MARFIN FINAL REPORT

Discrimination Among U.S. South Atlantic and Gulf of Mexico King Mackerel Stocks With Otolith Shape Analysis and Otolith Microchemistry

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Abstract

Natural tags were developed from king mackerel, *Scomberomorous cavalla*, otolith shape and microchemistry to distinguish landings contributed by U.S. Gulf of Mexico (Gulf) and Atlantic Ocean (Atlantic) migratory groups to south Florida winter mixed-stock landings. King mackerel otoliths were sampled from the Gulf and Atlantic in late spring through summer 2001 (n = 201) and 2002 (n = 231) when stocks were separate. Otolith shape was analyzed with an image analysis system and otolith microchemistry (Ba, Ca, Mg, Mn, and Sr) was analyzed with sector field-inductively coupled plasma-mass spectrometry (SF-ICP-MS). Sex- and year-specific linear discriminant functions (LDFs) were computed with otolith shape and otolith microchemistry data to distinguish stocks. Jackknifed classification accuracies from otolith shape LDFs ranged from 66% to 76%, while classification accuracies from otolith microchemistry LDFs ranged from 68% to 91%. Maximum likelihood stock mixing models then were parameterized with migratory group-specific otolith shape data or otolith elemental signatures and applied to shape and microchemistry data of samples from winter mixed-stock landings. In 2001/02, maximum likelihood estimates from otolith shape data indicated a gradient of Atlantic migratory group contribution for both females and males from 80% to 90% in SE Florida to approximately 60% in SW Florida. Estimates from otolith elemental signatures ranged from 86% to 21% Atlantic migratory group across the same zones for females and from 83% to 40% for males. Estimates of Atlantic migratory group contribution to 2002/03 winter landings were not consistent between sexes nor models derived from shape versus microchemistry data. From shape analysis, males in 2002/03 samples were estimated to be 72% Atlantic fish off SE Florida to 46% Atlantic fish off SW Florida, while females were estimated to be 40% Atlantic fish off SE Florida to 15% Atlantic fish off SW Florida. From otolith microchemistry analysis, males were estimated to be 27% Atlantic fish off SE Florida to 75% Atlantic fish off SW Florida, while females were estimated to be 21% Atlantic fish off SE Florida to 61% Atlantic fish off SW Florida. Overall, results of this study indicate the current management practice of assigning all south Florida winter landings to the Gulf migratory group may greatly overestimate the contribution of the Gulf migratory group to winter mixed-stock landings.

Executive Summary

Natural tags were developed from king mackerel, *Scomberomorous cavalla*, otolith shape and microchemistry to distinguish landings contributed by U.S. Gulf of Mexico (Gulf) and Atlantic Ocean (Atlantic) migratory groups to south Florida winter mixed-stock landings. Currently, all landings taken from November through March in a management zone off southeast Florida are assumed to be 100% Gulf fish. Although the Atlantic migratory group was estimated to contribute a high percentage of winter mixed-stock landings, this management strategy was implemented in the early 1980s with the expectation that conservative regulations set in winter would aide the overfished Gulf migratory group. Recent simulations have shown, however, that assuming all winter mixing zone landings are Gulf fish may in fact overestimate the health of the Gulf migratory group and lead to overfishing.

King mackerel were sampled from Gulf and Atlantic landings in late spring through summer 2001 (n = 201) and 2002 (n = 231) when stocks were separate. Landings also were sampled in winter 2001/02 and 2002/03 from mixed-stock fisheries prosecuted in three zones around south Florida. Sampled fish had their length measured, their sex determined by examination of gonad tissue, and their sagittal otoliths extracted. Age was estimated for all individuals by counting opaque zones in whole or sectioned otoliths. One otolith from each fish then was digitized with an image analysis system. Shape parameters were estimated from otolith digital images with Fourier analysis. Linear discriminant functions (LDFs) were computed with shape data using a stepwise model-building algorithm to distinguish samples from each migratory group; sexes and years were modeled separately. Jackknifed classification accuracies from otolith shape LDFs ranged from 66% to 76%. Following shape analysis, otolith microchemistry (Ba, Ca, Mg, Mn, and Sr) was analyzed with sector field-inductively coupled plasma-mass spectrometry (SF-ICP-MS). Sex- and year-specific LDFs then were computed with otolith microchemistry data. Jackknifed classification accuracies from otolith microchemistry LDFs were higher than classification accuracies obtained with shape data, ranging from 68% to 91%.

Maximum likelihood stock mixing models were employed to estimate the contribution of the Atlantic migratory group to winter mixed-stock fisheries. Models first were parameterized with otolith shape data or otolith elemental signatures and then applied to shape and microchemistry data of samples from winter mixed-stock landings. In 2001/02, maximum likelihood estimates from otolith shape data indicated a gradient of Atlantic migratory group contribution for both females and males from 80% to 90% in SE Florida to approximately 60% in SW Florida. Estimates from otolith elemental signatures ranged from 86% to 21% Atlantic group contribution across the same zones for females and from 83% to 40% for males. Estimates of Atlantic group contribution to 2002/03 winter landings were not consistent between sexes nor models derived from shape versus microchemistry data. From shape analysis, males in 2002/03 samples were estimated to be 72% Atlantic fish off SE Florida to 46% Atlantic fish off SW Florida, while females were estimated to be 40% Atlantic fish off SE Florida to 15% Atlantic fish off SW Florida. From otolith microchemistry analysis, males were estimated to be 27% Atlantic fish off SE Florida to 75% Atlantic fish off SW Florida, while females were estimated to be 61% Atlantic fish off SE Florida to 21% Atlantic fish off SW Florida.

Otolith shape parameters and otolith elemental signatures proved to be effective natural tags to distinguish king mackerel migratory groups. Temporal variability between study years in otolith shape may indicate natural tags derived from otolith shape should be computed annually if the goal is to estimate the contribution of each migratory group to south Florida landings on an annual basis. Otolith elemental signatures, on the other hand, may be accurately distinguish migratory groups when applied to winter data from more than one year. Future studies should further examine the temporal stability of otolith shape and elemental signature markers, as well as examine the potential of other natural markers, such as otolith stable isotope composition, to distinguish migratory groups. Overall, results of this study indicate the current management practice of assigning all south Florida winter landings to the Gulf migratory group may greatly overestimate the contribution of the Gulf migratory group to winter mixed-stock landings.

Purpose

Background:

King mackerel are large, piscivorus scombrids that occur in the western Atlantic from Massachusetts to Brazil, including the Gulf of Mexico (Gulf) and Caribbean Sea (Collette and Nauen 1983). Adults display sexual dimorphism with females attaining significantly larger sizes at age than males (DeVries and Grimes 1997). Females may reach fork lengths (FL) greater than 1.5 m and weigh nearly 40 kg, while large males are rarely longer than 1 m FL or heavier than 25 kg (DeVries and Grimes 1997). Maximum longevity for king mackerel appears to be around 25 yr in both the Atlantic Ocean (Atlantic) and Gulf, but Gulf fish (both males and females) are larger at age than their Atlantic counterparts (DeVries and Grimes 1997; Sutter et al. 1991). Despite morphological differences between Gulf and south Atlantic fish, mixing does occur between purported stocks[2]. Tagging studies conducted in the 1970s and 1980s demonstrated king mackerel in the eastern Gulf and Atlantic migrate along the Florida peninsula in late fall and overwinter in south Florida where commercial gillnet and recreational hook-and-line fisheries are prosecuted on the mixed stock. As water temperatures warm in spring, fish migrate northward and return to summer spawning grounds (Powers and Eldridge 1983; Sutter et al. 1991) (Fig. 1).

Throughout its range king mackerel supports important commercial and recreational fisheries. Concerns over fluctuations and declines in U.S. landings in the late 1970s and early 1980s lead to the creation of the Coastal Pelagics Management Plan (CPMP), which originally treated the species as a single stock in U.S. waters (GMFMC and SAFMC 1983). Currently, king mackerel in U.S. waters are assumed to constitute two separate migratory groups (Gulf and Atlantic), but remain jointly managed by the Gulf of Mexico and South Atlantic Fishery Management Councils (GMFMC and SAFMC, respectively). This division into two migratory groups was implemented with Amendment 1 to the CPMP (GMFMC and SAFMC 1985) and was based on tag recapture data that indicated two distinct migratory groups (stocks) existed (Powers and Eldridge 1983; Sutter et al. 1991). Subsequent genetic analyses have confirmed Gulf and Atlantic fish are genetically distinct (Gold et al. 1997; Gold 2002).

The impetus for creating a federal management plan for king mackerel was the perception the species was subjected to overfishing in the 1970s. Regulations were implemented to decrease fishing mortality and increase spawning stock size beginning in the mid 1980s. The Atlantic migratory group experienced increased spawning stock size through the late 1990s and is estimated to be above its target biomass level (MSAP 2003). Routine overruns of total allowable catch (TAC) coupled with the absence of a clearly defined rebuilding strategy for the Gulf migratory group resulted in it not recovering above an overfished threshold during the 1990s (MSAP 1999, 2000; Powers 1996)[3]. Following the most recent full assessment of Gulf

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[2] The Gulf of Mexico and South Atlantic Fishery Management Councils adopted the term “migratory group” in the 1980s to refer to king mackerel populations in the Gulf of Mexico and Atlantic Ocean that had unique migratory pathways as inferred from tagging data. More recent evidence of population genetics and dynamics differences between Gulf and Atlantic fish strongly suggests these “migratory groups” are, in fact, unique genetic stocks. In this report, the convention of using the term “migratory group” is maintained in most places, but occasionally “stock” is used as a synonym.

[3] Prior to the 2000 MSAP report, overfished was defined for Gulf king mackerel as having a transitional spawning potential ratio (SPR) less than 30%. Currently, overfished is defined as biomass having a greater than 50% probability of being less than the minimum stock size threshold (MSST), which is equal to $(1-M)*(B_{MSY})$ or

king mackerel, the SEDAR5 Review Panel estimated that migratory group was not overfished (probability $B_{2002} < MSST = 17\%$) nor did it experience overfishing in the previous fishing year (probability $F_{2001/02} < F_{MSY} = 18\%$). However, it had yet to recover fully from being overfished (i.e., stock biomass remained below B_{MSY}) (Ortiz et al. 2004; SEDAR5 2004).

Mixing Zone:

A winter mixing zone in southeast Florida was defined in the mid 1980s from the Collier/Monroe County line in the southwest to the Flagler/Volusia County line in the northeast as a conservation measure to aid recovery of the overfished Gulf migratory group (Fig. 2). Although stock mixing was not well understood at the time of the zone's creation, all fish harvested in it from November through March have since been attributed to the Gulf migratory such that management regulations can limit winter mixing zone landings as added protection for that group. Results of simulation modeling demonstrated, however, that estimates of Gulf group biomass and health (relative to a benchmark SPR of 30%) actually were overestimated when the Atlantic migratory group was assumed not to contribute to winter mixing zone landings (Legault 1998). Legault (1998) estimated that increasing the percentage of fish in the winter mixing area attributed to the Atlantic migratory group had no effect on the status of the Atlantic group (i.e., no effect on transitional SPR), but both estimated Atlantic group stock size and allowable biological catch (ABC) increased as the percentage of fish in the mixing area assigned to it increased. Conversely, estimated Gulf migratory group stock size and ABC decreased as Atlantic group contribution to the mixed fishery increased. Worse yet, estimated SPR for the Gulf group decreased as the percentage of fish assigned to the Atlantic increased.

Ortiz (2004) conducted additional simulations of the effect of Atlantic migratory group contribution to winter mixing zone landings. He simulated the effect of assuming 0% (base 2004 VPA model), 50%, and 98% of winter mixing zone landings were Atlantic fish. The estimated stock size of the Atlantic migratory group increased as the percentage of winter landings contributed by it increased. Atlantic group stock status estimates were unaffected by the simulation values because it currently is not estimated to be overfished or undergoing overfishing, but, as might be expected, increasing the contribution of the Atlantic group to winter landings had a negative effect on Gulf group stock status. As stated above, results from the base VPA model (0% Atlantic group contribution) indicated there was only an estimated 17% probability B_{2002} was less than MSST and a 18% probability $F_{2001/02}$ was less than F_{MSY}. In the 50% Atlantic group contribution simulation, the probability B_{2002} was less than MSST increased to 44%, the probability $F_{2001/02}$ was less than F_{MSY} increased to 65%, and more than 2 million pounds would have to be cut (10.2 million to 8.1 million lbs) from the base VPA 2003/04 allowable biological catch (ABC) to avoid further overfishing. Gulf group stock status was further diminished when 98% of winter mixing zone landings were assumed to be contributed by the Atlantic group. Under that scenario, the probability B_{2002} was less than MSST increased to 60%, the probability $F_{2001/02}$ was less than F_{MSY} increased to 90%, and more than 3 million pounds from the base VPA 2003/04 ABC would have to be cut (10.2 million to 7.0 million lbs). Thus, not only would the Gulf group be undergoing overfishing, it also would be estimated to be overfished if most winter mixing zone landings were Atlantic fish.

The implication of these results for management is obvious: if the Atlantic migratory group contributes significantly to winter landings off south Florida, an ABC recommended for

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$0.8B_{30\%SPR}$. Overfishing is defined as current F have greater than a 50% probability of being greater than F_{MSY}, which if $F_{30\%SPR}$ for Gulf migratory group king mackerel.

the Gulf stock assuming the present mixing scenario likely would lead to overfishing (Legault 1998; Ortiz 2004). Moreover, the Atlantic migratory group could be fished harder than it currently is being fished and still remain healthy. Thus, even prior to the most recent simulation results, the South Atlantic Fishery Management Council had been vocal in its desire to change how the winter mixing zone is managed, which they articulated in a proposed amendment to the CPMP (Draft Amendment 13, SAMFC 2001) calling for separate management of Gulf and Atlantic king mackerel stocks. Passage, or even serious consideration, of such an amendment would necessitate readdressing current seasonally varying stock boundaries.

Estimating Migratory Group Mixing:

To address these issues, several recent studies have examined differences between Gulf and Atlantic king mackerel genetics, otolith shape, and otolith elemental signatures, with the common goal of developing natural markers that could be used to estimate migratory group identity of mixing zone fish. Gold et al. (2002) reported patterns of genetic variability found in nuclear DNA microsatellites indicated weakly divergent genetic stocks; however, less than 0.2% of the total genetic variance occurred between purported Gulf and Atlantic stocks. The authors estimated the stock composition of landings from several regions around the southern tip of Florida based on stock-specific microsatellite signatures. They reported approximately half of fish sampled in each region had a Gulf or Atlantic genetic signature regardless of the month samples were taken. Their results may indicate the stock composition of winter mixed stock fisheries in all regions around south Florida is evenly split between the two stocks, or, alternatively, microsatellite markers were such weak discriminators that results did not deviate from expectation under random assignment (i.e., a 1:1 ratio of outcomes).

While genetic differences may be insufficient to estimate stock identity of mixing zone landings, recent studies employing otoliths as natural stock markers have shown great promise (DeVries et al. 2002; Patterson et al. unpub. MS). Reasons why otoliths are ideal natural makers of fish populations or stocks are straightforward. Otoliths are calcium carbonate and protein matrices that are deposited in the vestibular system of bony fishes as they grow (Casselman 1987). Otoliths grow or accrete relative to somatic growth and form concentric opaque and translucent zones with which the age of the fish may be estimated; increments in otoliths are deposited sub-daily, daily, and annually. Otoliths are metabolically inert once formed and are never resorbed under natural conditions (Campana and Neilson 1985; Casselman 1987). Therefore, otolith characteristics that are unique to individual species or stocks have proven to serve as ideal, permanent natural tags.

Differences in otolith morphology have been reported among closely related species (Johnson 1995) and among stocks of single species (Bird et al. 1996; Begg and Brown 2000), and are thought to reflect genotypic variability as well as differential environmental histories and growth rates (Campana and Casselman 1993). These differences have been used as stock-specific natural tags in many species (e.g., Begg and Brown 2000; Bird et al. 1996; Campana and Casselman 1993) and otolith shape analysis recently has been used to discriminate among Gulf and Atlantic king mackerel. DeVries et al. (2002) reported differences in sagittal otolith shape parameters were significant between Atlantic and Gulf females in summer 1996 (when stocks were separate). They developed a quadratic discriminant function (with otolith perimeter, area, and 10 harmonics of Fournier amplitude as independent variables) that classified 71% of Atlantic fish and 78% of Gulf fish accurately. The authors then parameterized a maximum likelihood stock mixing model with the same set of variables to estimate the stock composition of 463 fish sampled during winter 1996/97 off southeast Florida. They estimated 99.8% (SE = 3.4%) of winter samples belonged to the Atlantic migratory group. Furthermore, the authors concluded results from otolith shape analysis suggested the migratory groups did not mix off southeast Florida in winter 1996/97.

An equally promising otolith-based approach to estimate movement patterns or stock mixing of adult fishes involves using otolith elemental and/or isotopic signatures as natural biogeochemical tags of fish from different water bodies, geographic areas, or stocks (Begg et al. 1998; Kennedy et al. 2000; Patterson et al. 1998, 2002; Thorrold et al. 1998, 2001). As otoliths grow, minor and trace metals are incorporated into their matrices from the water in which the fish lives (Bath et al. 2000; Hoff and Fuiman 1995; Kalish 1989). Because otoliths are metabolically inert once formed and the chemistry and environmental parameters of seawater vary geographically, analysis of otolith microchemistry reveals the environmental history of fish and can be used as a natural biogeochemical tag of fish populations or stocks (Campana et al. 1999; Patterson et al. 1998, 2002; Thorrold et al. 1998, 2001). Patterson et al. (unpub. MS) demonstrated Gulf and Atlantic king mackerel collected on their summer spawning grounds in 1995 had otolith elemental signatures that were migratory group-specific. Classification accuracies computed from linear discriminant functions (LDFs) with elemental concentrations (Ba, Mn, Mg, and Sr) as dependent variables were 85.3% for females and 76.8% for males.

The purpose of the current study was to continue lines of research aimed at developing natural tags derived from otolith shape analysis and otolith elemental signatures of Gulf and Atlantic king mackerel. Our objectives were to test if accurate migratory group-specific tags could be developed from otolith shape or elemental signatures; to test if shape parameters or elemental signatures were significantly different between migratory groups, sexes, and sampling years; to use shape parameters or elemental signatures to estimate the percentage of winter landings in south Florida contributed by the Atlantic migratory group; and, to estimate if migratory group composition estimates from winter samples differed between sexes.

Approach

Otolith Shape Analysis:

King mackerel were sampled from recreational landings caught in the U.S. south Atlantic and eastern Gulf during summer 2001 and 2002 when migratory groups were separate (Fig. 3). Fish were measured to the nearest mm fork length (FL) and sex was determined by macroscopic examination of gonads. We attempted to extract both sagittal otoliths from each fish sampled, but for some samples only one sagitta was taken. Extracted otoliths were cleansed of adhering tissue and placed in plastic vials for storage.

Fish age was estimated by Doug DeVries and Chris Palmer of the National Marine Fisheries Service's Panama City Laboratory following the methods of DeVries and Grimes (1997). Opaque zones generally could be counted in whole otoliths of females less than 900 mm FL and males less than 800 mm. Otoliths of fish larger than those sizes generally had to be sectioned to estimate age. Therefore, we only were able to conduct otolith shape and otolith microchemistry analyses on otoliths of large individuals from which both sagittae were collected.

After age estimation, otolith shape analysis was conducted following the methods of DeVries et al. (2002). The proximal lateral surface of otoliths was digitized with an Image-Pro® image analysis system. The left otolith was digitized when available; otherwise, the right otolith was digitized and inverted to approximate the left otolith (DeVries et al. 2002). Otolith perimeter was traced by the software prior to estimation of shape parameters. The rostrum of king mackerel otoliths is fragile and often broken during extraction (Fig. 4). Therefore, the anterior portion of otolith perimeter was estimated around the tip of the antirostrum and then from its ventral posterior terminus across the posterior portion of the rostrum in a line perpendicular to the transverse axis of the otolith (DeVries et al. 2002).

Otolith shape parameters were computed for each sample using an algorithm in Image-Pro®. The software was used to compute otolith perimeter, area, roundness, circularity, and rectangularity, as well as amplitudes of the first twenty Fourier harmonics. All 25 shape parameters were standardized by removing the common pooled group slope of the linear relationship between each parameter and fish length. Variables were tested for normality with Shapiro-Wilkes' test and for homogeneity of variances with an F_{max} test, and were transformed when necessary to meet parametric assumptions (Sokal and Rohlf 1981). Differences between migratory groups, collection years, and sexes were tested with multivariate analysis of variance (MANOVA) of shape data to determine if significant differences in otoliths shape existed (SAS, Inc. 1996). Linear discriminant function models (LDFs) were computed to distinguish Atlantic and Gulf fish. First, a stepwise discriminant model building procedure in SAS was used to compute parsimonious models that also controlled for potential collinearity resulting from correlations among shape parameters (PROC STEPDISC; SAS, Inc., 1996). Models were computed separately for each sex in each year. In the model building procedure, the significance level to enter or retain a given shape parameter was set to 0.15 and maximum tolerance was set to 0.80 to avoid potential problems with correlations among parameters. Classification success of LDFs was computed with the jackknife crossvalidation option in SAS (PROC DISC; SAS, Inc. 1996).

King mackerel landings were sampled from three zones around south Florida in winter 2001/02 and 2002/03 to estimate the contribution of the Atlantic migratory group (Figure 5). Samples from zone I were of fish landed in the commercial gillnet fishery operating around and north of the Dry Tortugas. Zone II samples were of fish landed by hook-and-line recreational fisheries centered in Islamorada. Zone III samples were from troll commercial fisheries operating between West Palm Beach and Melbourne. Otolith shape analysis of winter samples was conducted as detailed above. Maximum likelihood models then were parameterized in S-Plus with shape parameters resulting from discriminant function analysis of summer-sampled fish to estimate the stock composition of winter mixed stock samples (DeVries et al. 2002). Models were computed to estimate the percentage of samples from each zone contributed by the Atlantic migratory group; 95% confidence intervals were bootstrapped (n = 500) about each estimate in S-Plus (DeVries et al. 2002).

Otolith Elemental Signatures:

Otolith microchemistry was analyzed once shape analyses were completed. Samples were prepared for analysis in a class-100 clean room at the Department of Geological Sciences, Louisiana State University. Otoliths were cleaned of any remaining tissue by rinsing with ultrapure water (18.3 megaohm polished water) and lightly scrubbing their surface with an acid-leached synthetic bristle brush. Otolith surfaces then were alternately flooded with 1% ultrapure nitric acid and rinsed with ultrapure water. Cleaned samples were air-dried in a laminar flow class-10 clean hood and then weighed.

Otoliths were dissolved in 1% ultra-pure nitric acid at a near constant ratio of acid volume to otolith weight (dilution factor ~1000x). Solutions were spiked with Indium as an internal standard and then analyzed with a Finnigan MAT Element II sector field-inductively coupled plasma-mass spectrometer (SF-ICP-MS) in the Department of Chemistry and Biochemistry at Old Dominion University. Precision and accuracy of sample analyses were determined by periodic analysis of an otolith certified reference material (Japan National Institute for Environmental Studies fish otolith CRM; Yoshinaga et al. 2000).

Otolith microchemistry data obtained from SF-ICP-MS analysis were analyzed statistically following the same methods applied to shape data except for one departure. Results from analysis of the Japan NIES otolith CRM indicated small differences existed in SF-ICP-MS performance between 2001 and 2002 analyses (see Results in Findings section below). Therefore, to remove any potential bias when making between year comparisons, statistical tests were performed on within-year residuals instead of the raw data. Once residuals were calculated, MANOVA, LDF, and maximum likelihood models were computed as detailed above.

Project Management:

Dr. Robert Shipp ultimately was responsible for all phases of project management, Dr. Will Patterson administered all day to day project operations. This included procurement of samples; data acquisition, assimilation and analysis; project reporting; and, preparation of scientific publications. The project received significant cooperation from the NMFS and the North Carolina Department of Environment and Natural Resources, Division of Marine Fisheries (NCDMF) in obtaining otolith samples. Mr. Doug DeVries of the NMFS Southeast Science Center, Panama City Laboratory coordinated collection of fish sampled from the Atlantic in summer 2001 and 2002. This involved several personnel of the NCDMF and NMFS port agents in South Carolina and NE Florida. National Marine Fisheries Service port agent Ms. Debbie Fable and others collected samples from NW Florida in summer 2001 and 2002. Several University of South Alabama (USA) graduate and undergraduate students collected samples from the north central Gulf in summer 2001 and 2002. Winter sample collections were facilitated by Mr. Guy Davenport of the NMFS. National Marine Fisheries Service port agent Mr. Ed Little aided sample collection from winter zones I and II, and agents Mr. Charlie Schaefer and Ms. Michelle Gamby aided sample collection from winter zone III. Otolith shape analysis and associated statistical analysis were performed by USA graduate student Mr. Todd Clardy. Otolith microchemistry sample preparation was performed by Dr. Will Patterson. Analysis of otolith microchemistry samples was performed by project collaborator Dr. Zhongxing Chen at Old Dominion University. Statistical analysis of otolith elemental signatures was performed by Dr. Will Patterson.

Project Findings

Results:

Otolith shape analysis was performed for 201 samples collected in summer 2001 and 231 samples collected in summer 2002 (Fig. 6). The ratio of females to males for summer samples of both stocks was approximately 1:1 during both years except for Atlantic samples in summer 2002 (1.49:1). Fork length and age distributions were similar within year between stocks for both males and females (Fig.s 6 & 7).

Standardized perimeter data were log-transformed and standardized amplitudes of harmonics 13 through 16 were square root-transformed to meet parametric assumptions of normality and homogeneity of variances. Multivariate analysis of variance computed with shape parameters as dependent variables indicated there was a significant difference in otolith shape between sexes (Pillai's Trace $F_{d.f.=30;401} = 2.93$; $p < 0.001$) and stocks (Pillai's Trace $F_{d.f.=30;175} = 2.18$; $p < 0.001$) but not years (Pillai's Trace $F_{d.f.=30;401} = 0.763$; $p = 0.813$). Linear discriminant function analysis of otolith shape parameters yielded jackknifed classification accuracies ranging from 60.4% to 76.4% (Table 1). Classification accuracies generally were higher when sexes were modeled separately, despite there being no significant difference in otolith shape between sexes. Therefore, maximum likelihood models employed to estimate the percentage of winter landings contributed by the Atlantic migratory group were computed separately for each sex.

Otolith shape analysis was performed for 350 king mackerel sampled from three zones in south Florida in winter 2001/02 and for 389 fish sampled in winter 2002/03 (Fig.s 8 & 9). Sex- and year-specific maximum likelihood models parameterized with shape data from summer-sampled fish indicated a high percentage of samples from each winter zone in each year of the study were Atlantic stock fish; however, 95% confidence intervals about the estimates were wide (Table 2). The trend observed for all models was an east to west increase in the estimated percent Atlantic migratory group contribution to landings.

Of the 201 otoliths collected in summer 2001, only 176 were judged to be suitable for chemical analysis (n = 52 females and 49 males from the Atlantic and 38 females and 37 males from the Gulf). Only 196 of the 231 otoliths collected in summer 2002 (n = 50 females and 48 males from the Atlantic and 51 females and 47 males from the Gulf) were judged to be suitable for chemical analysis. Unsuitable otoliths either were stored with excessive amounts of tissue on their surface or were broken following shape analysis. Removing these samples did not alter the FL or age distributions relative to samples used for otolith shape analysis.

Five elements were quantified in king mackerel otolith solutions: Ba, Ca, Mg, Mn, and Sr. Limits of detection for all five elements were well below concentrations in king mackerel otolith solutions (Table 3). Comparison of Japan NIES CRM certified elemental concentration values with values measured during analysis of 2001 and 2002 samples indicated otolith microchemistry analyses were within acceptable tolerances ($< \pm 5\%$; Table 4). However, elemental concentration differences were greater between years for Ca and Sr in the current study than differences between certified values and ones we measured. Therefore, to eliminate potential bias when making between year comparisons, statistical tests were performed on within-year residuals instead of the raw data.

Residuals of Mg, and Sr were log-transformed to meet parametric assumptions of normality (Mg and Sr) and homogeneity of variances (Mg). Multivariate analysis of variance computed with elemental concentration residuals as dependent variables indicated there was a significant difference in otolith elemental signatures between stocks (Pillai's Trace $F_{d.f.=4;358} = 71.86$; $p < 0.001$) but not between sexes (Pillai's Trace $F_{d.f.=4;358} = 2.14$; $p = 0.075$) or years (Pillai's Trace $F_{d.f.=4;358} = 0.97$; $p = 0.422$). [Note: Results of a MANOVA run on the raw microchemistry data were exactly the same as those of the residuals model for sex and stock effects but the year effect was significant (Pillai's Trace $F_{d.f.=4;358} = 57.08$; $p < 0.001$)]. Linear discriminant function models were computed for males and females separately, as well as jointly for both sexes, in 2001 and 2002. Jackknifed classification accuracies from computed LFDs ranged from 64.7% to 90.9% (Table 5).

Otolith microchemistry analysis was performed for 323 of the 350 king mackerel sampled from south Florida in winter 2001/02 and 306 of the 389 fish sampled in winter 2002/03. Sample sizes for winter 2001/02 were 140 for zone I (female = 77; male = 63), 49 for zone II (female = 44; male = 5), and 134 for zone III (female = 65; male = 69). Sample sizes for winter 2002/03 were 119 for zone I (female = 67; male = 52), 74 for zone II (female = 51; male = 23), and 113 for zone III (female = 62; male = 51). Maximum likelihood models parameterized with otolith elemental signatures derived from summer-sampled fish indicated nearly all winter 2001/02 fish of both sexes sampled in zone III were estimated to be Atlantic fish, while most fish sampled in zone I were not (Table 6). That same trend was estimated for winter 2002/03 females, but for males, Atlantic fish were estimated to contribute a greater percentage to zone III landings than zone I landings.

Discussion:

Results from this study indicate both otolith shape analysis and analysis of otolith elemental signatures provided effective natural tags of king mackerel stocks. Otolith shape analysis has several advantages over analyzing otolith elemental signatures in that is less costly, less time consuming, and nondestructive. However, otolith elemental signatures provided higher classification success which was affected only minimally by modeling sexes jointly or separately.

Otolith shape discriminant function classification accuracies were similar to those reported by DeVries et al. (2002) for female king mackerel sampled in summer 1996 despite lower sample sizes in the present study. Otolith shape classification accuracies were slightly lower for males than females in this study, which might be expected given greater differences in female growth between stocks (DeVries and Grimes 1997). One also might expect differences between sexes in shape parameters retained in stepwise discriminant functions given that otolith shape was estimated to be significantly different between sexes. The fact that such different models resulted within sex between years is difficult to explain, however, because year was not a significant year effect in the MANOVA model and there was significant overlap in year classes between 2001 and 2002.

Otolith elemental signatures were more accurate than otolith shape analysis in distinguishing king mackerel migratory groups. We are further encouraged that no significant difference in elemental signatures existed between sexes, which we interpret as males and females sharing migration pathways. A finding of no significant difference between years might suggest environmental parameters driving differences in otolith shape or elemental signatures were temporally stable. An alternate interpretation is there was only one cohort difference among five cohorts sampled in summer 2001 versus summer 2002 fish, thus there should be high correspondence in otolith elemental signatures between sampling years even if environmental parameters driving signatures varied among all years represented in our samples.

Maximum likelihood estimates of percent Atlantic stock contribution to 2001/02 winter samples were similar between methods for both males and females despite differences in discriminant function classification success between otolith shape analysis and otolith microchemistry approaches. Both methods estimated the majority of zone III landings were contributed by the Atlantic migratory group. This finding was somewhat consistent with results reported by DeVries et al. (2002) from their shape analysis study; however, DeVries et al. (2002) reported nearly all fish (99.8%) of their large sample (n = 463) from SE Florida were contributed by the Atlantic stock. Differences between their results and those from our study may indicate changes exist in king mackerel migrations among years or that Gulf fish contributions to winter landings in SE Florida have increased as stock size has increased in the past decade.

In the current study, maximum likelihood models based on otolith shape data estimated over half the fish sampled in zone I were Atlantic fish, while estimates based on otolith elemental signatures indicated the majority of zone I samples were not Atlantic fish. Without corroborating evidence of stock composition it is difficult to assess which estimates were closer to true mixing conditions. Perhaps more weight should be given to the elemental signature estimates given the greater classification success with summer samples. It is difficult to have much confidence in point estimates derived from either method, however, given the wide confidence intervals estimated with each.

Otolith shape derived estimates of the percent Atlantic group contribution to sampled landings differed somewhat between sexes in winter 2002/03. Shape derived estimates for males followed an east-west gradient similar to that estimated with otolith microchemistry for both males and females in 2001/02. The same trend was nearly true of shape derived female estimates in 2002/03 except that Atlantic group contribution to zone III landings was estimated to be less than 50%. By far the greatest deviation from an east-west trend of decreasing estimated Atlantic group contribution to landings existed for males' 2002/03 otolith microchemistry derived estimates. In that case, a higher percent Atlantic group contribution actually was estimated for zone I than for zone III. Examination of mean concentrations of individual elementals for males sampled in summer 2002 and winter 2002/03 reveals Mn and Sr concentrations drove the resultant winter classifications. It is unclear, however, why differences existed in information from otolith shape versus otolith microchemistry data for males in 2002/03.

Results from this study add to a growing body of evidence that assigning all winter mixing zone landings to the Gulf migratory group does not reflect real mixing conditions (DeVries et al. 2002; Gold et al. 2002). Study results indicate both otolith shape analysis and analysis of otolith elemental signatures hold promise as effective tools to estimate stock composition of winter landings off south Florida. Otolith elemental signatures more accurately distinguished migratory groups than shape analysis, but further research is required to remove uncertainties resulting from both techniques discussed above. For now, it appears there is sufficient evidence to compute stock assessment models assuming at least half and perhaps more of the king mackerel caught in the winter mixing zone are contributed by the Atlantic migratory group.

Further Research:

Results from this study clearly demonstrate the utility of otolith-derived natural tags in distinguishing Gulf from Atlantic migratory group king mackerel. If the two approaches we employed are to be used to monitor changes in winter mixing between king mackerel groups, several aspects of each approach require further research. First, a study should be conducted to determine the optimal sample size needed to distinguish fish from the Gulf versus Atlantic accurately, as well as what effect increased sample size has on analysis cost. For example, there are greater costs per sample associated with otolith microchemistry analysis than shape analysis, but much greater sample sizes might be required for shape analysis to achieve the same level of accuracy as distinguishing migratory groups with otolith microchemistry. The temporal variation in each method also should be further examined, as it may prove that natural tags derived from otolith microchemistry can be accurately applied to winter samples across several years, thus decreasing analysis costs.

Evaluation

Delays in sample processing and otolith microchemistry analysis necessitated a one-year no-cost extension for the project. Following that extension, all goals and objectives of the study have been met. We feel this work is a significant contribution to understanding king mackerel stock mixing dynamics and has the potential to affect management profoundly. It also establishes a new method (otolith microchemistry) for distinguishing coastal pelagic stocks in U.S .waters that may be applied to other species such as cobia or amberjack.

Project results have been and will continue to be disseminated in a variety of ways. Dr. Patterson presented project results at the December 2003 MaRFIN Panel meeting in Biloxi, MS and prepared a report on project findings for the 2004 king mackerel SEDAR panel meetings. Todd Clardy presented otolith shape analysis results at the February 2004 meeting of the Alabama Fisheries Association meeting in Gulf Shores, Alabama, as well as at the 3rd International Conference for Otolith Research and Application held in Townsville, Australia in June 2004. Dr. Patterson plans to present a paper on total project results at the 2005 Annual Meeting of the American Fisheries Society in Anchorage, Alaska. The otolith shape analysis portion of the study is the subject of Todd Clardy's master's thesis (University of South Alabama) and a manuscript based on shape analysis results will be submitted in winter 2005 to *US Fishery Bulletin*. A second manuscript on the otolith microchemistry portion of the study is being prepared by Dr. Patterson for submission to *Canadian Journal of Fisheries and Aquatic Sciences*.

Acknowledgements

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Table 4. Comparison of Japan NIES fish otolith certified reference material elemental concentration values ± 95% confidence intervals with values ± 95% confidence intervals measured during analysis of king mackerel otolith solutions. Year 2001 analysis refers to analysis of samples collected in summer 2001 and winter 2001/02, and year 2002 refers to analysis of samples collected in summer 2002 and winter 2002/03. Percent difference was computed as the difference between NIES certified values and current study values divided by the NIES values and then multiplied by 100.

Table 5. Linear discriminant function models computed with otolith elemental concentrations to distinguish Atlantic and Gulf migratory group king mackerel sampled in summer 2001 and 2002. Classification success was computed with the jackknife crossvalidation option in PROC DISC of SAS.

Table 6. Results of maximum likelihood models computed with otolith elemental signatures to estimate the percentage Atlantic migratory group king mackerel sampled from three zones around south Florida in winter 2001/02 and 2002/03. Models were parameterized with elements listed in Table 5 for each sex/year combination.

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Table 1. Resultant linear discriminant function models computed with otolith shape variables to distinguish Atlantic and Gulf migratory group king mackerel sampled in summer 2001 and 2002. Classification success was computed with the jackknife crossvalidation option in PROC DISC of SAS.

Model	Parameters Included	Classification %		
		Gulf	Atl.	Total
2001 Females	Harmonics 3,5,6, 8, 9, and 10	81.7	71.1	76.4
2001 Males	Roundness, Rectangularity, and Harmonic 3,7, and 20	69.7	67.6	68.7
2001 Both Sexes	Stan3 Stan5 Stan6 Stan8 Stan9	69.8	70.7	70.3
2002 Females	Perimeter, Roundness, and Harmonics 2, 9, 13, 15, and 16	67.9	70.8	69.4
2002 Males	Perimeter, Rectangularity, and Harmonics 2, 8, 11, and 13	61.2	70.4	65.8
2002 Both Sexes	TCPerim CorrRound Stan2 Stan4 TStan13 TStan15	64.6	56.2	60.4

Table 2. Results of maximum likelihood models computed with otolith shape data to estimate the percentage Atlantic migratory group king mackerel sampled from three zones around south Florida in winter 2001/02 and 2002/03. Models were parameterized with variables listed in Table 1 for each sex/year combination.

Year	Zone	Sex	% Atlantic	95% CI	Sex	% Atlantic	95% CI
2001/02	1	Females	60.1	40-74	Males	61.0	32-82
	2	Females	48.6	20-67	Males	99.9	61-100
	3	Females	76.0	57-98	Males	83.8	63-100
2002/03	1	Females	14.5	0-29	Males	45.5	21-70
	2	Females	41.3	21-69	Males	83.1	49-100
	3	Females	40.4	24-60	Males	71.9	52-99

Table 3. Limits of detection for isotopes of five elements analyzed in king mackerel otolith solutions. Year 2001 refers to analysis of samples collected in summer 2001 and winter 2001/02 and year 2002 refers to analysis of samples collected in summer 2002 and winter 2002/03. Limits were computed as 3 times the standard deviation of mean blank values.

Year	Ca42 µg g^{-1}	Ba137 ng g^{-1}	Mg24 ng g^{-1}	Mn55 ng g^{-1}	Sr88 µg g^{-1}
2001 n = 36 blanks	0.051	0.0039	0.140	0.025	0.020
2002 n = 18 blanks	0.64	0.085	0.203	0.005	0.044

Table 4. Comparison of Japan NIES fish otolith certified reference material elemental concentration values ± 95% confidence intervals with values ± 95% confidence intervals measured during analysis of king mackerel otolith solutions. Year 2001 analysis refers to analysis of samples collected in summer 2001 and winter 2001/02, and year 2002 refers to analysis of samples collected in summer 2002 and winter 2002/03. Percent difference was computed as the difference between NIES certified values and current study values divided by the NIES values and then multiplied by 100.

Element	Standard	2001 Analysis n = 10	% Difference	2002 Analysis n = 8	% Difference
Ca	38.8 % ± 0.5	37.7 % ± 0.98	-2.84	40.2 % ± 0.84	6.63
Ba	2.89 µg g^{-1} ± 0.09	2.85 µg g^{-1} ± 0.060	-1.40	2.90 µg g^{-1} ± 0.020	-0.35
Mg	21.1 µg g^{-1} ± 1.0	21.3 µg g^{-1} ± 0.75	0. 95	22.5 µg g^{-1} ± 0.54	1.90
Mn	NA	0.099 µg g^{-1} ± 0.0085	NA	0.101 µg g^{-1} ± 0.0022	NA
Sr	2.36 mg g^{-1} ± 0.05	2.39 mg g^{-1} ± 0.077	-1.27	2.49 mg g^{-1} ± 0.082	4.24

Table 5. Linear discriminant function models computed with otolith elemental concentrations to distinguish Atlantic and Gulf migratory group king mackerel sampled in summer 2001 and 2002. Classification success was computed with the jackknife crossvalidation option in PROC DISC of SAS.

Model	Parameters Included	Classification %		
		Gulf	Atl.	Total
2001 Females	Ba, Mg, Mn, and Sr	100	72.4	86.2
2001 Males	Ba, Mg, Mn, and Sr	98.0	83.8	90.9
2001 Both Sexes	Ba, Mg, Mn, and S	97.8	70.7	84.3
2002 Females	Ba, Mg, Mn, and Sr	70.0	67.4	68.7
2002 Males	Ba, Mg, Mn, and Sr	74.0	61.5	67.8
2002 Both Sexes	Ba, Mg, Mn, and S	71.0	58.3	64.7

Table 6. Results of maximum likelihood models computed with otolith elemental signatures to estimate the percentage Atlantic migratory group king mackerel sampled from three zones around south Florida in winter 2001/02 and 2002/03. Models were parameterized with elements listed in Table 5 for each sex/year combination.

Year	Zone	Sex	% Atlantic	95% CI	Sex	% Atlantic	95% CI
2001/02	1	Females	21.1	7-35	Males	39.7	19-62
	2	Females	38.7	21-59	Males	73.8	16-99
	3	Females	85.6	68-99	Males	83.1	66-99
2002/03	1	Females	21.3	9-37	Males	74.8	33-100
	2	Females	68.1	20-91	Males	7.3	0-27
	3	Females	61.1	19-86	Males	27.2	12-42

Figure 1. Maps of generalized annual fall and spring migrations of king mackerel in the western Gulf of Mexico, eastern Gulf of Mexico, and Atlantic Ocean inferred from tagging data.

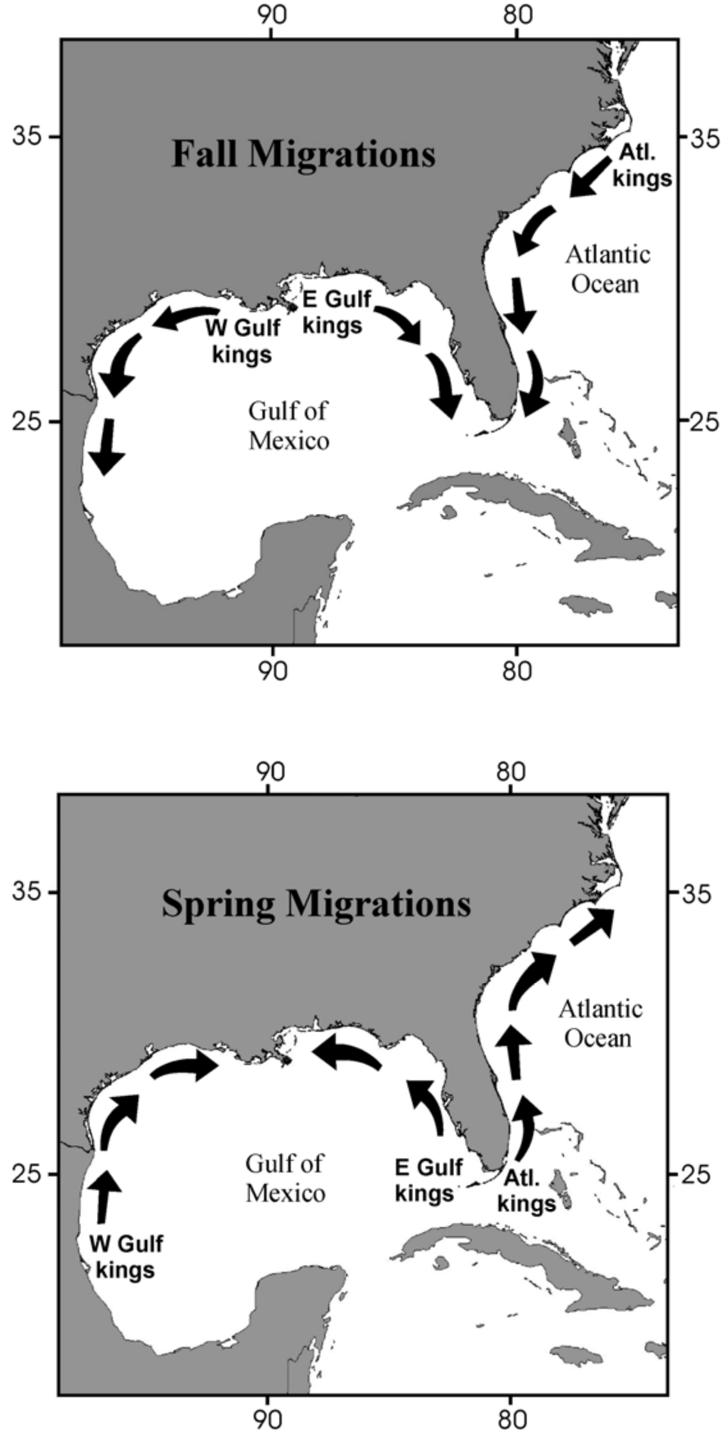

Figure 2. Map depicting boundaries of the king mackerel winter mixing area off south Florida. All landings from this area made during November through March are attributed to the Gulf migratory group. During all other months mixing zone landings are attributed to the Atlantic migratory group. The seaward boundary of the mixing zone is the edge of U.S. exclusive economic zone. Most king mackerel are caught over the continental shelf, the edge of which is denoted by the 200 m isobath.

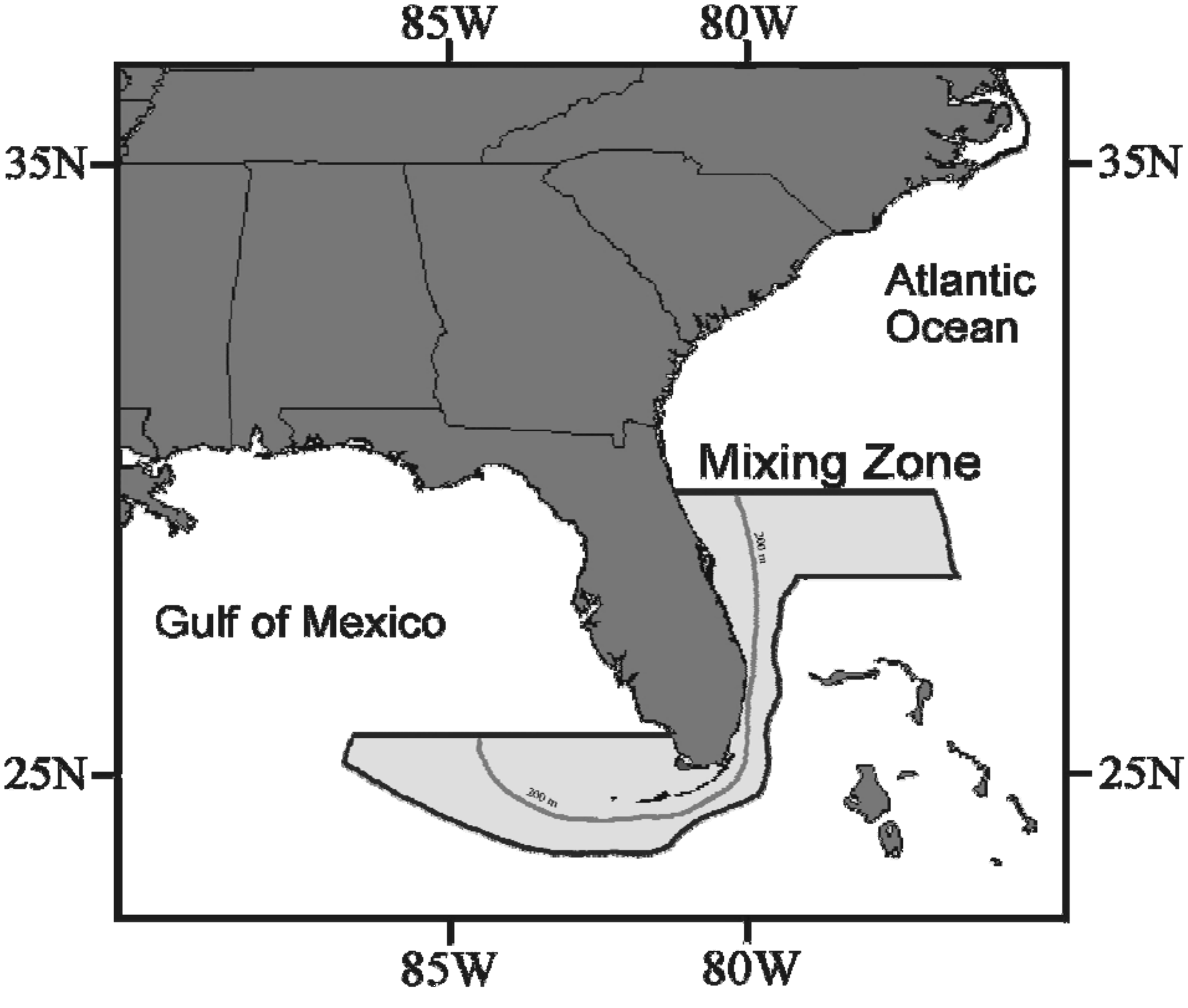

Figure 3. Map of sampling locations for king mackerel sampled in summer 2001 and 2002. Sample locations from east to west in the Gulf of Mexico were Dauphin Island, Alabama, Destin, Florida and Panama City, Florida. In the Atlantic Ocean, sample locations from north to south were southeastern North Carolina, Charleston, South Carolina, and Jacksonville, Florida.

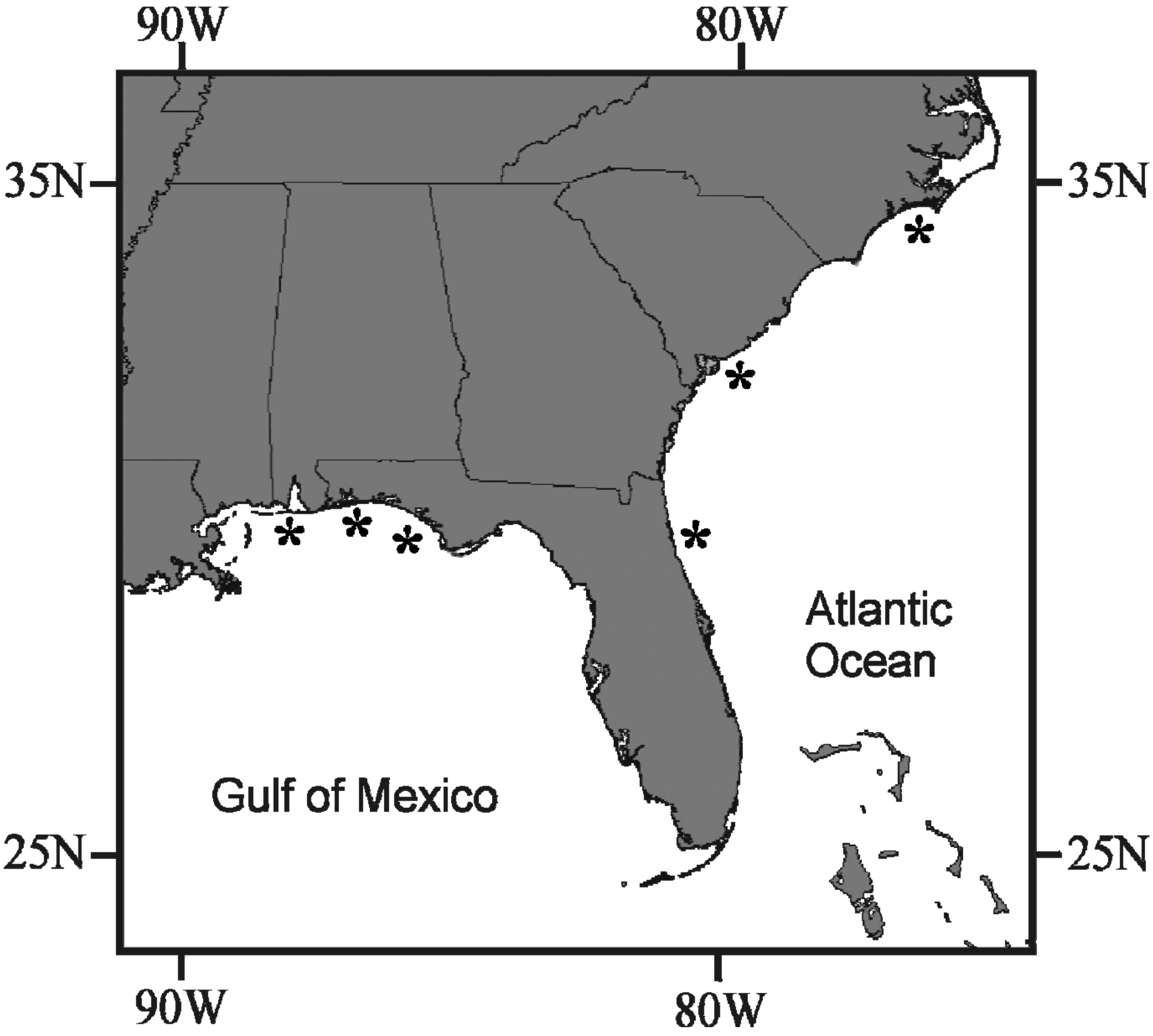

Figure 4. Digital image of a pair of king mackerel sagittae. The right sagitta (bottom) was extracted and stored with its rostrum intact. The left sagittae (top) had its rostrum broken during extraction or subsequent storage.

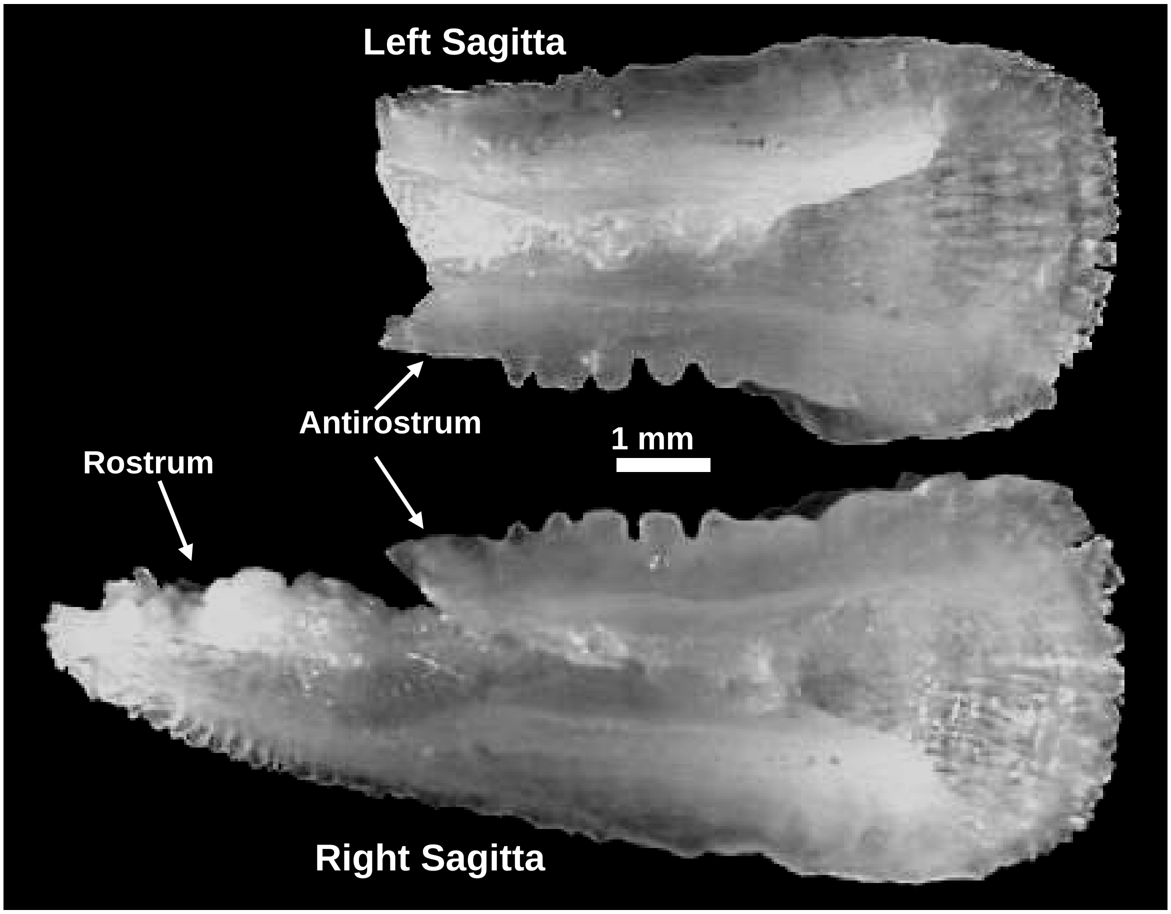

Figure 5. Map of three zones around south Florida where king mackerel were sampled from commercial and recreational landings in winter 2001/02 and 2002/03.

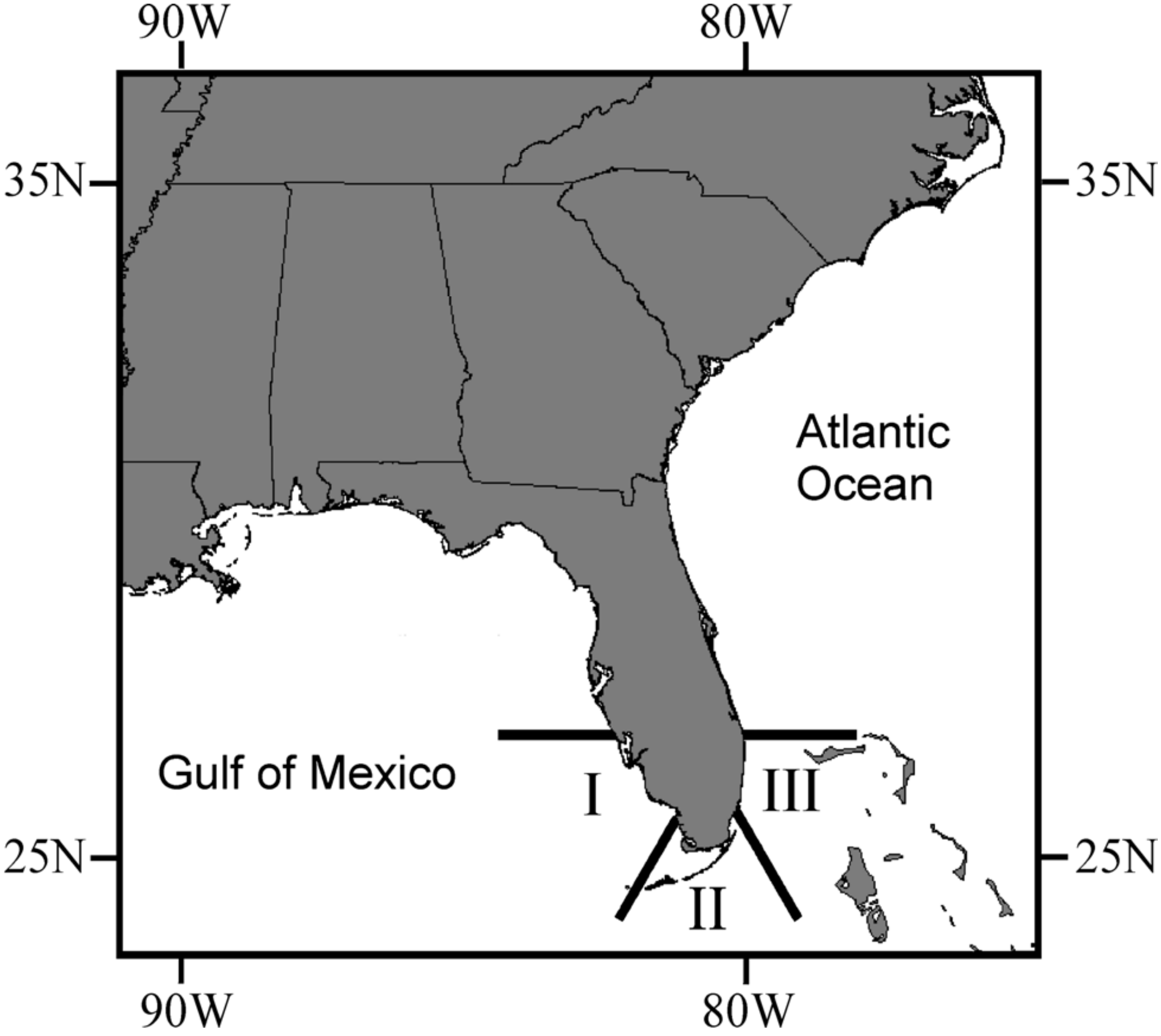

Figure 6. Fork length distributions and sample sizes of king mackerel sampled in the Gulf of Mexico and U.S. south Atlantic Ocean in summer 2001 and 2002.

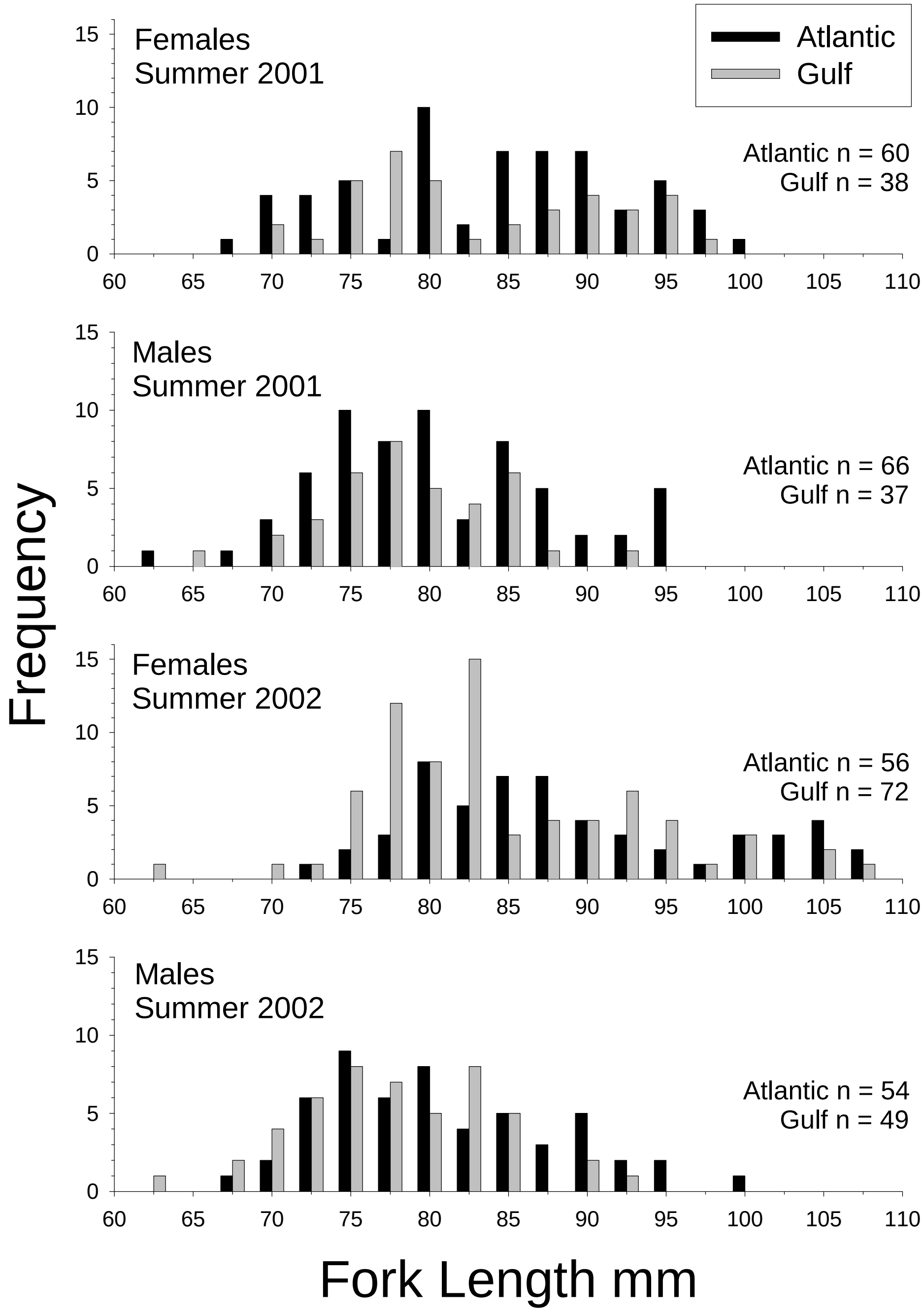

Figure 7. Age distributions of king mackerel sampled in the Gulf of Mexico and U.S. south Atlantic Ocean in summer 2001 and 2002.

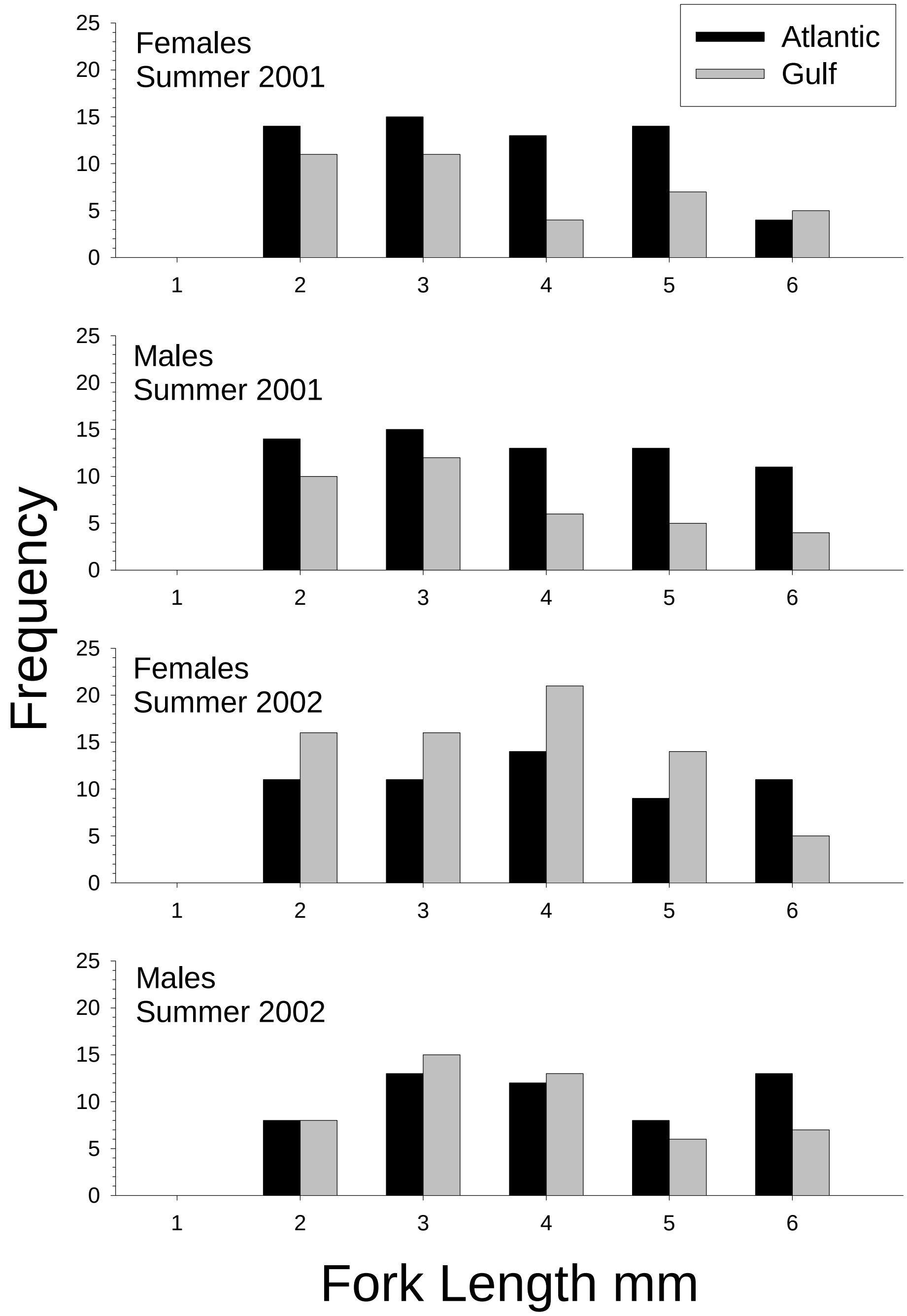

Figure 8. Fork length distributions and sample sizes of king mackerel sampled from three zones around south Florida in winter 2001/02 and 2002/03.

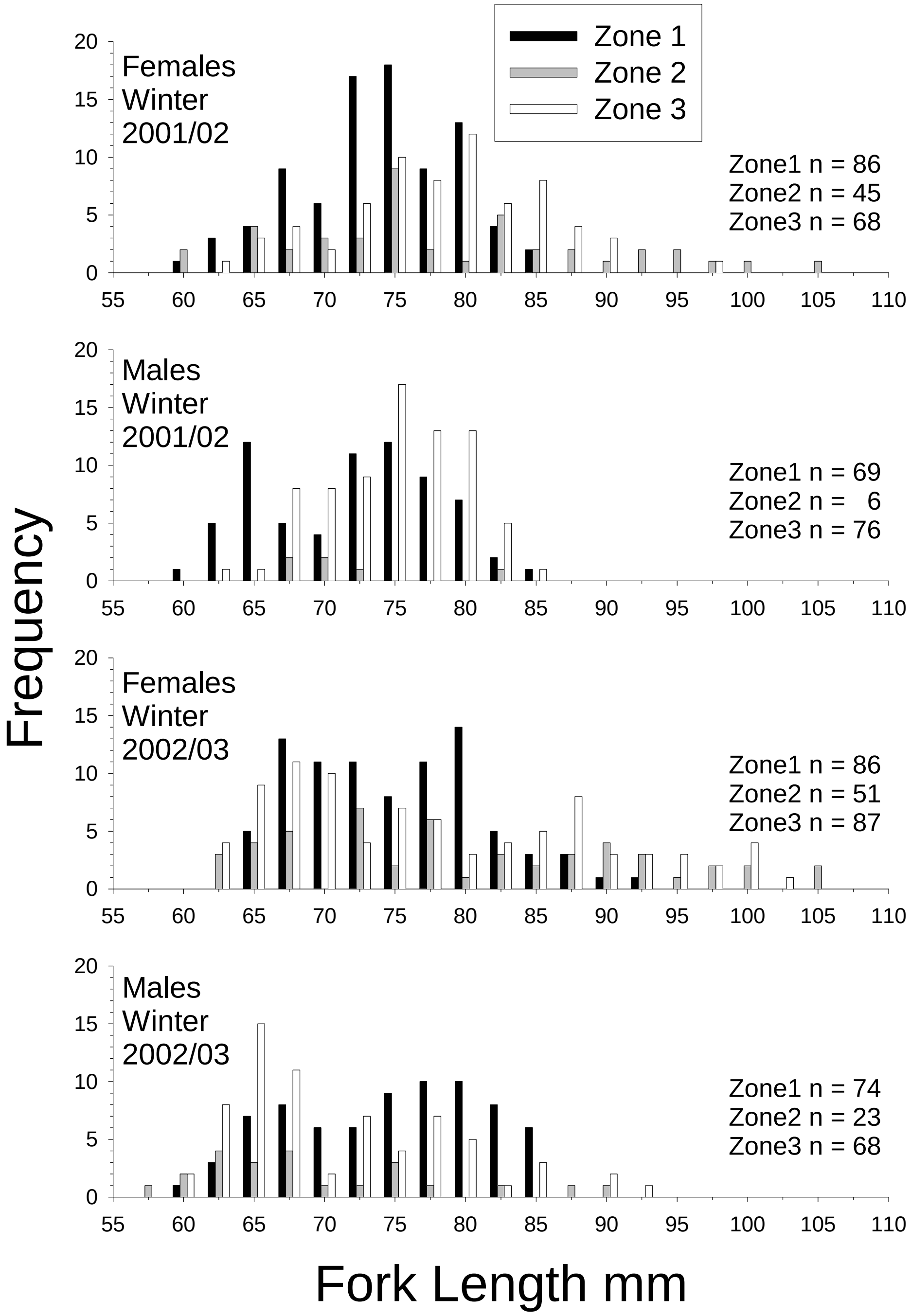

Figure 9. Age distributions of king mackerel sampled from three zones around south Florida in winter 2001/02 and 2002/03.

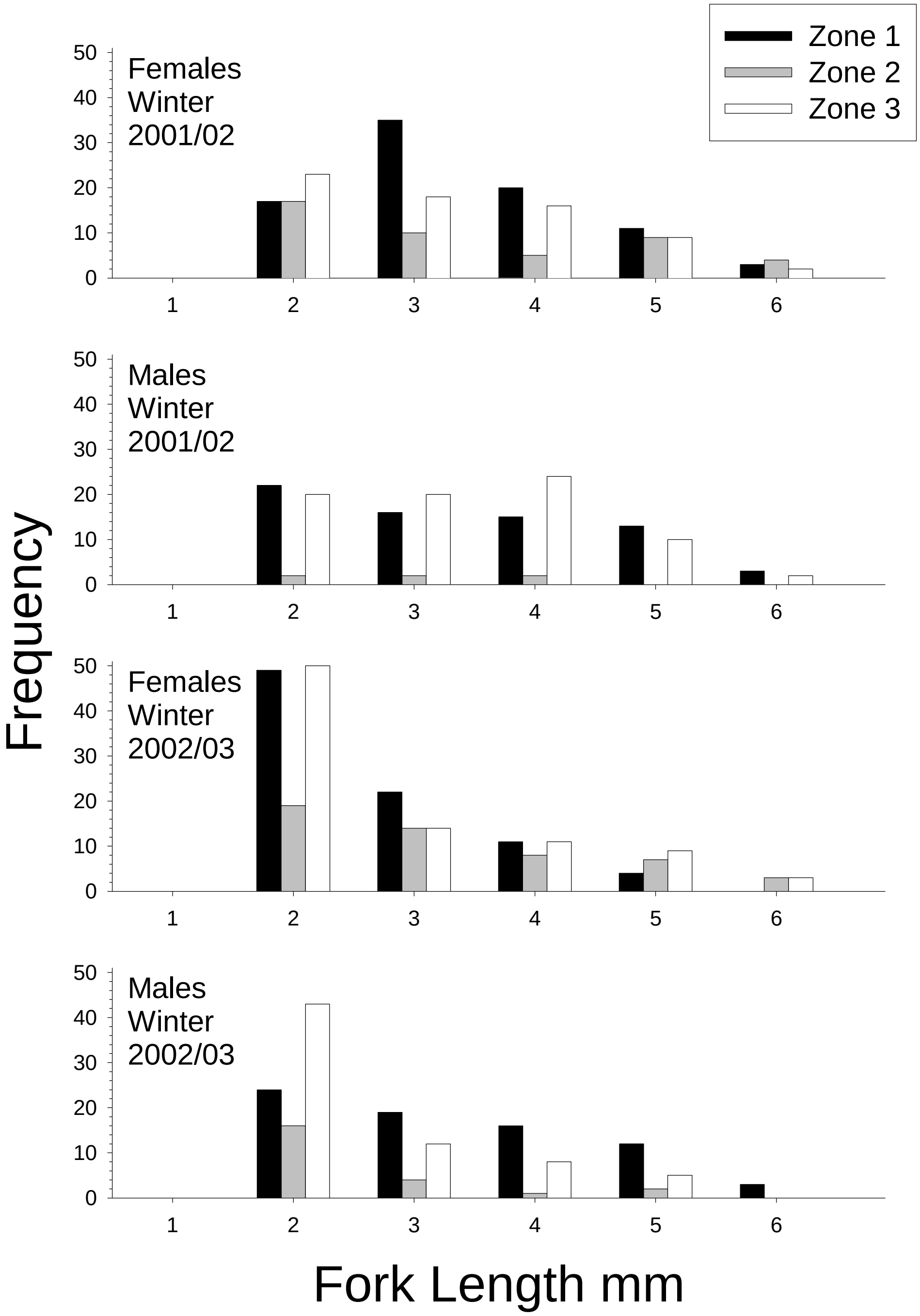

Figure 10. A) Elemental concentrations of king mackerel otoliths sampled from the Gulf of Mexico and Atlantic Ocean during summer 2001 and 2002. B) Year-specific residuals of summer 2001 and 2002 otolith elemental concentrations.

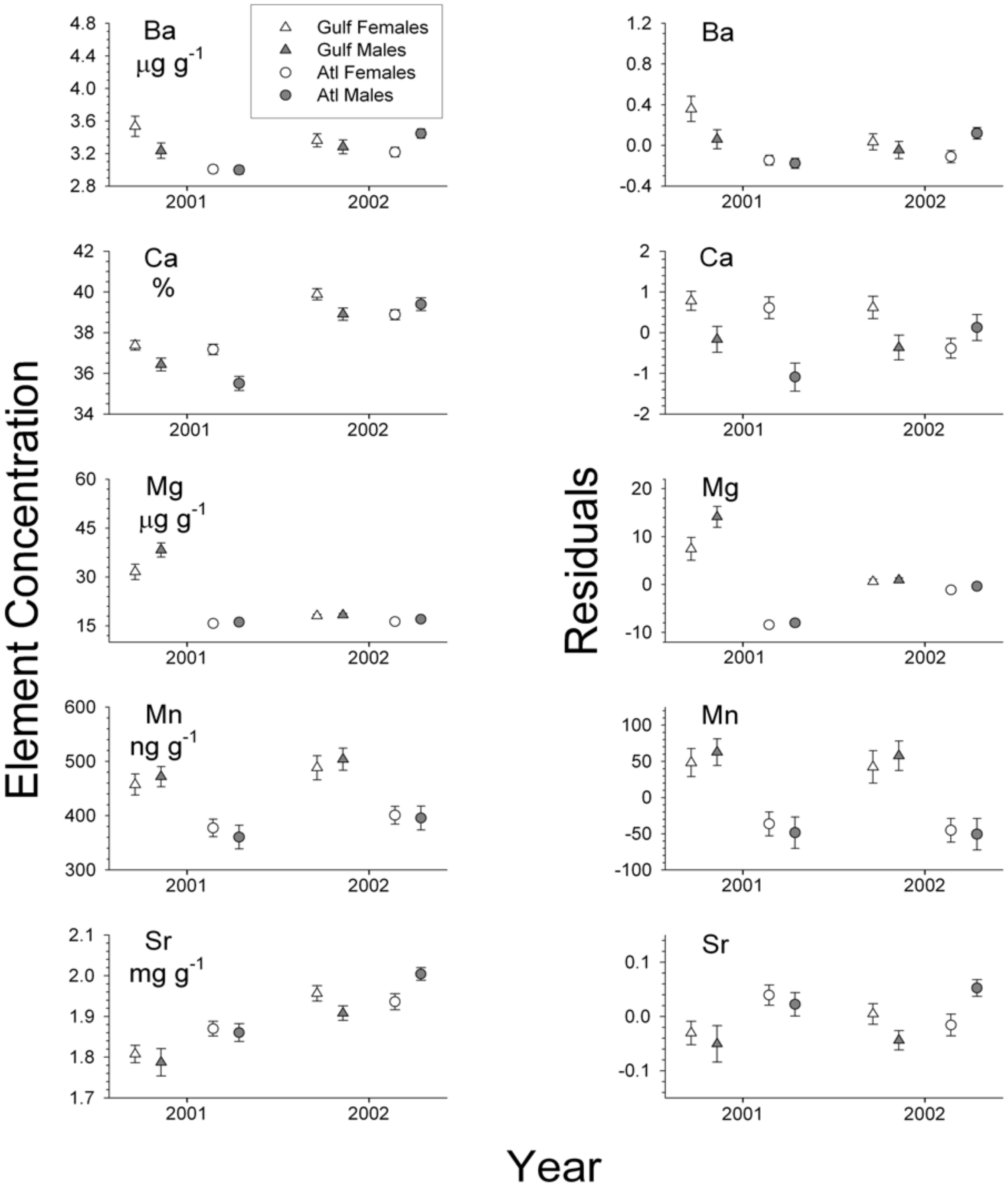

Figure 11. A) Elemental concentrations of king mackerel otoliths sampled from three zones around south Florida during winter 2001/02 and 2002/03. B) Year-specific residuals of winter 2001/02 and 2002/03 otolith elemental concentrations.