# Variation in the isotopic signatures of juvenile gray snapper (*Lutjanus griseus*) from five southern Florida regions

**Trika Gerard (contact author)**

**Barbara Muhling**

Email address for contact author: trika.gerard@noaa.gov

National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southeast Fisheries Science Center
75 Virginia Beach Dr.
Miami, Florida 33149

**Abstract**—Measurements of $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios in the carbonate of juvenile gray snapper (*Lutjanus griseus*) sagittal otoliths collected during 2001–2005 from different southern Florida regions indicated significant variations in the ratios between Florida Bay and surrounding areas. Annual differences in isotopic composition were also observed. Classification accuracy of individual otoliths to a region averaged 80% (63% to 96%), thereby enabling the probability of assigning an unknown individual to the appropriate juvenile nursery habitat. Identification of isotopic signatures in the otoliths of gray snapper from Florida Bay and adjacent ecosystems may be important for distinguishing specific portions of the bay that are crucial nursery grounds for juveniles. Separation of gray snapper between geographic regions and nursery sites is possible and has the potential to establish a link between adult gray snapper present on offshore reefs and larvae and juveniles at nursery habitats in Florida Bay or adjacent areas.

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Identification of nursery habitats is important for effective management of coral reef fishes. Many reef fish are commercially, recreationally, and ecologically important and are believed to migrate to reefs from juvenile nursery areas such as seagrass and mangrove habitats. Little is known about the nature of these nursery areas or the migration corridors that exist between nursery areas and coral reefs. Otolith microchemistry is a proven method for determining the early stage habitats of juvenile fishes, particularly if tagging studies are not feasible (Gao et al., 2004). Trace elements and stable isotopes obtained from the otoliths of teleost fish have been well documented as useful tools for providing a wealth of information on environmental variations and stock structure of fish throughout their life history (Stephenson et al., 2001). New research has shown that the stable isotopic composition of otoliths provides a way of identifying fish that have inhabited different water masses, therefore serving as a tracer for distinguishing various nursery habitats of fish populations (Ashford and Jones, 2006).

The application of the stable isotopic composition of otoliths as a natural chemical tag can help in the evaluation of the significance of estuaries as nursery grounds for coral reef fish, and determine which habitats, such as seagrass beds, mangrove islands, and mangrove shorelines, within an estuary are most important as nursery areas for producing adult coral reef fish. Each of these habitats supplies juvenile reef fish with nutrition and shelter from predators. Gray snapper (*Lutjanus griseus*) juveniles are often present in a variety of estuarine ecosystems with soft and hard bottom habitats and with a wide range of temperatures and salinities (Bortone and Williams[1]).

Gray snapper are an economically important Florida reef fish. They make up a large proportion of the commercial and recreational finfish landings for the state and are the most popular game fish, with the highest recreational landings in Florida Bay (Starck and Schroeder, 1971; Lara et al., 2008). After spawning, larvae spend approximately 20–35 days as pelagic plankton at sizes ranging from 10–20 mm standard length (SL). They then settle as early juveniles in seagrass beds at sizes of approximately 40–50 mm SL (Richards et al., 2005) and migrate to coral reef habitats as adults.

The goal of this study was to analyze and assess the spatial and temporal variation of stable isotopic carbon and oxygen composition in the otoliths of juvenile gray snapper from southern Florida. This analysis will help determine the importance of Florida Bay as a nursery ground

[1] Bortone, S., and J. Williams. 1986. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (South Florida)—gray, lane, mutton, and yellowtail snappers. U. S. Fish and Wildlife Service Biol. Rep. 82(11.52), 18 p.

before juvenile gray snapper make an ontogenetic shift to the nearby Florida reef tract. We examined variation in the otolith stable isotopic chemistry ($\delta^{13}C$ and $\delta^{18}O$) of juvenile gray snapper among various regions of southern Florida, and among sites within these regions, over four years. Differences in isotopic composition of the otoliths for regions and sites were used to determine if, and at what spatial scale, specific nursery grounds for juvenile gray snapper exist.

## Materials and methods

### Sample collection

A total of 317 juvenile gray snapper were collected between 2001 and 2005 from 27 sites within five geographical regions of Southern Florida: Ten Thousand Islands, Biscayne Bay, Florida Bay, Lower Florida Keys, and Dry Tortugas (Fig. 1). Eight to ten fish per site collected in 2002 and 2003 were used for an analysis of spatial variation and ten each from two sites collected in 2001, 2002, 2004, and 2005 were used for an analysis of temporal variation. The fish sampled ranged from 50 to 210 mm SL. For consistency, only gray snapper measuring 100 mm SL or larger, collected between May and September, were used in statistical analyses. Habitats at the sites varied from seagrass beds to mangrove islands and mangrove-lined coastlines. All samples were obtained by using traditional hook-and-line gear, baited with fresh shrimp, except for the collection of gray snapper from the Ten Thousand Islands region that was obtained from bycatch of crab traps. Juvenile gray snapper were collected between March and November. Samples remained frozen until dissection for removal of otoliths.

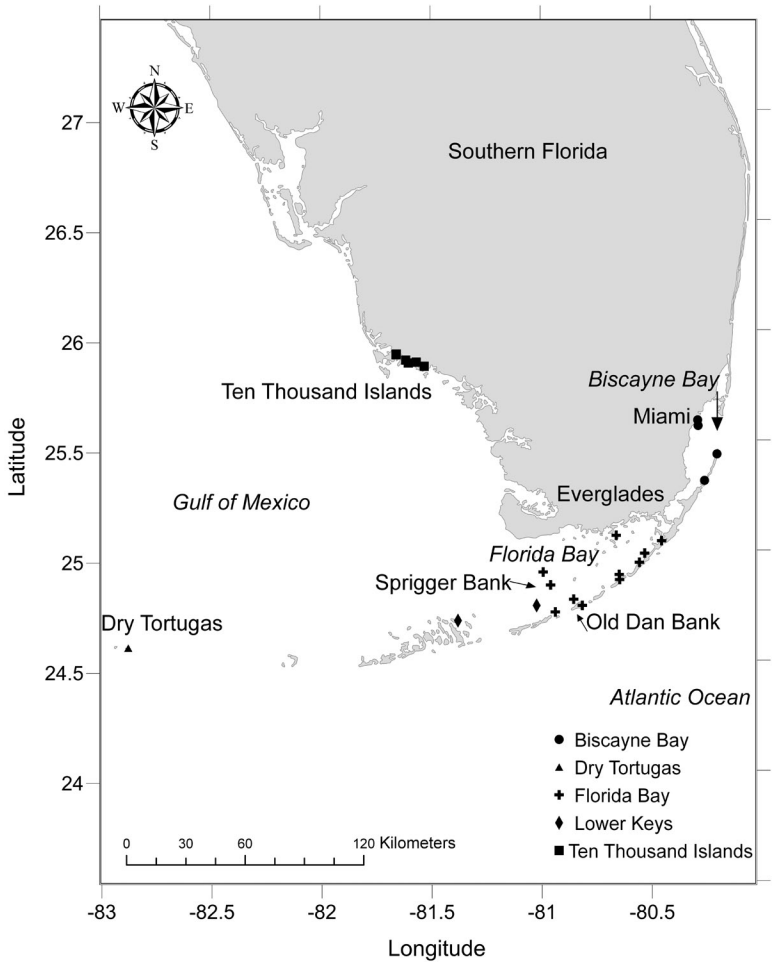


**Figure 1**

Collection sites for juvenile gray snapper (*Lutjanus griseus*) from five southern Florida regions: Ten Thousand Islands, Florida Bay, Biscayne Bay, Lower Keys, and Dry Tortugas. Gray snapper were collected in the summer of 2002 and 2003 for analysis of otolith microchemistry in order to identify individuals to specific nursery sites.

### Sample analysis

According to Campana (1999), there is no significant difference in the chemical composition between the left or right otolith of a fish; therefore either otolith was randomly chosen. All dissection, cleaning, and drying of otoliths were conducted in a class-100 laminar flow clean hood by using acid-washed glass knives and probes. After the removal of all extraneous tissue, the otoliths were rinsed with milli-Q water.

Otoliths are composed of 96% calcium carbonate in the form of aragonite, 3% protein, and 1% inorganics (Arslan and Paulson, 2003); therefore, deproteination procedures were conducted on all otoliths. During deproteination, the mass of the whole otolith was obtained; then whole otoliths were crushed to a fine powder with an agate mortar and pestle and transferred to a 15 ml plastic centrifuge tube. For otoliths larger than 17 mg, only one half or one quarter of the crushed whole otolith powder was used. Depending on the otolith mass, various volumes of 10% sodium hypochlorite were added to the crushed otolith and the mixture was allowed to sit for 48 hours. Samples were rinsed with milli-Q water and centrifuged for 1.5 minutes between rinses before being dried in an oven at 60°C for six hours (Gaughan et al.[2]). Stable isotope analyses were carried out at the University of Miami Stable Isotope Laboratory, Miami,

[2] Gaughan, D. J., G. A. Baudains, R. W. D. Mitchell, and T. I. Leary. 2001. Pilchard *(Sardinops sagax)* nursery areas and recruitment process assessment between different regions in southern Western Australia. Fish. Res. Rep. Dep. of Fish 131:1–44. Government of Western Australia, Dep. Fisheries, Fisheries Research Division, WA Marine Research Laboratories, PO Box 20 North Beach, Western Australia 6920.

Florida, by using a ThermoQuest Finnigan Delta Plus mass spectrometer (Thermo Fisher Scientific, Inc., Bremen, Germany) with a Kiel III device. Standard mass spectrometric techniques were applied after the carbonate was decomposed to $CO_2$ with 100% phosphoric acid. ISODAT software (Thermo Fisher Scientific, Inc., Waltham, MA) indicated all isotopic values, which are reported with standard delta notation (‰):

$$\delta^{18}\mathrm{O} = \left[\left(\left(^{18}\mathrm{O}/^{16}\mathrm{O}\right)_x / \left(^{18}\mathrm{O}/^{16}\mathrm{O}\right)_s\right) - 1\right] \times 1000,$$

where $X$ = sample; and
$S$ = the marine carbonate standard Vienna Peedee belemnite, VPDB (Gao et al. 2001).

### Statistical analysis

To account for any effect of fish size on $\delta^{13}C$ and $\delta^{18}O$ isotope ratios, the relationship between each isotope and otolith mass (which is considered to be proportional to fish age) was examined within each regional group. Where a significant linear relationship was found, isotopic ratios were corrected by using the following formula:

$$R_{\mathrm{corr}} = R - (B \times Mass),$$

where $R$ = isotopic ratio;
$B$ = slope; and
$Mass$ = otolith mass.

Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in isotopic signatures among years, regions, and sites within regions. PERMANOVA is a "semiparametric," multivariate version of a univariate one-way ANOVA (Anderson, 2001), producing a pseudo $F$-statistic, and a $P$-value. Canonical analysis of principal coordinates (CAP), with the "leave-one-out" method of cross-validation was used to assess whether juvenile snapper from South Florida marine ecosystems could be accurately classified to one of the five regions. Sample collection between years, regions, and times of year was not consistent, and therefore only data from "summer" (May to September) were included. The four larger regions (Biscayne Bay, Florida Bay, Lower Keys, and Ten Thousand Islands) were sampled at least once in 2002 and 2003 only. Therefore, only data from these two years were used to assess spatial variation in isotope signatures. Old Dan Bank and Sprigger Bank in Florida Bay were sampled in late May–June in 2001, 2002, 2004, and 2005; thus, temporal variation was also investigated. Age-length estimates for juvenile gray snapper from Florida were used to ensure that the otolith samples reflected juvenile isotopic signatures within the year of collection (Allman and Grimes, 2002; Gerard, 2007). Isotope data were normalized before analysis, and all multivariate analyses were completed in Primer-6, (Plymouth Marine Laboratory, Ivybridge, U.K.) with PERMANOVA add-on.

## Results

### Spatial variation

An initial PERMANOVA between years and regions (except the Dry Tortugas) for 2002 and 2003 data combined showed significant differences between regions (pseudo-$F$=358.3, $P$<0.001) and years (pseudo-$F$=41.8, $P$<0.001). Because this analysis showed that data from the two years could not be pooled, further analyses were performed on each year separately. Across both years, the Dry Tortugas and Florida Bay regions had the highest stable isotopic carbon ratios, and Ten Thousand Island had the lowest (Fig. 2). Stable oxygen isotopic ratios were lowest at the Ten Thousand Islands and highest in Florida Bay. Pairwise PERMANOVA on 2002 and 2003 data that were treated separately showed significant differences between all regions in both years at $P$<0.05.

More than one site was sampled within the same region and in the same month on only three occasions: in Florida Bay in June 2002 and September 2003, and in the Ten Thousand Islands in September 2002. PERMANOVA indicated that isotope signatures from fish collected at different sites within these regions were significantly different (Florida Bay June 2002: pseudo-$F$=8.90, $P$<0.001; Florida Bay September 2003: pseudo-$F$=7.60, $P$=0.003; Ten Thousand Islands September 2002: pseudo-$F$=10.66, $P$<0.001. Pairwise PERMANOVA tests between sites within a region showed that the strength of differences in isotope signatures among sites was variable (Table 1). Differences between the same sites within Florida Bay in June 2002 tended to be stronger than in September 2003.

Leave-one-out cross validation analysis for 2002 data only yielded an average classification accuracy of 79.7%. Classification success rates were 68.0% (Florida Bay), 50.0% (Biscayne Bay), 100.0% (Ten Thousand Islands), 83.3% (lower Florida Keys), and 90.0% (Dry Tortugas). The same analysis on 2003 data returned much lower classification successes, with 63.5% overall (Florida Bay [69.4%], Biscayne Bay [60.0%], Ten Thousand Islands [90.0%], lower Florida Keys [38.9%]). This result was largely due to the absence of samples from the Dry Tortugas in 2003 (which were strongly distinct from other regions in 2002) and to the lower classification success in the Lower Keys in 2003 than in 2002. Classification success rates for Biscayne Bay, Florida Bay, and the Ten Thousand Islands were similar between years.

### Temporal variation

Samples were taken at both Old Dan Bank and Sprigger Bank in late May or June of 2001, 2002, 2004, and 2005. Scatter plots showed significant overlap between samples from different years (Figs. 3 and 4). PERMANOVA revealed significant differences between samples from 2004 and 2002, and 2004 and 2005 at both sites (Table 2). At Old Dan Bank, results for 2001 were also significantly different from those for 2004,

and at Sprigger Bank, results for 2002 were also distinct from those for 2005. Where interannual differences were present, they were usually most discernible with respect to carbon, and less so for oxygen (Figs. 3 and 4). The annual mean SL of fish used in this analysis ranged from 135 mm (±2.6 mm standard error [SE]) in 2002 to 169 mm (±3.4 mm SE) in 2001 at Old Dan Bank, and from 156 mm (±13.5 mm SE) in 2001 to 160 mm (±16.1 mm SE) in 2005 at Sprigger Bank. However, the linear correlation between fish standard length and carbon isotope signature for samples included in temporal analyses was very weak, and only marginally significant (correlation coefficient $r^2$=0.03, $P$=0.04).

## Discussion

Otoliths can be used to separate fish by region on the basis of isotopic composition. This study revealed spatial separation for carbon and oxygen isotopes between five geographical regions in southern Florida. In any marine ecosystem, temperature and salinity interact to influence the concentrations of oxygen isotopes. Carbon and oxygen isotopic concentrations typically increase with salinity and decrease with increasing temperature (Elsdon and Gillanders, 2002).

Oxygen isotopes in the otolith are deposited in equilibrium with the carbon isotopic composition of the ambient water in which the fish resides (Campana, 1999). Carbon isotopes in the otolith, however, are deposited in disequilibrium to ambient carbon isotopic composition of the water mass. This disequilibrium is explained as an effect of metabolism and therefore provides information on the position of a fish in the food chain and on changes in metabolic rate over the life of a fish (Gauldie, 1996; Dufour et al., 2005; Huxham et al., 2007). The regional variation in the otolith isotopic composition of gray snapper indicates the regional differences in the environmental conditions and nutrient sources available to these fish throughout their early life. Regional isotopic differences provide a unique chemical signature that can be used to identify where individual adult gray snapper spend their time as juveniles.

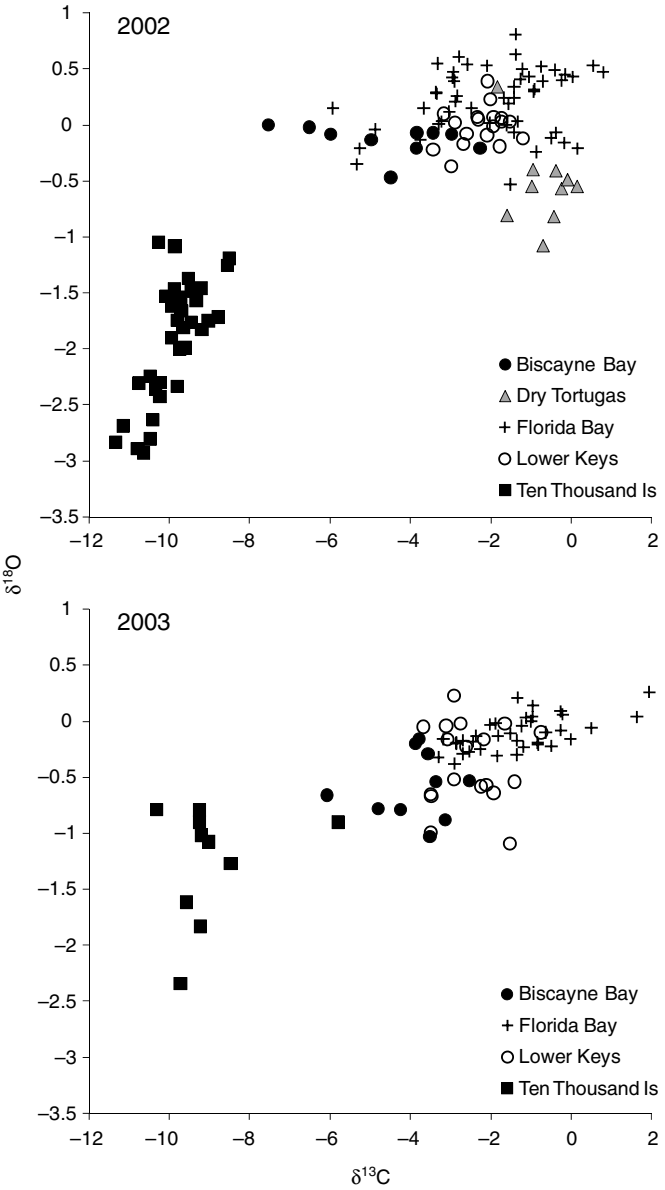


**Figure 2**

Scatter plot depicting spatial separation of juvenile gray snapper (*Lutjanus griseus*) stable isotope otolith signatures ($\delta^{18}$O and $\delta^{13}$C) collected in 2002 and 2003 among five southern Florida regions: Ten Thousand Islands, Florida Bay, Biscayne Bay, Lower Keys and Dry Tortugas.

Results show spatial separation of stable oxygen and carbon isotopic ratios among **collection** sites within a region. Significant differences in carbon and oxygen isotopic composition among sites (approximately 10 km apart) indicate high site fidelity and a high classification accuracy for specific sites at this scale. Classification success with the leave-one-out cross validation procedure for a single observation in a region was approximately 80%, thereby allowing for the possibility classifying an unknown fish with reasonable certainty to a region. The spatial stable isotopic variation, coupled with a successful cross validation result, provides a means of substantiating the hypothesis that juvenile gray snapper exhibit strong site fidelity, and provides a specific isotopic tag to determine whether Florida Bay is a significant source of adults on the reef.

Elemental composition of otoliths has been shown to be valuable for identifying the relative contribution of juveniles from different nursery habitats to adult

**Table 1**

Results of pairwise permutational multivariate analysis of variance (PERMANOVA) of juvenile gray snapper (*Lutjanus griseus*) otolith stable isotope signatures among sampling sites, within sampled region, for Florida Bay (June 2002 and September 2003), and the Ten Thousand Islands (September 2002).

| Region and month | Sites compared | Test statistic (pseudo-*F*) | *P*-value | Number of permutations |
|---|---|---|---|---|
| Florida Bay | | | | |
| June 2002 | Black Betsy Key, Old Dan Bank | 3.69 | 0.002 | 997 |
| | Black Betsy Key, Sprigger Bank | 4.07 | 0.001 | 993 |
| | Black Betsy Key, Schooner Bank | 0.38 | 0.864 | 991 |
| | Old Dan Bank, Sprigger Bank | 2.57 | 0.008 | 990 |
| | Old Dan Bank, Schooner Bank | 2.97 | 0.001 | 991 |
| | Sprigger Bank, Schooner Bank | 3.07 | 0.002 | 993 |
| Sept 2003 | Old Dan Bank, Sprigger Bank | 4.04 | 0.001 | 995 |
| | Old Dan Bank, Schooner Bank | 1.79 | 0.083 | 994 |
| | Sprigger Bank, Schooner Bank | 1.86 | 0.073 | 991 |
| Ten Thousand Is. (west coast) | | | | |
| Sept 2002 | Blackwater Bay, Faka Bay | 4.34 | 0.003 | 637 |
| | Blackwater Bay, Palm Bay | 1.29 | 0.252 | 35 |
| | Blackwater Bay, Pumpkin Bay | 1.70 | 0.132 | 441 |
| | Faka Bay, Palm Bay | 3.03 | 0.015 | 275 |
| | Faka Bay, Pumpkin Bay | 3.74 | 0.004 | 986 |
| | Palm Bay, Pumpkin Bay | 0.56 | 0.625 | 165 |

populations (Martin and Wuenschel, 2006). Lara et al. (2008), using trace elemental analysis, found an overall cross validation success rate of 82% for classifying randomly selected samples to a juvenile nursery habitat signature in one of five regions: Biscayne Bay, Florida Bay, lower Florida Keys, Ten Thousand Islands, and Dry Tortugas. However, that study had a lower percentage for successfully classifying unknown samples to two regions, Dry Tortugas (50%) and Ten Thousand Islands (52%). In the current study, isotopes allowed us to distinguish between sites that the elemental ratios in the Lara et al. study were unable to separate and provided a clearer successful classification for all regions. The Dry Tortugas and Ten Thousand Islands regions showed the highest percentage for successful classification, 90% and 100%, respectively, compared to other regions analyzed. These two regions are geographically the farthest separated, Dry Tortugas has the most oceanic influence, and Ten Thousand Islands has the most freshwater influence from Taylor Slough and Shark River Slough through the Florida Everglades. There was also some interannual variability in classification success in the Lower Keys.

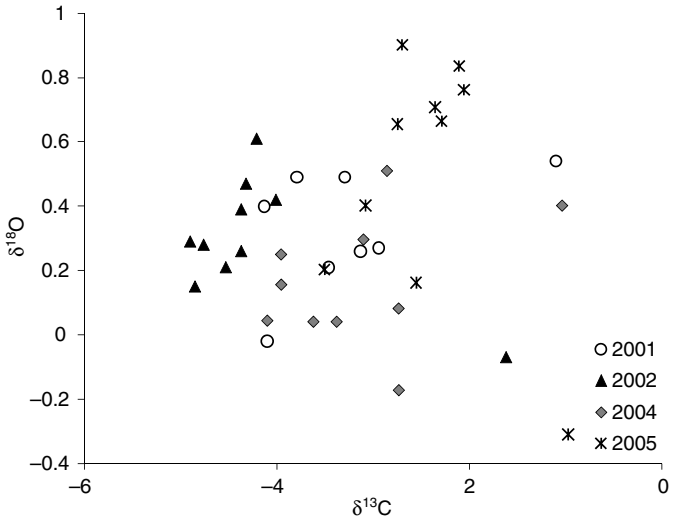


**Figure 3**

Scatter plot depicting variation in juvenile gray snapper (*Lutjanus griseus*) stable isotope otolith signatures ($\delta^{18}O$ and $\delta^{13}C$) from the summer of 2001, 2002, 2004, and 2005 from Sprigger Bank, Florida Bay.

Temporal variation of stable isotopic ratios of carbon and oxygen in the otoliths of gray snapper has not been previously studied in southern Florida. This study indicates that temporal variation in carbon and oxygen isotopic ratios was expressed in otolith samples collected from years 2001 through 2005. Swearer et al. (2003) tested temporal variation of three species (topsmelt [*Atherinops affinis*], arrow goby [*Clevelandia ios*], and California halibut [*Paralichthys californicus*]), collected in spring and autumn of 1996 from Carpinteria Marsh in southern

**Table 2**

Results of pairwise permutational multivariate analysis of variance (PERMANOVA) of juvenile gray snapper (*Lutjanus griseus*) otolith stable isotope signatures among sampled years, for sampling sites Old Dan Bank, and Sprigger Bank, Florida Bay.

| | Years compared | Test statistic (pseudo-*F*) | *P*-value | Number of permutations |
|---|---|---|---|---|
| Old Dan Bank | 2001, 2002 | 1.53 | 0.09 | 995 |
| | 2001, 2004 | 1.84 | 0.05 | 993 |
| | 2001, 2005 | 1.11 | 0.30 | 992 |
| | 2002, 2004 | 2.76 | <0.001 | 992 |
| | 2002, 2005 | 1.50 | 0.10 | 981 |
| | 2004, 2005 | 2.13 | 0.01 | 979 |
| Sprigger Bank | 2001, 2002 | 1.65 | 0.07 | 990 |
| | 2001, 2004 | 1.27 | 0.23 | 984 |
| | 2001, 2005 | 1.52 | 0.11 | 979 |
| | 2002, 2004 | 2.26 | 0.01 | 989 |
| | 2002, 2005 | 2.86 | <0.001 | 989 |
| | 2004, 2005 | 2.26 | 0.02 | 988 |

California. Their results showed a substantial degree of temporal variation in chemical signatures between collection periods. Assessing the temporal variation of isotopic signatures is useful for potential determination of environmental change or lack of site fidelity in fishes within Florida Bay. Preliminary results from acoustic telemetry on juvenile gray snapper in a Florida coastal Atlantic marine estuary, Loxahatchee, and otolith chemical signature analysis of juvenile gray snapper in Florida Bay indicate high site fidelity (Lara et al., 2008). Based on these results, it is probable that the variations shown during annual sampling of the same sites in Florida Bay are a result of differences in environmental conditions such as temperature and salinity, possibly resulting from changes in water management practices or weather events.

Significant differences between both oxygen and carbon isotopic signatures in otoliths were present between sampled years. However, differences with respect to carbon were more pronounced than differences in respect to oxygen. Otolith carbon isotopic signatures can be affected by water temperature and salinity (Elsdon and Gillanders, 2002), and by the trophic position and diet of the fish (Shiao et al., 2009).

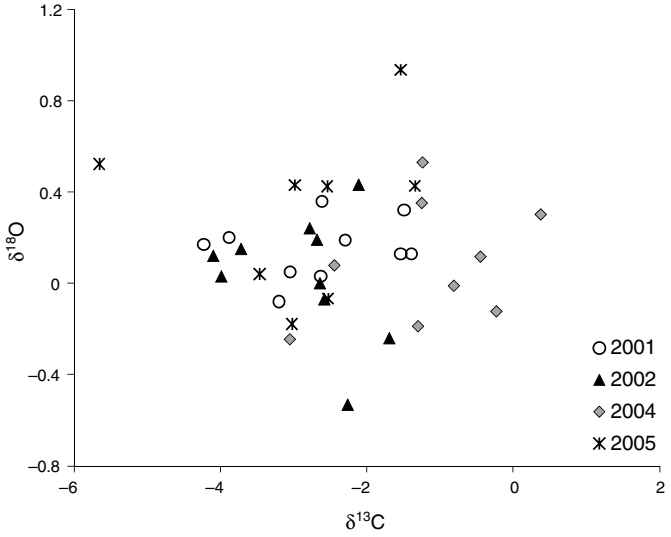


**Figure 4**

Scatter plot depicting variation in juvenile gray snapper (*Lutjanus griseus*) stable isotope otolith signatures ($\delta^{18}O$ and $\delta^{13}C$) from the summer of 2001, 2002, 2004, and 2005 from Old Dan Bank, Florida Bay.

Historically, Florida Bay has experienced dramatic changes in salinity levels as a result of changes in water management practices and land use upstream. The impact of salinity changes on juvenile fish habitats, such as seagrass beds and mangroves, and on fishes is still unknown. The positive relationship between oxygen isotopic ratios and salinity makes it possible to look closer at salinity levels of portions of Florida Bay from 2000 to 2005. Kelble et al. (2007) examined mean annual Florida Bay salinity from 1998 through 2004 using flow-through system data from survey cruises. Results showed low salinity levels in the summer of 2002 (29–34‰), and much higher salinity levels in the summer of 2004 (37–42‰). Otolith carbon isotopic signatures from both Sprigger Bank and Old Dan Bank were significantly lower in 2002 than in 2004—a result that may have reflected the lower salinity conditions in 2002. Oxygen isotopic signatures, however, were similar between these two years at both sites.

The formation of otolith carbonate is an inorganic reaction where calcium carbonate is precipitated from

the endolymphatic fluid. The carbon isotopic ratios in the otolith, in addition to pure kinetic effects, are controlled by only those metabolic pathways that modify the $\delta^{13}C$ of the endolymphatic fluid (Radtke, 1996). Diet has a pronounced and significant effect on carbon isotopic ratios in the otoliths. The markedly different mean salinity values for summer 2002 and summer 2004 indicate differences in freshwater inflow to Florida Bay. These differing regimes would likely have influenced primary productivity in the bay, in terms of chlorophyll biomass and food-web structure, which could ultimately be recorded as differences in carbon isotopic ratios in juvenile fishes. Autotrophs, mangrove detritus, seagrass, and particulate organic matter play a pivotal role as important sources of nutrition for juvenile gray snapper (Melville and Connolly, 2003). Different plant groups display different carbon isotopic ratios in their organic material depending on the photosynthetic pathway used, and carbon isotopic ratios for the otoliths of prey organisms act as labels that can be measured through to higher trophic level consumers. Recorded variations of carbon isotopic ratios for the otoliths may therefore constitute a record of the food that these gray snapper consumed and show potential for assessing the health of the Florida Bay ecosystem.

Mulcahy (1979) found that stable carbon isotopic ratios for the otoliths of a benthopelagic fish increased with fish age. This increase with maturity of the fish was attributed to a decrease in the metabolic production rate of dissolved inorganic carbon. Because we examined only juvenile and small adult fish of a relatively narrow size range, it is unlikely that the observed differences in carbon isotopic ratios were due to differences in age-dependent metabolism.

Spatial separation of regions and collection sites as shown in this study by using isotopic analysis of otoliths has the potential for establishing habitat linkages between adult gray snapper on offshore reefs to those in nursery habitats in Florida Bay and other surrounding areas. Results indicate that fishery managers should address the whole ecosystem and connectivity between habitats of a known species rather than site-specific management. Managing and protecting a particular species requires spatially explicit characterization of all habitats and processes encountered during the life history of a species for the design of marine protected areas in coastal regions (Thorrold et al., 2001). Connectivity must be taken into consideration when assessing management effectiveness of economically important reef fish species that use multiple habitats throughout their life.

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