Estimation of the Stock Composition of Winter King Mackerel Fisheries off South Florida with Natural Tags Based on Otolith Stable Isotope Chemistry

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Introduction

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

While genetic differences may be insufficient to estimate stock identity of mixing zone landings, recent studies employing otoliths as natural stock markers in that application has shown great promise (DeVries et al. 2002; Patterson et al. 2004; Clardy et al. in press). Reasons why otoliths are ideal natural makers of fish populations or stocks are straightforward. Otoliths are calcium carbonate and protein matrices that are deposited in the vestibular system of bony fishes as they grow (Casselman 1987). Otoliths grow or accrete relative to somatic growth and form concentric opaque and translucent zones with which the age of the fish may be estimated; increments in otoliths are deposited sub-daily, daily, and annually. Otoliths are metabolically inert once formed and are never resorbed under natural conditions (Campana and Neilson 1985; Casselman 1987). Therefore, otolith characteristics that are unique to individual species or stocks have proven to serve as ideal, permanent natural tags. Differences in otolith morphology have been reported among closely related species (Johnson 1995) and among stocks of single species (Bird et al. 1996; Begg and Brown 2000), and are thought to reflect genotypic variability as well as differential environmental histories and growth rates (Campana and Casselman 1993). These differences have been used as stock-specific natural tags in many species (e.g., Campana and Casselman 1993; Bird et al. 1996; Begg and Brown 2000) and otolith shape analysis recently has been used to discriminate between GOM and Atlantic king mackerel stocks (DeVries et al. 2002; Clardy et al. in press).

An equally promising otolith-based approach to estimate movement patterns or stock mixing of adult fishes involves using otolith elemental and/or isotopic signatures as natural biogeochemical tags of fish from different water bodies, geographic areas, or stocks (Begg et al. 1998; Thorrold et al. 1998, 2001; Patterson et al. 1998, 2002; Kennedy et al. 2000). As otoliths grow, minor and trace metals are incorporated into their matrices from the water in which the fish lives (Hoff and Fuiman 1995; Kalish 1989; Bath et al. 2000). Because otoliths are metabolically inert once formed and the chemistry and environmental parameters of seawater vary geographically, analysis of otolith microchemistry reveals the environmental history of fish and can be used as a natural biogeochemical tag of fish populations or stocks (Thorrold et al. 1998, 2001; Campana 1999).

We and several collaborators have been employing otolith techniques to derive natural tags to estimate stock mixing in king mackerel. DeVries et al. (2002) reported differences in sagittal otolith shape parameters were significant between Atlantic and GOM females in summer 1996 (when stocks were separate), and that discriminant function analysis of shape data classified 71% of Atlantic and 78% of GOM fish accurately. The authors then parameterized a maximum likelihood mixing model with the same set of variables to estimate the stock composition of fish sampled during winter 1996/97 off southeast Florida. They estimated 99.8% of king mackerel sampled in winter from that region were contributed by the Atlantic migratory group or stock. Furthermore, the authors concluded otolith shape analysis suggested the Atlantic and GOM stock effectively did not mix off southeast Florida in winter 1996/97. In a similar approach, Clardy et al. (in press) were able to distinguish female and male mackerel between Atlantic and GOM groups sampled in summer 2001 and 2002 with between 65 and 82% accuracy with otolith shape characteristics. Maximum likelihood estimates of the stock identity of fish collected in three zones around southern Florida in winter 2001/02 and 2002/03 indicated the GOM stock contributed up to 85% of winter landings off southwest Florida, while up to 84% of fish landed off southeast Florida were contributed by the Atlantic stock.

Patterson et al. (2004) examined differences in king mackerel migratory group-specific otolith elemental signatures. Classification accuracies computed from sex-specific linear discriminant functions (LDFs) with elemental concentrations (Ba, Mn, Mg, and Sr) as dependent variables ranged from 69 - 91%. Otolith chemistry-based maximum likelihood estimates of the stock identity of fish collected from three south Florida winter zones mirrored results from otolith shape analysis: fish landed in the southwestern zone were mostly GOM fish and fish landed in the southeastern zone were predominantly Atlantic fish. One important difference between results from otolith shape versus chemistry analyses was that distinguishing stocks with chemistry was about 10% more accurate than with shape parameters, which resulted in tighter confidence intervals around winter mixing points estimates for the chemistry approach. More recently, we analyzed the stable isotope ($\delta^{13}C$ and $\delta^{18}O$) composition of a subset (n = 100) of otoliths from the summer 2002-collected fish and found our ability to distinguish stocks increased 10% on average among female, male, and combined sex models (WFP, unpublished data). Therefore, it is reasonable to assume combining stable isotope signatures with elemental signatures in future analyses will increase our ability to distinguish stocks, as well as decrease confidence limits of stock mixing estimates.

The objective of this study is to estimate the temporal and spatial variability in king mackerel stock mixing with natural tags derived from otolith chemistry analysis. To accomplish that objective, we began on a two-year study in summer 2006 to examine spatial and temporal variability in otolith elemental and stable isotope (jointly, chemical) signatures of Atlantic and GOM king mackerel stocks, with the goal of applying stock-specific chemical signatures to estimate the temporal and spatial variability in the stock composition of winter landings off south Florida. Data presented here represent only the stable isotope portion of the first year (2006/07) of the study. Elemental chemistry results from the 2006/07 year should be available in late February and resultant stock composition estimates will be computed thereafter.

Methods

King mackerel were sampled in the US Atlantic and GOM in summer 2006 when stocks were separate (Fig. 1). Summer sampling consisted of both fishery-independent and fishery-

dependent sampling in the Atlantic and eastern GOM. Fishery-independent samples were collected by Captain Ben Hartig, Captain Jeff Thierry, and University of West Florida (UWF) personnel. Fishery-dependent samples were collected by UWF personnel, as well as by NMFS port agents and North Carolina Department of Environment and Natural Resources, Division of Marine Fisheries (NCDMF) personnel. Sampled fish were measured to the nearest mm fork length (FL), weighed to the nearest 0.1 kg, and had their sex determined by macroscopic examination of gonads. We extracted both sagittal otoliths from each fish sampled. Extracted otoliths were cleansed of adhering tissue and placed in plastic vials for storage.

King mackerel were sampled in three south Florida sample zones during winter 2006/07. Fish were sampled around the tip of the Florida Peninsula to estimate region-specific stock mixing proportions (Fig. 2), and specifically to estimate the temporal variability in stock mixing off southeast Florida monthly during the winter mixing zone period (December through March). Fish and otolith samples were handled the same as summer-sampled individuals.

Fish age was estimated from one sagitta of each sampled fish in the Fisheries Laboratory at UWF following the methods of DeVries and Grimes (1997). After ageing and otolith shape analysis (see Shepard et al. in press) was completed, a sub-sample of aged fish was selected for otolith chemistry analysis with stratified random sampling. Forty-five males and females were selected from each stock with approximately even distributions of age 2 - 6 years. This age range was chosen because winter landings predominantly consist of these year class. The member of each otolith pair not selected for ageing was prepared for otolith chemistry analysis under a class-10 clean hood in the Fisheries Laboratory at UWF. Otoliths were cleaned of any remaining tissue by rinsing with ultrapure water (18.3 megaohm cm^{-1} polished water) and lightly scrubbing their surface with an acid-leached synthetic bristle brush. Otolith surfaces then were alternately flooded with 1% ultrapure nitric acid and rinsed with ultrapure water. Cleaned samples were air-dried in a laminar flow class-10 clean hood and then weighed.

Otoliths were prepared for analysis with high resolution inductively coupled plasma-mass spectrometry (HR-ICP-MS) and isotope ratio-mass spectrometry (IR-MS) following cleaning. Samples were pulverized with an acid-leached glass mortar and pestle. Pulverized and homogenized otolith material from each sample was split and sample aliquots were prepared for either HR-ICP-MS or IR-MS analysis. (Note: Elemental analysis [Ba, Ca, Mg, Mn, and Sr] by HR-ICP-MS analysis will not be completed until the end of February; therefore, this paper will only detail IR-MS methods and results.)

Otolith powder from each sample was stored in a polypropylene microcentrifuge tube. Samples were shipped to the Department of Geology at the University of California at Davis (UC Davis) where they were analyzed by project collaborator Mr. David Winter with a GV Instruments Optima mass spectrometer. Otolith samples first were roasted in a vacuum at 375º C for 0.5 hour. Using a common phosphoric acid bath at 90º C, 20-50 µg of sample were reacted and analyzed in a directly coupled dual inlet of the mass spectrometer. Stable isotope values of C and O were reported in the standard parts per mille notation as delta values ($\delta^{13}C$ and $\delta^{18}O$, respectively) relative to Vienna-Pee Dee Belemnite (VPDB) through the use of the UC Davis' working standard, a carrara marble, which has been calibrated by repeated direct measurement against the International Atomic Energy Agency's carbonate standard NBS-19.

Univariate and multivariate statistics were employed to test for differences in otolith stable isotope signatures between stocks and sexes for fish sampled in summer 2006. A multivariate analysis of variance (MANOVA) was computed with $\delta^{13}C$ and $\delta^{18}O$ as dependent variables and stock and sex as independent variables in SAS (SAS, vers. 6.11, SAS Inst., Inc.,

Cary, NC); analysis of variance (ANVOA) tested for the effect of both independent variables on $\delta^{13}C$ and $\delta^{18}O$ individually. Linear discriminant function models were computed for females, males, and combined sexes, with classification accuracy being estimated with the jackknifed crossvalidation procedure in SAS's Proc Discrim.

Otolith $\delta^{13}C$ and $\delta^{18}O$ values were input into a two-step expectation-maximization (EM) algorithm written for the S-Plus statistical package (Insightful Corp., Seattle, WA) to estimate the stock composition of fish sampled among winter sampling zones off south Florida (Millar, 1987; DeVries et al., 2002). Sex-specific and combined sex ML models first were parameterized with $\delta^{13}C$ and $\delta^{18}O$ data from summer-sampled fish. Then, the EM algorithm computed estimates of the percentage of landings within a given winter sampling zone that were members of the Atlantic stock based on their $\delta^{13}C$ and $\delta^{18}O$ values. A bootstrap procedure (n = 500 bootstraps) was used to compute bias-corrected 90% confidence intervals around the maximum likelihood estimate (MLE) of Atlantic stock contribution.

Results

The age distribution of summer 2006 male and female king mackerel samples analyzed for stock-specific stable isotope signatures were similar between Atlantic and GOM samples, as well as between sexes (Fig. 2). Stock-specific $\delta^{13}C$ and $\delta^{18}O$ values of the samples were distinctly different between Atlantic and GOM king mackerel sampled in summer 2006 (Fig. 3A&C). MANOVA results indicated stable isotope signatures were significantly different between stocks ($p < 0.001$) but not between sexes ($p = 0.06$). Results from ANOVA models of individual stable isotope delta values indicated differences in $\delta^{13}C$ values were significant between stocks ($p < 0.001$) but not sexes ($p = 0.212$), which was also true of differences in $\delta^{18}O$ values (stock: $p < 0.001$; sex: $p = 0.166$). Results of LDF models indicated otolith stable isotope signatures serve as robust natural tags of king mackerel stocks, as mean jackknifed classification accuracy was greater than 80% for sex-specific and combined sex models (Table 1).

Stable isotope signatures of winter-sampled king mackerel were intermediate to $\delta^{13}C$ and $\delta^{18}O$ values of summer-sampled fish (Fig. 3B&D). Results from maximum likelihood stock composition modeling indicate an east-west gradient in percent Atlantic stock contribution to winter mixed-stock king mackerel fisheries existed for winter 2006-07 samples (Fig. 4). The lowest estimate of percent Atlantic contribution (21.4%) was for males in zone I (sampled in mid to late January 2007), while the highest estimate (93.6%) was for females sampled in zone III during February 2007. The trend in stock composition estimates of zone III landings among winter months was lowest Atlantic contribution (i.e., highest GOM contribution) occurring in December and January and highest Atlantic contribution occurring in March.

Discussion

King mackerel otolith stable isotope signatures proved to be robust natural stock-specific tags. Classification accuracies from linear discriminant function analysis were on the higher end of the range of those estimates resulting from prior otolith shape and otolith elemental chemistry studies (DeVries et al. 2002; Patterson et al. 2004; Clardy et al. in press). While stable isotope signatures originally were examined simply to enhance the resolution of otolith elemental chemistry signatures, it is apparent from results presented here that they provide accurate tags of Atlantic and GOM king mackerel stocks in their own right. However, the combination of

elemental data with stable isotope values should prove to be an even more powerful natural marker to distinguish Atlantic and GOM king mackerel stocks when otolith elemental data are available for statistical analysis in late February 2007.

Differences in stable isotope signatures between Atlantic and GOM king mackerel follow expectations we had prior to analysis. Otolith $\delta^{13}C$ has been shown to reflect the stable isotope composition of dissolved inorganic C in seawater, but also to be affected by trophic fractionation (Thorrold et al. 1997; Wurster and Patterson 2003). It remains untested what factors most affected $\delta^{13}C$ values we observed in king mackerel otoliths, but Roelke and Cifuentes (1997) reported population-specific differences in $\delta^{13}C$ values of king mackerel muscle samples from different regions of the GOM and the US south Atlantic, thus indicating the existence of trophic differences among regions. Otolith $\delta^{18}O$ is driven by temperature and by latitudinal effects of the hydrologic cycle, and is incorporated into otoliths in near isotopic equilibrium with its value in seawater (Thorrold et al. 1997). Distinct differences in otolith $\delta^{18}O$ values between GOM and Atlantic king mackerel likely resulted from temperature differences they experienced during their disparate migrations, as well due to latitudinal difference in their migration routes.

Spatial and temporal patterns observed in estimates of the percent Atlantic stock contribution to winter king mackerel fisheries off south Florida are consistent with those estimated with otolith shape data from the same samples (Shepard et al. in press), as well as those observed in earlier otolith shape and otolith elemental chemistry studies (DeVries et al 2002; Patterson et al. 2004; Clardy et al. in press). The preponderance of evidence now accumulated on king mackerel winter mixing indicates that the current management practice of assigning all winter mixing zone fish to the GOM stock is inaccurate. However, results from the various stock mixing studies also demonstrate that mixing between Atlantic and GOM king mackerel is spatially and temporally (both intra- and inter-annually) variable. It appears unrealistic that a single mixing percentage can be applied to south Florida winter mixed-stock fisheries. Instead, a gradient approach to estimating stock identity of mixed-stock landings should be incorporated into routine sampling of the stocks, the results of which can be incorporated into assessment models to model real and not perceived or imposed mixing scenarios.

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Table 1. Jackknifed classification accuracies (percent) from linear discriminant function models computed with king mackerel otolith stable isotope delta values ($\delta^{13}C$ and $\delta^{18}O$) for females, males, and combined sex models of fish sampled in the U.S. south Atlantic Ocean and the eastern Gulf of Mexico during summer 2006.

Stock	Females Accuracy %	Males Accuracy %	Combined Sexes Accuracy %
Atlantic	91.1	84.2	88.0
GOM	77.8	73.4	73.4
Overall Accuracy	84.4	78.8	80.6

Figure 1. Map of Atlantic Ocean and Gulf of Mexico sample sites for king mackerel. Asterisks indicate sample sites for fish sampled in summer 2006. Fish were sampled from the three south Florida sampling zones in winter 2006/07.

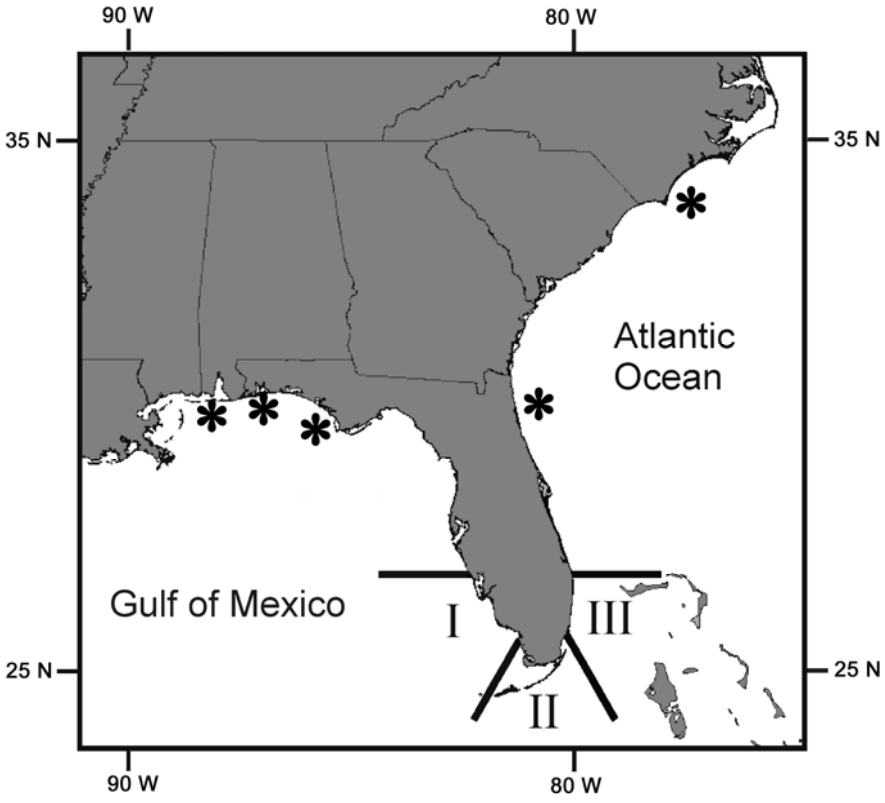

Figure 2. Age distribution of king mackerel sampled in A) the Atlantic Ocean and Gulf of Mexico in summer 2006, B) females sampled in south Florida winter sampling zones, and C) males sampled in south Florida winter sampling zones. Abbreviations for summer samples are AF = Atlantic females, AM = Atlantic males, GF = Gulf of Mexico females, and GM = Gulf of Mexico males. Winter sampling zone abbreviations are Z1 = zone I, Z2 = zone II, Z3DecJan = zone III in December and January, Z3Feb = zone III in February, and Z3Mar = zone III in March. Zone I and II fish were sampled in mid to late January 2007.

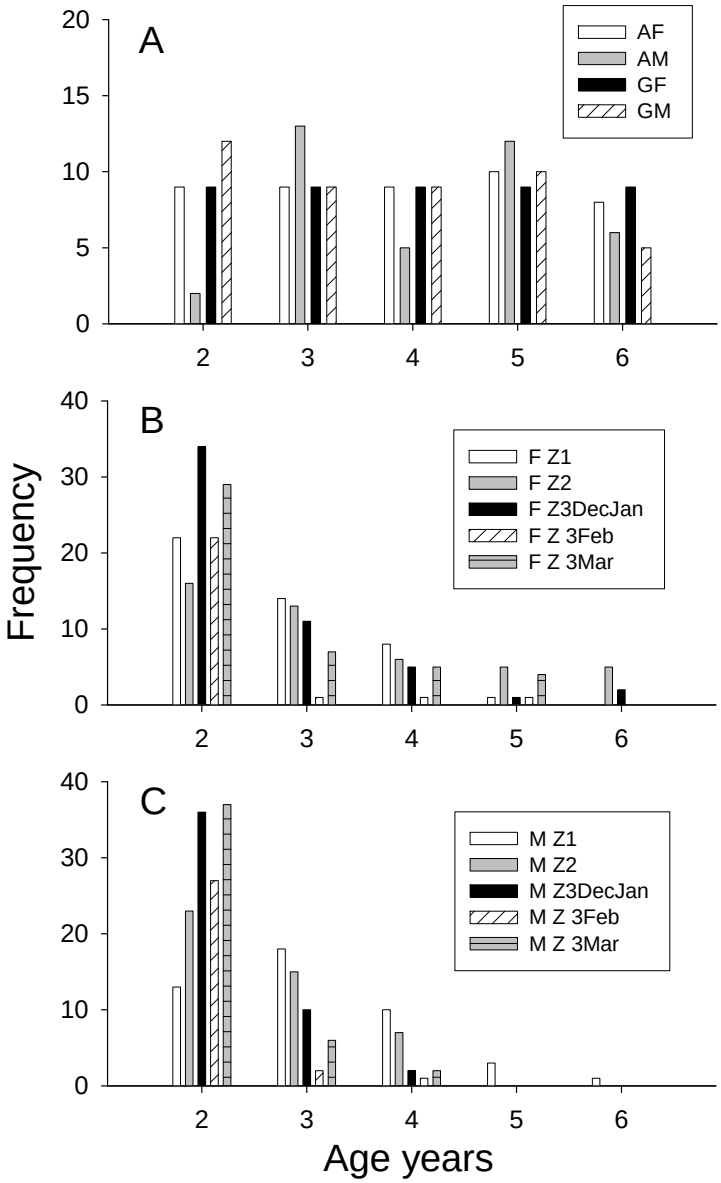

Figure 3. Mean (± SE) king mackerel otolith stable isotope values for fish sampled in summer 2006 and winter 2006/07. Panel A contains $\delta^{13}C$ values of summer-sampled fish and B contains $\delta^{13}C$ values for winter samples. Panel C contains $\delta^{18}O$ values of summer-sample fish and D contains $\delta^{18}O$ values of winter samples. Tick label abbreviations are the same as indicated in the caption of Figure 2. The legend in Panel B applies to all panels.

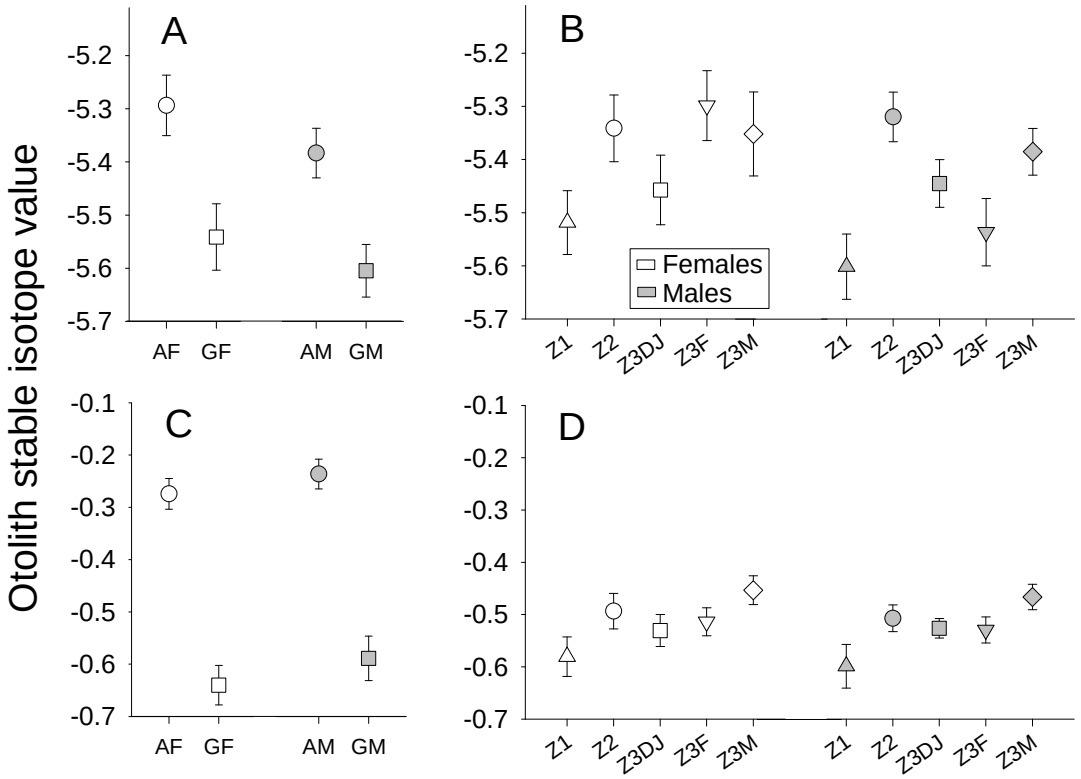

Figure 4. Results of maximum likelihood stock composition models computed with stable isotope data to estimate the percent contribution of Atlantic stock king mackerel to winter south Florida fisheries for A) female, B) male, and C) combined sex models. Error bars indicate asymmetrical bias-corrected 90% confidence intervals around point estimates of percent Atlantic stock contribution. Tick label abbreviations are the same as presented in the caption for Figure 2.