King mackerel population dynamics and stock mixing in the United States Atlantic Ocean and Gulf of Mexico

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KING MACKEREL POPULATION DYNAMICS AND STOCK MIXING IN THE UNITED STATES ATLANTIC OCEAN AND GULF OF MEXICO

by

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ABSTRACT

KING MACKEREL POPULATION DYNAMICS AND STOCK MIXING IN THE U.S. ATLANTIC AND GULF OF MEXICO

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Atlantic Ocean (Atlantic) and Gulf of Mexico king mackerel, *Scomberomorus cavalla*, populations are genetically distinct but have overlapping winter ranges around south Florida. This study was designed to estimate growth functions for each population using fishery-dependent and -independent data, and to evaluate spatial and temporal variability in the Atlantic population's contribution to mixed-stock winter landings in 2006/07 and 2007/08. Estimated von Bertalanffy growth functions were significantly different between sexes and populations (χ^2, $p<0.01$). Analysis of otolith shape and stable isotope ($\delta^{13}C$ and $\delta^{18}O$) chemistry in summer-sampled king mackerel revealed significant sex and stock effects (MANOVA, $p<0.001$). Discriminant function analysis conducted with otolith shape or stable isotope ratios as classification variables produced average stock-specific jackknifed classification success rates of 65.8% and 79.3%, respectively. Maximum likelihood estimates from otolith shape- and stable isotope-based models of the Atlantic population's contribution to mixed-stock winter landings among three south Florida winter sampling zones indicated a spatial gradient existed, with the lowest Atlantic contribution off southwest Florida and the highest off southeast Florida. Monthly estimates of Atlantic contribution to the eastern-most zone increased from December through March, possibly reflecting the northward spring migration of both populations.

INTRODUCTION

King mackerel (*Scomberomorus cavalla*) is a large, coastal pelagic fish that ranges from waters off Massachusetts in the Atlantic Ocean (Atlantic) to northern Brazil, including the Gulf of Mexico (GOM) and Caribbean Sea (Collette and Nauen 1983). King mackerel exploited in United States (US) waters of the Atlantic and GOM were originally believed to constitute a single stock (Sutter et al. 1991). However, tagging efforts in the 1970s and 1980s provided evidence for three distinct migratory groups (Figure 1), with one spawning in summer off the mid-Atlantic coast and two spawning in the northern GOM (Sutter et al. 1991). Western GOM fish migrate south along the Texas coast in late fall and winter, while eastern GOM fish migrate south along the west Florida shelf and mix with Atlantic fish around southern Florida in winter. Analysis of mitochondrial DNA (mtDNA) and nuclear DNA microsatellites suggested the existence of genetically distinct Atlantic and GOM populations (Gold et al. 1997, 2002), but no significant genetic difference was found between the eastern and western GOM migratory groups.

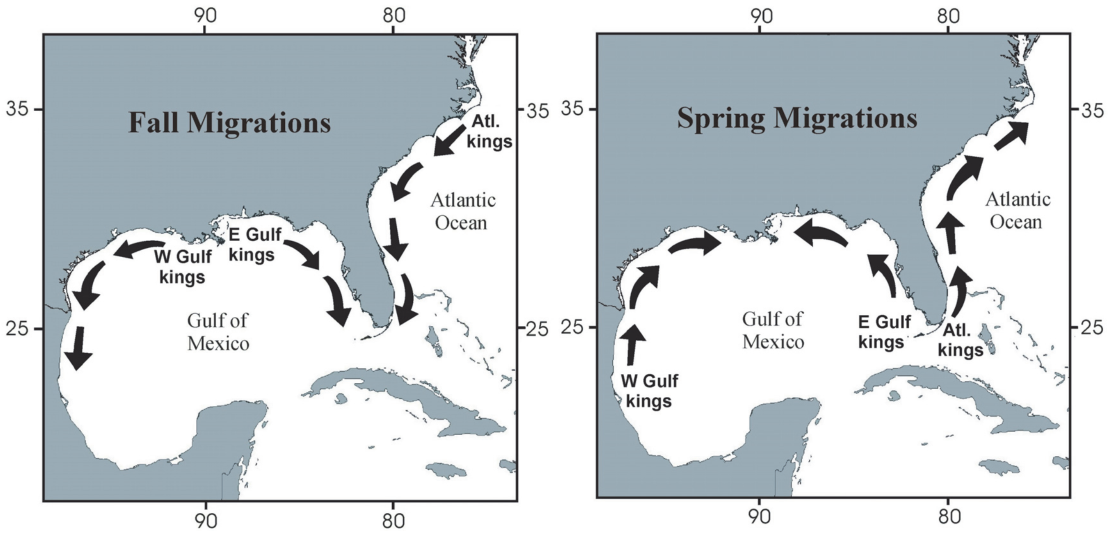

Figure 1. Map of the Gulf of Mexico and US South Atlantic Ocean indicating idealized fall and spring migratory pathways of three king mackerel, *Scomberomorous cavalla*, migratory groups (western Gulf of Mexico, eastern Gulf of Mexico, and Atlantic Ocean) inferred from historic tagging data.

Important commercial and recreational fisheries exist for king mackerel throughout their range in US waters. Fisheries management is complicated for eastern GOM and Atlantic fish, however, due to high catch levels off south Florida during winter when those migratory groups are mixed. Thus, the South Atlantic and Gulf of Mexico Fishery Management Councils (SAFMC and GMFMC) have managed eastern GOM and Atlantic king mackerel jointly under the Coastal Migratory Pelagics Fishery Management Plan (CMPFMP) since 1983. In 1985, Amendment 1 to the FMP recognized the existence of separate GOM and Atlantic migratory groups and established a winter mixing zone for management purposes (GMFMC and SAFMC 1985). The boundary between the two populations is assumed to be the Volusia-Flagler County line from November to March and the Monroe-Collier County line from April to October (Figure 2). All fish caught from November to March in the region between these two lines (i.e., the winter mixing zone) have been attributed to the GOM population. This convention was originally adopted in the 1980s such that conservative winter catch allocations could be assigned to protect the GOM population. However, simulations conducted by Legault (1998) indicated that assigning all winter mixing zone fish to the GOM population actually resulted in overestimates of its biomass and health. The most recent stock assessment (SEDAR16 2008) addressed this issue by assigning 50% of winter landings to each population.

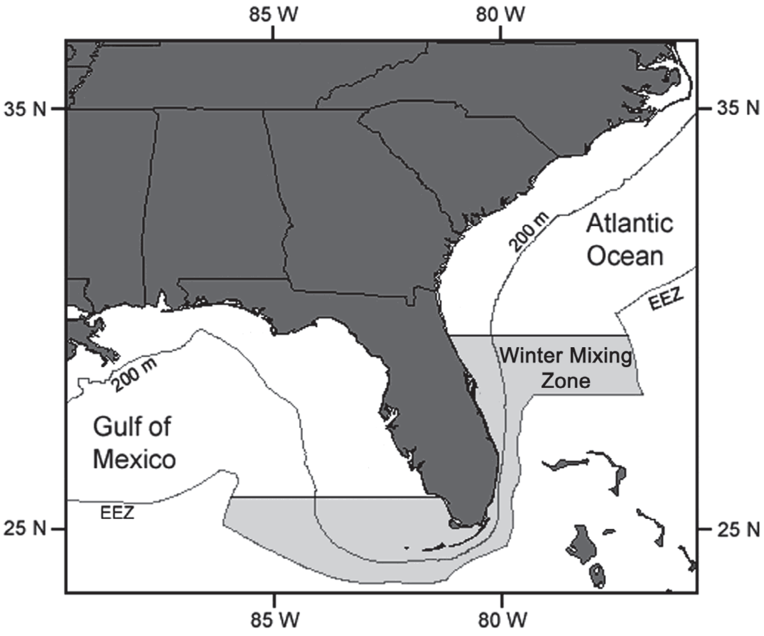

Figure 2. Map of the southeastern USA that indicates the winter mixing zone specified for king mackerel, *Scomberomorous cavalla*, within the Coastal Pelagics Management Plan. The seaward boundary is the edge of the exclusive economic zone (EEZ), but most fish occur and are caught over the shelf (200 m isobath).

The GOM population historically was fished at a higher rate than the Atlantic population, with landings serially exceeding catch limits until 1997, long after the populations came under management (Powers 1996; SEDAR5 2004). In 1998, the GOM population was estimated to be below its minimum stock size threshold (MSST), which is the biomass level below which a stock is estimated to be overfished (GMFMC and SAFMC 1998; Ortiz 2004)[1]. In 2001, the GOM population was estimated to be above the MSST, thus was reclassified as not overfished (SEDAR 5 2004). The most recent stock assessments indicated the GOM spawning stock biomass has increased dramatically since 2001 and that the Atlantic population has declined, but neither stock is currently estimated to be overfished (SEDAR16 2008).

The 2004 stock assessments of Atlantic and GOM king mackerel conducted by NOAA Fisheries identified several data needs for more effective assessment of king mackerel populations in US waters. The most significant needs were more precise estimates of the Atlantic stock's contribution to mixed winter landings and estimates of the spatial and temporal variability in Atlantic stock contribution to landings in the winter mixing zone. Also identified was the need for population-specific von Bertalanffy growth functions (VBGFs) fitted to fishery-dependent and -independent samples (SEDAR5 2004).

Reliable estimates of the relative contribution of each population to the mixed winter landings are essential for accurate stock assessment and effective fisheries management. Historic tagging studies suggested the existence of distinct migratory groups but were not designed to estimate population mixing rates (Sutter et al. 1991). Genetic differences found between GOM and Atlantic fish, while statistically significant, suggest only weak divergence between populations (Gold et al. 1997, 2002).

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[1] The MSST for GOM king mackerel is defined as the biomass capable of supporting maximum sustainable yield (B_{MSY}) reduced by natural mortality (M) (MSST = (1-M)*B_{MSY}), with a 30% spawning potential ratio adopted as the proxy for B_{MSY} for GOM king mackerel (GMFMC and SAFMC 1998)

Calculating mixing rates with a weak population-specific marker would result in an estimated contribution of roughly 50% from each population. This is consistent with the results achieved when attempting to distinguish winter landings from south Florida with genetics markers indicating they are not useful as a natural tag in estimating mixing rates (Gold et al. 2002). Analysis of otolith shape and otolith chemistry have shown promise as natural markers in discriminating between GOM and Atlantic king mackerel, thus suggesting these methods can be useful for studying mixing rates (DeVries et al. 2002; Clardy et al. 2008)

Otolith Applications

Otoliths are aragonite and protein structures found in the vestibular system of all bony fishes. They are metabolically inert once formed, and their rate of deposition varies seasonally with periods of slow and rapid growth. This leads to the formation of discrete opaque and translucent zones, with each opaque zone representing one year of life in adults (Campana and Neilson 1985). This property has resulted in otoliths being the most commonly used hard part in aging fish, with age determined by counting the opaque zones.

King mackerel are sexually dimorphic with females exhibiting higher growth rates and reaching larger sizes than males (DeVries and Grimes 1997). Females can reach sizes of at least 158 cm fork length (FL) in the GOM and 152 cm FL in the Atlantic, while males reach at least 127 cm and 121 cm FL in the GOM and Atlantic, respectively (DeVries and Grimes 1997). Longevity is consistent between the sexes with maximum reported ages of 26 in the Atlantic and 24 in the GOM (DeVries and Grimes 1997).

Age estimates have been used with length data to calculate von Bertalanfy growth functions (VBGFs) for GOM and Atlantic king mackerel:

$$L_t = L_\infty (1 - e^{-k(t-t_0)}) \quad (1)$$

where L_t = estimated length at age t, L_∞ = asymptotic length, k = growth coefficient, t = age in years, and t_0 = hypothetical age at zero length. Growth functions calculated from king mackerel sampled in 1977-78 and 1986-92 indicated that GOM fish grow significantly faster and reach larger sizes than Atlantic fish for both sexes (DeVries and Grimes 1997). Regional variation in growth rate may be the result of latitudinal effects due to temperature, metabolic effects based on prey availability, or genotypic differences between populations. However, no work has been conducted to examine whether population-specific growth functions have changed over the past 15 years.

Historic king mackerel growth functions were estimated from fishery-dependent age and length data (DeVries and Grimes 1997). Fishery-dependent samples tend to display truncated size and age distributions due to the exclusion of young fish that have not fully recruited to the fishery (Taylor et al. 2005; Lombardi-Carlson et al. 2008). Estimating VBGF parameters without data from the smallest and youngest individuals leads to unrealistic estimates of t_0 (DeVries and Grimes 1997). Including fishery-independent data for the smallest size classes and estimating growth functions based on a truncated size-distribution for fishery-dependent samples should improve estimates of t_0 and produce VBGFs that more accurately reflect population growth, rather than just that of the landed catch.

Managed fisheries can artificially select for slow growth by targeting large, fast-growing individuals due to minimum size limits aimed at protecting small, young fish while maximizing yield per recruit (Conover and Munch 2002). Results from laboratory experiments with Atlantic silverside (*Menidia menidia*) suggest these effects are difficult to reverse even after the selective pressure is removed for several generations (Conover and Munch 2002). Long term declines in population growth rate have been reported for heavily exploited populations of red porgy (*Pagrus pagrus*) and vermilion snapper (*Rhomboplites aurobens*) in the eastern GOM and South Atlantic Bight (Harris and McGovern 1997; Zhao et al. 1997; Hood and Johnson 1999, 2000). A comparison of long-term trends in growth between the chronically overexploited GOM king mackerel population and the Atlantic population, which has never been classified as overfished, would be valuable for evaluating possible evolutionary consequences of high fishing pressure.

Otolith Shape Analysis

A number of techniques exist for deriving natural tags to examine mixing between fish populations that have overlapping ranges. One common method is otolith shape analysis. An otolith's shape is affected by the rate at which it is deposited (Campana and Neilson 1985). The rate of otolith accretion is linked to somatic growth, and variation in otolith morphology between stocks is driven by genotypic and phenotypic effects on growth rates (Smith 1992; Campana and Casselman 1993; Cardinale et al. 2004). Such variation has been used to derive otolith shape-based natural tags for stock discrimination since the early 1950s (Einarsson 1951).

A number of advancements in otolith shape analysis techniques have lead to an increase in the precision and accuracy of shape descriptors. Authors of early studies examined scale and otolith shape by manually measuring linear distances under a microscope and tracing projections of the scale or otolith outline (Jarvis et al. 1978; L'Abee-Lund 1988). Outlines were digitized for analysis by either placing a grid over the traced outline and assigning Cartesian coordinates to a series of points along the contour or by manually tracing the contour with a digitizer, which records its coordinates at a set time interval as it moves along the outline (Jarvis et al. 1978; Casselman et al. 1981). Computers have allowed researchers to automatically trace and digitize otolith outlines from digital images captured with image analysis software integrated with cameras on microscopes, thus enabling more accurate representations of the otolith for measurement (Campana and Casselman 1993; Galley et al. 2006). Image analysis software has dramatically increased the speed of shape analysis with automated features that calculate a number of linear measurements, shape indices, and Fourier series harmonics. (Castonguay et al. 1991; Smith 1992; Begg and Brown 2000)

Otolith shape is most simply described in terms of conventional morphometric variables such as area, perimeter, box width, and box height. Area is calculated for the region inside an otolith contour. Perimeter is the length of the contour traced counter-clockwise from a distinct landmark. Box width and height are defined as the dimensions of an otolith's smallest enclosing rectangle. Shape indices further describe an otolith's morphology by quantifying its general shape. Roundness, circularity, ellipticity, and rectangularity are common indices of morphology (Pothin et al. 2006). Roundness and circularity, as their names suggest, describe how closely an otolith resembles a perfect circle. Ellipticity quantifies the proportionality of an otolith's box width and height, and rectangularity is an index of the difference between the area of an otolith and the area of its smallest enclosing rectangle.

Fourier series harmonics are the most sophisticated method for quantifying otolith shape. Jarvis et al. (1978) first applied Fourier analysis to fish scales in an attempt to address questions about stock mixing, but temporal instability in scale shape limits its utility as a natural tag. Fourier harmonics calculated from a digitized otolith outline, however, can be used to reconstruct otolith shape or to create a natural tag for stock discrimination (Bird et al. 1986; Campana and Casselman 1993).

There are several steps to calculating Fourier harmonics. First, a number of radii of varying lengths are drawn from the calculated centroid of an otolith to coordinates along the contour at regular angular intervals. The radii are then unrolled from a distinct landmark and the radii lengths are plotted against the angle at which they were drawn. A low-frequency cosine wave is fitted to the undulation in radii length. Successive higher-frequency cosine waves are added to the first to explain the radii length undulation in finer and finer detail (Campana and Casselman 1993). Each cosine wave added to the Fourier series is referred to as a harmonic and can be described in terms of its amplitude and phase angle. The height of radius R at polar angle θ is calculated:

$$R(\theta) = A_0 + \sum_{n=1}^{\infty} A_n \cos(n\theta - \phi_n) \tag{2}$$

where θ is the angle from the chosen landmark, A_0 is the amplitude of the 0th harmonic (the height of the mean radius), A_n is the amplitude of the nth harmonic, and ϕ_n is the phase angle of the nth harmonic (Bird et al. 1986). Phase angles are difficult to normalize for use in statistical comparisons and, therefore, typically excluded from analysis (Campana and Casselman 1993).

Otolith shape analysis is a simple and commonly used method to derive natural tags in stock discrimination studies. Galley et al. (2006) distinguished Atlantic cod *Gadus morhua* caught in Moray Firth from other spawning groups in the North Sea with >70% classification success. Pothin et al. (2006) correctly classified >90% of juvenile yellowstripe goatfish *Mulloidichthys flavolineatus* to their island of origin in the Southwest Indian Ocean. Cardinale et al. (2004) reported success rates between 79% and 85% discriminating between Atlantic cod from Faroe Bank and Faroe Plateau based on otolith shape.

DeVries et al. (2002) and Clardy et al. (2008) examined the problem of mixed-stock king mackerel fisheries in south Florida during winter by using otolith shape analysis to distinguish Atlantic from GOM fish. The authors of both studies sampled fish in summer when stocks were separated and then estimated how accurately otolith shape parameters distinguished GOM from Atlantic fish from the results of linear discriminant function analysis. DeVries et al. (2002) reported 78% of GOM females and 71% of Atlantic females were correctly classified to their population of origin. Clardy et al. (2008) correctly classified 66%-76% of fish (both males and females sampled) to their sample region, with highest classification success resulting from sex-specific discriminate functions.

DeVries et al. (2002) and Clardy et al. (2008) measured the first twenty Fourier harmonics for each otolith and derived natural tags from the harmonics with the greatest difference between populations. Lower order harmonics (1-10) describe gross otolith morphology, while the higher harmonics (>10) describe fine detail of the contour (Bird et al. 1986; Campana and Casselman 1993). DeVries et al. (2002) reported that higher order harmonics were more useful in stock discrimination of king mackerel than lower order harmonics; however, Clardy et al. (2008) found both low and high order harmonics, as well as perimeter length, circularity and rectangularity, useful in estimating king mackerel mixing rates.

Both studies applied the natural tags derived from otolith shape analysis to estimate the stock composition of fish landed off south Florida in winter. DeVries et al. (2002) reported that the Atlantic population contributed 99.8% of females landed in southeast Florida during the 1996/97 fishing season. Clardy et al. (2008) divided the winter mixing area into three zones and reported that otolith shape analysis indicated a gradient in Atlantic contribution with a lower percentage of Atlantic fish landed in the west and a higher percentage in the east. This trend was consistent between sexes and years, but the estimated Atlantic contribution decreased between the 2001/02 and 2002/03 fishing seasons. The estimated Atlantic contribution to female landings caught off southeastern Florida was 82.8% in 2001/02 and 40.4% in 2002/03, both of which are lower than the 99.8% estimated by DeVries et al. (2002) for the 1996/97 fishing year.

Otolith Stable Isotope Analysis

Otolith carbon and oxygen stable isotope analysis also has been successfully used in stock delineation (Edmonds and Fletcher 1997; Edmonds et al. 1999; Newman et al. 2000) and to estimate mixing rates between populations with overlapping ranges (Thorrold et al. 1998; Rooker and Secor 2004). Carbon isotopes from muscle tissue have been used for decades to estimate trophic position, and oxygen stable isotopes in otoliths and other aragonitic structures (e.g., foraminifera and corals) are commonly analyzed in temperature reconstruction studies (McConnaughey and McRoy 1979; Patterson 1999). However, the application of otolith stable isotope analysis in studying fish population structure is a promising tool that has only developed within the last decade.

The abundance of heavier to lighter stable isotopes varies with environmental conditions for carbon and oxygen (Kennedy et al. 1997; Bastow et al. 2002). This creates regionally distinct isotopic signatures in separate water masses. Fish incorporate carbon and oxygen from the water into the otolith matrix; however, the isotope ratio is altered in an otolith in response to a number of internal and external conditions (e.g. temperature, diet, and metabolic rate) (Kalish 1991; Thorrold et al. 1997). Many complex factors contribute to the carbon and oxygen isotopic composition of a given otolith, but if stable isotope ratios of fish from geographically separate populations are distinct, absolute knowledge of the source of variation is not necessary to use the chemical signature as a natural tag (Edmonds et al. 1999; Newman et al. 2000; Bastow et al. 2002).

Stable isotope ratios are measured using isotope ratio mass spectrometry (IR-MS) and reported as $\delta^{x}E$, where x is the mass number of the heavier isotope and E is the elemental symbol. The resulting δ value describes the ratio of heavier to lighter isotopes relative to the ratio found in a standard.

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) x 1000 \qquad (3)$$

where R is the isotope ratio (Kalish 1991). A sample is said to be enriched relative to a second sample if its δ value is lower than the second sample and depleted if its δ value is higher than the second sample .

Oxygen stable isotopes are incorporated in an otolith in equilibrium with the $\delta^{18}O$ of the surrounding water, although they do fractionate somewhat with water temperature (Kalish 1991; Thorrold et al. 1997). This makes understanding the source of variation in otolith $\delta^{18}O$ straightforward. Differences in otolith $\delta^{18}O$ values are caused by differences in water $\delta^{18}O$, water temperature, or both. Lighter ^{16}O isotopes evaporate more readily than ^{18}O, thus water masses with high evaporation rates tend to be isotopically enriched. Freshwater is often depleted in ^{18}O, creating a positive correlation between water $\delta^{18}O$ and salinity (Thorrold et al. 1997; Bastow et al. 2002).

Temperature drives $\delta^{18}O$ fractionation making otolith $\delta^{18}O$ signatures most useful for stock discrimination between populations that inhabit areas with different mean water temperatures (Edmonds and Fletcher 1997). Sea surface temperatures vary strongly with latitude, and the northern limits of the GOM and Atlantic king mackerel populations are distinct. GOM fish migrate to summer spawning grounds in the northern GOM at approximately 30°N latitude, while there is no land barrier preventing Atlantic king mackerel from migrating farther north into cooler waters.

Carbon stable isotope fractionation and incorporation into otoliths is more complex than oxygen. Carbon isotopes are correlated with $\delta^{13}C$ of the dissolved inorganic carbon (DIC) in the ambient water but with a high degree of variability (Thorrold et al. 1997; Begg and Weidman 2001). This disequilibrium is caused by incorporation of metabolically derived carbon into otoliths (Kalish 1991; Soloman et al. 2006). Trophic enrichment of $\delta^{13}C$ yields carbon values that are enriched as trophic level increases. The deposition of carbon obtained from prey items causes otolith $\delta^{13}C$ to be higher than one would expect if it were derived solely from DIC. Otolith $\delta^{13}C$ is negatively correlated with temperature, however. This is likely caused by the relationship between metabolic rate and temperature, because otolith $\delta^{13}C$ is also correlated with somatic growth (Thorrold et al. 1997; Begg and Weidman 2001).

Despite the trophic effects noted above, variation in otolith $\delta^{13}C$ is principally driven by the $\delta^{13}C_{DIC}$ of seawater with some metabolic effects; thus, populations inhabiting distinct water masses and with clear differences in metabolic rate should be distinguishable based on their otolith $\delta^{13}C$ values. GOM and Atlantic king mackerel migrate to separate summer spawning grounds with separate temperature and hydrologic regimes. Also, DeVries and Grimes (1997) demonstrated a difference in growth rate between the two populations. This suggests that $\delta^{13}C$ will likely be useful as a natural tag in US king mackerel populations.

Otolith carbonate has been shown to display ontogenetic changes in carbon and oxygen isotopic composition in some fishes. Shifts in $\delta^{18}O$ are likely the result of size- and age-specific differences in distribution, habitat preference, and migratory pathway that cause fish to come into contact with different water masses (Edmonds and Fletcher 1997; Thorrold et al. 1998; Begg and Weidman 2001). Ontogenetic effects on $\delta^{13}C$ are likely the result of increasing trophic position and changes in metabolic rate with age (Kalish 1991; Solomon et al. 2006). Kalish (1991) estimated that >30% of otolith carbon is metabolically derived, and shifting from a plankton-based diet to a fish-based diet would result in a 3‰ enrichment in the $\delta^{13}C$ of otolith material deposited. Otolith $\delta^{13}C$ has also been shown to exhibit a peak at sexual maturity, suggesting metabolic rate is a significant factor in $\delta^{13}C$ ontogenetic shifts (Begg and Weidman 2001).

Stable isotope signatures have been shown to be highly accurate natural tags. Rooker and Secor (2004) reported classification success rates of 98% using oxygen isotope ratios alone to distinguish eastern Atlantic Ocean from Mediterranean Sea bluefin tuna (*Thynnus thynnus*). Thorrold et al. (1998) reported classification success for weakfish *Cynoscion regalis* to nursery estuaries along the east coast of the US was only 63% with otolith elemental signatures, but increased to 87% when $\delta^{13}C$ and $\delta^{18}O$ values also were modeled as part of otolith chemical signatures. Therefore, king mackerel otolith stable isotope signatures may produce higher classification accuracies than otolith shape signatures, which have been used in the past.

OBJECTIVES AND HYPOTHESES TO BE TESTED

The overall goal of my thesis research is to improve king mackerel growth function estimates using fishery-dependent and fishery-independent data and to examine spatial and temporal variability in stock composition of winter landings in south Florida. The first aspect of my study involves examining age and growth in GOM and Atlantic king mackerel caught in the 2006 and 2007 summer spawning seasons compared to historic, fishery-dependent age and growth data. Historic growth functions exist in the literature from king mackerel collected between 1977 and1992 (DeVries and Grimes 1997). I update king mackerel growth analysis with fishery-dependent and fishery-independent samples collected from the eastern GOM and Atlantic populations during the 2006 and 2007 summer spawning seasons. Furthermore, I test for significant shifts in king mackerel growth functions since the early 1990s. With current and historic sex-specific VBGFs for each population I test the following hypotheses:

$H_{0,1}$: There is no difference between male and female VBGFs for each population.

$H_{A,1}$: Sex-specific differences in growth suggest it is more appropriate to model growth separately for each sex.

$H_{0,2}$: There is no difference between GOM and Atlantic VBGFs for each sex.

$H_{A,2}$: Distinct population-specific growth functions reflect differences in genetics and migration patterns between GOM and Atlantic fish.

$H_{0,3}$: There is no difference between current and historic VBGFs for each sex and population.

$H_{A,3}$: An increase in estimates of t_0 reflect an improved VBGF fit for the youngest age classes due to the incorporation of fishery-independent data.

$H_{0,4}$: There is no difference between current and historic mean size-at-age for each sex and population.

$H_{A,4}$: A decrease in size-at-age over time in the GOM population that is not mirrored in the Atlantic indicates a decline in growth rate due to artificial selection from fishing.

Next, I examine stock contribution to winter landings in south Florida with natural, population-specific tags derived from otolith shape and chemistry. I use these tags to estimate the Atlantic contribution to landings from three spatial zones and monthly sampling events during the 2006/07 and 2007/07 winter fishing seasons. I derive otolith shape signatures for each population and use those signatures to parameterize maximum likelihood models to estimate the Atlantic contribution to winter landings in the three mixing zones defined by Clardy et al. (2008) over two fishing seasons. I sampled zone 3 each month from December to March to examine trends in intra-annual variability. I examine not only whether the Atlantic contribution to winter landings is negligible, as it is currently assumed to be by management, but also whether it varies spatially and temporally (both within and between years). I test the following hypotheses:

$H_{0,5}$: There is no difference in otolith shape parameters between sexes.

$H_{A,5}$: Differences in otolith shape between sexes suggest sex-specific mixing models are more appropriate than combined-sex models.

$H_{0,6}$: There is no difference in otolith shape parameters between sampling years.

$H_{A,6}$: Differences between sampling years indicate Atlantic contribution should be estimated for each year separately based on the otolith shape data from only the preceding summer.

$H_{0,7}$: There is no difference in otolith shape parameters between populations.

$H_{A,7}$: Differences between populations indicate otolith shape can successfully be used as a natural tag to build mixing models.

$H_{0,8}$: The proportion of winter landings in each zone and month (in zone 3) attributed to the Atlantic population based on otolith shape is zero.

$H_{A,8}$: There is a significant Atlantic contribution to the winter mixing area that is not being accounted for under the current management regime.

The last aspect of my study was designed to increase the precision of mixing estimates by creating sex- and stock-specific chemical fingerprints from stable carbon and oxygen isotope ratios. I complement the otolith shape-based portion of the study by using the same study design to test the following hypotheses with otolith chemistry data:

$H_{0,9}$: There is no difference in otolith chemical signatures between sexes.

$H_{A,9}$: Differences in otolith chemistry between sexes suggest sex-specific mixing models are more appropriate than combined-sex models.

$H_{0,10}$: There is no difference in otolith chemical signatures between sampling years.

$H_{A,10}$: Differences between sampling years indicate Atlantic contribution should be estimated for each year separately based on the otolith chemistry data from only the preceding summer.

$H_{0,11}$: There is no difference in otolith chemical signatures between populations.

$H_{A,11}$: Differences between populations indicate otolith chemistry can successfully be used as a natural tag to build mixing models.

$H_{0,12}$: The percentage of landings in each zone and month (in zone 3) attributed to the Atlantic population based on otolith chemistry is zero.

$H_{A,12}$: There is a substantial Atlantic contribution to the winter mixing area that is not being accounted for under the current management regime.

METHODS

Sampling

I sampled king mackerel that were landed in the northern GOM (from Dauphin Island, AL to Panama City, FL) and Atlantic (Cape Canaveral, FL to Morehead City, NC) during three successive summer spawning seasons (2006, 2007, and 2008 from May to October) when GOM and Atlantic populations were separated. Data from summer-sampled fish can be used reliably to represent each population with negligible mixing; thus, are used here to estimate growth functions and derive stock-specific natural tags. I measured FL to the nearest millimeter (all subsequent references herein will be in those units), determined sex macroscopically and removed both saggital otoliths for each individual sampled. For the GOM population, juveniles and adults under the legal size limit, as well as legal-sized fish, were caught on fishery-independent research cruises with a permit from NOAA Fisheries to retain undersized fish and approval from the Animal Care and Use Committee to collect live animals (Appendix A). Age-0 and age-1 king mackerel were collected on NOAA Fisheries Southeast Area Monitoring and Assessment Program (SEAMAP) summer and fall trawl surveys. Fish across a wide size range above the legal limit were sampled from recreational charter boat landings, and large fish, which are difficult to obtain using other methods, were sampled at fishing tournaments. Atlantic samples were collected through cooperation with NOAA Fisheries port agents in Morehead City, NC and Cape Canaveral, FL. Under-sized Atlantic samples were collected by a cooperating commercial captain, Ben Hartig, holding a permit from NOAA fisheries to retain sub-legal fish.

The management-designated winter mixing area was divided into three sampling zones (Figure 3). Zone 1 encompassed the southwest coast of Florida. I sampled fish from a commercial gill-net fishery prosecuted in zone 1 in January and landed in Stock Island (Key West), FL. Zone 2 included the Florida Keys and was represented by samples collected in January from recreational charter boat landings in Islamorada, FL. Each month (December-March) individuals from zone 3 off southeast Florida were sampled from commercial handline landings in Jupiter and Cape Canaveral, FL. This study was designed to estimate the Atlantic contribution to landings in each zone rather than true mixing rates between the two populations. Thus, the fishery that produces the bulk of king mackerel landings in each zone was sampled. While differences in gear selectivity between the fisheries may affect estimates of Atlantic contribution, those estimates should reflect the percentage of landings from the Atlantic population.

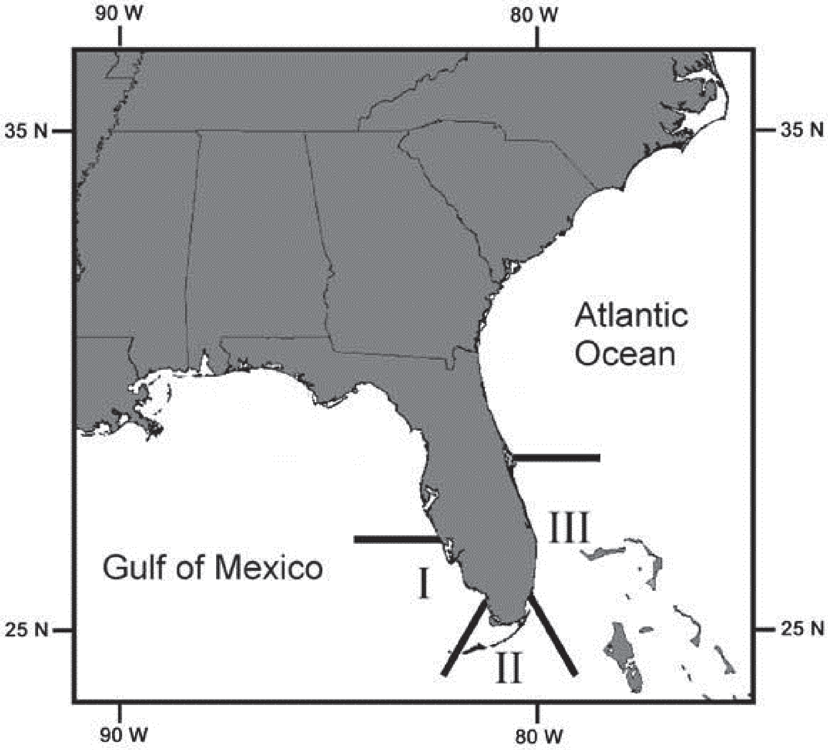

Figure 3. Map of the southeastern United States depicting the three winter zones sampled to examine spatial variability in the percentage of Atlantic Ocean population contribution to south Florida winter king mackerel, *Scomberomorus cavalla,* landings.

Age and Growth

Otolith annuli were counted to determine age (Johnson et al. 1983; DeVries and Grimes 1997). Otoliths from males > 800 mm FL and females > 900 mm FL were sectioned for aging. Otoliths to be sectioned were embedded in epoxy and three thin (~0.5mm) transverse sections were made through the nucleus using a low speed Isomet saw. The sections were fixed to a microscope slide with Cytoseal adhesive and their annuli counted under a dissection microscope at 10x magnification with transmitted light. Otoliths from males < 800 mm FL and females < 900mm FL were immersed in water in an opaque, black dish and aged whole under a dissection microscope at 7x magnification with reflected light. For both whole and sectioned otoliths, the number of opaque zones were recorded and the margin (area between the last opaque zone and the edge of the otolith) was classified as nonexistent, small, average, or large relative to the previous increment (distance between the penultimate and final annuli). The margin was classified as nonexistent if the last opaque zone was on the edge of the otolith. A small margin was less than 1/3 the width of the previous increment. An average margin was 1/3 to 2/3 the width of the previous increment, and a large margin was greater than 2/3 the width of the previous increment.

Annuli count was converted to age based on margin classification with the following algorithm established by the NOAA Fisheries Panama City Laboratory for aging king mackerel. From January 1st to May 31st, age was equivalent to the number of annuli if the margin was nonexistent or small. If the margin was average or large, age was advanced 1 year. From June 1st to July 20th, age was advanced if the margin was large or if it was average and there were more than 2 annuli. From July 21st to December 31st, age was always equivalent to the number of annuli. Integer ages were converted to fractional ages for analysis according to DeVries and Grimes (1997).

To estimate precision between my aging results and that of the NOAA Fisheries Panama City Laboratory, 10% of the otoliths aged were read a second time by one of their experienced technicians. Average percent error (APE) was calculated:

$$APE = \frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{\left|X_{ij} - X_{j}\right|}{X_{j}}\right] \tag{4}$$

where N = number of fish aged, R = number of times each fish was aged, X_{ij} = *i*th age determination of the *j*th fish, and X_j = average age determination of the *j*th fish (Beamish and Fournier 1981). The calculated APE served as a measure of between-reader agreement and aging precision.

Fish age and length data from samples collected between May and November in 2006, 2007, and 2008 were used to fit population- and sex-specific von Bertalanffy growth functions using a maximum likelihood procedure in EXCEL with a truncated size distribution to account for the minimum size limit (609.6 mm) in the fishery-dependent samples (M. Ortiz, NOAA Fisheries, personal communication). The minimum size for fishery-independent samples was set to 0 mm. Differences in growth function parameters were tested between years and populations with likelihood ratio tests (Cerrato 1990). The likelihood ratio χ^2 statistic was calculated:

$$\chi^2 = -N \times \ln\left(\frac{SS_\Omega}{SS_\omega}\right) \qquad (9)$$

where N = combined sample size for the two data sets being tested, SS_Ω = sum of squared residuals when fitting the two VBGFs separately, and SS_ω = sum of squared residuals when fitting a single VBGF to the two data sets combined (Kimura 1980).

Additional tests were conducted between contemporary samples and historic data that were provided by Doug DeVries of NOAA Fisheries. Growth functions from fish collected in the 1970s, 1980s and 1990s were compared with functions computed from fish sampled during the current study. A minimum size limit of 304.8 mm was first enacted on August 20, 1990; therefore, historic samples collected after that date were analyzed with a truncated size distribution to account for sub-legal fish that were caught but not retained in the landings. Samples collected in south Florida between 1986 and 1992 were excluded from analysis due to uncertainty about the origin population.

Analysis of size at age over time was used to test for differences in growth rate between contemporary and historic data over ages displaying linear growth. Mean size-at-age was estimated for ages 2-10 for all sex/stock combinations from data collected during three time periods (1977-1979, 1986-1992, and 2006-2008). Analysis of covariance (ANCOVA) test of equal slopes was used to test for differences in mean size-at-age among the three time periods.

Otolith Shape Analysis

Otolith shape analysis was performed for all summer- and winter-sampled king mackerel with an image analysis system consisting of an Olympus SZX12 dissecting microscope fitted with an Olympus DP71 camera connected to a Dell personal computer running Image Pro (version 6.0) image analysis software. The distal lateral surface of each otolith first was magnified 7x and a digital image captured (Figure 4). Left otoliths were analyzed whenever possible. However, otoliths are fragile, especially the rostrum, and were sometimes broken during extraction. Thus, the right otolith was used and the image reversed whenever the left was damaged (Friedland and Reddin 1994). The rather high frequency of otoliths with broken rostra made it necessary to measure and obtain shape parameters from only the posterior portion of the otolith. (DeVries et al. 2002; Figure 4). The otolith perimeter was digitally traced with the auto trace feature in Image Pro.

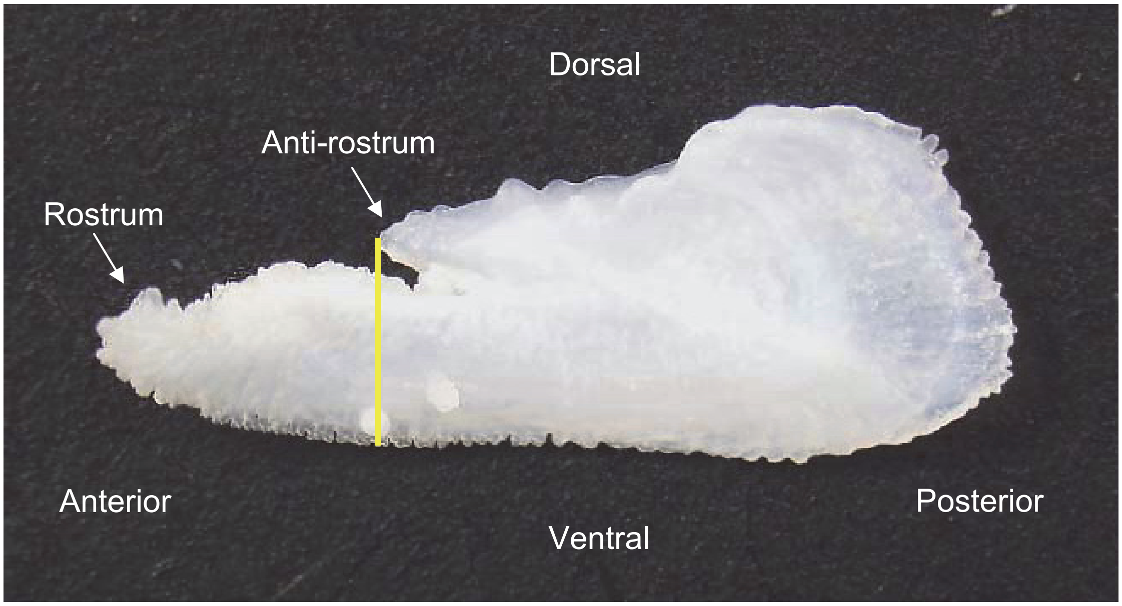

Figure 4. The distal lateral view of a left king mackerel, *Scomberomorous cavalla*, otolith with the area to the right of the line representing the portion used for shape analysis.

Image Pro was used to measure the following gross morphometric parameters automatically: area, perimeter, length, width, and roundness. Those measurements were used to calculate circularity, ellipticity, and rectangularity (i.e., derived parameters), which were calculated as follows:

$$\text{Roundness} = \frac{4\pi * \text{otolith area}}{\sqrt{\text{otolith perimeter}}} \tag{5}$$

$$\text{Circularity} = \sqrt{\frac{\text{otolith area}}{\text{otolith perimeter}}} \tag{6}$$

$$\text{Ellipticity} = \frac{\text{otolith length} - \text{otolith width}}{\text{otolith length} + \text{otolith width}} \tag{7}$$

$$\text{Rectangularity} = \frac{\text{otolith area}}{\text{area of its minmal enclosing rectangle}} \;. \tag{8}$$

The digitized contour of each otolith posterior was used to calculate Fourier series harmonics. ImagePro determined the mathematical centroid and then drew 256 radii at equiangular intervals to the otolith contour to approximate its shape. These radii were used to calculate the first nineteen Fourier harmonics, and ImagePro automatically reported the harmonic amplitudes and mean radius. Mean radius was reported as harmonic 1; therefore, harmonics 2-20 represent the first nineteen harmonics used in analysis.

Otolith shape is affected by the rate of deposition, which is a function of somatic growth. Therefore, fish size and age can be confounding effects in otolith shape analysis and several precautions were taken to account for these effects while conducting otolith shape analysis. Only individuals aged from 2 to 6 years were included because approximately 85% of winter landings are derived from those age classes. All shape parameters were standardized by dividing each parameter by the mean radius (amplitude of the 0^{th} harmonic). Any significant correlation between each parameter and FL was removed by subtracting the product of FL and the slope of the least squares linear relationship from the standardized parameter. After sorting by sampling year, all parameters continued to be significantly correlated with FL ($p<0.001$), thus were detrended to remove variation due to size.

Otolith shape data were used to derive sex- and population-specific natural tags, which were used to parameterize maximum likelihood mixing models to estimate the Atlantic contribution to the mixed winter landings. First, each parameter was standardized to the mean radius and corrected for any correlation with FL, as described above. The data were then tested for sex, year, and population effects with multivariate analysis of variance (MANOVA). Each individual parameter was tested with analysis of variance (ANOVA) to determine whether overall differences were driven by the indices and low-order harmonics (gross morphology) or high-order harmonics (fine-scale variation). Only first order interactions were tested for otolith shape parameters due to limited degrees of freedom. Stepwise year- and sex-specific discriminant functions (DFs) were computed in SAS (Proc STEPDISC; SAS Institute, Inc. 1996) with the 20 harmonic amplitudes and the gross and derived shape parameters of summer-sampled fish serving as dependent variables. Quadratic DFs were computed due to heterogeneity among variance-covariance matrices. Jackknifed population- and sex-specific classification accuracies were computed for resultant models (Proc DISCRIM; SAS Institute, Inc. 1996).

Maximum likelihood stock mixing models were parameterized with the significant shape variables and applied to the mixed winter samples to estimate the percentage of landings contributed by the Atlantic population in each sampling zone (and month for zone 3) (DeVries et al. 2002; Clardy et al. 2008). The standard error (SE) of estimates was calculated from 500 bootstraps. All maximum likelihood modeling was conducted in S-Plus® (version 6.0).

Otolith Chemistry

A sub-sample of otoliths used for shape analysis was randomly selected for chemical analysis. All preparations were conducted under a laminar flow class-10 clean hood to prevent contamination. One otolith from each pair was weighed. Right and left otoliths were not available for all samples, thus one was selected at random from each pair. Thorrold et al. (1997) reported finding no significant difference between the carbon and oxygen isotopic composition of left and right otoliths. An otolith's rostrum was removed if it survived the extraction process intact in order to maintain consistency among samples; otoliths were reweighed following rostrum removal. Otolith samples were cleaned with 1% nitric acid and rinsed with ultrapure water. After drying, otoliths were reweighed to determine the amount of material lost during cleaning.

Clean otoliths were pulverized with acid-leached mortar and pestles. The pulverized material was transferred to a centrifuge tube and stored dry. Stable isotope analysis was conducted with a Finnigan MAT 251 IR-MS by Mr. David Winter at the University of California-Davis. The machine was calibrated daily against the International Atomic Energy Agency's carbonate standard, NBS-19. Accuracy of analytical runs was measured through routine analysis of an in-house check standard which had been stringently calibrated against NBS-19. Results were reported as $\delta^{18}O$ and $\delta^{13}C$ expressed as per mil (‰) relative to the international carbonate standard: Vienna Peedee Belemnite. Following analysis, otolith stable isotope signatures were analyzed with the same statistical techniques described above for the otolith shape data, and estimates of Atlantic stock contribution to winter sampling zones were compared between methods.

Simulations were conducted to examine the effects of absolute difference in stable isotope delta values, interpopulational variance in values, and sample size on the precision and accuracy of population mixing estimates. Five simulated data sets were generated based on the calculated mean and variance of 2006/07 stock-specific stable isotope signatures. In simulation 1, stable isotope values were simulated with a random number generator for 90 Atlantic and 90 GOM samples from a normally distributed population with a mean and standard deviation equal to those of the 2006/07 stock specific samples. In simulation 2, values were simulated similarly to simulation 1 except that distribution means were increased by 1‰ for the Atlantic and decreased by 1‰ for the GOM to increase the absolute difference between population-specific mean values such that there was no overlap in the range of values for each population. In simulation 3, the standard deviations from simulation 1 were halved, and the means were unchanged. Simulation 4 generated data from the same distributions used in simulation 1. However, in simulation 4 sample sizes were increased to 180 individuals from each population. The population-specific data set in simulation 5 was identical to that of simulation 1, but the application of simulated signatures differed as detailed below.

Mixed-stock samples were simulated for hypothetical winter landings by randomly generating data from the population distributions in each simulation. Mixed-stock samples for simulations 1-4 included values simulated for 70 individuals from the Atlantic population and 30 from the GOM population. The simulated mixed-stock values for simulation 5 included 105 Atlantic individuals and 45 GOM individuals. Simulated data were analyzed with statistical methods identical to those employed with the actual 2006/07 and 2007/08 stable isotope data.

RESULTS

Age and Growth

A total of 2,316 king mackerel was sampled for age and growth analysis (Table 1). Samples were collected between May and November in 2006, 2007, and 2008 from the northern GOM and the Atlantic Ocean off the southeastern US. Juveniles from SEAMAP surveys were collected in November. GOM females ranged from 75 to 1,672 mm FL and GOM males from 75 to 1,254 mm FL (Figure 5, panels A and B); Atlantic females from 90 to 1,380 mm FL and Atlantic males from 90 to 1,220 mm FL (Figure 5, panels C and D). Juveniles were defined as sub-legal individuals that could not be sexed macroscopically and were included in the models for both sexes. Maximum observed age was 19, 20, 19, and 23 years for GOM females, GOM males, Atlantic females, and Atlantic males, respectively (Figure 6). The APE computed between my age estimates and the age estimates from NOAA Fisheries personnel for a sub-set of 237 individuals was 3.39 %, which is well below the accepted standard of 5% for between reader aging precision.

Table 1. The Number of Female, Male, and Juvenile King Mackerel, *Scomberomorus cavalla,* Sampled from the Northern Gulf of Mexico (GOM) and Atlantic Ocean Each Year for Age and Growth Analysis from Southeast Area Monitoring and Assessment Program (SEAMAP) Trawls, Fishery-independent Hook-and-line Cruises, and Fishery-dependent Hook-and-line Commercial, Recreational, and Tournament Landings.

	GOM			Atlantic		
	Female	Male	Juvenile	Female	Male	Juvenile
2006						
SEAMAP	1	0	36	0	0	0
Fishery-Ind H&L	17	13	0	0	0	22
Recreational	137	64	2	279	118	0
Tournament	166	28	0	0	0	0
2007						
SEAMAP	0	0	85	0	0	0
Fishery-Ind H&L	86	46	0	0	0	0
Recreational	341	146	1	7	7	0
Tournament	173	43	0	306	120	0
Commercial	0	0	0	10	0	0
2008						
SEAMAP	0	0	62	0	0	0
Total	921	340	186	602	245	22

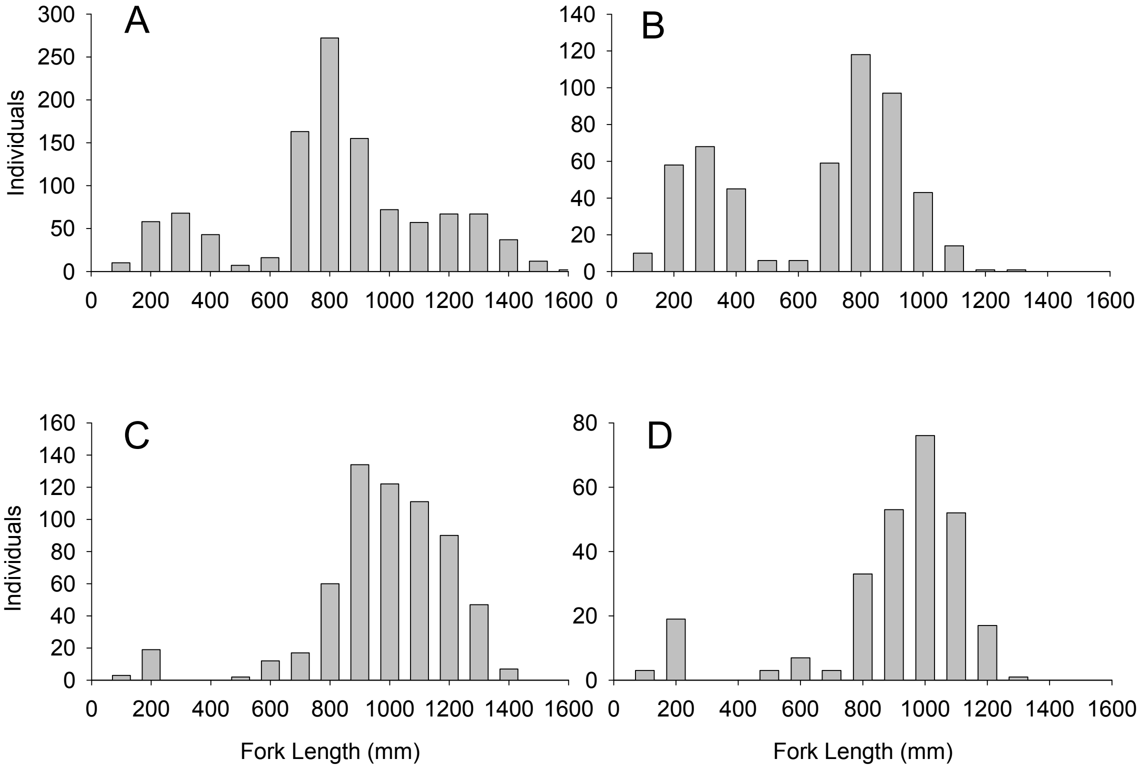

Figure 5. Sex- and stock-specific king mackerel, *Scomberomorus cavalla,* size distributions for samples collected in summer 2006, 2007, and 2008 and used to estimate von Bertalanffy growth functions for A=Gulf of Mexico females, B=Gulf of Mexico males, C=Atlantic Ocean females, and D=Atlantic Ocean males.

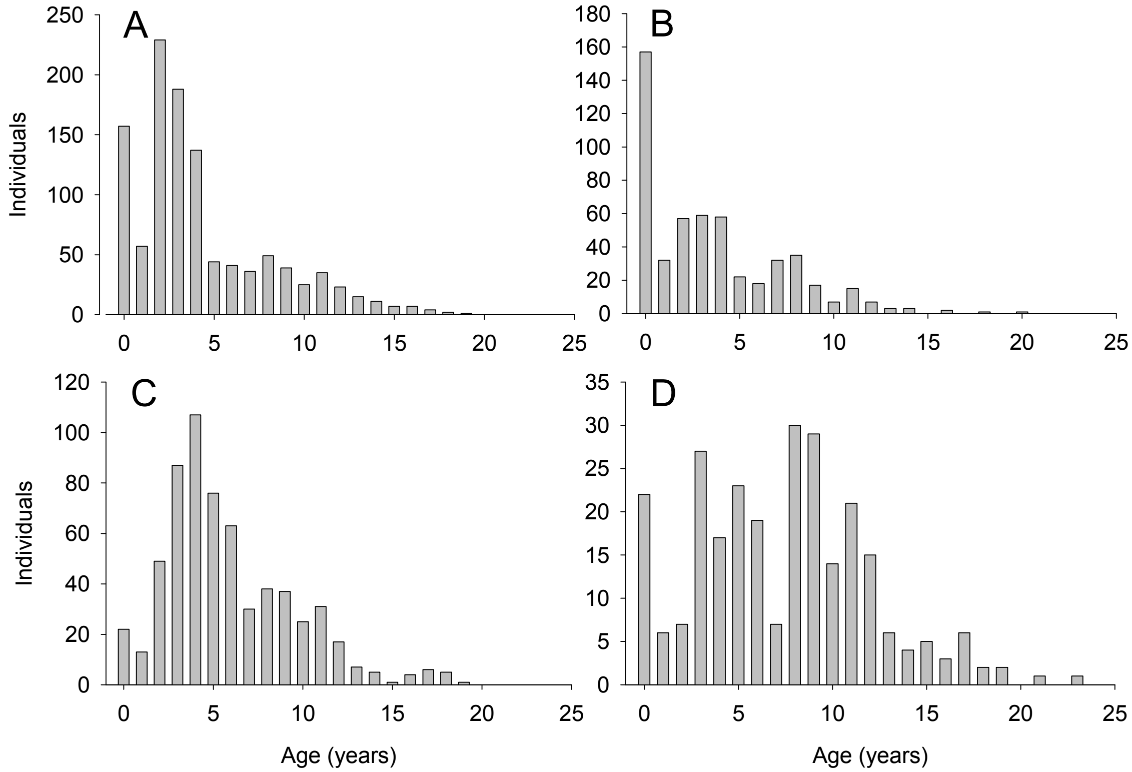

Figure 6. Sex- and stock-specific king mackerel, *Scomberomorus cavalla.* age distributions samples collected in summer 2006, 2007, and 2008 and used to estimate von Bertalanffy growth functions for A=Gulf of Mexico females, B=Gulf of Mexico males, C=Atlantic Ocean females, and D=Atlantic Ocean males.

The fitted VBGFs display clear sex- and stock-specific differences. Females in both populations had higher estimates of L_∞ and lower estimates of k and t_0 than males (Figure 7, Table 2). Females from the GOM had a larger L_∞ and smaller k and t_0 than Atlantic females, while GOM males displayed lower values for L_∞ and t_0 and a higher value for k than Atlantic males. Likelihood ratio tests on the 2006-2008 VBGFs (Figure 7, Table 2) indicated sex and stock effects were significant (Table 3).

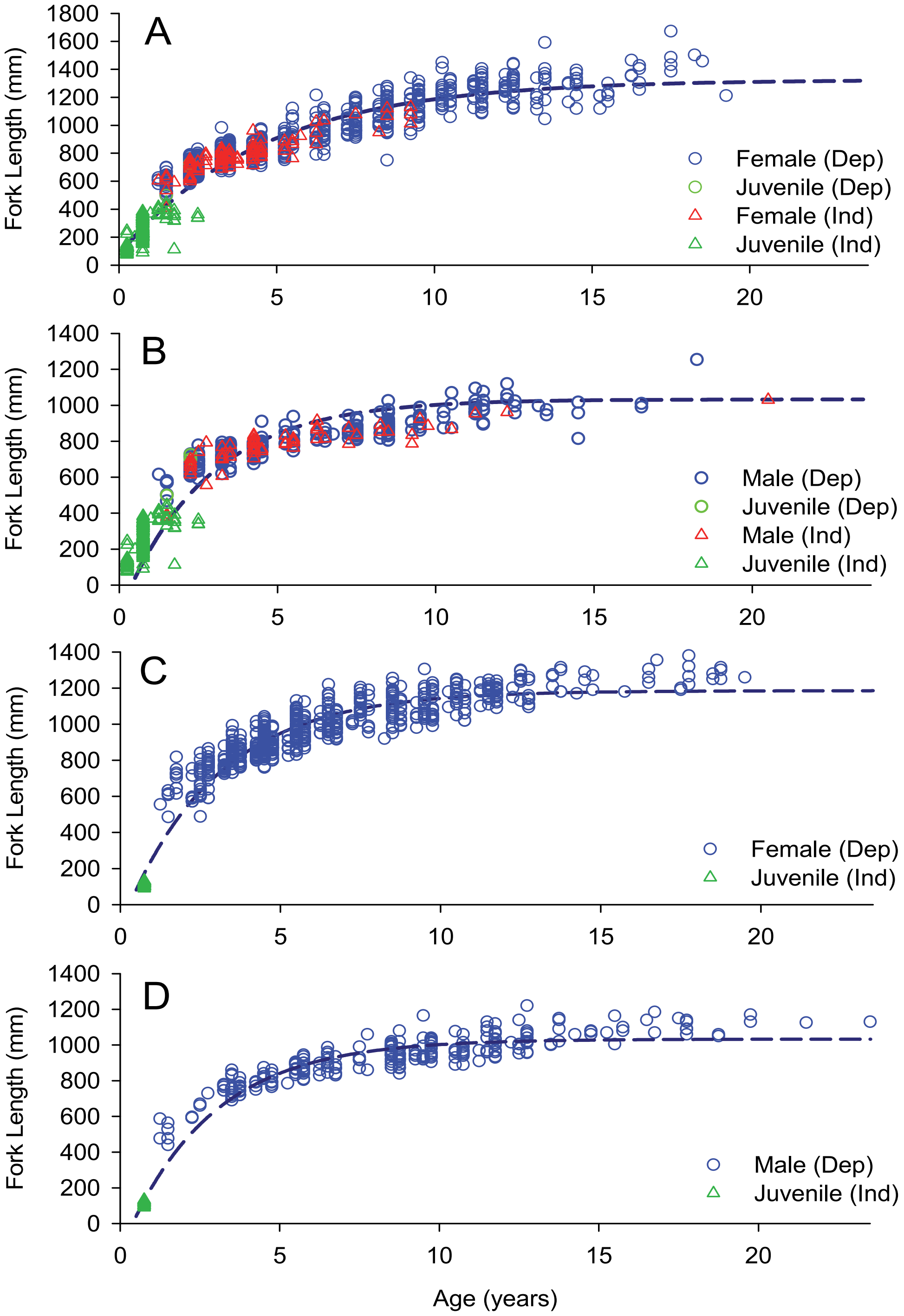

Figure 7. Size at age data for king mackerel, *Scomberomorus cavalla,* sampled during summer 2006, 2007, and 2008 (Dep=fishery-dependent, Ind=fishery-independent). Fitted lines are von Bertalanffy growth functions; function parameters are provided in Table 2. (A=Gulf of Mexico females, B=Gulf of Mexico males, C=Atlantic Ocean females, D=Atlantic Ocean males).

Table 2. Estimated von Bertalanffy Growth Function Parameters for Female (F) and Male (M) King Mackerel, *Scomberomorus cavalla,* Sampled in 2006-2008 from the Gulf of Mexico (G) and Atlantic Ocean (A) Populations.

	GF	GM	AF	AM
n	1107	526	624	267
L_∞	1325.078	949.9535	1185.689	1033.124
k	0.219597	0.372193	0.342373	0.365496
t_0	-0.26653	-0.08307	0.292574	0.392768

Table 3. Results of Likelihood Ratio Tests for Differences in the Estimated von Bertalanffy Growth Functions for Female (F) and Male (M) King Mackerel, *Scomberomorus cavalla,* from the Gulf of Mexico (G) and Atlantic Ocean (A) Populations.

	GF-GM	AF-AM	GF-AF	GM-AM
n	1633	891	1731	793
χ^2	682.7829	331.5726	11.79537	139.1478
df	3	3	3	3
p	<0.001	<0.001	<0.001	<0.001

Residuals from the VBGFs modeling growth for contemporary fishery-dependent data exhibited a distinctly different pattern from that of the fishery-independent samples (Figure 8). Fishery-dependent residuals tended to be positive for the youngest and oldest individuals and neutral or negative for intermediate ages. Fishery-independent samples from the GOM population displayed more evenly distributed residuals for the younger year classes. All Atlantic fishery-independent-residuals were negative.

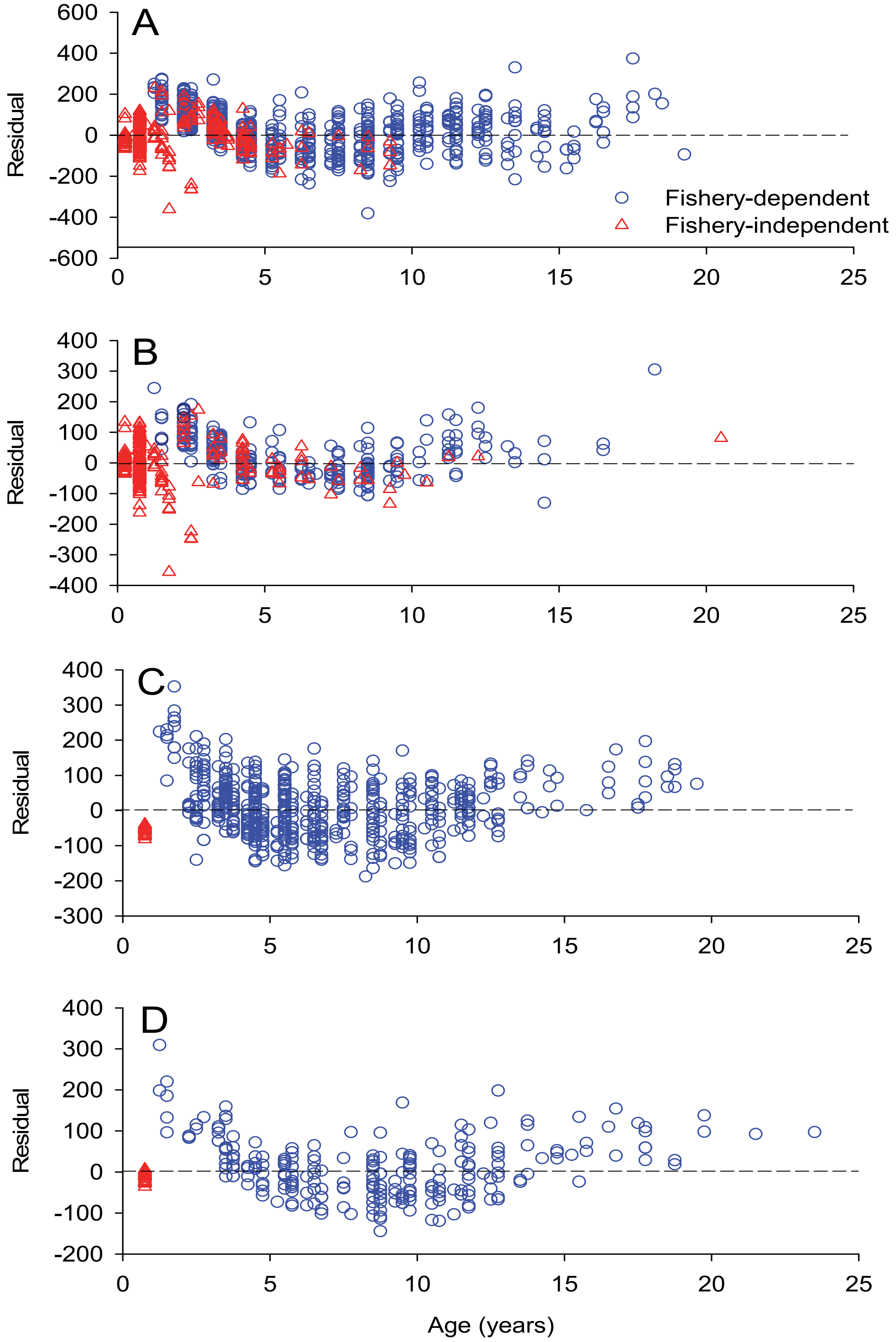

Figure 8. Residual distributions for von Bertalanffy growth functions estimated from king mackerel, *Scomberomorus cavalla,* samples collected from fishery-dependent (blue

circles) and fishery –independent (red triangles) sources for A=Gulf of Mexico females, B=Gulf of Mexico males, C=Atlantic Ocean females, and D=Atlantic Ocean males.

VBGFs fitted to contemporary data displayed increased estimates of t_0 over historic growth functions (Figure 9). Fitting t_0 closer to the origin also resulted in a slight decrease in L_∞ and an increase in the estimated value of k in current versus historic VBGFs except in Atlantic males, which exhibited an increase in both k and L_∞. The ANCOVA results revealed significant or near significant (α=0.05) differences in mean size at age between current and historic data for all sex/stock combinations (GOM female p=0.014, GOM male p=0.089, Atlantic female p=0.022, Atlantic male p=0.005). Mean size-at-age in the GOM is lower in the 2006-2008 samples than the historic data, while contemporary mean size-at-age in the Atlantic appears higher than historic estimates (Figure 10).

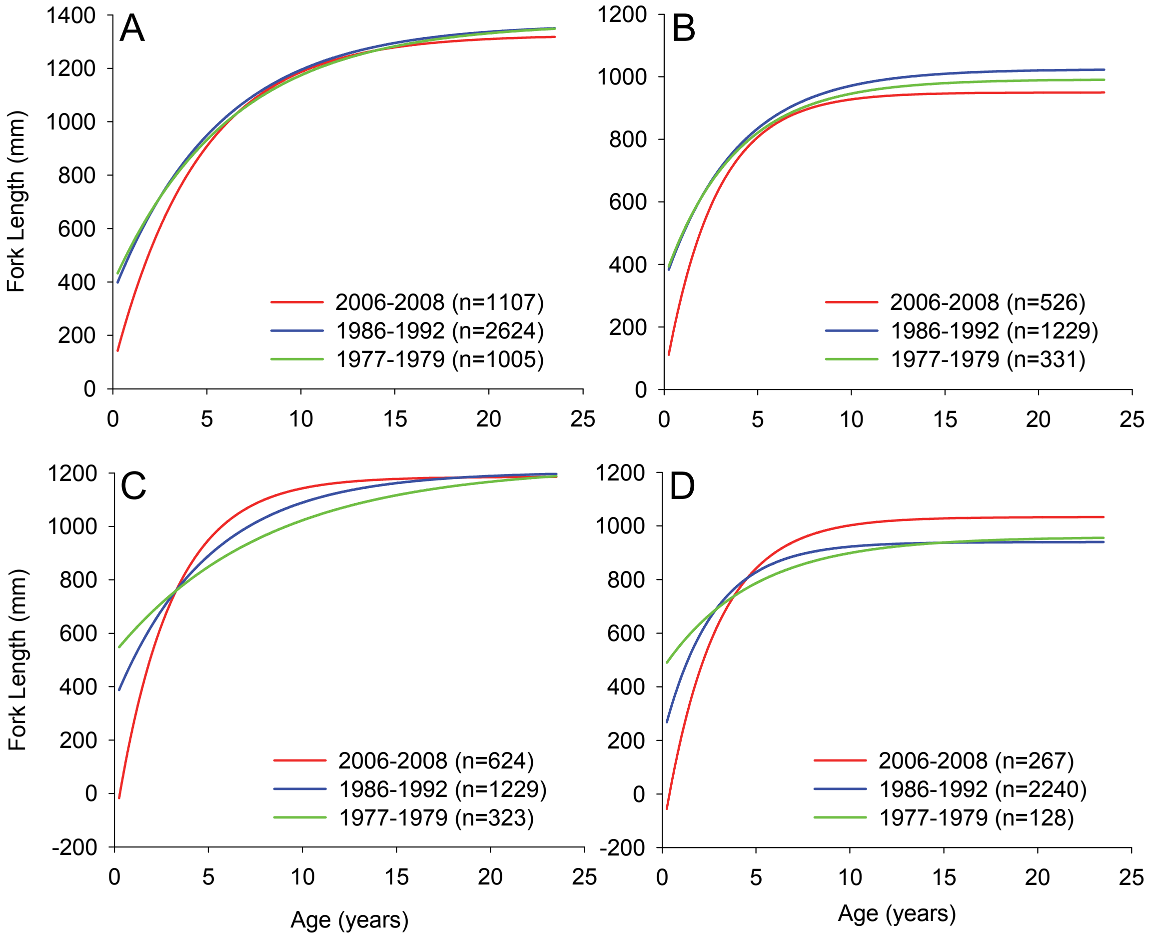

Figure 9. Von Bertalanffy growth functions estimated for king mackerel, *Scomberomorus cavalla,* collected during the summer spawning seasons over three time periods for A=Gulf of Mexico females, B=Gulf of Mexico males, C=Atlantic Ocean females, D=Atlantic Ocean males. Data for 1986-1992 and 1977-1979 originally reported by DeVries and Grimes (1997).

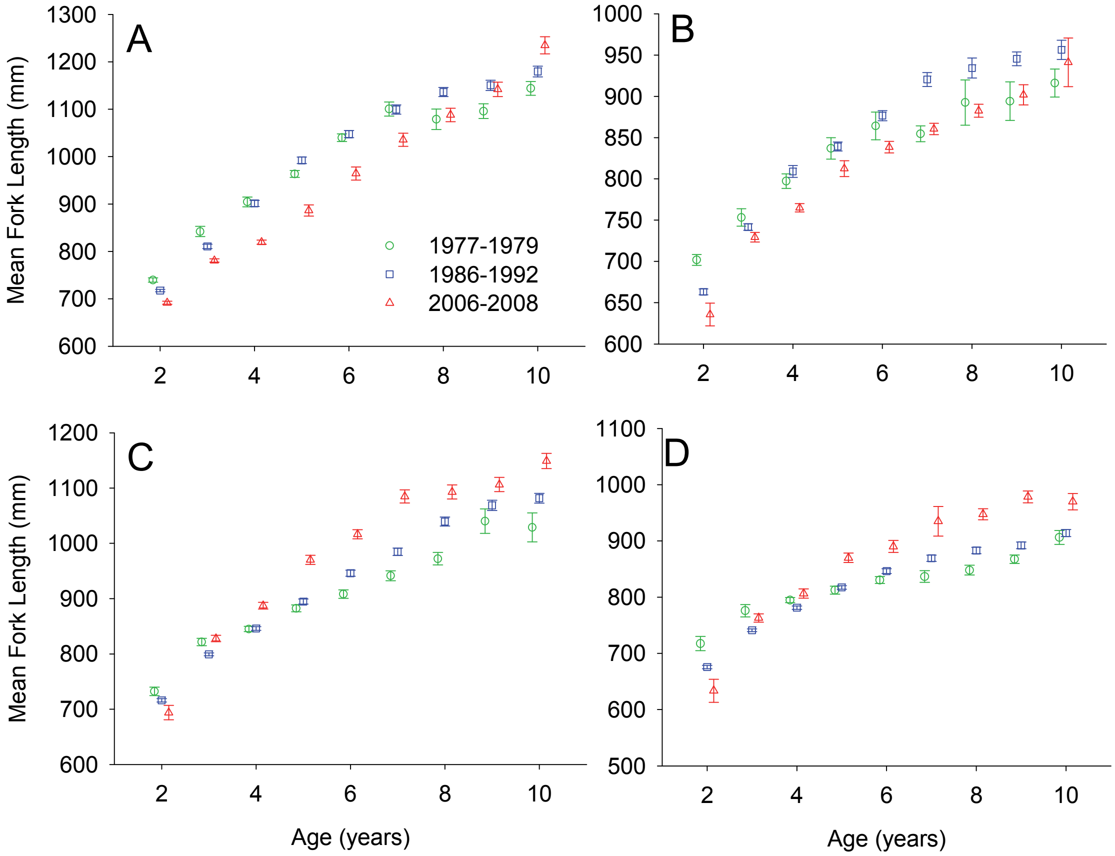

Figure 10. Mean size at age for Gulf of Mexico female (A) and male (B) and Atlantic Ocean female (C) and male (D) king mackerel, *Scomberomorus cavalla,* ages 2-10 sampled during the summer spawning seasons over three time periods (2006-2008=red, 1986-1992=blue, 1977-1979=green). Data for 1986-1992 and 1977-1979 originally reported by DeVries and Grimes (1997). Error bars are standard error.

Otoliths shape analysis

Totals of 965 and 1,309 king mackerel that were sampled in the 2006/07 and 2007/08 fishing years, respectively, were between ages 2 and 6 and yielded at least one otolith intact for shape analysis (Table 4). The 11 samples collected in zone 3 in December (zone3A in Table 4) of 2006 were added to the samples collected in January 2007 (zone3B in Table 4) for analysis.

Table 4. King Mackerel, *Scomberomorus cavalla,* Samples Sizes for Otolith Shape Analysis from 2006/07 and 2007/08 Gulf of Mexico (GOM) and Atlantic Ocean Summer Samples and Winter Samples from Zones 1-3. For Zone 3, Monthly Samples Are Given as A=December, B=January, C=February, and D=March.

	GOM	Atlantic	Zone 1	Zone 2	Zone 3A	Zone 3B	Zone 3C	Zone 3D
2006/07								
Female	155	133	119	73	8	49	25	59
Male	51	38	54	61	3	44	31	62
2007/08								
Female	399	130	102	69	27	43	59	36
Male	141	30	51	47	23	38	67	47

GOM and Atlantic samples from summer 2006 had similar sample sizes and somewhat similar age distributions, with the exception of a higher proportion of age-2 fish from the GOM and a slightly higher proportion of age-4 and age-5 fish from the Atlantic (Figure 11). The 2007 summer samples were sub-sampled to mitigate bias due to uneven sample sizes and age distributions (Figure 11). Wherever possible, ten samples were randomly selected for each age-class in each sex and stock resulting in a sample size of 50 individuals for each sex/stock combination with the exception of the Atlantic males, which were not sub-sampled due to already low sample sizes. The majority of 2006/07 winter-sampled fish were ages 2 and 3, with the former making up the bulk of the zone 3 landings sampled (Figure 12). The 2007/08 winter samples displayed a broader age distribution with the majority of samples between age-2 and age-4 except for zone 3 samples collected in March, the majority of which were age-2 (Figure 13).

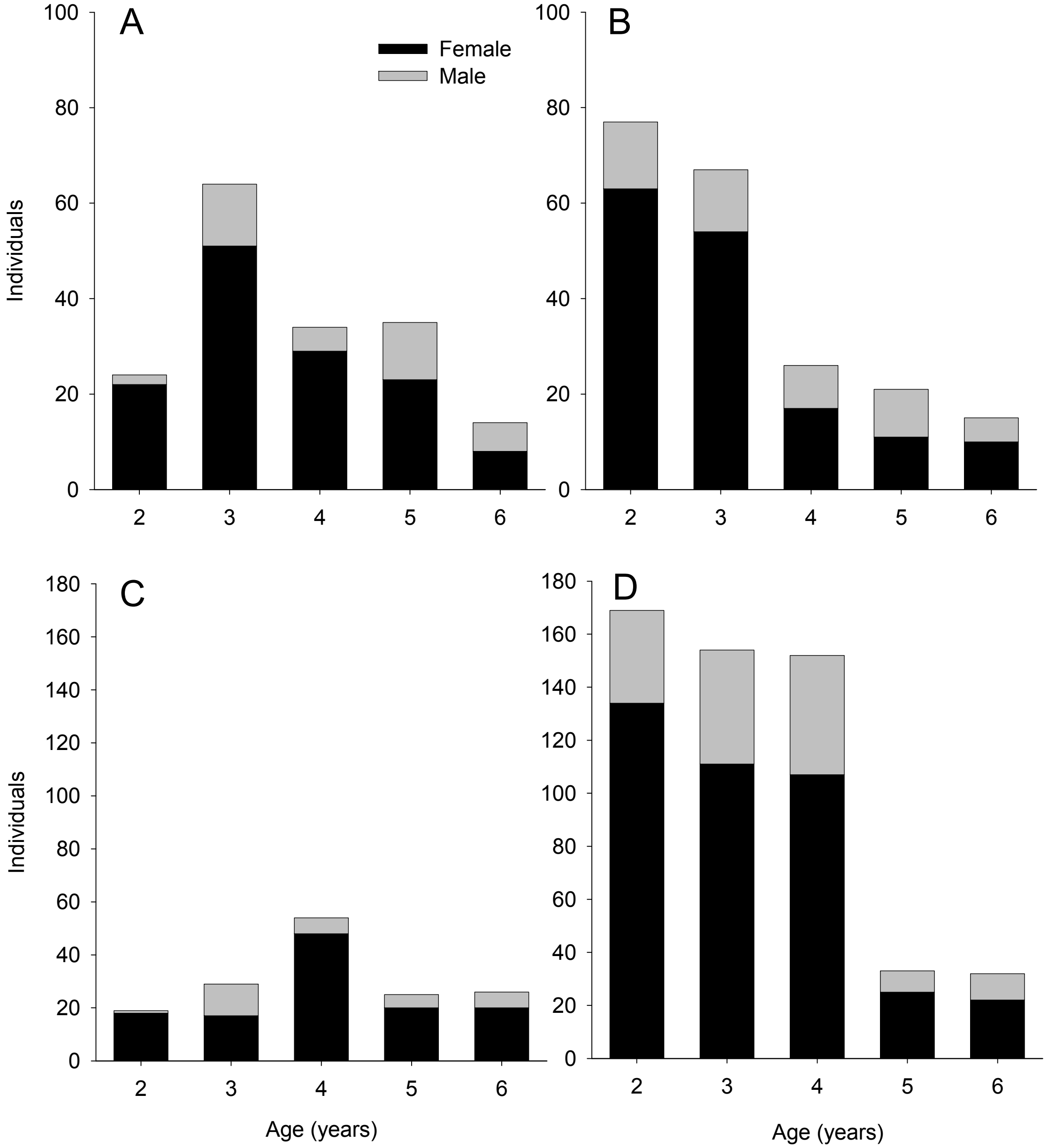

Figure 11. Age distributions of king mackerel, *Scomberomorus cavalla,* samples used to derive stock specific otolith shape signatures for A=2006 Atlantic Ocean, B=2006 Gulf of Mexico, C=2007 Atlantic Ocean, and D=2007 Gulf of Mexico.

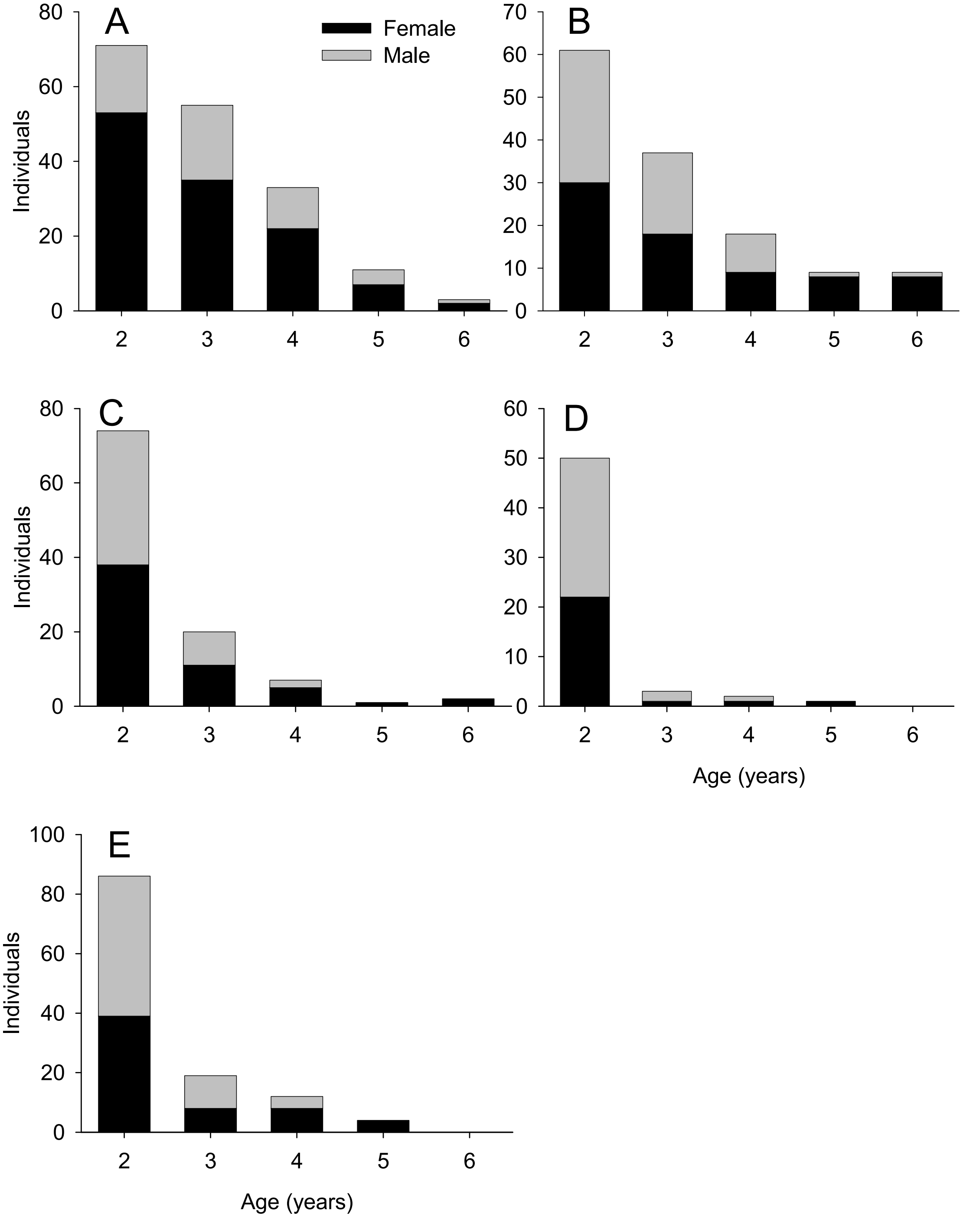

Figure 12. Sample age distributions for 2006/07 mixed-stock king mackerel, *Scomberomorus cavalla,* used in otolith shape based maximum likelihood models to estimate the Atlantic Ocean stock contribution to each south FL sampling zone for A=zone 1, B=zone 2, C=zone 3 December & January, D=zone 3 February, and E=zone 3 March.

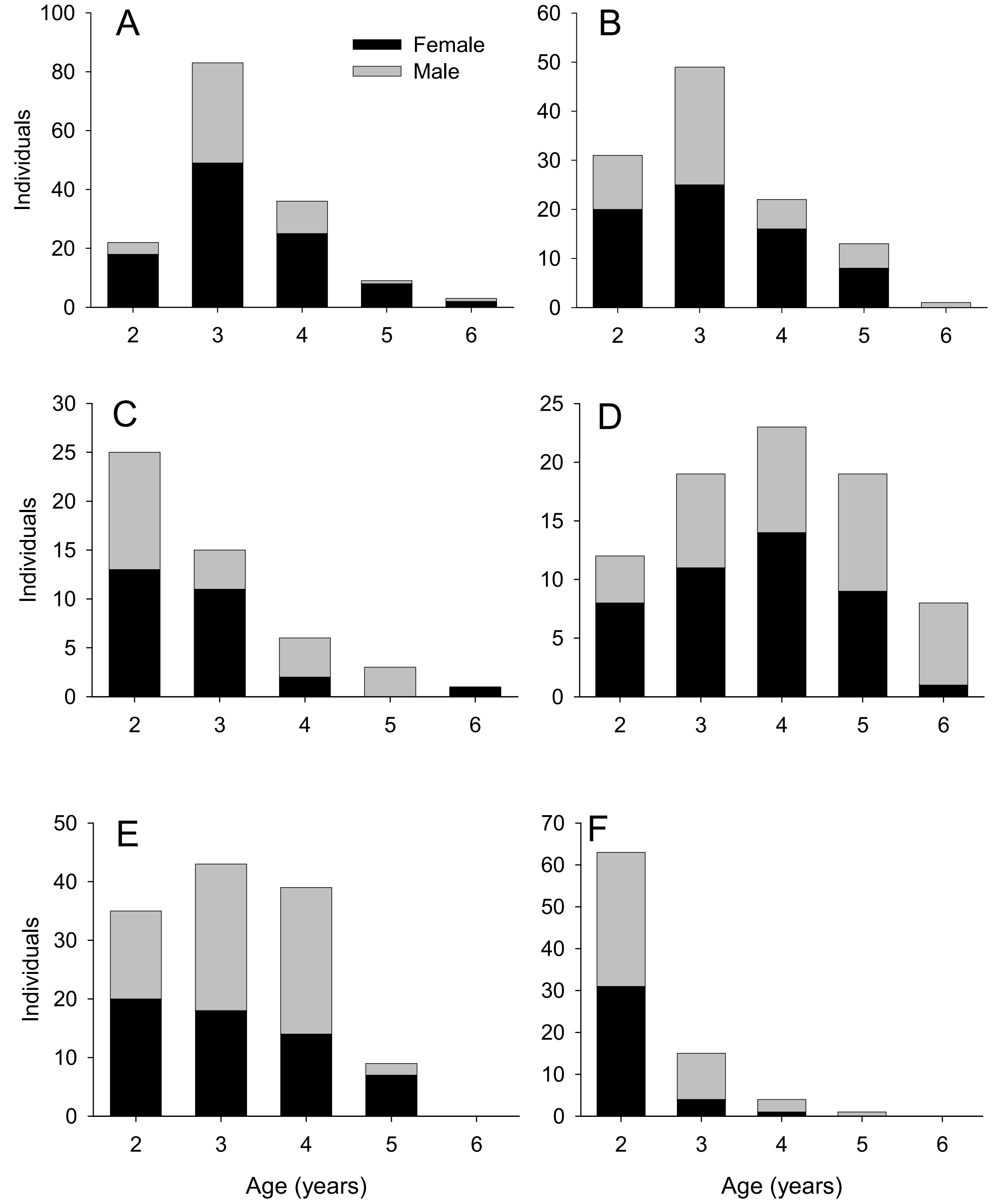

Figure 13. Sample age distributions for 2007/08 mixed-stock king mackerel, *Scomberomorus cavalla,* used in otolith shape based maximum likelihood models to estimate the Atlantic Ocean stock contribution to each south FL sampling zone for A=zone 1, B=zone 2, C=zone 3 December, D=zone 3 January, E=zone 3 February, and F=zone 3 March.

All 27 otolith shape parameters were significantly correlated with FL (MANOVA, $p<0.001$), and each parameter was detrended to remove the size effect. Variance-covariance matrices were heteroscedastic and area, perimeter, roundness, and harmonic 8 were leptokurtic, thus violating normality. Several common transformations were attempted to normalize the data; these resulted in further deviation from normality. Pillai's Trace was used as the test statistic in the MANOVA because it is robust to violations of homoscedasticity and normality.

The MANOVA revealed that fishing year, sex, stock, and age and the year*age interaction term were all significant effects (Table 5). The significant stock effect is evidence for the potential of otolith shape to be used as a natural tag in GOM and Atlantic king mackerel. Separate rule functions were developed from the 2006/07 and 2007/08 summer samples to account for variation in otolith shape between fishing years. Using separate rule functions for each fishing year also accounted for the significant year*age interaction, which were likely driven by differences in the age distributions between years. Sex-specific differences in the MANOVA results also supported analyzing each sex separately to improve classification success and precision in estimates of Atlantic contribution. The significant age effect is evidence for the importance of using uniform sample age distributions between populations.

Table 5. Multivariate Analysis of Variance Results from Otolith Shape Data from Summer-sampled King Mackerel, *Scomberomorus cavalla,* to Determine Differences between the 2006/07 and 2007/08 Fishing Years, Gulf of Mexico (GOM) and Atlantic Ocean Stocks, Sexes, and Ages 2-6.

Source	Pillai's Trace	F	Num DF	Den DF	p
Year	0.91184013	1.82	27	508	0.008
Group	0.17023817	3.86	27	508	<0.001
Sex	0.18642982	4.31	27	508	<0.001
Age	0.45205475	2.41	108	2044	<0.001
Year*Group	0.06997433	1.42	27	508	0.082
Year*Sex	0.04720513	0.93	27	508	0.565
Year*Age	0.29879203	1.53	108	2044	<0.001
Group*Sex	0.04981261	0.99	27	508	0.486
Group*Age	0.23573475	1.19	108	2044	0.099
Sex*Age	0.2383521	1.2	108	2044	0.084

Individual ANOVAs for each shape parameter revealed differences in gross and fine-scale otolith morphology between stocks, sexes, and age-classes while differences between fishing years were only apparent in gross morphological features (Appendix B). Shape indices and low-order harmonics as well as high-order harmonics displayed significant stock, sex, and age effects. Fishing year was only a significant factor in box width, ellipticity, and harmonics 3 and 6, all of which describe otolith shape on a relatively broad scale. The year*age interaction was a significant effect in gross otolith morphology (box width, ellipticity, and harmonics 3-5) and harmonic 14.

Stepwise DF analysis of detrended shape data separated by fishing year revealed that both gross and fine-scale otolith shape parameters were significant in discriminating between GOM and Atlantic king mackerel, which is consistent with the ANOVA results (Table 6). Only box width and roundness were significant in the DF analysis but did not also exhibit a significant stock effect in the individual ANOVA results. Classification success ranged from 60.0% to 72.6% with higher success rates for the Atlantic population than the GOM population (Table 6). Sex-specific DFs also resulted in higher classification success than combined sex DFs.

Totals of 588 and 609 king mackerel were sampled from the south Florida sampling zones in winter 2006/07 and 2007/08, respectively, with zone 3 sampled monthly across the winter mixing period (Table 5). Estimates of Atlantic stock contribution to winter landings were greater than zero across all zones and among all models (Figure 14). All 2006/07 models and the 2007/08 combined sex model displayed a consistent spatial pattern in the estimated percentage of landings contributed by the Atlantic stock, with lower contributions in zone 1 and higher contributions in zone 3. Results also showed an increasing trend in Atlantic contribution to zone 3 landings from December to March in 2006/07. Sex-specific results for 2007/08 do not display any apparent trends in the percentage of landings estimated to have been contributed by the Atlantic stock. Atlantic contribution was lower in 2007/08 than 2006/07 in all models for each estimate except zone 3B. The zone 3A 2007/08 estimates were not included in this comparison.

Table 6. King Mackerel, *Scomberomorus cavalla,* Year- and Sex-specific Stepwise Discriminant Function Analysis Results Identifying Otolith Shape Parameters Significant for Discrimination between the Gulf of Mexico and Atlantic Ocean Populations and the Associated Jackknifed Classification Success Rates.

		Classification Success	
	Significant Parameters	GOM	Atlantic
2006/07			
Female	Roundness, Circularity, Harmonics 3, 4, 12, 15, 16, 18	62.4	66.5
Male	Harmonics 4, 9, 14, 17	71.1	72.6
Combined	Harmonics 4, 5, 10, 12, 15, 16	61.2	62.8
2007/08			
Female	Area, Harmonics 2, 14, 16	70.0	70.0
Male	Harmonics 2, 4, 12, 13	60.0	66.0
Combined	Box width, Ellipticity, Rectangularity, Harmonics 4, 7, 12, 16	62.5	65.0

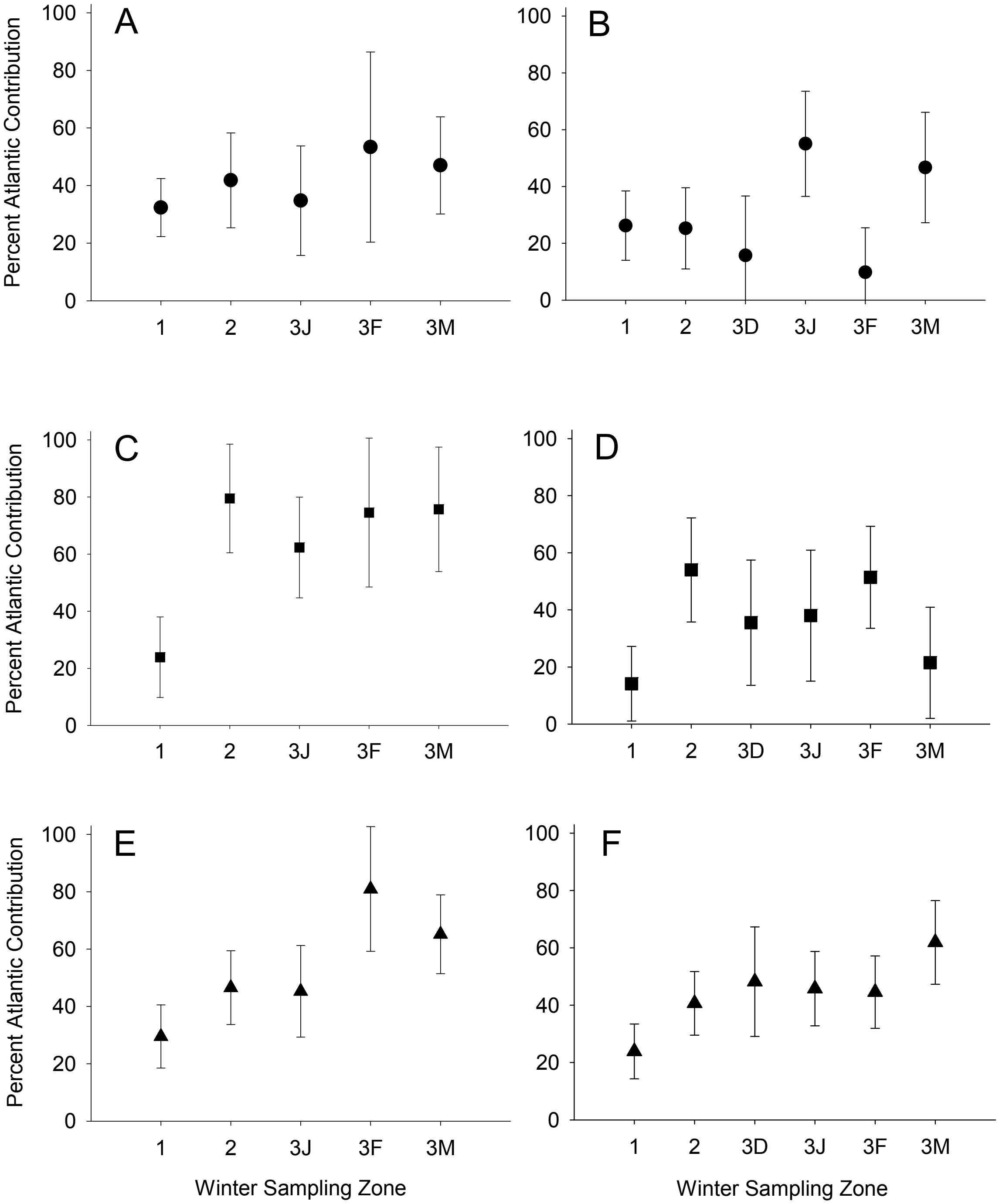

Figure 14. Otolith shape based maximum likelihood estimates of the proportion of female (A=2006/07, B=2007/08), male (C=2006/07, D=2007/08), and combined sex (E, F) king mackerel, *Scomberomorus cavalla,* landings contributed by the Atlantic Ocean stock to each south FL sampling zone (and month in zone 3). Zone 3 samples were collected in December (3A), January (3B), February (3C), and March (3D). Error bars are standard error.

Otolith Chemistry

Totals of 601 and 657 king mackerel were selected for stable isotope analysis from the samples collected in the 2006/07 and 2007/08 fishing years, respectively (Table 7). The 12 samples collected in zone 3 in December (zone3A in Table 7) of 2006 were added to the samples collected in January 2007 (zone3B in Table 7) for analysis. Sample sizes were not sufficient in all cases to randomly select 9 individuals from each age class between age-2 and age-6. Ultimately, wherever sample sizes were sufficiently large (i.e., ≥45 males or females), samples from each stock and sampling zone were selected with stratified random sampling to achieve as even an age distribution as possible. Summer samples for GOM and Atlantic females were evenly distributed across ages; however, Atlantic male age distributions were more variable due to sample size limitations (Figure 15). The 2006/07 winter-sampled fish displayed skewed age distributions in all zones and both sexes with a higher frequency of younger fish than older fish (Figure 16). The 2007/08 winter-sample age distributions were highly variable. Zone 3 samples from December and March were skewed similar to the 2006/07 samples, and zone 1, zone 2, and zone 3 January and February samples included more older fish than the other mixed-stock sample sets (Figure 17).

Stable isotope results from both fishing years displayed stock-specific differences in $\delta^{18}O$ and $\delta^{13}C$ values. Mean $\delta^{18}O$ and $\delta^{13}C$ values were very similar between sexes with mean $\delta^{18}O$ slightly higher in males than females (except for 2007 GOM samples) and mean $\delta^{13}C$ virtually identical between sexes in both populations and both years (Figure 18). In both fishing years, mean $\delta^{18}O$ displayed greater variation between stocks in both sexes than did mean $\delta^{13}C$.

Table 7. King Mackerel, *Scomberomorus cavalla,* Samples Sizes for Otolith Stable Isotope Chemistry from 2006/07 and 2007/08 Gulf of Mexico (GOM) and Atlantic Ocean Summer Samples and Winter Samples from Zones 1-3. For Zone 3, Monthly Samples Are Given as A=December, B=January, C=February, and D=March.

	GOM	Atlantic	Zone 1	Zone 2	Zone 3A	Zone 3B	Zone 3C	Zone 3D
2006/07								
Female	45	45	45	45	7	45	25	45
Male	45	38	45	45	5	45	30	45
2007/08								
Female	45	45	45	45	27	45	45	37
Male	45	38	45	45	23	40	45	45

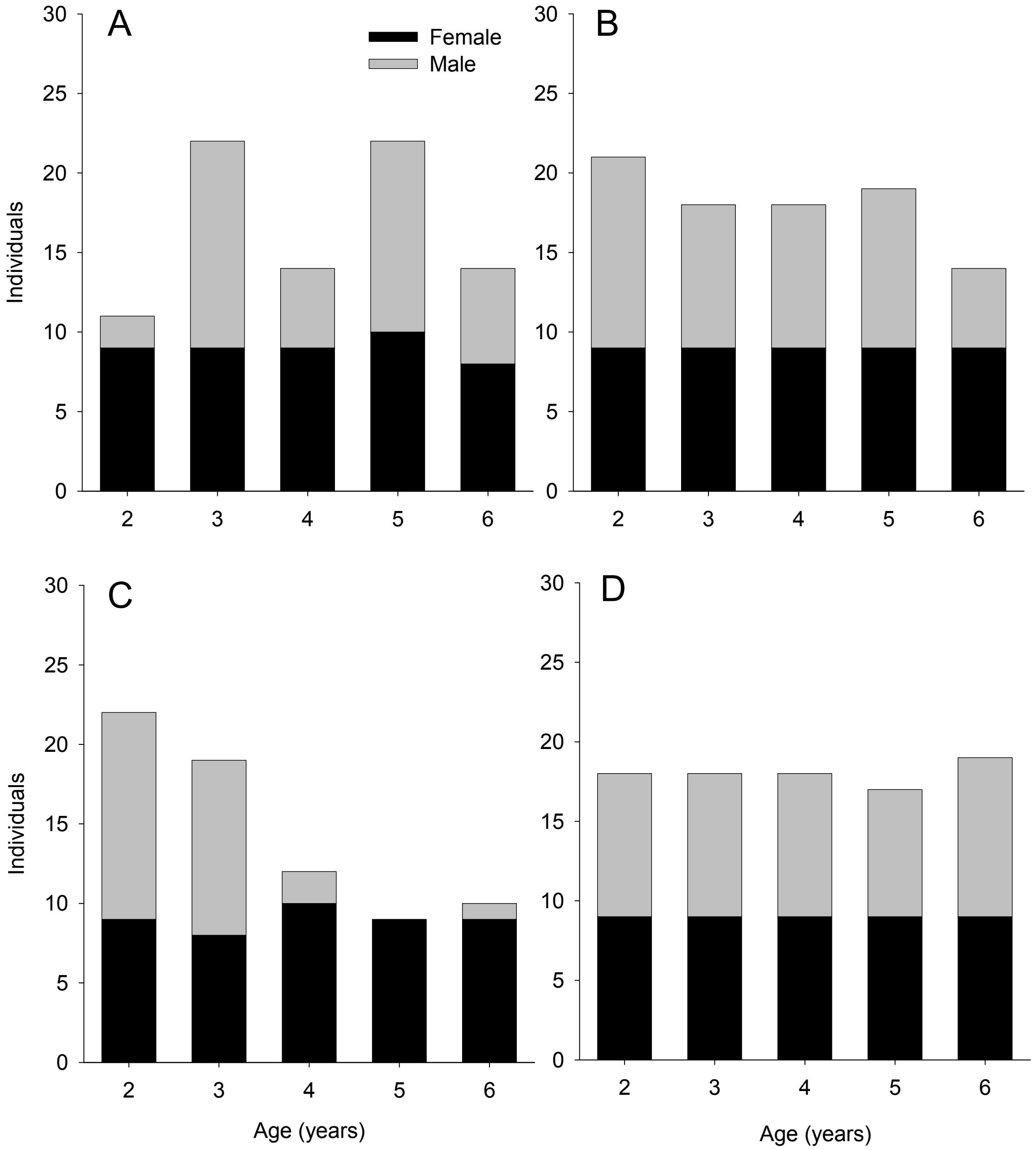

Figure 15. Age distributions of king mackerel, *Scomberomorus cavalla,* samples used to derive stock specific otolith stable isotope signatures for A=2006 Atlantic Ocean, B=2006 Gulf of Mexico, C=2007 Atlantic Ocean, and D=2007 Gulf of Mexico.

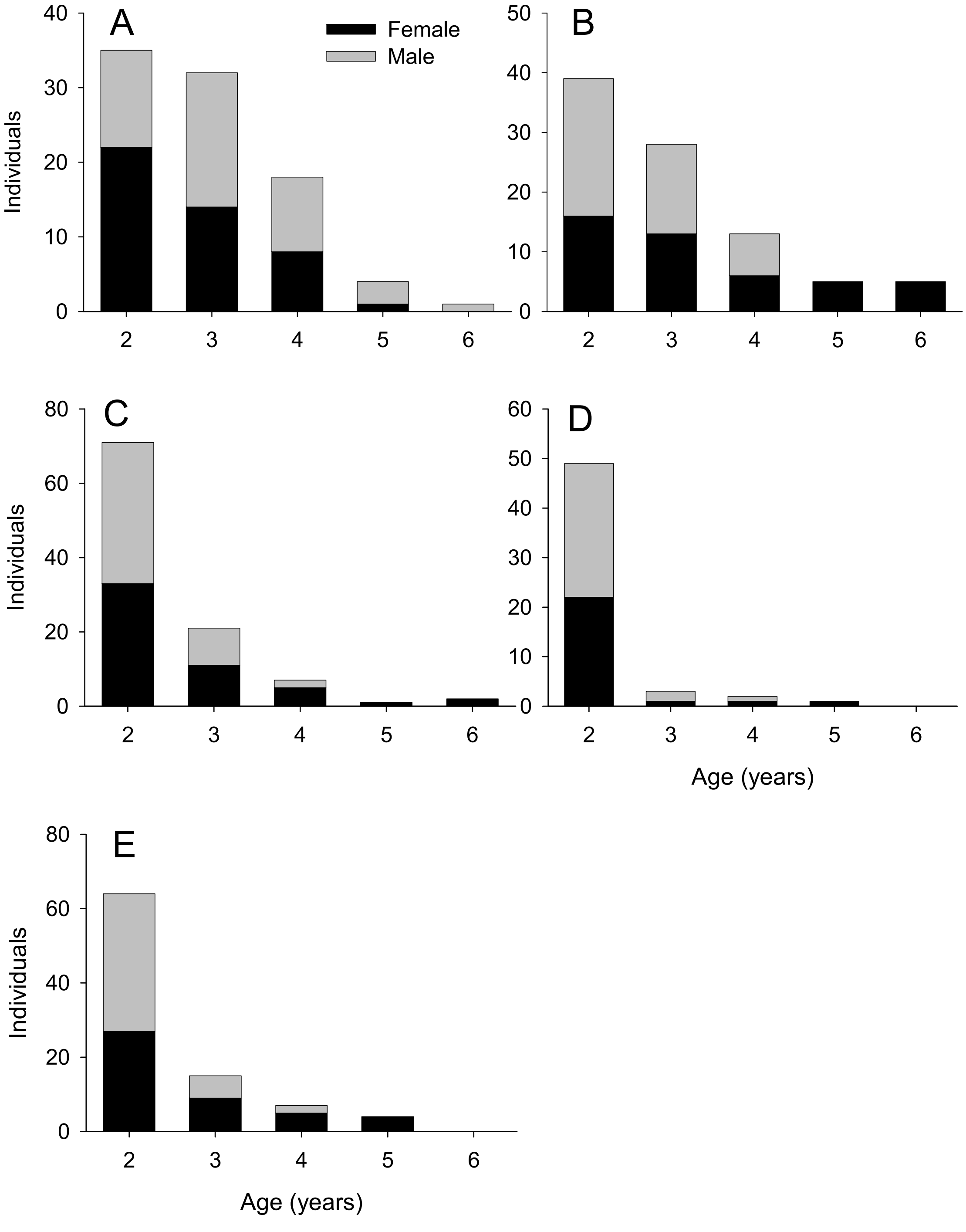

Figure 16. Sample age distributions for 2006/07 mixed-stock king mackerel, *Scomberomorus cavalla,* used in otolith stable isotope-based maximum likelihood models to estimate the Atlantic Ocean stock contribution to each south FL sampling zone for A=zone 1, B=zone 2, C=zone 3 December & January, D=zone 3 February, and E=zone 3 March.

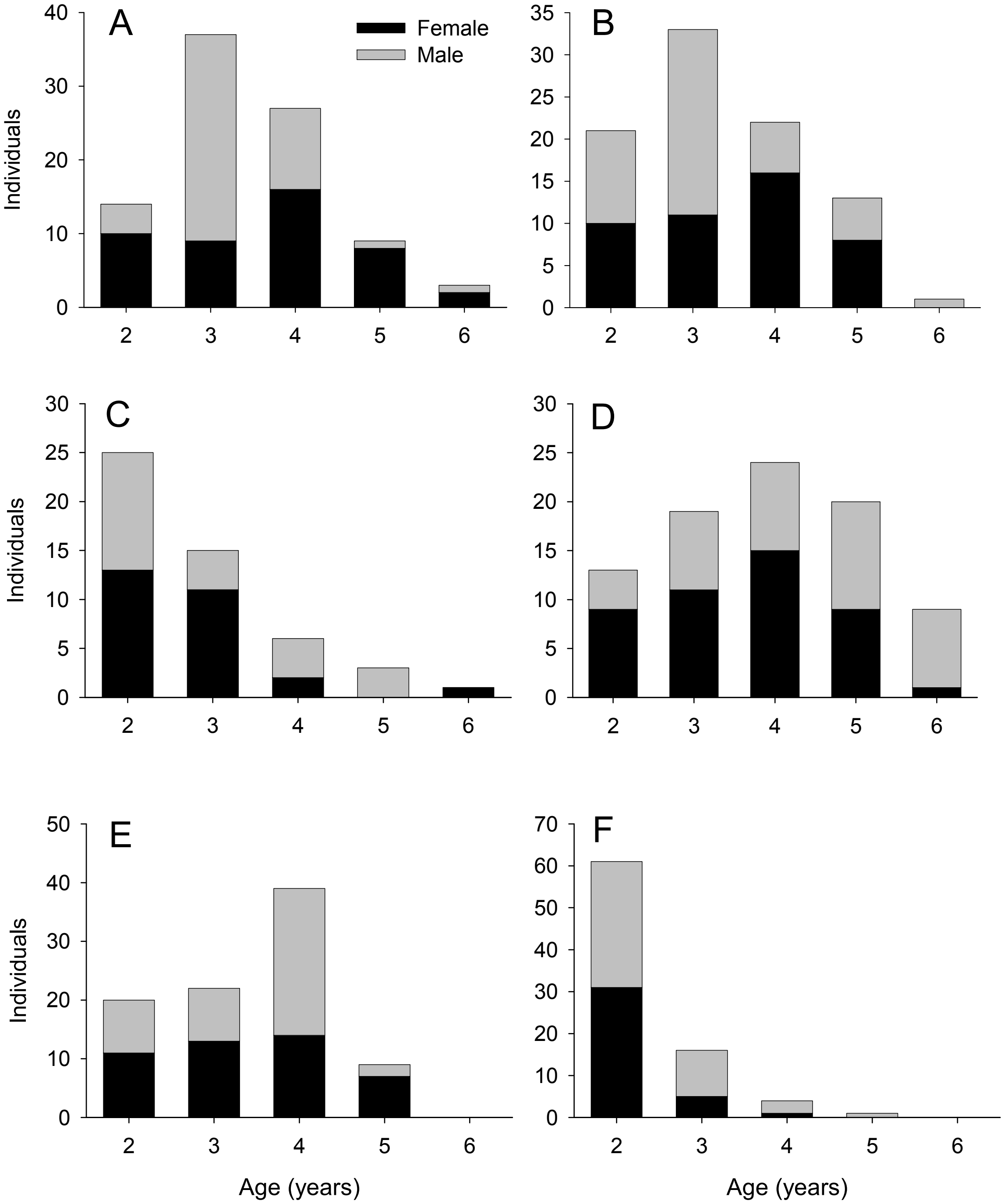

Figure 17. Sample age distributions for 2007/08 mixed-stock king mackerel, *Scomberomorus cavalla,* used in otolith stable isotope-based maximum likelihood models to estimate the Atlantic Ocean stock contribution to each south FL sampling zone for A=zone 1, B=zone 2, C=zone 3 December, D=zone 3 January, E=zone 3 February, and F=zone 3 March).

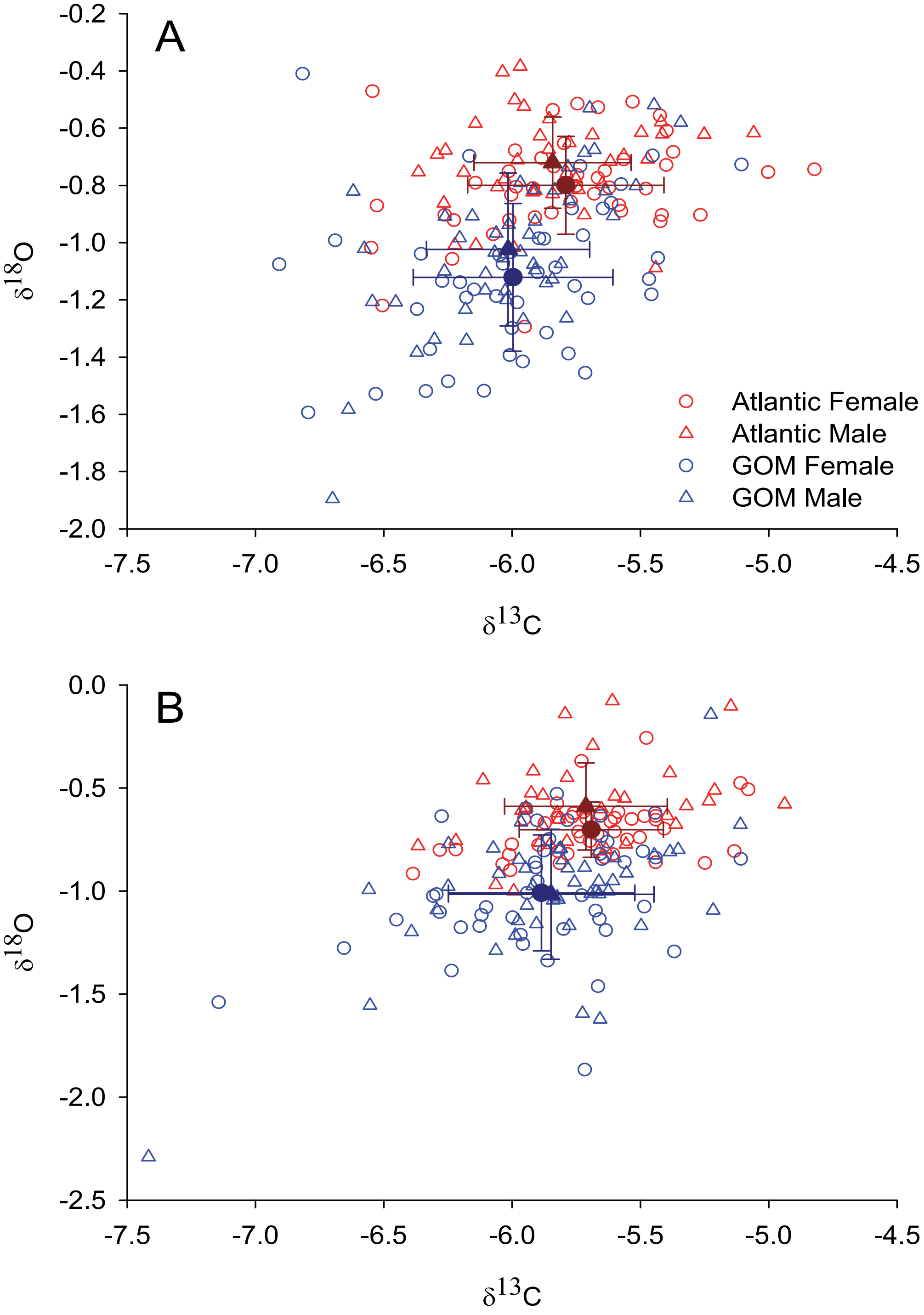

Figure 18. Results from stable isotope analysis of Gulf of Mexico (GOM) and Atlantic Ocean (Atlantic) king mackerel, *Scomberomorus cavalla,* otoliths collected in summers 2006 (A) and 2007 (B) to derive natural tags for stock discrimination. Solid symbols represent mean values with error bars to one standard deviation. Delta values were detrended to remove variation due to fish size.

Both $\delta^{18}O$ and $\delta^{13}C$ were significantly correlated with FL so they were detrended to remove the resulting variation. Otolith $\delta^{18}O$ and $\delta^{13}C$ values from summer 2007 deviated from normality; however, this appears to have been driven by one or two samples with particularly low $\delta^{18}O$ and $\delta^{13}C$ values. All attempted transformations resulted in worse violation of parametric assumptions. Also, the variance-covariance matrices were heteroscedastic. Thus, Pillai's Trace was used as the MANOVA test statistic, and quadratic, rather than linear, DFs were estimated. MANOVA indicated year, group, sex, age and the year*age, group*age, and group*sex*age interaction terms all significantly affected stable isotope signatures (Table 8). The significant group effect suggests otolith stable isotope signatures may be used as natural stock-specific tags. Separate rule functions were calculated from summer data in each fishing year, and both sex-specific as well as combined sex analyses were conducted. Any possible bias due to the age effect was mitigated by the stratified selection of stock-specific samples. These measures also accounted for the three significant interaction effects. Individual ANOVAs revealed significant year, group, sex and group*age effects on $\delta^{18}O$ values, and significant group, age, year*age, group*sex, group*age, group*sex*age, and year*group*sex*age effects on $\delta^{13}C$ values (Table 9). Otolith $\delta^{18}O$ values from summer 2006 increased with age in all sex/stock/year combinations, while 2007 GOM $\delta^{18}O$ values decreased with age (Figure 19). In summer 2006, $\delta^{13}C$ values increased with age in GOM samples, but displayed no clear trend in samples from the Atlantic population (Figure 20). This pattern is less apparent in the summer 2007 data.

Table 8. Multivariate Analysis of Variance Results from Otolith Stable Isotope Data from Summer-sampled King Mackerel, *Scomberomorus cavalla,* to Determine Differences Between Years (2006/07 and 2007/08), Stocks (Gulf of Mexico and Atlantic Ocean), Sexes, and Ages (2-6).

Source	Pillai's Trace	F	Num DF	Den DF	p
Year	0.07650141	12.51	2	302	<0.001
Group	0.31515822	69.49	2	302	<0.001
Sex	0.04535173	7.17	2	302	<0.001
Age	0.06555591	2.57	8	606	0.009
Year*Group	0.00854016	1.3	2	302	0.274
Year*Sex	0.00436319	0.66	2	302	0.517
Year*Age	0.06076085	2.37	8	606	0.016
Group*Sex	0.01926573	2.97	2	302	0.053
Group*Age	0.14043425	5.72	8	606	<0.001
Sex*Age	0.03194658	1.23	8	606	0.279
Year*Group*Sex	0.01010226	1.54	2	302	0.216
Year*Group*Age	0.0436415	1.69	8	606	0.0978
Year*Sex*Age	0.01792031	0.68	8	606	0.705
Group*Sex*Age	0.06862508	2.69	8	606	0.006
Year*Group*Sex*Age	0.04392997	1.7	8	606	0.095

Table 9. Analysis of Variance Results from Otolith Stable Isotope Data from Summer-sampled King Mackerel, *Scomberomorus cavalla,* to Determine Differences between Years (2006/07 and 2007/08), Stocks (Gulf of Mexico and Atlantic Ocean), Sexes, and Ages (2-6).

$\delta^{18}O$

Source	DF	SS	MS	F	p
Model	39	14.3995	0.369218	7.26	<0.001
Year	1	1.074213	1.074213	21.12	<0.001
Group	1	6.938043	6.938043	136.4	<0.001
Sex	1	0.303407	0.303407	5.96	0.015
Age	4	0.485622	0.121406	2.39	0.051
Year*Group	1	0.104188	0.104188	2.05	0.153
Year*Sex	1	0.026111	0.026111	0.51	0.474
Year*Age	4	0.324837	0.081209	1.6	0.175
Group*Sex	1	0.003903	0.003903	0.08	0.782
Group*Age	4	0.544672	0.136168	2.68	0.032
Sex*Age	4	0.238936	0.059734	1.17	0.322
Year*Group*Sex	1	0.137674	0.137674	2.71	0.101
Year*Group*Age	4	0.470546	0.117637	2.31	0.058
Year*Sex*Age	4	0.045721	0.01143	0.22	0.924
Group*Sex*Age	4	0.209975	0.052494	1.03	0.391
Year*Group*Sex*Age	4	0.326413	0.081603	1.6	0.173
Error	303	15.41198	0.050865		
Total	342	29.81148			

$\delta^{13}C$

Source	DF	SS	MS	F	p
Model	39	12.95281	0.332123	3.23	<0.001
Year	1	0.000353	0.000353	0	0.953
Group	1	1.042551	1.042551	10.15	0.002
Sex	1	0.279785	0.279785	2.72	0.099
Age	4	1.224277	0.306069	2.98	0.019
Year*Group	1	0.000998	0.000998	0.01	0.922
Year*Sex	1	0.028906	0.028906	0.28	0.596
Year*Age	4	1.054111	0.263528	2.57	0.038
Group*Sex	1	0.45229	0.45229	4.4	0.037
Group*Age	4	3.311973	0.827993	8.06	<0.001
Sex*Age	4	0.533652	0.133413	1.3	0.270
Year*Group*Sex	1	0.001158	0.001158	0.01	0.915
Year*Group*Age	4	0.911349	0.227837	2.22	0.067
Year*Sex*Age	4	0.519032	0.129758	1.26	0.284
Group*Sex*Age	4	1.890041	0.47251	4.6	0.001
Year*Group*Sex*Age	4	1.172088	0.293022	2.85	0.024
Error	303	31.11465	0.102689		
Total	342	44.06746			

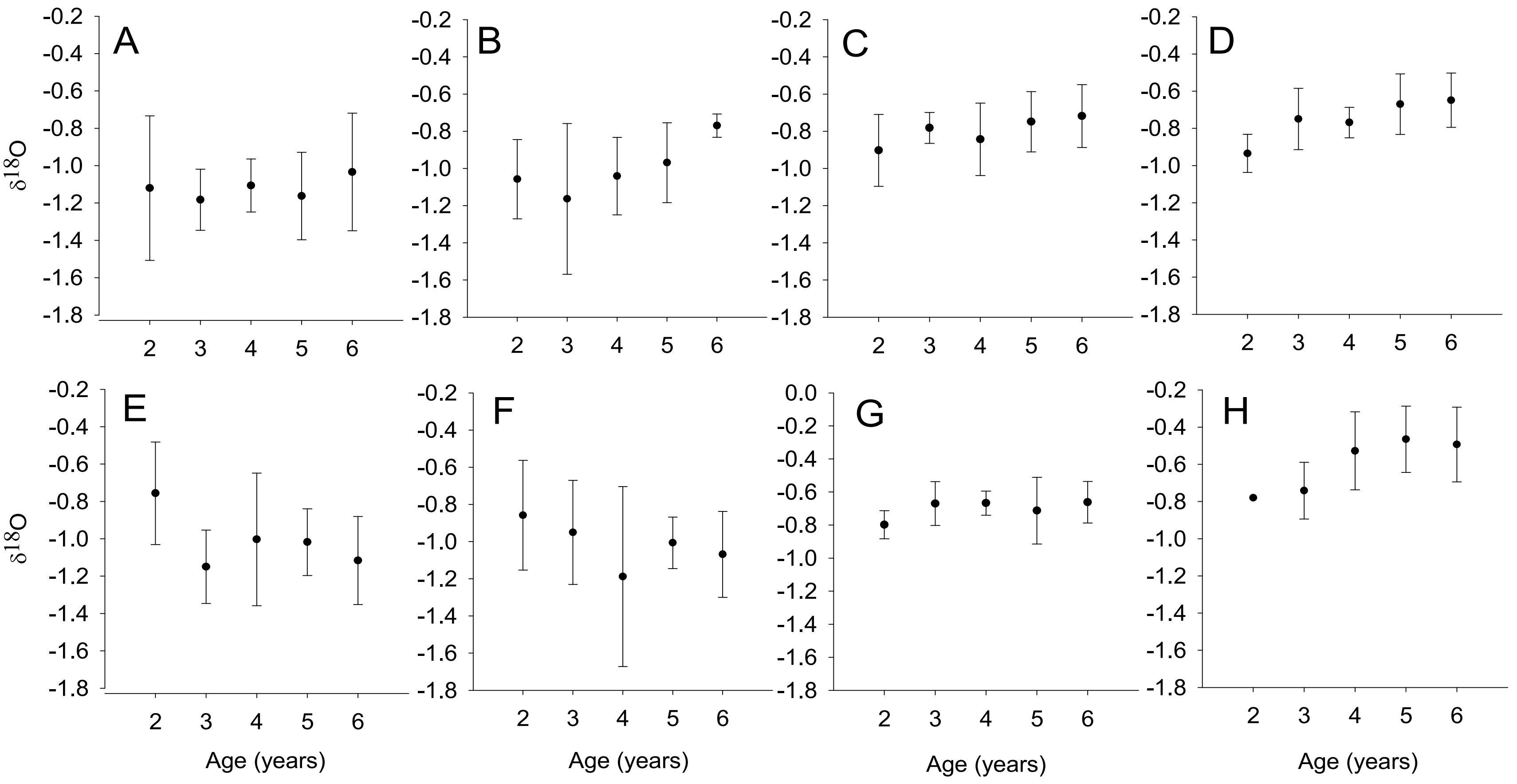

Figure 19. Mean otolith δ^{18}O values by age from Gulf of Mexico (GOM) and Atlantic Ocean (Atlantic) king mackerel, *Scomberomorus cavalla,* sampled in summer 2006 and 2007. A=2006 GOM female, B=2006 GOM male, C=2006 Atlantic female, D=2006 Atlantic male, E=2007 GOM female, F=2007 GOM male, G=2007Atlantic female, and H=2007 Atlantic male. Error bars represent one standard deviation. Delta values were detrended to remove variation due to fish size.

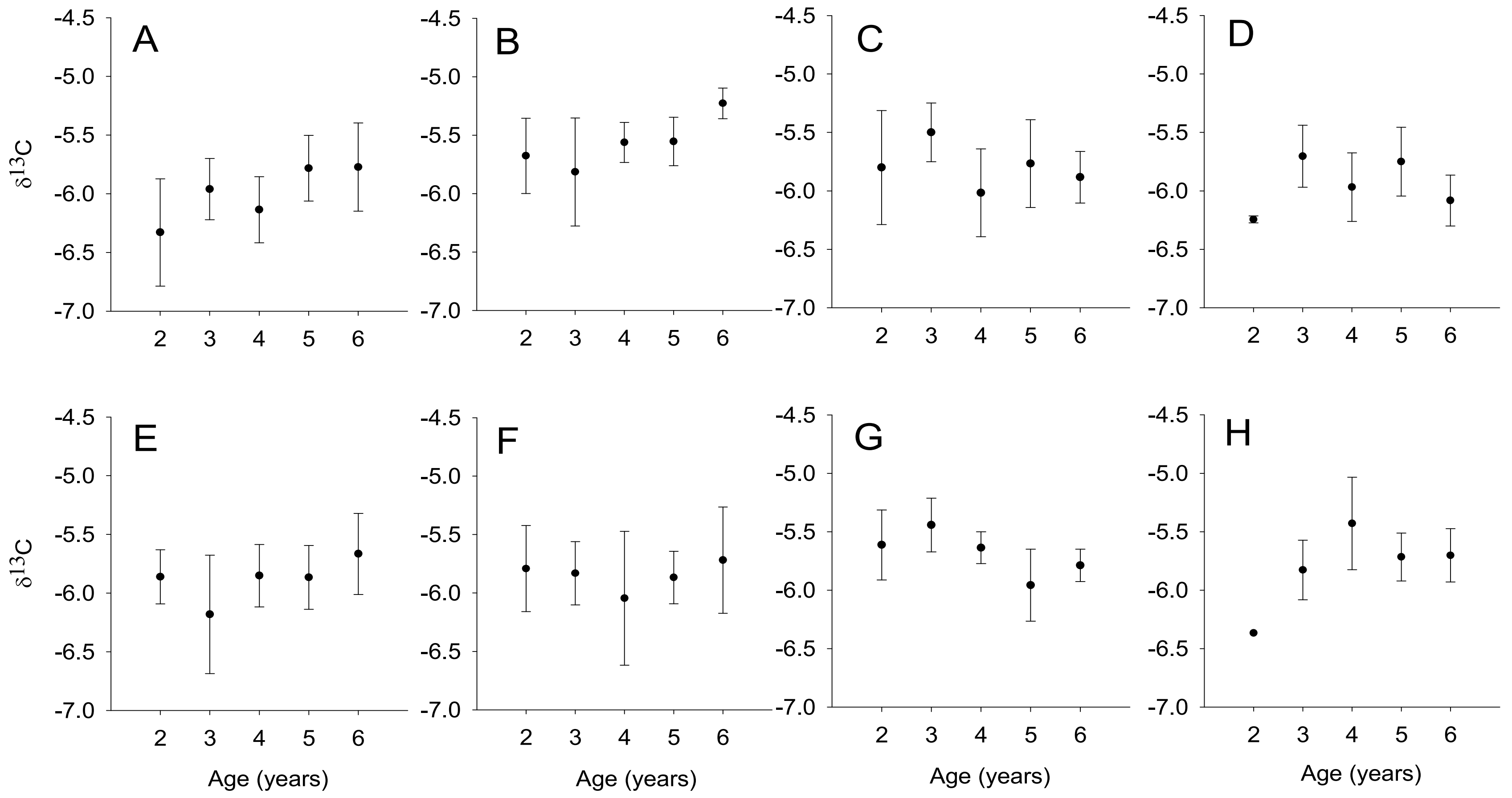

Figure 20. Mean otolith $\delta^{13}C$ values by age from Gulf of Mexico (GOM) and Atlantic Ocean (Atlantic) king mackerel, *Scomberomorus cavalla,* sampled in summer 2006 and 2007. A=2006 GOM female, B=2006 GOM male, C=2006 Atlantic female, D=2006 Atlantic male, E=2007 GOM female, F=2007 GOM male, G=2007Atlantic female, and H=2007 Atlantic male. Error bars represent one standard deviation. Delta values were detrended to remove variation due to fish size.

Discriminant functions calculated with otolith stable isotope signatures resulted in mean classification success ranging from 71.1 to 86.5% (Table 10). The summer 2006 combined sex analysis resulted in a mean classification success greater than the male only run and lesser than the female only run. The summer 2007 male only analysis produced the highest classification success and the female only run resulted in the lowest. Atlantic classification success was higher than the GOM in all cases.

Table 10. Jackknifed Percent Classification Success for Gulf of Mexico (GOM) and Atlantic Ocean King Mackerel, *Scomberomorus cavalla,* with Sex-specific and Combined-sex Otolith Stable Isotope-based Discriminant Function Analysis of Samples Collected in Summers 2006 and 2007.

	2006			2007		
	Female	Male	Combined	Female	Male	Combined
GOM	77.8	66.7	71.1	60.0	84.4	68.9
Atlantic	88.9	86.8	89.2	82.2	88.6	87.5
Mean	83.4	76.8	80.2	71.1	86.5	78.2

Maximum likelihood results for both years indicated some level of Atlantic contribution in all zones. The estimated percentage of Atlantic fish in the landings increased from west to east with the lowest percentage in zone 1 and the highest in zone 3 (Figure 21). This trend was less apparent in the male only estimates for both years. Atlantic contribution in zone 3 also increased over the course of the mixing season (December-March). Point estimates of Atlantic contribution in 2006/07 were consistently higher for females than males, and the combined sex model results were intermediate between the sexes. In 2007/08 the Atlantic contributed more heavily to male landings in zones 1 and 2 and to female landings in zone 3. In both fishing years, the mean SE about combined sex mixing estimates was slightly lower than that of female only estimates, while the mean error about male only estimates was at least 5 percentage points higher than the female only and combined sex estimates.

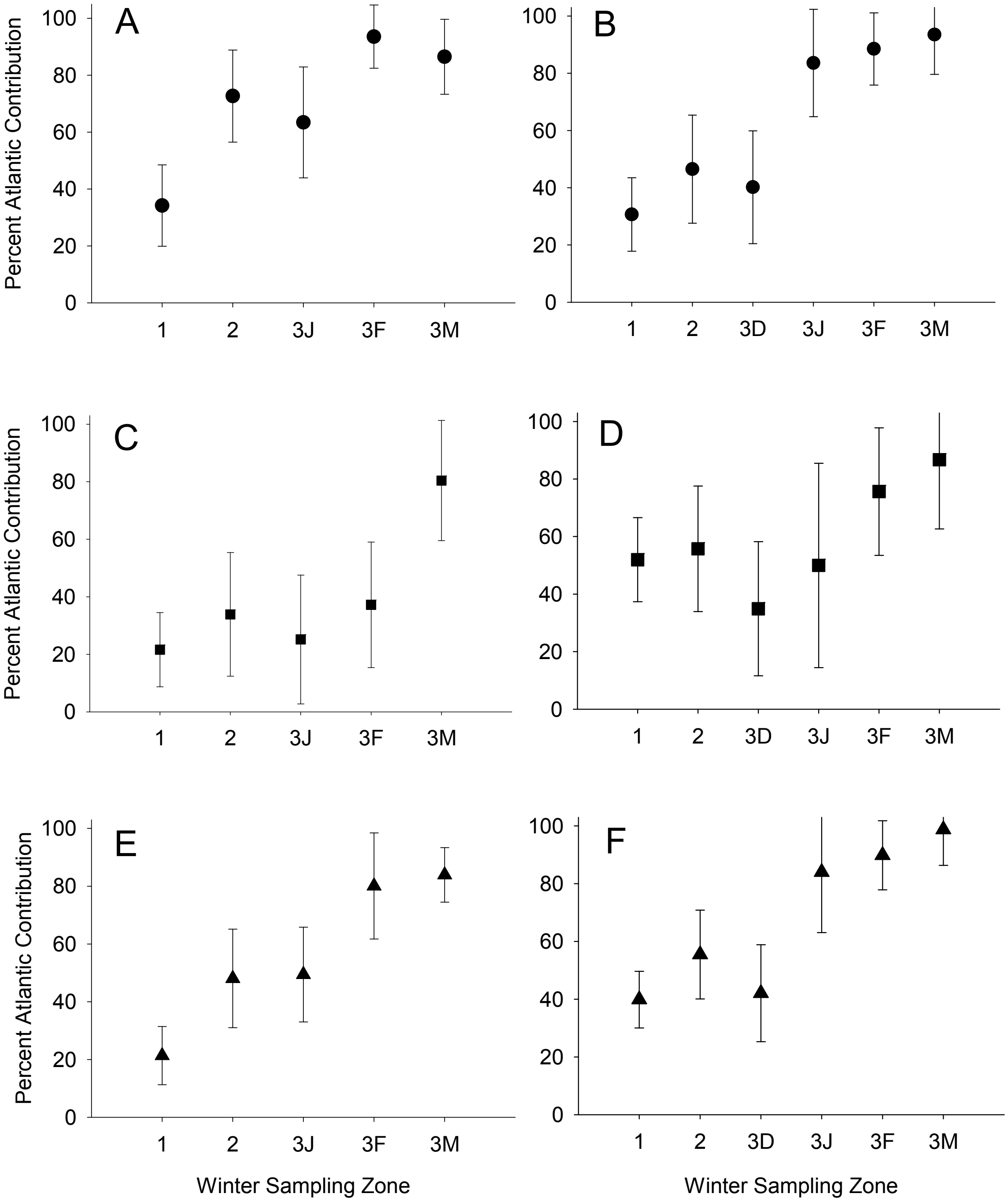

Figure 21. Otolith chemistry based maximum likelihood estimates of the proportion of female (A=2006/07, B=2007/08), male (C=2006/07, D=2007/08), and combined sex (E, F) king mackerel, *Scomberomorus cavalla,* landings contributed by the Atlantic Ocean stock to each south FL sampling zone (and month in zone 3). Zone 3 samples were collected in December (3A), January (3B), February (3C), and March (3D). Error bars are standard error.

Simulated stable isotope signatures in exercises 1 (and 5) (Figure 22 panel A) and 4 (Figure 22 panel D) resembled those of the wild population. Simulation 2 (Figure 22 panel B) displayed the greatest contrast between the isotope signatures of the two populations. Simulation 3 (Figure 22 panel C) resulted in reduced overlap in the range of $\delta^{18}O$ values, although contrast in $\delta^{13}C$ values between the two population continued to be low.

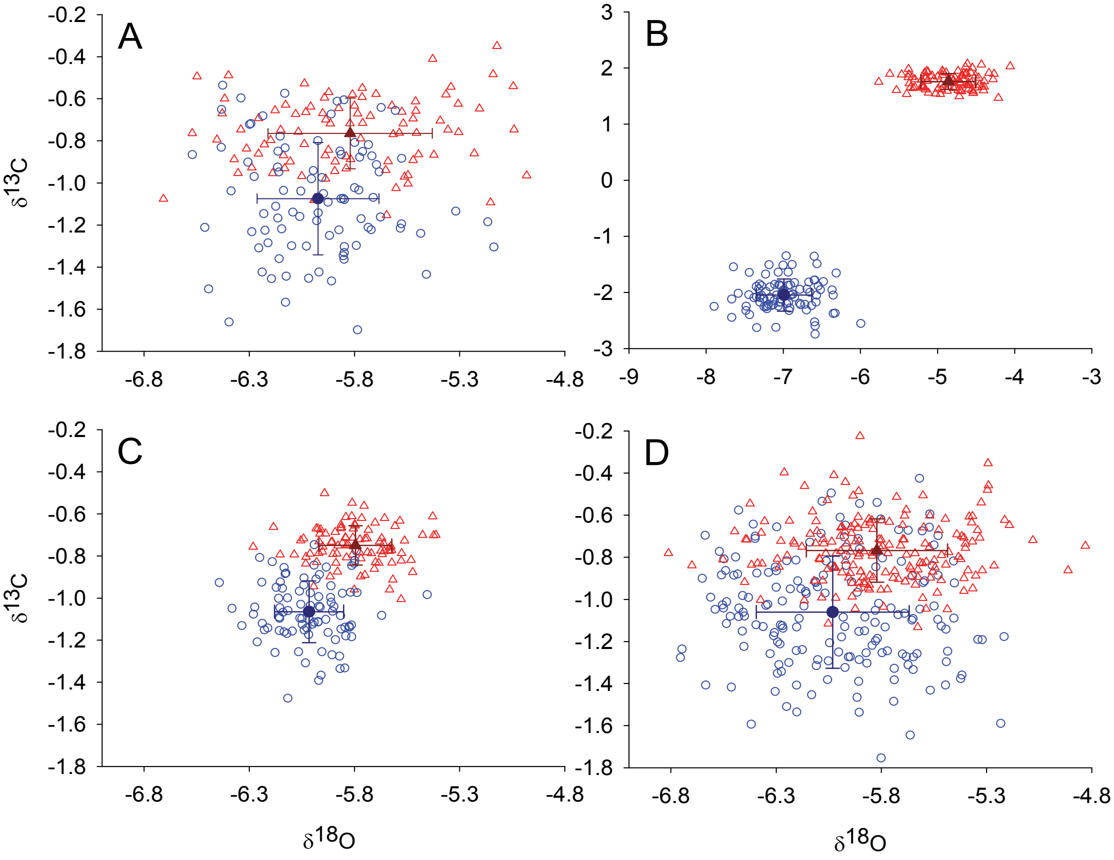

Figure 22. Randomly generated otolith stable isotope signatures from Gulf of Mexico (blue circles) (GOM) and Atlantic Ocean (red triangles) king mackerel, *Scomberomorus cavalla,* in multiple simulation exercises: (A) mean and standard deviation of isotope values equal to those estimated from wild-caught samples, (B) the distance between the population means increased by 2‰ and standard deviations equal to the wild populations, (C) the means equal to the wild populations and standard deviation halved, and (D) the means and standard deviations equal to the wild populations and sample sizes doubled. Solid symbols represent means with error bars equal to one standard error. Note the scale of axes is different on panel B.

Population-specific classification success was higher in simulations 2, 3, and 4 than simulations 1 and 5 (Table 11). Increasing the difference in mean values between the two populations (simulation 2) resulted in 100% classification success. Reducing variance about the means (simulation 3) increased success by more than 15 percentage points, while increasing the stock-specific sample size only increased mean classification success by 5 percentage points (simulation 4).

Table 11. Jackknifed Percent Classification Success for Gulf of Mexico (GOM) and Atlantic Ocean King Mackerel, *Scomberomorus cavalla,* from Discriminant Function Analysis of Stable Isotope Signatures Randomly Generated in Five Simulation Exercises. Stock-specific Simulated Signatures Were Identical in Simulations 1 and 5.

Simulation	1,5	2	3	4
GOM	67.8	100.0	91.1	74.4
Atlantic	82.2	100.0	93.3	83.3
Mean	75.0	100.0	92.2	81.4

Estimates of Atlantic contribution in all five simulated mixed samples were fairly accurate and displayed higher precision relative to results from wild-caught samples (Figure 23). The estimate in simulation 2 was exactly 70% and displayed the greatest precision. The estimate in simulation 4 was farthest from 70% and least precise but was still only inaccurate by 7.4% with a SE less that 10% (similar to the SE in simulation 1). Increasing the distance between population means (simulation 2), decreasing variance about those means (simulation 3) in the stock-specific samples, and increasing mixed-stock sample size (simulation 5) all appeared to improve precision about mixing estimates. It is not clear why increasing stock-specific sample size would not also increase precision or why accuracy decreased in this simulation.

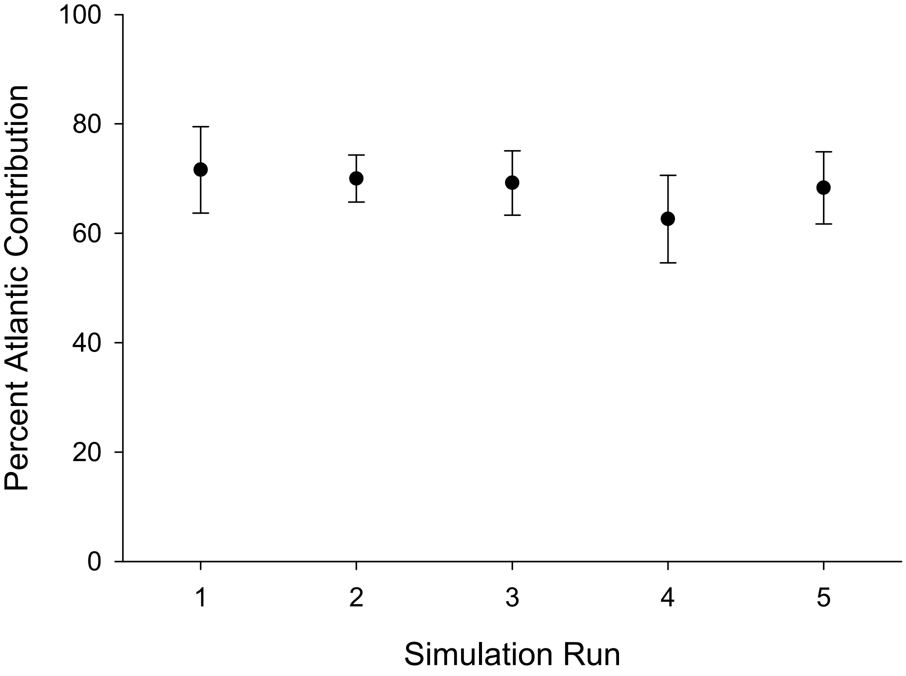

Figure 23. Maximum likelihood estimates of Atlantic Ocean contribution to mixed-stock samples of king mackerel, *Scomberomorus cavalla,* otolith stable isotope signatures in five simulation exercises with standard error bars. The ratio of Atlantic Ocean to Gulf of Mexico king mackerel in all mixed-stock samples was 70:30.

DISCUSSION

Age and Growth

Patterns in the VBGF parameters between sexes and populations were consistent with historic results with the exception of the male growth parameters for the two populations, which displayed a reversed pattern. Females in both populations are estimated to grow slower, but achieve larger sizes than males, and GOM females grow slower, but achieve larger sizes than Atlantic females. However, the current estimates suggest that GOM males grow faster and to smaller sizes than Atlantic males. This is contrary to the historic pattern reported by DeVries and Grimes (1997). The reversal appears to be driven by an increase in the estimated asymptotic length of Atlantic males relative to historic estimates. This increase in L_∞ may be due to bias from the large proportion of samples collected from tournament landings. However, historic age and growth studies also relied heavily on fishing tournaments to collect samples (DeVries and Grimes 1997).

The contemporary (2006-2008) growth functions computed in this study likely more accurately describe population growth patterns than historic functions due to the presence of fishery-dependent and -independent data, particularly for the GOM population. Collecting samples from fishery-independent as well as fishery-dependent sources resulted in broader size and age distributions than would be available if all samples were collected from the landed catch. This is evidenced by the large number of age-0 fish included in the analysis as well as the number of samples below the legal size limit (<606.9 mm). Including sub-legal fish, and age-0 fish in particular, anchored the VBGF by estimating values for t_0 closer to the origin than those reported by DeVries and Grimes (1997). Improving estimates of t_0 affects the fit of the overall growth function, decreasing estimates of L_∞ and increasing estimates of k. This effect is apparent in comparing the current GOM and Atlantic female VBGFs to historic growth functions, which were estimated from exclusively fishery-dependent data. The current Atlantic male VBGF demonstrated an increase in L_∞ and t_0 and a slight decrease in k relative to historic growth functions. This effect may be due to the use of tournament landings.

The importance of incorporating fishery-independent samples to mitigate bias from the truncated size distribution of fishery landings is highlighted in the pattern of residuals for the current VBGFs (Figure 9). Fishery-dependent residuals form a U-shaped pattern with the youngest and oldest age classes having mostly positive residuals. The positive residuals for the oldest fish are likely due to the fact that those samples were collected from tournament landings. Tournament anglers target the largest size classes; thus, it is not surprising that their catch would display positive residuals around the growth curve. The larger than expected size-at-age for the youngest fishery-dependent samples likely results from the tendency of hook-and-line fisheries to selectively remove fast-growing, young fish, particularly in the presence of minimum size limits (Conover and Munch 2002, Taylor et al 2005). Only the young individuals that have grown fast enough to recruit to the gear or reach the minimum size limit will be represented in the landings, and age-0 king mackerel tend to be absent entirely. The addition of fishery-independent hook and line samples reduces bias in the youngest year classes by including young fish that have recruited to the gear, but remain under the legal size limit; inclusion of age-0 and age-1 samples collected in scientific trawl surveys has a similar effect. The scarcity of negative residuals for age-2 and age-3 fish may be due to the difficulty in collecting slow-growing individuals in those year classes that are no longer vulnerable to the trawling gear but have not fully recruited to the hook-and-line fishery. It might also reflect a bias in the trawl survey toward smaller, slow-growing age-0 and age-1 fish that swim more slowly than larger fish and are unable to escape the gear. Overall, including age and length data for juvenile and sub-legal adult samples improved estimates of t_0 for all sex/stock combinations.

The ANCOVA results indicate fishing pressure may have impacted king mackerel size-at-age in the GOM and Atlantic through artificial selection and compensatory mechanisms. Heavy fishing pressure in the GOM lead to a decline in the biomass of that population in the 1970s and 1980s (Powers 1996). Greater size at age displayed by GOM fish in the 1980s may have resulted from increased growth due to density dependence. Contemporary estimates of GOM size-at-age are lower than those from the 1970s and 1980s, which may reflect a long-term genotypic response to the removal of large, fast-growing individuals (Conover and Munch 2002). Artificial selection for slow growth would be further exacerbated by minimum size limits implemented in 1990, which protect small, slow growing king mackerel. Atlantic mean size-at-age has exhibited an increase in recent years compared to historic estimates. Although neither overfished nor undergoing overfishing, the Atlantic population biomass estimates have steadily declined over the last two decades (SEDAR16 2008).

The higher size-at-age of contemporary Atlantic estimates may be evidence of a density dependent feedback on growth, with smaller mean size-at-age in the youngest age classes possibly showing initial signs of a genotypic response to artificial selection. It is also possible that higher size at age for older fish is an artifact of tournament sampling.

Age and growth results presented here display clear differences when compared to historic age and growth data. Improving estimates of t_0 with the use of fishery-independent data for the youngest year classes affects the fit of the growth function as a whole; however, general patterns in growth between sexes and stocks appear consistent with fishery-dependent based estimates. Finally, fishing pressure does appear to have affected size at age for GOM king mackerel and the Atlantic population may also be demonstrating initial signs of a fishing effect.

Otolith Shape Analysis

Results from king mackerel otolith shape analysis were consistent with those of historic studies. The variety of factors contributing to otolith shape reduces its power as a natural tag, as evidenced by low classification success, which limits the precision of estimates of Atlantic contribution. However, the fact that spatial trends appear consistent over time, in spite of the imprecision, further supports the existence of an east-west gradient in Atlantic contribution to south Florida winter landings.

Variation in otolith shape due to fishing year, sex, and age complicate its use as a natural stock-specific tag. The effects of fishing year and sex are easily accounted for by modeling years and sexes separately. However, age continued to be a significant factor in determining otolith shape after the data were detrended to correct for fish size. This implies a year-class effect that may result from inter-annual variation in somatic growth with periods of poor or favorable environmental conditions and prey availability. The age range of samples included for analysis was limited to reduce any age effect, but it appears that examining individual year class models would be necessary to fully account for variation due to age. Sample sizes were deemed insufficient to examine that in the current study.

Combining age classes for analysis may reduce the resolution of otolith shape signatures, likely contributing to the low classification success rates in this and historic studies of king mackerel mixing (DeVries et al. 2002, Clardy et al. 2008). An otolith shape-based stock discrimination study of Atlantic mackerel (*Scomber scombrus*) produced an average classification success of 82.4% when year classes were tested individually (Castonguay et al. 1991). However, it is possible that classification success might be inflated in a combined age analysis if sample age distributions vary between stocks (Castonguay et al. 1991). In such a case, stock-specific otolith shape signatures would reflect both variation due to stock and variation due to age. The consistency in classification success rates between 2006/07, when age distributions varied slightly, and 2007/08, when the data were sub-sampled to create uniform age distributions, suggests classification success was not inflated by combining ages in this study.

The otolith shape based-classification success rates of between 60% and 73% in this study are similar to but somewhat lower than historic mean classification success: 74.5% (DeVries et al. 2002) and 68.5% (Clardy et al. 2008). The higher classification success using sex-specific over combined sex models is likely due to the removal of variation in otolith shape caused by sex-specific differences in growth rate. Overall, consistently low classification success rates indicate otolith shape is not a powerful stock-specific tag. Classification success was similar between fishing years in this study, but 2007/08 point estimates displayed higher precision than 2006/07 estimates, as evidenced by lower SEs. This increase in precision may be the result of an increase in mixed-stock sample sizes as was seen in the stable isotope simulation results. Larger stock-specific sample sizes might also increase the robustness of otolith shape as a natural tag by permitting separate year class models.

The estimated Atlantic contribution was greater than zero in each zone for all model results, thus indicating the current management strategy, which assigns all landings from the winter mixing area to the GOM population, does not accurately reflect king mackerel migratory patterns. The results presented here suggest a spatial pattern in Atlantic contribution from west to east with a lower proportion of landings from the Atlantic stock in zone 1 than zones 2 and 3. This pattern is consistent with historic otolith shape based mixing estimates (Clardy et al. 2008). The absence of any spatial pattern in the sex-specific 2007/08 models may be due to the broader age distribution of the samples. It is possible that older and larger fish move in less predictable ways than the age-2 and age-3 fish that made up the majority of mixed-stock winter samples in 2006/07.

The estimated Atlantic contribution to zone 3 landings exhibited an increasing trend across months during the winter mixing season. As with trends in spatial variability, this pattern is not apparent in the 2007/08 sex-specific model results, but is clearly demonstrated in the 2007/08 combined sex and all 2006/07 models. It is likely that the increase in the percentage of Atlantic fish landed later in the season is evidence of early spring migration to the summer spawning grounds. Thus, the period of time in which mixed-stock landings were collected should be taken into account when interpreting any mixing estimates for zone 3.

A comparison between otolith shape-based estimates of Atlantic contribution to winter landings reported here and historic estimates indicates a possible long-term decline in Atlantic contribution to mixed-stock winter landings. DeVries et al. (2002) estimated that 99.8% of female king mackerel in southeast Florida (Cape Canaveral to Palm Beach) in 1996/97 were from the Atlantic population. This is striking considering the majority of 1996/97 mixed-stock samples were collected in December when Atlantic contribution would be lowest based on current temporal trends. Estimated Atlantic contribution to zone 3 female landings declined to 82.8% in 2001/02 and 40.4% in 2002/03 (Clardy et al. 2008). Mixed-stock 2001/02 and 2002/03 samples were collected in February and March when Atlantic contribution is expected to be highest. In the current study the average percentage across all winter months of zone 3 female landings contributed by the Atlantic population was 45.1% in 2006/07 and 31.8% in 2007/08. Zone 3 male landings also declined from 76.0% in 2001/02 and 71.9% in 2002/03 (Clardy et al. 2008) to 21.8% and 20.6% in 2006/07 and 2007/08, respectively.

Similar declines can be seen between estimated Atlantic contribution reported by Clardy et al. (2008) for zones 1 and 2 in 2001/02 and 2002/03 and those reported here for 2006/07 and 2007/08. Mixed-stock zone 1 and zone 2 samples were collected in January in both studies. Zone 1 female Atlantic contribution dropped from 61% in 2001/02 to 14.5% in 2002/03 then increased to 32.4% in 2006/07 and fell to 26.2% in 2007/08. Estimated Atlantic contribution to zone 1 male landings declined from 61%

and 45% in 2001/02 and 2002/03, respectively to 23.9% and 14.1% in 2006/07 and 2007/08, respectively. The estimated zone 2 female Atlantic contribution remained fairly stable at 48.6%, 41.3%, and 41.8% in 2001/02, 2002/03, and 2006/07, respectively but declined to 25.3% in 2007/08. Finally, the percentage of Atlantic zone 2 male landings was estimated as high as 99% in 2001/02 and dropped to 83.1% in 2002/03 then to 79.5% in 2006/07 and 54.0% in 2007/08.

Current estimates of Atlantic contribution are consistently lower than historic otolith shape-based estimates for all zones and both sexes. The decline in Atlantic contribution may reflect an increase in the GOM stock's presence in the mixing area, a decline in the Atlantic stock's presence, or both. During the period between 1996 and 2008 the GOM stock biomass has increased in response to conservation measures and decreased consumption due to public concern over mercury warnings (Powers 1996, SEDAR16 2008). The Atlantic stock's biomass has steadily declined over the same period. It is also possible that the trend is in response to long-term environmental changes affecting king mackerel migration patterns. However, the imprecision about point estimates of Atlantic contribution prevents drawing any concrete conclusions about the cause of the decline.

Otolith chemistry

Otolith stable isotope chemistry is a more powerful natural stock-specific tag than otolith shape, although trends in estimated Atlantic contribution to south Florida winter landings were consistent between approaches. Atlantic contribution to winter landings was estimated to have increased across sampling zones from west to east and across months in zone 3 from December to March. However, precision about the estimates of Atlantic contribution does not appear to reflect the increase in classification success using stable isotope chemistry (mean SE=17.3) rather than otolith shape signatures (mean SE=17.0) in discriminating between the two populations.

Age was a significant factor in both $\delta^{18}O$ and $\delta^{13}C$ variation, indicating ontogenetic shifts likely due to changes in habitat utilization and trophic position (Begg and Weinman 2001, Soloman et al. 2006). Otolith $\delta^{18}O$ is negatively correlated with temperature, suggesting the increase in $\delta^{18}O$ with age in both populations in 2006 samples and in the Atlantic in 2007 samples was likely due to older king mackerel inhabiting cooler water masses (Kalish 1991). It is unclear why mean $\delta^{18}O$ appeared to decline with age in 2007 GOM samples.

The increase in GOM $\delta^{13}C$ values with age in summer 2006 samples is evidence of a possible ontogenetic shift in trophic position as well as habitat temperature. Otolith $\delta^{13}C$ has been reported to be negatively correlated with water temperature but somewhat positively correlated with trophic position (Thorrold et al. 1997). Neither 2006 Atlantic samples nor samples from either population collected in 2007 displayed a clear relationship between $\delta^{13}C$ values and age. As trends in otolith $\delta^{18}O$ values indicated a likely shift to cooler water masses with age, it is possible the lack of $\delta^{13}C$ variation is due to limitations on the availability of higher trophic level prey items. However, analysis of muscle $\delta^{13}C$ or gut contents over a range of age classes would be necessary to test such a hypothesis.

Otolith $\delta^{18}O$ varied more between populations than $\delta^{13}C$. The difference between GOM and Atlantic mean $\delta^{18}O$ values was 0.31‰ in 2006/07 and 0.36‰ in 2007/08, while the difference in mean $\delta^{13}C$ was only 0.19‰ in 2006/07 and 0.17‰ in 2007/08. As one would expect, simulation results indicated higher contrast in chemical signatures resulted in a more powerful natural tag with higher classification success. In fact, when stock-specific otolith chemistry analysis was repeated with $\delta^{18}O$ mean classification success was comparable (within 1%) to combined stable isotope signatures with similar estimates of Atlantic contribution and precision around those estimates. Thus, it is possible that future analysis of Atlantic contribution to mixed-stock king mackerel landings could be conducted using otolith $\delta^{18}O$ alone.

Otolith stable isotopes appear to be a more powerful stock-specific tag than otolith shape. Classification success using stable isotope-based DFs averaged 13.5 percentage points higher than otolith shape-based classification success. Summer 2006 male samples were correctly classified at a higher rate than female and combined sex samples using otolith shape, while males displayed the lowest classification success using stable isotope signatures. The following year for females, classification accuracy was highest with otolith shape and lowest with stable isotopes. Combined sex-analyses were stable between years and methods with mean classification success near 80% using stable isotope signatures, which was approximately 15 percentage points higher than the associated otolith shape-based results.

Sex-specific analyses did not consistently result in higher classification success over combined sex analyses despite a significant sex effect revealed by MANOVA. The 2006/07 male-specific and 2007/08 female-specific DFs produced a lower mean classification success than the combined sex analyses in those years. Low discriminatory power in male 2006/07 samples suggests the difference in age distributions between populations did not artificially inflate classification accuracies. However, it is not clear what drove the low classification success for 2006/07 male and 2007/08 female samples.

Otolith shape- and stable isotope-based methods both classified Atlantic samples more accurately than GOM samples. This is likely due to lower variance around stock-specific signature values in the Atlantic than the GOM as stated above. The lower variance in Atlantic signatures may be the result of lower variability in environmental conditions (i.e. temperature) of areas where Atlantic king mackerel reside. Otolith shape is driven by the somatic growth rate, and the mean standard deviation about size at age was 67.8 for the GOM population and 54.6 for the Atlantic in the age and growth portion of this study. Genetics and prey availability play a role in determining somatic growth; however, growth rate is also strongly linked to temperature. That fact, combined with the lower variance about temperature-driven $\delta^{18}O$ values in the Atlantic than the GOM population, suggests Atlantic king mackerel might encounter a narrower range of temperatures than GOM king mackerel.

Maximum likelihood estimates of the Atlantic population contribution to winter landings based on otolith stable isotope data displayed trends similar to those seen in current and historic otolith shape-based results (Clardy et al. 2008). The percentage of landings contributed by the Atlantic population was consistently greater than zero and increased from west to east. Stable isotope-based estimates also provided further evidence of an increase in Atlantic contribution to landings off southeast Florida across the mixing season from December to March as the populations begin their spring migration northward. The 2007/08 combined sex and male only estimates were greater than those for 2006/07, while the percent contribution to female landings decreased between fishing years. Sex-specific inter-annual variability in estimated Atlantic contribution suggests the sexes may be migrating independently and that migratory pathways may also vary, possibly in response to variation in water temperature and migration of prey species. Distinct sex-specific migration is further supported by differences in estimated Atlantic contribution between the sexes within each fishing year. The percentage of female landings attributed to the Atlantic population was generally greater than that of males, except in zones 1 and 2 in 2007/08, for which the reverse was true. Combined sex estimates of Atlantic contribution generally fell between the estimates for male and female only landings, except in 2007/08 zone 3, when they were slightly greater than those for females only. The SEs about estimates of Atlantic contribution to combined sex landings were lower than those of sex-specific estimates in both years. This increase in precision is likely a function of doubling the mixed-stock sample size and is similar to results observed in simulation 5 when winter sample sizes were doubled. Thus, combined sex stable isotope signatures present a straightforward, accurate natural tag for estimating Atlantic contribution.

Simulation results suggest stable isotope-based estimates of Atlantic contribution would be accurate under a variety of scenarios, and that precision is driven by both classification success and mixed-stock sample size. Consistent accuracy in estimating Atlantic contribution across simulation exercises is evidence of the robustness of the maximum likelihood models used in this study. However, variation in precision about estimates of Atlantic contribution in the wild population is not consistent with the simulation results. The SE about the estimated mixing rate in simulation 1 was 7.9, which is much lower than the SE (14.3) from the 2006/07 combined sex model, despite the facts that they had similar $\delta^{18}O$ and $\delta^{13}C$ distributions, comparable sample sizes, and the wild-sampled model yielded higher mean classification success.

The much larger SE about the point estimate of Atlantic contribution from the 2006/07 combined sex model than that of from simulation 1 might be explained by the presence in winter of some contingent of king mackerel that inhabit the mixing area year round. Early tagging data indicated some king mackerel released off south Florida remained in the region rather than migrating north during the spring (Sutter et al. 1991). The presence of resident king mackerel around south Florida that are members of neither from the GOM nor the Atlantic migratory contingents could impact the precision about estimated Atlantic contribution. The SEs about mixing estimates displayed consistent spatial and temporal patterns that indicate a resident contingent may not be present in all zones of the mixing area at all times. Zone 1 and late season zone 3 maximum likelihood results displayed lower SEs that more closely resembled those of the simulation exercise. Also, mean SE about male only estimates of Atlantic contribution were higher than those of female samples in 2007/08 (male=23.6, female=16.11) in spite of a higher male classification success rate (male=86.5, female=71.1). Ultimately, no concrete conclusions can be drawn regarding the existence and distribution of a south Florida resident contingent of king mackerel without collecting samples in the mixing area during the summer, when the GOM and Atlantic populations have migrated to their distinct spawning grounds.

Conclusions

The sex- and population-specific growth functions estimated in this study display an improved fit over historic estimates through the inclusion of small individuals collected using fishery-independent methods. Age-0 and age-1 samples proved useful in producing more realistic estimates of the von Bertalanffy parameter t_0 (i.e., closer to the origin), which in turn decreased estimates of L_∞ and increased estimates of k. Historic VBGFs were re-estimated based on a truncated distribution to allow a comparison with the current results. However, differences between current and historic growth functions indicate that estimating growth based on a truncated distribution may not fully account for the absence of juveniles in the sample. Growth rates appear to have declined over time in the GOM, likely as a result of several decades of intense fishing. This decline may be a genetic response which could affect GOM king mackerel productivity for several generations (Conover and Munch 2002). Estimates of Atlantic growth have increased relative to historic data, which may be an initial response to increased fishing pressure or may be an artifact caused by the use of data from tournament landings, which tend to be size selective. Further research is needed to assess how data from tournament fish impacts growth rate estimates, and any future work should include a larger proportion of non-tournament samples from the Atlantic.

Discriminant function analysis of otolith shape and stable isotope chemistry indicated both are useful in discriminating between GOM and Atlantic king mackerel. However, stable isotope signatures appear to be more powerful natural stock-specific tags than otolith shape based on jackknifed classification success rates. Furthermore, $\delta^{18}O$ signatures display greater variation between the two populations than $\delta^{13}C$ values.

Future analyses using otolith $\delta^{18}O$ alone as a natural tag may be sufficient to discriminate between the two populations.

Estimates of Atlantic contribution reported here are consistent with historic spatial trends, but indicate both inter- and intra-annual variability. Atlantic contribution appears to be consistently greater than zero throughout the mixing area, but lower off southwest Florida than southeast Florida. Analysis of temporal variability in zone 3 indicated an increase in the percentage of landings contributed by the Atlantic population from December to March. This shift is attributed to the beginning of the regular spring migration northward to the spawning grounds. Instability in Atlantic contribution to zone 3 landings within the winter fishing season will complicate any management measure aimed at establishing a set mixing rate for assigning landings to each population.

Consistency in spatial and temporal patterns in estimated Atlantic contribution between the two methods used in this study and between current and historic estimates further supports the validity of these results. Otolith shape and stable isotope chemistry are independently derived characteristics, and yet natural tags derived from these characters indicate similar patterns of Atlantic contribution. Also, the otolith shape based estimates display a spatial pattern consistent with estimates from samples collected five years earlier indicating the east-west gradient is stable over time despite variation in the degree of Atlantic contribution.

A comparison of the otolith shape-based estimates from this study and historic estimates exhibits a clear decline in Atlantic contribution over time, possibly reflecting shifts in biomass in the two populations. In 1996/97, when DeVries et al. (2002)

estimated a 99.8% Atlantic contribution to zone 3 female landings, the GOM population was overfished and had undergone overfishing for many years (Powers 1996). Since then a combination of factors including conservation measures, reduced demand for GOM king mackerel, and a series of strong year classes has lead to a dramatic increase in the estimated GOM population size (SEDAR16 2008). Simultaneously, the Atlantic population has experienced an estimated decline in biomass (SEDAR16 2008). It is likely the decreased Atlantic contribution to mixed-stock landings is a byproduct of this shift in the Atlantic population size relative to that of the GOM. Such changes in the contribution of each population to winter landings with trends in biomass further complicate the application of a fixed mixing rate for management and assessment purposes. Preliminary results from this study were included in the 2008 stock assessments, which resulted in changing the mixing rate assumed in the stock assessment model. The historic assumption that all mixed-stock winter landings were from the GOM was replaced by a 50:50 ratio of GOM to Atlantic contribution. While this decision is more reflective of natural stock dynamics than the historic assumption, it still does not fully account for the spatial and temporal variability in Atlantic contribution to mixed-stock winter landings.

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APPENDIXES

APPENDIX A

Animal Care and Use Committee Approval Letter

University of West Florida

Research and Sponsored Programs
Building 11, Room 109
11000 University Parkway
Pensacola, FL 32514-5750

MEMORANDUM

May 7, 2007

TO: Ms. Kate Shepard
Biology Department
Building 58

FROM: Animal Care and Use Committee
Christine Cavanaugh, Interim Director
Research and Sponsored Research

Christine K. Cavanaugh

SUBJECT: ACUC Approval

The Animal Care and Use Committee has completed its review of your proposal titled "King Mackerel Population Dynamics and Stock Mixing in the U.S. Atlantic and Gulf of Mexico" and has granted approval for you to proceed with your study. Please note the following:

- Prior ACUC approval is required for significant changes to your protocol.
- The maximum approval period is three years. Should your project continue beyond the three year period, you must request ACUC approval prior to the end of the approval period, you must request ACUC approval prior to the end of the approval period.
- Annual status reports may be required by the ACUC. These reports must include a complete description of any and all changes in your project.

Please take time to review our ACUC web page at: http://research.uwf.edu/boards-committees/acuc.htm which includes the ACUC Policies and Procedures, the PHS Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and other pertinent ACUC documents.

Good luck in your research endeavors.

CC: Dr. William Patterson
Dr. George Stewart

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APPENDIX B

Analysis of Variance Results from Otolith Shape Data from Summer-sampled King Mackerel, Scomberomorus cavalla, to Determine Differences between Years (2006/07 and 2007/08), Stocks (Gulf of Mexico and Atlantic Ocean), Sexes, and Ages (2-6).

Area

Source	DF	SS	MS	F	p
Model	22	8.61887185	0.3917669	3.32	<0.001
Year	1	0.05473591	0.05473591	0.46	0.496
Group	1	1.32133065	1.32133065	11.21	<0.001
Sex	1	0.85014099	0.85014099	7.21	0.008
Age	4	2.70056639	0.6751416	5.73	<0.001
Year*Group	1	0.00211509	0.00211509	0.02	0.894
Year*Sex	1	0.01593414	0.01593414	0.14	0.713
Year*Age	4	1.07502331	0.26875583	2.28	0.060
Group*Sex	1	0.00392753	0.00392753	0.03	0.855
Group*Age	4	0.53820296	0.13455074	1.14	0.336
Sex*Age	4	0.0874554	0.02186385	0.19	0.946
Error	534	62.93708278	0.11785971		
Total	556	71.55595463			

Perimeter

Source	DF	SS	MS	F	p
Model	22	3.23701022	0.14713683	3.69	<0.001
Year	1	0.0221266	0.0221266	0.56	0.457
Group	1	0.19699178	0.19699178	4.94	0.027
Sex	1	1.03046404	1.03046404	25.86	<0.001
Age	4	0.43680364	0.10920091	2.74	0.028
Year*Group	1	0.06444059	0.06444059	1.62	0.204
Year*Sex	1	0.01606734	0.01606734	0.4	0.526
Year*Age	4	0.35038524	0.08759631	2.2	0.068
Group*Sex	1	0.03001532	0.03001532	0.75	0.386
Group*Age	4	0.26777559	0.0669439	1.68	0.153
Sex*Age	4	0.14020673	0.03505168	0.88	0.476
Error	534	21.27908517	0.03984847		
Total	556	24.5160954			

Box Width

Source	DF	SS	MS	F	p
Model	22	0.24534219	0.01115192	1.65	0.032
Year	1	0.04396823	0.04396823	6.51	0.011
Group	1	0.00364486	0.00364486	0.54	0.463
Sex	1	0.08463162	0.08463162	12.54	<0.001
Age	4	0.02531435	0.00632859	0.94	0.442
Year*Group	1	0.00312899	0.00312899	0.46	0.496
Year*Sex	1	0.00211274	0.00211274	0.31	0.576
Year*Age	4	0.05650369	0.01412592	2.09	0.081
Group*Sex	1	0.00067191	0.00067191	0.1	0.753
Group*Age	4	0.00946828	0.00236707	0.35	0.844
Sex*Age	4	0.00652189	0.00163047	0.24	0.915
Error	534	3.60424107	0.00674952		
Total	556	3.84958326			

Box Height

Source	DF	SS	MS	F	p
Model	22	0.08142876	0.00370131	2.61	<0.001
Year	1	0.00215734	0.00215734	1.52	0.218
Group	1	0.03665181	0.03665181	25.84	<0.001
Sex	1	0.00012784	0.00012784	0.09	0.764
Age	4	0.00694758	0.0017369	1.22	0.299
Year*Group	1	0.00000813	0.00000813	0.01	0.940
Year*Sex	1	0.00086677	0.00086677	0.61	0.435
Year*Age	4	0.01801572	0.00450393	3.18	0.014
Group*Sex	1	0.0016444	0.0016444	1.16	0.282
Group*Age	4	0.00998971	0.00249743	1.76	0.135
Sex*Age	4	0.00111664	0.00027916	0.2	0.940
Error	534	0.75746479	0.00141847		
Total	556	0.83889355			

Roundness

Source	DF	SS	MS	F	p
Model	22	0.01649118	0.0007496	1.55	0.053
Year	1	0.00100421	0.00100421	2.08	0.150
Group	1	0.00015246	0.00015246	0.32	0.575
Sex	1	0.00523285	0.00523285	10.81	0.001
Age	4	0.0021438	0.00053595	1.11	0.352
Year*Group	1	0.00150707	0.00150707	3.11	0.078
Year*Sex	1	0.00015463	0.00015463	0.32	0.572
Year*Age	4	0.00331621	0.00082905	1.71	0.146
Group*Sex	1	0.00019021	0.00019021	0.39	0.531
Group*Age	4	0.00095525	0.00023881	0.49	0.741
Sex*Age	4	0.00124412	0.00031103	0.64	0.632
Error	534	0.25842895	0.00048395		
Total	556	0.27492013			

Circularity

Source	DF	SS	MS	F	p
Model	22	0.00184769	0.00008399	4.97	<0.001
Year	1	0.00000526	0.00000526	0.31	0.577
Group	1	0.00021964	0.00021964	12.99	<0.001
Sex	1	0.00046322	0.00046322	27.39	<0.001
Age	4	0.00024366	0.00006091	3.6	0.007
Year*Group	1	0.00000892	0.00000892	0.53	0.468
Year*Sex	1	0.00001899	0.00001899	1.12	0.290
Year*Age	4	0.0001211	0.00003027	1.79	0.129
Group*Sex	1	0.00001797	0.00001797	1.06	0.303
Group*Age	4	0.00015738	0.00003935	2.33	0.055
Sex*Age	4	0.00006769	0.00001692	1	0.407
Error	534	0.00902977	0.00001691		
Total	556	0.01087746			

Ellipticity

Source	DF	SS	MS	F	p
Model	22	0.00203134	0.00009233	1.6	0.041
Year	1	0.00034557	0.00034557	6	0.015
Group	1	0.00048847	0.00048847	8.49	0.004
Sex	1	0.00011301	0.00011301	1.96	0.162
Age	4	0.00014921	0.0000373	0.65	0.628
Year*Group	1	0.00000814	0.00000814	0.14	0.707
Year*Sex	1	0.00002704	0.00002704	0.47	0.493
Year*Age	4	0.00064297	0.00016074	2.79	0.026
Group*Sex	1	0.00002655	0.00002655	0.46	0.497
Group*Age	4	0.00019317	0.00004829	0.84	0.501
Sex*Age	4	0.00000543	0.00000136	0.02	0.999
Error	534	0.03073732	0.00005756		
Total	556	0.03276865			

Rectangularity

Source	DF	SS	MS	F	p
Model	22	0.00624589	0.0002839	4.42	<0.001
Year	1	0.00001939	0.00001939	0.3	0.583
Group	1	0.00227991	0.00227991	35.48	<0.001
Sex	1	0.00099537	0.00099537	15.49	<0.001
Age	4	0.0008242	0.00020605	3.21	0.013
Year*Group	1	0.00000025	0.00000025	0	0.951
Year*Sex	1	0.00000793	0.00000793	0.12	0.726
Year*Age	4	0.00037908	0.00009477	1.47	0.208
Group*Sex	1	0.00000684	0.00000684	0.11	0.744
Group*Age	4	0.00043899	0.00010975	1.71	0.147
Sex*Age	4	0.00025852	0.00006463	1.01	0.404
Error	534	0.03431859	0.00006427		
Total	556	0.04056448			

Harmonic 1

Source	DF	SS	MS	F	p
Model	22	0.00152905	0.0000695	0.99	0.480
Year	1	0.00001086	0.00001086	0.15	0.695
Group	1	0.00011201	0.00011201	1.59	0.208
Sex	1	0.00000021	0.00000021	0	0.956
Age	4	0.00063763	0.00015941	2.26	0.061
Year*Group	1	0.00025179	0.00025179	3.57	0.059
Year*Sex	1	0	0	0	0.998
Year*Age	4	0.00029985	0.00007496	1.06	0.374
Group*Sex	1	0.00000022	0.00000022	0	0.956
Group*Age	4	0.00003642	0.0000091	0.13	0.972
Sex*Age	4	0.00006709	0.00001677	0.24	0.917
Error	534	0.03763029	0.00007047		
Total	556	0.03915934			

Harmonic 2

Source	DF	SS	MS	F	p
Model	22	0.00543119	0.00024687	1.33	0.146
Year	1	0.00121373	0.00121373	6.53	0.011
Group	1	0.00130893	0.00130893	7.04	0.008
Sex	1	0.00034	0.00034	1.83	0.177
Age	4	0.00093077	0.00023269	1.25	0.288
Year*Group	1	0.0002323	0.0002323	1.25	0.264
Year*Sex	1	0.00004818	0.00004818	0.26	0.611
Year*Age	4	0.00141751	0.00035438	1.91	0.108
Group*Sex	1	0.00000575	0.00000575	0.03	0.860
Group*Age	4	0.00038879	0.0000972	0.52	0.719
Sex*Age	4	0.00000995	0.00000249	0.01	0.996
Error	534	0.09932142	0.000186		
Total	556	0.10475262			

Harmonic 3

Source	DF	SS	MS	F	p
Model	22	0.00359319	0.00016333	2.25	0.001
Year	1	0.00004565	0.00004565	0.63	0.428
Group	1	0.0002173	0.0002173	2.99	0.084
Sex	1	0.00079053	0.00079053	10.88	0.001
Age	4	0.00059301	0.00014825	2.04	0.087
Year*Group	1	0.00007189	0.00007189	0.99	0.320
Year*Sex	1	0.00000456	0.00000456	0.06	0.802
Year*Age	4	0.00122091	0.00030523	4.2	0.002
Group*Sex	1	0.00018531	0.00018531	2.55	0.111
Group*Age	4	0.00011665	0.00002916	0.4	0.808
Sex*Age	4	0.00034154	0.00008538	1.18	0.321
Error	534	0.03878827	0.00007264		
Total	556	0.04238146			

Harmonic 4

Source	DF	SS	MS	F	p
Model	22	0.00316854	0.00014402	1.64	0.034
Year	1	0.00019456	0.00019456	2.22	0.137
Group	1	0.00047591	0.00047591	5.42	0.020
Sex	1	0.00001	0.00001	0.11	0.736
Age	4	0.00052072	0.00013018	1.48	0.206
Year*Group	1	0.00021337	0.00021337	2.43	0.120
Year*Sex	1	0.00003927	0.00003927	0.45	0.504
Year*Age	4	0.00088865	0.00022216	2.53	0.040
Group*Sex	1	0.00001162	0.00001162	0.13	0.716
Group*Age	4	0.00027793	0.00006948	0.79	0.531
Sex*Age	4	0.00027806	0.00006952	0.79	0.531
Error	534	0.04689267	0.00008781		
Total	556	0.05006121			

Harmonic 5

Source	DF	SS	MS	F	p
Model	22	0.00483442	0.00021975	3.37	<0.001
Year	1	0.00051384	0.00051384	7.87	0.005
Group	1	0.00060023	0.00060023	9.2	0.002
Sex	1	0.00057931	0.00057931	8.88	0.003
Age	4	0.0009911	0.00024777	3.8	0.005
Year*Group	1	0.00005712	0.00005712	0.88	0.350
Year*Sex	1	0.00002663	0.00002663	0.41	0.523
Year*Age	4	0.00066358	0.00016589	2.54	0.039
Group*Sex	1	0.00003717	0.00003717	0.57	0.451
Group*Age	4	0.00023032	0.00005758	0.88	0.474
Sex*Age	4	0.0002275	0.00005688	0.87	0.481
Error	534	0.03485582	0.00006527		
Total	556	0.03969023			

Harmonic 6

Source	DF	SS	MS	F	p
Model	22	0.00401678	0.00018258	2.94	<0.001
Year	1	0.00000041	0.00000041	0.01	0.935
Group	1	0.00074494	0.00074494	11.98	<0.001
Sex	1	0.0010256	0.0010256	16.5	<0.001
Age	4	0.00056273	0.00014068	2.26	0.061
Year*Group	1	0.00002021	0.00002021	0.32	0.569
Year*Sex	1	0.0000113	0.0000113	0.18	0.670
Year*Age	4	0.00048618	0.00012155	1.95	0.100
Group*Sex	1	0.00001443	0.00001443	0.23	0.630
Group*Age	4	0.00017183	0.00004296	0.69	0.598
Sex*Age	4	0.00035198	0.000088	1.42	0.228
Error	534	0.03320167	0.00006218		
Total	556	0.03721845			

Harmonic 7

Source	DF	SS	MS	F	p
Model	22	0.00382727	0.00017397	3.29	<0.001
Year	1	0.00020724	0.00020724	3.92	0.048
Group	1	0.00077955	0.00077955	14.75	<0.001
Sex	1	0.0004702	0.0004702	8.9	0.003
Age	4	0.00059431	0.00014858	2.81	0.025
Year*Group	1	0.00001332	0.00001332	0.25	0.616
Year*Sex	1	0.00000063	0.00000063	0.01	0.913
Year*Age	4	0.00049581	0.00012395	2.35	0.054
Group*Sex	1	0.00001951	0.00001951	0.37	0.544
Group*Age	4	0.00020518	0.0000513	0.97	0.423
Sex*Age	4	0.00020705	0.00005176	0.98	0.418
Error	534	0.02821624	0.00005284		
Total	556	0.03204351			

Harmonic 8

Source	DF	SS	MS	F	p
Model	22	0.00364231	0.00016556	3.06	<0.001
Year	1	0.00004131	0.00004131	0.76	0.382
Group	1	0.00065913	0.00065913	12.2	<0.001
Sex	1	0.00053035	0.00053035	9.82	0.002
Age	4	0.0006876	0.0001719	3.18	0.013
Year*Group	1	0.00005113	0.00005113	0.95	0.331
Year*Sex	1	0.00000141	0.00000141	0.03	0.872
Year*Age	4	0.00044852	0.00011213	2.08	0.083
Group*Sex	1	0.00006865	0.00006865	1.27	0.260
Group*Age	4	0.00022678	0.00005669	1.05	0.381
Sex*Age	4	0.00014422	0.00003605	0.67	0.615
Error	534	0.02885152	0.00005403		
Total	556	0.03249383			

Harmonic 9

Source	DF	SS	MS	F	p
Model	22	0.00395131	0.00017961	3.47	<0.001
Year	1	0.00005338	0.00005338	1.03	0.311
Group	1	0.00092828	0.00092828	17.91	<0.001
Sex	1	0.0005976	0.0005976	11.53	<0.001
Age	4	0.00066894	0.00016724	3.23	0.012
Year*Group	1	0.00003733	0.00003733	0.72	0.396
Year*Sex	1	0.00000827	0.00000827	0.16	0.690
Year*Age	4	0.00045238	0.00011309	2.18	0.070
Group*Sex	1	0.00002543	0.00002543	0.49	0.484
Group*Age	4	0.00017248	0.00004312	0.83	0.505
Sex*Age	4	0.0001737	0.00004342	0.84	0.502
Error	534	0.02767684	0.00005183		
Total	556	0.03162816			

Harmonic 10

Source	DF	SS	MS	F	p
Model	22	0.00356324	0.00016197	3.53	<0.001
Year	1	0.00010638	0.00010638	2.32	0.129
Group	1	0.00079732	0.00079732	17.35	<0.001
Sex	1	0.00044449	0.00044449	9.67	0.002
Age	4	0.0006646	0.00016615	3.62	0.006
Year*Group	1	0.00002988	0.00002988	0.65	0.420
Year*Sex	1	0.00000185	0.00000185	0.04	0.841
Year*Age	4	0.00042032	0.00010508	2.29	0.059
Group*Sex	1	0.00000261	0.00000261	0.06	0.812
Group*Age	4	0.0002419	0.00006047	1.32	0.263
Sex*Age	4	0.0002265	0.00005663	1.23	0.296
Error	534	0.02453379	0.00004594		
Total	556	0.02809703			

Harmonic 11

Source	DF	SS	MS	F	p
Model	22	0.00410623	0.00018665	3.79	<0.001
Year	1	0.00008186	0.00008186	1.66	0.198
Group	1	0.00083214	0.00083214	16.9	<0.001
Sex	1	0.0005302	0.0005302	10.77	0.001
Age	4	0.00090495	0.00022624	4.59	0.001
Year*Group	1	0.00000696	0.00000696	0.14	0.707
Year*Sex	1	0.00000545	0.00000545	0.11	0.740
Year*Age	4	0.00040101	0.00010025	2.04	0.088
Group*Sex	1	0.00003647	0.00003647	0.74	0.390
Group*Age	4	0.00023211	0.00005803	1.18	0.319
Sex*Age	4	0.0002648	0.0000662	1.34	0.252
Error	534	0.02629414	0.00004924		
Total	556	0.03040037			

Harmonic 12

Source	DF	SS	MS	F	p
Model	22	0.00386314	0.0001756	3.82	<0.001
Year	1	0.0001221	0.0001221	2.65	0.104
Group	1	0.00091851	0.00091851	19.96	<0.001
Sex	1	0.00037403	0.00037403	8.13	0.004
Age	4	0.00071094	0.00017773	3.86	0.004
Year*Group	1	0.00003832	0.00003832	0.83	0.362
Year*Sex	1	0.00001867	0.00001867	0.41	0.524
Year*Age	4	0.00040775	0.00010194	2.22	0.066
Group*Sex	1	0.000016	0.000016	0.35	0.556
Group*Age	4	0.00026864	0.00006716	1.46	0.213
Sex*Age	4	0.00015957	0.00003989	0.87	0.484
Error	534	0.02456869	0.00004601		
Total	556	0.02843182			

Harmonic 13

Source	DF	SS	MS	F	p
Model	22	0.00384064	0.00017457	3.71	<0.001
Year	1	0.00010096	0.00010096	2.15	0.143
Group	1	0.00076017	0.00076017	16.17	<0.001
Sex	1	0.00057843	0.00057843	12.31	<0.001
Age	4	0.00071372	0.00017843	3.8	0.005
Year*Group	1	0.00003617	0.00003617	0.77	0.381
Year*Sex	1	0.00000378	0.00000378	0.08	0.777
Year*Age	4	0.00039107	0.00009777	2.08	0.082
Group*Sex	1	0.00001794	0.00001794	0.38	0.537
Group*Age	4	0.0002567	0.00006417	1.37	0.245
Sex*Age	4	0.00026731	0.00006683	1.42	0.225
Error	534	0.02509747	0.000047		
Total	556	0.02893811			

Harmonic 14

Source	DF	SS	MS	F	p
Model	22	0.00398497	0.00018114	3.8	<0.001
Year	1	0.00010787	0.00010787	2.26	0.133
Group	1	0.00092653	0.00092653	19.43	<0.001
Sex	1	0.00047674	0.00047674	10	0.002
Age	4	0.00068207	0.00017052	3.58	0.007
Year*Group	1	0.00004017	0.00004017	0.84	0.359
Year*Sex	1	0.000021	0.000021	0.44	0.507
Year*Age	4	0.00047769	0.00011942	2.5	0.041
Group*Sex	1	0.0000179	0.0000179	0.38	0.540
Group*Age	4	0.00022495	0.00005624	1.18	0.319
Sex*Age	4	0.00018278	0.0000457	0.96	0.430
Error	534	0.02545955	0.00004768		
Total	556	0.02944452			

Harmonic 15

Source	DF	SS	MS	F	p
Model	22	0.00385195	0.00017509	3.63	<0.001
Year	1	0.00006641	0.00006641	1.37	0.242
Group	1	0.00092308	0.00092308	19.11	<0.001
Sex	1	0.00050708	0.00050708	10.5	0.001
Age	4	0.00073891	0.00018473	3.82	0.004
Year*Group	1	0.0000822	0.0000822	1.7	0.193
Year*Sex	1	0.00000452	0.00000452	0.09	0.760
Year*Age	4	0.00045395	0.00011349	2.35	0.053
Group*Sex	1	0.00001284	0.00001284	0.27	0.606
Group*Age	4	0.00017498	0.00004374	0.91	0.460
Sex*Age	4	0.00022278	0.00005569	1.15	0.331
Error	534	0.02579207	0.0000483		
Total	556	0.02964402			

Harmonic 16

Source	DF	SS	MS	F	p
Model	22	0.00397602	0.00018073	3.69	<0.001
Year	1	0.00006815	0.00006815	1.39	0.240
Group	1	0.00097344	0.00097344	19.86	<0.001
Sex	1	0.00053124	0.00053124	10.84	0.001
Age	4	0.00072026	0.00018006	3.67	0.006
Year*Group	1	0.00001925	0.00001925	0.39	0.531
Year*Sex	1	0.00000356	0.00000356	0.07	0.788
Year*Age	4	0.00042365	0.00010591	2.16	0.072
Group*Sex	1	0.00000741	0.00000741	0.15	0.698
Group*Age	4	0.00020987	0.00005247	1.07	0.370
Sex*Age	4	0.00018905	0.00004726	0.96	0.427
Error	534	0.02617255	0.00004901		
Total	556	0.03014858			

Harmonic 17

Source	DF	SS	MS	F	p
Model	22	0.00381065	0.00017321	3.51	<0.001
Year	1	0.00006098	0.00006098	1.24	0.267
Group	1	0.0008907	0.0008907	18.05	<0.001
Sex	1	0.0004892	0.0004892	9.91	0.002
Age	4	0.00072562	0.00018141	3.68	0.006
Year*Group	1	0.00002076	0.00002076	0.42	0.517
Year*Sex	1	0.00000221	0.00000221	0.04	0.832
Year*Age	4	0.00038448	0.00009612	1.95	0.101
Group*Sex	1	0.00002475	0.00002475	0.5	0.479
Group*Age	4	0.00019053	0.00004763	0.97	0.426
Sex*Age	4	0.00021848	0.00005462	1.11	0.352
Error	534	0.02635451	0.00004935		
Total	556	0.03016516			

Harmonic 18

Source	DF	SS	MS	F	p
Model	22	0.00393171	0.00017871	3.62	<0.001
Year	1	0.00006837	0.00006837	1.39	0.239
Group	1	0.00087122	0.00087122	17.67	<0.001
Sex	1	0.00056344	0.00056344	11.43	<0.001
Age	4	0.00073839	0.0001846	3.74	0.005
Year*Group	1	0.00002126	0.00002126	0.43	0.512
Year*Sex	1	0.00000103	0.00000103	0.02	0.885
Year*Age	4	0.00043251	0.00010813	2.19	0.069
Group*Sex	1	0.0000174	0.0000174	0.35	0.553
Group*Age	4	0.0002218	0.00005545	1.12	0.344
Sex*Age	4	0.00018968	0.00004742	0.96	0.428
Error	534	0.02632871	0.0000493		
Total	556	0.03026042			

Harmonic 19

Source	DF	SS	MS	F	p
Model	22	0.00377649	0.00017166	3.62	<0.001
Year	1	0.00005995	0.00005995	1.27	0.261
Group	1	0.00079337	0.00079337	16.75	<0.001
Sex	1	0.00058362	0.00058362	12.32	<0.001
Age	4	0.00071781	0.00017945	3.79	0.005
Year*Group	1	0.00002594	0.00002594	0.55	0.460
Year*Sex	1	0.00000025	0.00000025	0.01	0.943
Year*Age	4	0.00038148	0.00009537	2.01	0.091
Group*Sex	1	0.0000205	0.0000205	0.43	0.511
Group*Age	4	0.0002213	0.00005533	1.17	0.324
Sex*Age	4	0.00019468	0.00004867	1.03	0.392
Error	534	0.02529771	0.00004737		
Total	556	0.02907419			