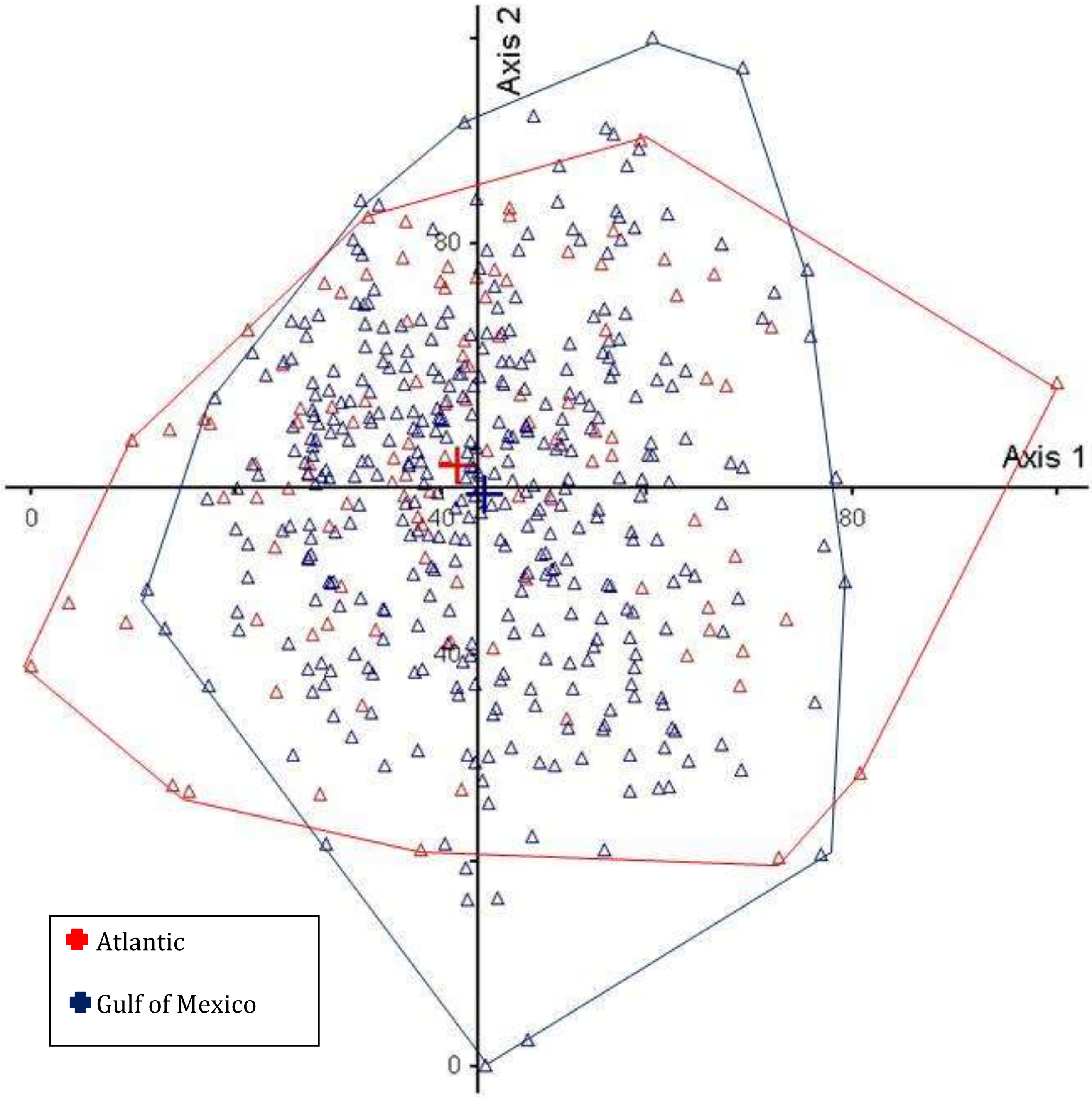

Figure 4. Two-dimensional Principal Component Analysis projection comparing otolith shape between males from the Gulf and Atlantic stocks of greater amberjack (Seriola dumerili), with maximum convex polygons enclosing the regions.

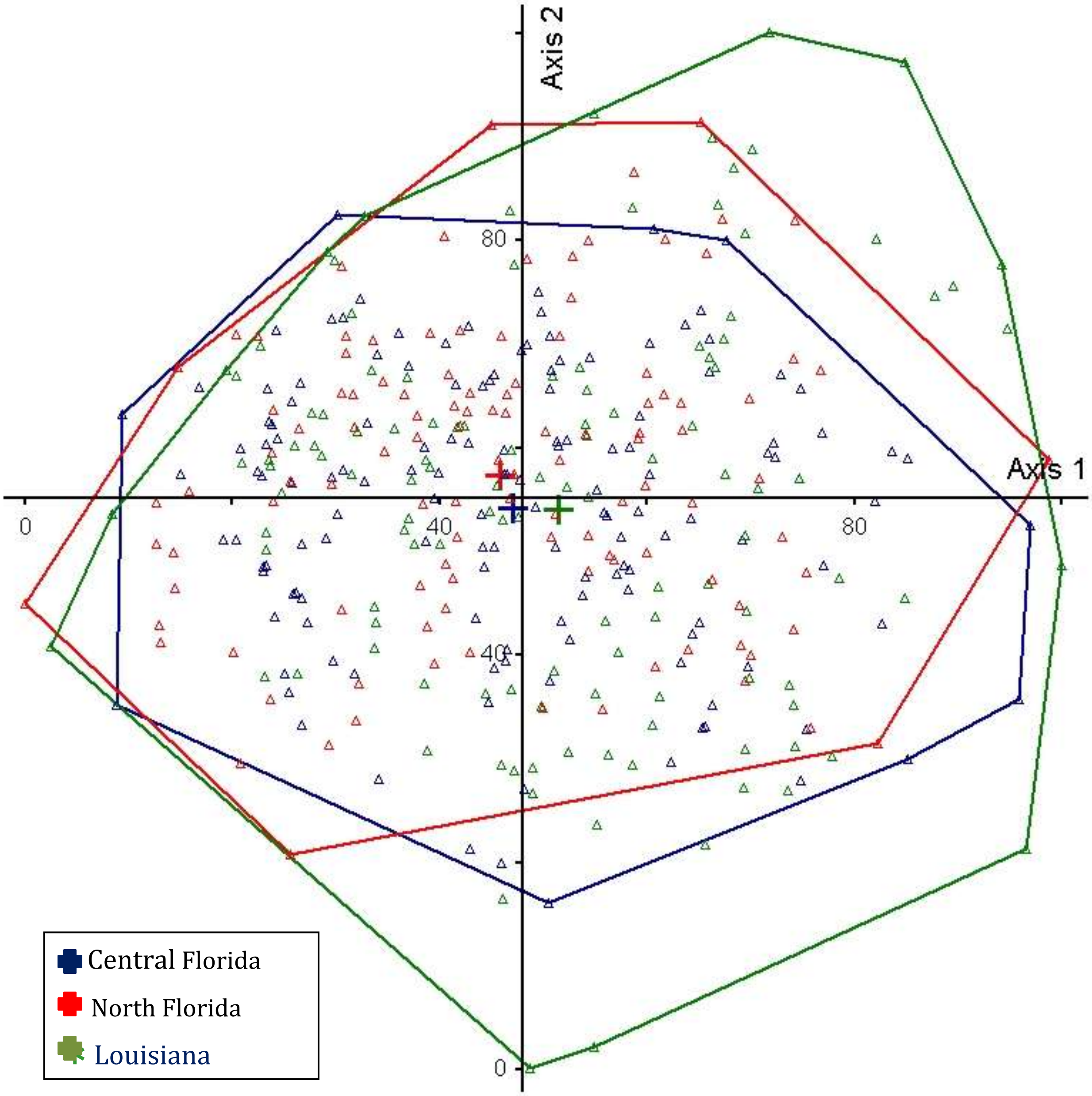

Figure 5. Two-dimensional Principal Component Analysis projection comparing otolith shape of greater amberjack (*Seriola dumerili*) among regions in the Gulf of Mexico, with maximum convex polygons enclosing the regions.

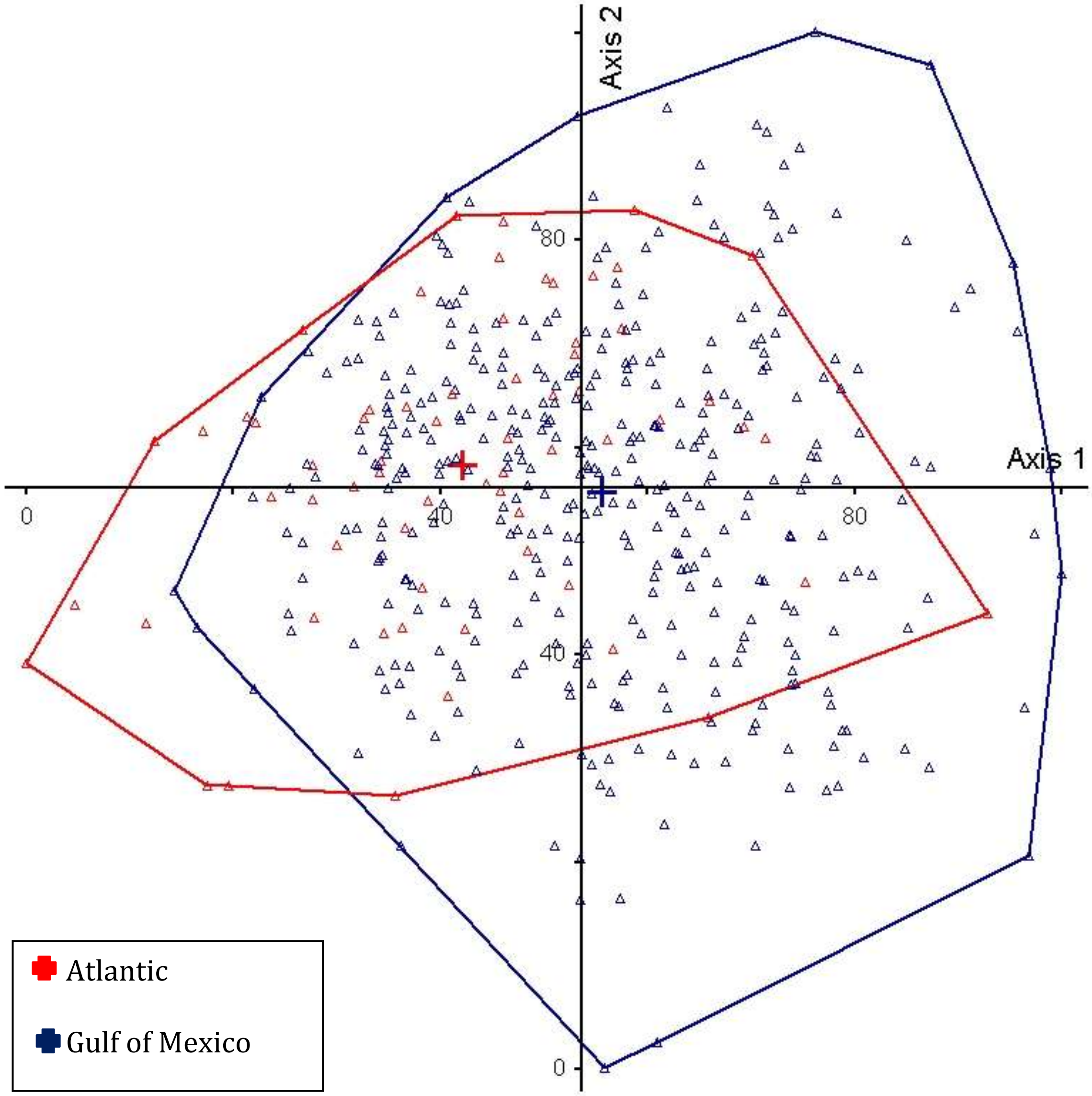

Figure 5. Two-dimensional Principal Component Analysis projection comparing otolith shape of greater amberjack (*Seriola dumerili*) among regions in the Gulf of Mexico, with maximum convex polygons enclosing the regions.