

Received Date : 13-Jan-2014

Revised Date : 08-May-2015

Accepted Date : 02-Jun-2015

Article type : Regular Paper

RUNNING HEADER: Diet of mesopelagic teleosts

Combined stomach content and $\delta^{13}\text{C}/\delta^{15}\text{N}$ analyses of oilfish, escolar, snake mackerel, and lancetfish in the western North Atlantic

Heidi Keller¹, Amy C. Hirons², David W. Kerstetter^{1, *}

¹Nova Southeastern University Oceanographic Center,
8000 North Ocean Drive, Dania Beach, FL 33004 USA

²Farquhar College of Arts and Sciences, Nova Southeastern University,
3301 College Avenue, Fort Lauderdale, FL 33314 USA

* Corresponding author: kerstett@nova.edu

ABSTRACT: Large, mesopelagic teleost fishes have a potentially keystone position in the ecology of the pelagic water column, yet remain relatively unstudied when compared to large, commercially important, epipelagic fishes. Here, the ecological roles of four, large, vertically migrating teleosts were examined. Stomach content analyses were performed on 48 oilfish (*Ruvettus pretiosus*), 35 escolar (*Lepidocybium flavobrunneum*), 32 snake mackerel (*Gempylus serpens*), and 7 lancetfish (*Alepisaurus* spp.) collected

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/maec.12317

This article is protected by copyright. All rights reserved

from pelagic longline in the western North Atlantic Ocean from 2007 to 2010. Of these specimens, stable carbon and nitrogen isotope analyses were also performed on white dorsal muscle tissue from 33 oilfish, 16 escolar, 27 snake mackerel, and 7 lancetfish. Based on literature length-at-maturity values, all escolar, snake mackerel, and lancetfish specimens were mature, while 13 of the 33 oilfish were juveniles. Crustaceans, annelids, salps, cephalopods, and teleosts were present in the stomachs and were presumed prey items. A Kruskal-Wallis test showed the four species to be isotopically segregated in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Escolar were the most depleted in $\delta^{13}\text{C}$, followed by adult oilfish, juvenile oilfish, lancetfish, with snake mackerel the most enriched. The depletion in $\delta^{13}\text{C}$ of adult oilfish and escolar may have been attributable to high C/N values which were strongly correlated with length in oilfish, weakly correlated with length in escolar, and moderately correlated with length in snake mackerel and lancetfish. The high C/N was likely due to the high lipid concentration of these fishes. Other factors which may have contributed to the depletion in $\delta^{13}\text{C}$ may have included spawning or a change in carbon source within the ecosystem. Large escolar occupied the highest trophic level ($\delta^{15}\text{N}=10.20$), followed by snake mackerel ($\delta^{15}\text{N}=9.66$), adult oilfish ($\delta^{15}\text{N}=9.32$), lancetfish ($\delta^{15}\text{N}=9.05$), and juvenile oilfish ($\delta^{15}\text{N}=7.83$). A marked change in oilfish $\delta^{13}\text{C}$ and C/N at 30-35 cm fork length coincided with a presumed length-at-maturity.

KEY WORDS: oilfish, escolar, snake mackerel, lancetfish, stomach content, stable isotope

INTRODUCTION

Top predatory fishes of commercial importance, such as thunnid tunas and swordfish *Xiphias gladius*, are known to include a broad suite of midwater animals in their diets (e.g., Potier *et al.*, 2007). However, the ecological roles of these prey items is poorly known. Net and light avoidance, migration, and lack of observations have led us to know more about the sessile benthos than midwater animals (Nybakken, 1993; Robison, 2004). Medium-bodied mesopelagic fishes, including oilfish *Ruvettus pretiosus*, escolar *Lepidocybium flavobrunneum*, snake mackerel *Gempylus serpens*, and lancetfish *Alepisaurus* spp., are occasionally caught as bycatch during pelagic longline fishing operations targeting swordfish and tuna in the epipelagic zone (0-200 m). Escolar are known to make diel vertical migrations to the surface at night before returning to the mesopelagic zone (200-1000 m) during the day (Kerstetter *et al.*, 2008), and limited diet data suggest similar behavior for these other mesopelagic species (e.g., Romanov *et al.*, 2008).

Trophic studies are used to define the prey items and nutritional and energy requirements of animals. The historical method for diet analysis was visual observation of undigested gut or fecal

contents. Biases in this method can occur due to the difference in the digestibility of prey items (i.e., what is assimilated versus ingested) and the inability for a single sample to capture variation within a diet (Bowen, 1996). Stable isotope analysis provides another technique that can overcome these problems. Consumers become enriched with heavier stable nitrogen and carbon isotopes in a predictable, stepwise fashion with increasing trophic level (Miyake and Wada, 1967; DeNiro and Epstein, 1978, 1981; Owens, 1987). Carbon stable isotope enrichment is relatively conservative and is used to trace carbon sources through a food web (DeNiro and Epstein, 1978; Fry and Sherr, 1984; France, 1995; Hecky and Hesslein, 1995; Wada *et al.*, 1993). Stable nitrogen isotope enrichment is stepwise and is used to determine trophic level (DeNiro and Epstein, 1978; Cabana and Rasmussen, 1996; van der Zanden and Rasmussen, 1999). Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios are increasingly used as natural tracers in ecosystem studies (Peterson and Fry, 1987; Hobson and Welch, 1992; Kling *et al.*, 1992; van der Zanden and Rasmussen, 1999). While stomach contents determine predator-prey interactions, stable isotope ratios provide quantitative values for prey material that are actually assimilated into consumers after feeding (Fry and Arnold, 1982).

Oilfish, escolar, and snake mackerel are members of Gempylidae (Collette *et al.*, 1984; Nakamura and Parin, 1993; Nelson, 2006), while lancetfishes are members of Alepisauridae (Robins, 1986). Oilfish and escolar have semi-fusiform body shapes while snake mackerel and lancetfish have anguilliform body shapes. Sharp, needle-like teeth are present in oilfish and escolar and to a greater degree in snake mackerels and lancetfish (Robins, 1986; Nakamura, 1991). Oilfish and anguilliform fishes like snake mackerel and lancetfish are thought to be ambush-type predators (Bone, 1972; Nakamura and Parin, 1993). Oilfish and escolar are kept and sold by commercial fishermen in the Gulf of Mexico and western North Atlantic Ocean. Other gempylid species are currently targeted in Asia with shore seining, set nets, and small trawls, and in Europe, southern Africa, Australia, New Zealand, and South America by recreational anglers and commercial trawls (FAO, 1978, 1980, 1982, 1992; Nakamura and Parin, 1993). Lancetfish have also been used for human consumption (Robins, 1986).

Oilfish, escolar, snake mackerel, and lancetfish are known to feed on fishes, squids, and crustaceans (Nakamura and Parin, 1993; Potier *et al.*, 2007; Vasilakopoulos *et al.*, 2011). Lancetfish from the Indian Ocean had crustacean-dominated diets followed by teleost fishes and cephalopods having a minor role. Diets varied geographically and Alepisauridae, including *A. ferox*, was the main fish family in the gut contents measured by the mean proportion reconstituted weight, which indicated some level of cannibalism (Potier, 2007). The linkages between these four mesopelagic fishes and the apex predatory commercial fishes have been examined with increasing detail (e.g., Romanov *et al.*, 2008). However, the descriptions of the diet and trophic position of the large mesopelagic fishes has not been done in a comprehensive manner, especially within the same geographic area. Using combined stomach content and

stable isotope analyses, we describe the diet and trophic position characteristics for these four co-occurring mesopelagic fishes for the first time.

MATERIALS AND METHODS

Samples were collected during commercial pelagic longline fishing operations targeting swordfish and tuna from January 2007 through January 2010 in U.S. waters of the western North Atlantic Ocean (between 28-36° N, west of 75° W). The hooks were predominantly baited with *Illex argentinus* squid ranging from 150-300 g in weight. Gear was set during sundown and retrieved shortly after dawn, and any intact mesopelagic bycatch was kept whole on ice upon retrieval. Each individual fish was weighed and morphometric measurements were recorded onboard or in the laboratory before dissection. Large escolar retained for sale by the vessels were measured, stomachs were removed, and the heads and dressed weights were later obtained from the fish house. *Alepisaurus ferox* and *A. brevirostris* were combined into a fourth species of *Alepisaurus* spp. for analysis due to difficulty in differentiation among partially damaged specimens obtained opportunistically from cooperating commercial vessels.

Tissue samples were taken from the white dorsal muscle tissue, placed into small vials, and frozen at 0°C for later stable isotopic analysis. Stomachs were removed, weighed, fixed in 10% buffered formalin, and then placed in isopropyl alcohol for final storage prior to manual content analysis. The muscle tissue samples for stable isotope analyses were placed in aluminum trays in a drying oven for a period of three days at 60° C. Large pieces were ground into smaller pieces with a mortar and pestle, and the remaining samples were pulverized with a Wig-L-Bug homogenizer before weighing. The extant literature does not include definitive values for size-at-maturity.

Simple gonadosomatic index (GSI) values were calculated per Crim and Glebe (2002) as the ratio of the gonad weight to the total weight. The resulting GSI values were used as an approximation of spawning periodicity and state to verify values obtained from the extant literature.

Stomach Content Analysis. Stomachs were opened using a dissecting knife or surgical scissors. Contents were separated, individually weighed, measured, and identified to the lowest possible taxa. Count of hard structures that only occurred once per prey item was used for identification of disarticulated prey items, and wet weight was obtained by blotting liquid from prey items before weighing. Prey items identified as bait (e.g., Atlantic mackerel *Scomber scombrus*, which is not found in the study area or squid of the bait size with damage consistent with being on a hook) were not included in stomach content analyses. Prey items were analyzed for frequency of occurrence, percent number, and percent weight (Bowen, 1996). Empty stomachs were excluded from calculations and partially digested material was included in percent weight calculations only. Partially digested material was defined as homogenous,

highly digested material that was unidentifiable as plant or animal matter. Unidentified animals were defined as animal tissues that were not further identifiable to taxa.

For each prey type within a predator species (Bowen, 1996; Hyslop 1980):

Frequency of Occurrence = Number of predators with prey type present / Total number of predators

Percent Number (%N) = Number of individuals of a prey type / Total number of prey items

Percent Weight (%W) = Total weight of a prey type / Total weight of stomach contents

The relative importance index was calculated using George and Hadley (1979):

$Ai_a = \% \text{ frequency of occurrence} + \% \text{ total number} + \% \text{ total weight}$, and

$$Ri_a = 100Ai_a / \sum Ai_a,$$

where Ai_a is the absolute importance index, and for food item a, Ai_a is summed over all food types.

The index of relative importance (IRI) was calculated to represent the proportion of each prey item in the diet of each species (George and Hadley, 1979):

$$IRI = \%O (\%W + \%N)$$

The IRI is then converted to a percentage (per Cortés, 1997):

$$\%IRI = 100 * IRI / \sum IRI$$

Stable Isotope Analysis. Stable isotope compositions vary among organisms and are expressed as the ratio of heavier to lighter isotopes over that of conventional standards. Conventional standards are atmospheric air for nitrogen and Pee Dee belemnite carbonate for carbon (DeNiro and Epstein, 1978, 1981). The formula for determining $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is as follows from DeNiro and Epstein (1978, 1981):

$$\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] * 10^3,$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.

Tissue samples were analyzed for carbon and nitrogen stable isotopes on a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to a Costech 4010 Elemental Analyzer (EA) via a Thermo ConFlo IV. Dehydrated muscle tissue was weighed to achieve a final sample weight ranging from 0.6-0.8 mg, and packed into tin capsules (Boutton, 1991; Paul et al., 1992). Each run included a set of standards for every 10-12 samples. Standards included USGS40 (L-glutamic acid), USGS41 (L-glutamic acid), and Costech acetanilide, and they were run with the same parameters as the samples. Reproducibility of the standards was $\leq 0.2\%$ (1SD) for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The error associated with all data points was $\pm 0.2\%$.

Statistical Analyses. The study region has a tropical-subtropical climate with a summer wet season and a winter dry season, especially in the offshore waters targeted by the commercial pelagic longline fishery. Historically, the median start date of the summer season on the Florida peninsula has been between 21-29

May and the winter season median start date has been between 17-24 October (Beidinger and Lushine, 1993). Samples were examined for seasonality using these seasonal divisions of the year.

Tests for normality, Kruskal-Wallis tests, and Mann-Whitney U tests were performed in SPSS (IBM, Inc.; version 19) and R (version 2.10.1), with figures generated in R. Percent occurrence, percent number, percent weight, and relative importance indices were calculated using programs within spreadsheets in Microsoft Excel 2007. Kruskal-Wallis tests were performed on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between species. Mann-Whitney U tests were performed within species to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ based on sex, seasonality, and maturity.

RESULTS

Stomach content analyses were performed on 48 oilfish, 35 escolar, 32 snake mackerel, and 7 lancetfish (*Alepisaurus* spp.) collected from the pelagic longline fishery in the western North Atlantic Ocean from 2007 to 2010. Stable carbon and nitrogen isotope analyses were performed on white dorsal muscle tissue of 33 oilfish, 16 escolar, 27 snake mackerel, and 7 lancetfish, all of which were also sampled for stomach content analysis.

GSI values calculated during the study compared with season were consistent with a spawning in mid- to late summer for oilfish (similar to Vasilakopoulos et al., 2011) and year-round spawning for longnose lancetfish (suggested in Nakamura and Parin, 1993). Spawning seasons were not determined for escolar and snake mackerel from the GSI data in the present study, although Nakamura and Parin (1993) suggested that snake mackerel spawn year-round. No length-at-maturity data exist for oilfish. Escolar reach sexual maturity at 30-35 cm or earlier (Maksimov, 1970). Snake mackerel males reach maturity at 43 cm and females at 50 cm (Nakamura and Parin, 1993). No maturity data exist for lancetfish, which are known to be hermaphroditic when young and sequential hermaphrodites as adults. Study individuals were separated into juveniles and adults based on visual examination of gonads. Oilfish are the sole species in the genus *Ruvettus* and escolar are a close relative to oilfish within the Family Gempylidae (Nishikawa, 1982); therefore, the same length-at-maturity for escolar was assumed for oilfish. All escolar, snake mackerel, and lancetfish captured were considered adults. Oilfish sampled for this study fell into distinct juvenile and adult groups.

Stomach Content Analyses. For oilfish, 52% of 29 stomachs examined were empty; 35% of 23 escolar stomachs were empty; 24% of 25 snake mackerel stomachs were empty; and 50% of 6 lancetfish stomachs were empty. The percentage of stomachs that contained only unidentifiable, partially digested material was relatively high for oilfish (66%) and escolar (35%) when compared with snake mackerel (4%) and lancetfish (0%). The percentage of stomachs with unidentifiable, partially digested material also

present with other identifiable prey items was low in oilfish (14%), escolar (17%), and lancetfish (17%), and a higher percentage in snake mackerel (36%). Stomach content data for each species are presented in Tables 1a-d as percent occurrence, percent number, percent weight, and percent index of relative importance (%IRI) for prey items.

Identifiable prey items in oilfish stomachs (Table 1a) included crustaceans (decapod shrimps and unidentified), cephalopods (squid), and teleosts (unidentified). Crustaceans were the prey group with the greatest percent occurrence (50.0%), percent number (61.3%) and RI (40.6). Percent weight for crustaceans was 9.2%. Squid were present with 42.9% occurrence, 19.4% number, 48.5% weight, and %IRI of 37.3. Teleosts were the least represented prey group present in percent occurrence (35.7%), percent number (16.1%), percent weight (3.1%), and %IRI (18.5).

Prey items found in escolar stomachs (Table 1b) included cephalopods (squid and unidentified) and teleosts (unidentified and larvae). Cephalopods made up the major portion of the escolar stomach contents by percent occurrence (60%), percent number (72.7%), percent weight (54.3%), and %IRI (68.0). Teleosts had an equal percent occurrence (60%), and lower percent number (27.3%), percent weight (0.9%), and %IRI (32.0) than those of cephalopods.

Crustaceans (unidentified), salps, cephalopods (squid and unidentified), teleosts (porcupinefish *Diodon* sp., flying fish, and unidentified), and *Sargassum* sp. were present in the stomachs of snake mackerel (Table 1c). Cephalopods comprised the largest portion of stomach contents in percent occurrence (78.9%), percent number (60.0%), percent weight (79.1%), and %IRI (68). Teleosts were the second largest group in the diet in percent occurrence (36.8%), percent number (22.5%), percent weight of (20.1%), and %IRI (24.5). Crustaceans and salps combined had a relatively low percent occurrence (5.3%), percent number (2.5%), percent weight ($\leq 0.1\%$), and %IRI (4.8).

Crustaceans (hyperiid amphipods, including *Phronima* sp., and isopods), polychaetes, mollusks (gymnosomes, squids, *Argonauta* sp., and unidentified cephalopods), salps, and teleosts (hatchetfish, lancetfish, larvae, and unidentified) occurred in the stomachs of lancetfish (Table 1d). Crustaceans had a 66.7% occurrence, comprised the greatest percent number (34.4%), and had a 7.1% weight, and a %IRI of 18.5. The parasitic hyperiid amphipod *Phronima* sp. was present in many host salps identified. Salps were an important prey group with a 66.7% occurrence, 20.8% number, 25% weight, and %IRI of 19.2. Mollusks occurred in 100% of the stomachs and had 15.6% number, 14.8% weight, and %IRI of 22.3. Teleosts had a percent occurrence of 66.7%, a percent number of 13.5%, a percent weight of 25.7%, and %IRI of 18.1. Polychaetes were present with a percent occurrence of 33.3%, percent number of 10.4%, percent weight of 3.0%, and %IRI of 8.0. A strip of a black plastic trash bag was also found in one stomach, which was excluded from analyses.

Stable Isotope Analyses. Stable carbon and nitrogen isotope analyses were performed on white dorsal muscle tissue of 33 oilfish, 16 escolar, 27 snake mackerel, and 7 lancetfish that were also sampled for stomach content. Carbon and nitrogen stable isotope values were compared with species, gender, and season, for all four species, and maturity as well for oilfish, the only species that included juveniles and adult specimens. Significance was determined at $\alpha \leq 0.05$.

Kruskal-Wallis tests were performed on $\delta^{13}\text{C}$ among species. Each species was isotopically segregated in $\delta^{13}\text{C}$ ($\chi^2=49.1$, $df=4$, $p<0.001$) (Figure 2). Mann-Whitney U tests showed statistically equal $\delta^{13}\text{C}$ distributions between genders for oilfish ($W=0$, $p=0.67$), escolar ($W=5$, $p=0.86$), and snake mackerel ($W=52$, $p=1.0$). Lancetfish was not analyzed for gender difference due to the species' hermaphroditism. Mann-Whitney U tests also showed statistically equal $\delta^{13}\text{C}$ distributions between seasons for oilfish ($W=133.5$, $p=0.97$), escolar ($W=34$, $p=0.71$), snake mackerel ($W=62$, $p=0.57$), and lancetfish ($W=7$, $p=0.25$). All escolar, snake mackerel, and lancetfish captured were considered adults. Adult and juvenile oilfish were both believed present due to the isotopic segregation in both $\delta^{13}\text{C}$ ($W=28$, $p<0.001$) (Figure 3A) and $\delta^{15}\text{N}$ ($W=239$, $p<0.001$) (Figure 3B).

Kruskal-Wallis tests performed on $\delta^{15}\text{N}$ indicated that each species was isotopically segregated ($\chi^2=15.6$, $df=4$, $p=0.001$) (Figure 2). Mann-Whitney U tests showed statistically equal $\delta^{15}\text{N}$ distributions between genders for oilfish ($W=1$, $p=1.0$), escolar ($W=6$, $p=1.0$), and snake mackerel ($W=49$, $p=0.84$). Lancetfish were not analyzed for gender difference due to the lack of males captured. Mann-Whitney U tests also showed no statistical differences in $\delta^{15}\text{N}$ between seasons for oilfish ($W=135.5$, $p=1.00$), escolar ($W=14$, $p=0.09$), snake mackerel ($W=62$, $p=0.60$), and lancetfish ($W=7$, $p=0.25$).

Because of the high lipid content present in the tissue of these species, a high C/N was anticipated as lipid is composed of chiefly carbon. Total body lipid content can also vary in fish as they become not only reproductively mature but it can also vary during spawning season with the production of eggs. Spearman's rank correlations were used to identify relationships between C/N with length for each species. Oilfish C/N was strongly correlated with fork length ($S=1289$, $\rho=0.78$, $p<0.001$). Escolar C/N was weakly correlated with fork length ($S=228$, $\rho=0.37$, $p=0.21$), snake mackerel C/N was moderately correlated with fork length ($S=2946$, $\rho=-0.46$, $p=0.03$), and lancetfish C/N was moderately correlated with length ($S=41$, $\rho=0.51$, $p=0.19$) (Figure 4).

DISCUSSION

A total of 122 individuals from these four mesopelagic fishes were collected for this project. These numbers do not reflect the actual abundance or size distributions of these species, as catch rates of these fishes are typically very low in this fishery (see Kerstetter and Graves, 2006), and even when hooked by the gear, they are rarely seen whole after gear retrieval. The inherent selectivity of the

sampling gear also resulted in presumed truncated size ranges due to the large hook (size 18/0 circle hook) used by the commercial pelagic longline vessels used to collect specimens in the study. However, the age distribution of these specimens may also be a result of distinct vertical distributions in the water column, horizontal distributions, foraging behaviors, or other distinctive behaviors of adults and juveniles within the species. These results nonetheless present the most complete trophic description of the four species to date.

The dominant prey items in all four fish species were largely benthic and demersal invertebrates. Based on the residence of prey in the stomachs, this would indicate these fish predominantly forage in deeper waters during the day. At night, the periods when these fishes were caught on pelagic longline gear, they may forage on softer bodied invertebrates that are digested rapidly and are not identifiable.

In particular, annelids were only present in lancetfish stomachs. Wasserung and Johnson (1976) reported that the stomachs of lancetfish are used for food storage as well as active digestion. We attribute the absence of annelids from oilfish and escolar stomachs to the high digestibility of these small, soft-bodied organisms and the higher rates of digestion found in oilfish and escolar stomachs. Similarly, salps were a very small portion of snake mackerel prey and yet an important part of lancetfish diets; this group was also absent from oilfish and escolar stomachs. Salps are the host organism for the amphipod crustacean *Phronima* sp., which occurred in high numbers in lancetfish stomachs. *Phronima* sp. amphipods have also been previously observed in lancetfish stomachs from the tropical-subtropical western North Atlantic Ocean (Satoh, 2004). The presence of identifiable salps – soft-bodied, planktonic tunicates – strongly suggests that lancetfish may be foraging for these invertebrates in the upper depths of the water column. The piece of plastic in the stomach of a lancetfish is also consistent with reports from stomachs in the Pacific (Jantz *et al.*, 2013)

Mollusks appeared to be an important prey group across all species; however, this prey group may be overrepresented due to the use of *Illex argentinus* squid as bait during many of the pelagic longline operations in the area. The relatively high digestibility of these soft-bodied animals may also have contributed to a portion of the partially digested material found in oilfish and escolar stomachs. Cephalopods were probably an important prey group for lancetfish in the area, with both gymnosomes and an octopod also present with squid in lancetfish stomachs.

Teleost fishes were a large portion of the total prey composition in all four species. Teleosts in snake mackerel stomachs included *Diodon* sp. porcupinefish and exocoetid flyingfishes, which along with buoyant *Sargassum* sp. macroalgae, suggests feeding at or near the surface. However, teleosts in lancetfish stomachs included sternoptychid hatchetfishes and *Alepisaurus* spp., indicating feeding which occurred deeper in the water column.

Lipids are an integral component of all four species tissues and given the fact that body lipid content can vary with age and reproductive status, we chose not to lipid correct the muscle $\delta^{13}\text{C}$. Additionally, no studies currently provide a reliable correction factor for any taxonomically similar species (see Logan *et al.*, 2008). Lipids within an organism are depleted in $\delta^{13}\text{C}$ relative to proteins and carbohydrates, and tissues with high lipid content will therefore show depleted $\delta^{13}\text{C}$ values relative to the whole animal. Strong relationships have been shown between C/N and lipid content, lipid content and $\delta^{13}\text{C}$, and between C/N and $\delta^{13}\text{C}$ in animals (Post *et al.*, 2007).

Escolar and oilfish had the most depleted $\delta^{13}\text{C}$ of the four species, averaging a depletion of more than 2‰. This disparity appears to be greater than would be explained by trophic shift alone. Oilfish showed increasingly depleted $\delta^{13}\text{C}$ values with maturity (Figure 2a), which did not coincide with any difference in prey items with maturity from stomach content analyses. The relatively high C/N observed in both adult oilfish (5.29 to 7.49) and escolar (5.78 to 10.10) when compared with juvenile oilfish (3.15 to 6.32), snake mackerel (3.07 to 6.26), and lancetfish (2.90 to 3.34) may be an effect of high lipid content, as oilfish and escolar tissues are known to contain high levels of lipid in the form of wax esters (Nakamura and Parin, 1993). Oilfish obtained from this study showed a marked increase in C/N at 30-35 cm that is strongly correlated with a significant shift in $\delta^{13}\text{C}$. This may indicate a marked increase in lipids in the tissue with maturity caused by an ontogenetic change at 30-35 cm fork length. Escolar $\delta^{13}\text{C}$ values showed a considerable spread, which were strongly correlated to C/N and weakly correlated with body length. All escolar captured were considered adults; therefore, variability in $\delta^{13}\text{C}$ of escolar may be explained by the capture of fish in differential spawning states (Box *et al.*, 2010) (Figure 3).

Juvenile oilfish had the most depleted $\delta^{15}\text{N}$ values (6.8 to 9.0‰), indicating the lowest trophic position of the group. Adult oilfish (7.06 to 10.81‰), snake mackerel (7.50 to 11.85‰), and lancetfish (8.39 to 10.79‰) had similar, intermediate values, and escolar had relatively high $\delta^{15}\text{N}$ (8.47 to 13.1‰) and the highest trophic position of the group. Oilfish $\delta^{15}\text{N}$ increased with maturity (Figure 2b), indicating an ontogenetic change in trophic predation with maturity.

Unfortunately, little stable isotope data exists for primary producers and consumers in this region of the southwestern North Atlantic Ocean. McClellan *et al.* (2010) reported stable isotope signatures for a limited number of organisms in the neritic and oceanic waters off North Carolina, where *Sargassum*-associated shrimps and crabs had a $\delta^{15}\text{N}$ of 4-5‰ and a $\delta^{13}\text{C}$ of -16. In this same study, yellow jack *Carangoides bartholomaei* was 5.4 and -17.8 and filefish *Stephanolepis hispidus* were 5.9 and -18.6, respectively. Neritic crabs were 10.7 and -17.6. Using conservative trophic fractionation adjustments (3‰ per trophic level for $\delta^{15}\text{N}$ and 1‰ per trophic level for $\delta^{13}\text{C}$), potential prey items would be dominantly in the range of 5 to 8‰ for $\delta^{15}\text{N}$ and -21 to -18‰ for $\delta^{13}\text{C}$. The values we present here for these four predatory mesopelagic fishes would be supported by the oceanic teleost data reported by

McClellan et al. (2010). Additional research at all trophic levels will provide further clarity regarding the ecological partitioning for the fish community of the pelagic ecosystem.

SUMMARY

This study examined the prey items, stable carbon and stable nitrogen isotopic compositions, C/N ratios, and GSIs of four large, mesopelagic teleosts for which little trophic or life history data exist. Stomach contents provided a “snapshot” into the diets, showing that they consumed crustaceans, annelids, salps, cephalopods, and teleosts. Stable carbon and nitrogen isotope analyses showed statistical segregation between all four species in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, providing information on what has been assimilated from prey items over a longer period of time, and combined with stomach content analyses, a finer resolution for analyzing differences in ecosystem use between the species. An ontogenetic shift in physiology and ecosystem use occurred within oilfish between juvenile and adults at 30-35 cm fork length, coinciding with a presumed length-at-maturity from the present study for oilfish. These data provide important information about the otherwise poorly known life histories of these four predatory fishes and suggest further research into ontogenetic behaviors into this mesopelagic complex.

ACKNOWLEDGEMENTS

This project was supported by NOAA Contract #8404-S-006 awarded to Nova Southeastern University. C. France at the Smithsonian Institution Museum Support Center performed the stable isotope analysis. Sample collection and measurements were assisted by members of the Nova Southeastern University Oceanographic Center Fisheries Laboratory, including S. Bayse, C. Cross, M. Taylor, and K. Bolow. Fishing vessels *Kristin Lee*, *Shady Lady*, *Southern Lady*, *Janice Ann*, and *Dakota*, and their respective captains and crews assisted in the fish specimen collections.

LITERATURE CITED

- Barausse A. (2007) *The Integrated Functioning of Marine Ecosystems* (Doctoral Dissertation). University of Padova (Italy), 95-98.
- Beidinger R., Lushine J.B. (1993) Duration of the Summer Season in South Florida. NOAA/NWS. Retrieved July 16, 2013, from http://www.srh.noaa.gov/mfl/?n=summer_season
- Bone Q. (1972) Buoyancy and hydrodynamic functions of integument in the castor oil fish, *Ruvettus pretiosus* (Pisces: Gempylidae). *Copeia*, **1**, 78-87.
- Boutton T.W. (1991) Stable carbon isotope ratios of natural materials: I. Sample preparation and mass spectrometric analysis. *Carbon isotope techniques*, **1**, 155.

- Bowen S.H. (1996) Quantitative Description of the Diet. In: B. R. Murphy and D. W. Willis (Eds). *Fisheries Techniques, 2nd edition*. American Fisheries Society, Bethesda, Maryland: 513-532.
- Box A., Deudero S., Blanco A., Grau A.M., Riera F. (2010) Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes in the pearly razorfish *Xyrichtys novacula* related to the sex, location and spawning period. *J. Fish Biol.* **76**, 2370–2381.
- Cabana G., Rasmussen J.B. (1996) Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences*, **93** (20), 10844-10847.
- Collette B.B., Potthoff T., Richards W.J., Ueyanagi S., Russo J.L., Nishikawa Y. (1984) Scombroidei: Development and relationships. In: Moser H.G., Richards W.J., Cohen D.M., Fahay M.P., Kendall A.W. Jr., Richardson S.L. (Eds). Ontogeny and systematic of fishes. *American Society of Ichthyologists and Herpetologists Special Publication*, **1**, 591-620.
- Cortés, E. (1997) A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**(3), 726–738.
- Crim L.W., Glebe B.D. (2002) Reproduction. In: C. B. Schreck and P. B. Moyle (Eds.) *Methods for Fish Biology*. American Fisheries Society, Bethesda (MD). 704 p.
- DeNiro M.J., Epstein S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495 – 506.
- DeNiro M.J., Epstein S. (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, **45**, 341 – 351.
- FAO (1978) *Yearbook of Fishery Statistics, 1976. Catches and Landings. FAO Statistics Series*, **46**, 25.
- FAO. (1980) *Yearbook of Fishery Statistics, 1978. Catches and Landings. FAO Statistics Series*, **50**, 38.
- FAO. (1982) *Yearbook of Fishery Statistics, 1980. Catches and Landings. FAO Statistics Series*, **54**, 52.
- FAO. (1992) *Yearbook of Fishery Statistics, 1990. Catches and Landings. FAO Statistics Series*, **70**, 105.
- France R.L. (1995) Source variability in $\delta^{15}\text{N}$ of autotrophs as a potential aid in measuring allochthony to freshwaters. *Ecography*, **18**, 318-320.
- Fry B., Arnold C. (1982) Rapid $\text{C}^{13}/^{12}\text{C}$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologica*, **54**, 200-204.
- Fry B., Sherr E.B. (1984) $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions to Marine Science*, **27**, 13-47.
- George E.L., Hadley W.F. (1979) Food and habitat partitioning between rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieu*) young of the year. *Transactions of the American Fisheries Society*, **108**, 253-261.

- Hecky R.E., Hesslein R.H. (1995) Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of North American Benthological Society*, **14**, 631-653.
- Hobson K.A., Welch H.E. (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Marine Ecology Progress Series*, **84**, 9-18.
- Hyslop E. J. (1980) Stomach contents analysis – a review of methods and their application. *Journal of Fish Biology*, **17** (4), 411-429.
- Jantz, L.A., Morishige, C.L., Bruland, G.L., Lepczyk, C.A. (2013) Ingestion of plastic marine debris by longnose lancetfish (*Alepisaurus ferox*) in the North Pacific Ocean. *Marine Pollution Bulletin*, **69**(1), 97-104.
- Kerstetter D.W., Rice P.H., Snodgrass D., Prince E.D. (2008) Behavior of an escolar *Lepidocybium flavobrunneum* in the Windward Passage as determined by popup satellite archival tagging. *Gulf and Caribbean Research*, **20**, 97-102.
- Kerstetter D.W., Graves J.E. (2006) Effects of circle versus J-style hooks on target and non-target species in a pelagic longline fishery. *Fisheries Research*, **80** (2), 239-250.
- Kling G.W., Fry B., O'Brien W.J. (1992) Stable isotopes and planktonic trophic structure in Arctic lakes. *Ecology*, **73** (2), 561-566.
- Logan J.M., Jardine T.D., Miller T.J., Bunn S.E., Cunjak R.A. (2008) Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, **77**, 838-846.
- Maksimov V.P. (1970) Some data on the biology of *Lepidocybium flavobrunneum* in the eastern Atlantic. *Voprosy Ikhtiologii*, **10** (1), 50-57.
- McClellan C.M., Braun-McNeill J., Avens L., Wallace B.P., Read A.J. (2011) Stable isotopes confirm a foraging dichotomy in juvenile loggerhead sea turtles. *Journal of Experimental Marine Biology and Ecology*, **387**, 44-51.
- Miyake Y., Wada E. (1967) The abundance ratio of $^{15}\text{N}/^{14}\text{N}$ in marine environments. *Records of Oceanographic Works in Japan*, **9**, 37-53.
- Nakamura I. (1991) Introduction to Scombriform fishes. *Aquabiology*, **74**, 249-225.
- Nakamura I., Parin N.V. (1993) *FAO Species Catalogue. Snake Mackerels and Cutlassfishes of the World (families Gempylidae and Trichiuridae). An annotated and illustrated catalogue of the Snake Mackerels, Snoeks, Escolars, Gemfishes, Sackfishes, Domine, Oilfish, Cutlassfishes, Scabbardfishes, Hairtails and Frostfishes known to date*, **15**, 1-129.
- Nelson J.S. (1996) *Fishes of the World*. John Wiley & Sons, Inc., Hoboken (NJ). 601 p.

- Nishikawa Y. (1982) Early development of the fishes of the family Gempylidae I. Larvae and juveniles of escolar, *Lepidocybium flavobrunneum* (Smith). *Bulletin of the Far Seas Fisheries Research Laboratory*, **19**, 1-19.
- Nybakken J.W. (1993) *Marine Biology: An Ecological Approach*. Harper Collins College Publishers, New York (NY): 159-163.
- Owens N.P. (1987) Natural variations in ^{15}N in the marine environment. *Advances in Marine Biology*, **24**, 389-451.
- Paul E.A., Melillo J., Knowles R., Blackburn H. (1992) *Nitrogen isotope techniques*. Academic Press. 11-54.
- Peterson B.J., Fry B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, **18**, 293-320.
- Post D.M., Layman C.A., Arrington D.A., Takimoto G., Quattrochi J., Montana C.G. (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, **152**(1), 179-189.
- Potier M., Marsac F., Cherel Y., Lucas V., Sabatie R., Maury O., Menard F. (2007) Forage fauna in the diet of three large pelagic fishes (lancetfish, swordfish, and yellowfin tuna) in the western equatorial Indian Ocean. *Fisheries Research*, **83**, 60-72.
- Robins R.C., Ray C.G., John D. (1986) *Atlantic Coast Fishes*. Houghton Mifflin Company, New York (NY): 1-354.
- Robison B.H. (2004) Deep pelagic biology. *Journal of Experimental Marine Biology and Ecology*, **300**, 253-272.
- Romanov E.V., Ménard F., Zamorov V.V., Potier M. (2008) Variability in conspecific predation among longnose lancetfish *Alepisaurus ferox* in the western Indian Ocean. *Fisheries Science*, **74** (1), 62-68.
- Satoh K. (2004) Occurrence of *Phronima sedentaria* (Forskal, 1775) (Amphipoda, Hyperiidea) in the stomach of the longnose lancetfish, *Alepisaurus ferox* (Lowe, 1833) (Aulopiformes, Alepisauroidae) in the North and tropical Atlantic Ocean. *Crustaceana*, **77**, 729-739.
- Van der Zanden M.J., Rasmussen J.B. (1999) Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology*, **80**, 1395-1404.
- Wada F., Kabaya Y., Kurihara Y. (1993) Stable isotopic structure of aquatic ecosystems. *Journal of Bioscience*, **18**, 483-499.
- Vasilakopoulos P., Pavlidis M., Tserpes G., (2011) On the diet and reproduction of the oilfish *Ruvettus pretiosus* (Perciformes: Gempylidae) in the eastern Mediterranean. *Journal of the Marine Biological Association of the United Kingdom*, **91**, 873-881.

Wassersug R. J., Johnson R.K. (1976) A remarkable pyloric caecum in the evermannellid genus *Coccorella* with notes on gut structure and function in alepisauroid fishes (Pisces, Myctophiformes)." *Journal of Zoology*, **179**, 273-289.

TABLES

Tables 1a-d. Percent occurrence (%O), percent number (%N), percent weight (%W), and percent index of relative importance (%IRI) of contents from 1a) oilfish *Ruvettus pretiosus* (n=14), 1b) escolar *Lepidocybium flavobrunneum* (n=15), 1c) snake mackerel *Gempylus serpens* (n=19), and 1d) lancetfish *Alepisaurus* spp. (n=3) stomachs sampled from the western North Atlantic Ocean. Note that n-values represent the number of stomachs with contents.

Table 2. Number, mean $\delta^{13}\text{C}$, mean $\delta^{15}\text{N}$, standard deviations (SD), and ranges of oilfish, escolar, snake mackerel, and lancetfish sampled from the western North Atlantic Ocean.

FIGURES

Figure 1. $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ of oilfish, escolar, snake mackerel, and lancetfish white dorsal muscle from the western North Atlantic Ocean, 2007-2010.

Figure 2. $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) vs. fork length of oilfish, escolar, snake mackerel, and lancetfish white dorsal muscle from the western North Atlantic Ocean, 2007-2010.

Figure 3. C/N vs. fork length of oilfish, escolar, snake mackerel, and lancetfish white dorsal muscle from the western North Atlantic Ocean, 2007-2010.

[Table 1a]

Prey Item	% O	% N	% W	%IRI
Crustacea	50.0	61.3	9.2	40.6
Decapoda				
Shrimps	35.7	54.8	6.0	
Unident. crustacean	14.3	6.5	3.2	
Mollusca	42.9	19.4	48.5	37.3
Cephalopoda				
Teuthida	42.9	19.4	48.5	
Teleostei	35.7	16.1	3.1	18.5
Unidentified teleost	35.7	16.1	3.1	
Partially digested material			38.9	
Unidentified Animals	7.1	3.2	0.4	3.6

[Table 1b]

Prey Item	% O	% N	% W	%IRI
Mollusca	60.0	72.7	54.3	68.0
Cephalopoda				
Teuthida	60.0	54.5	53.9	
Unidentified cephalopod	20.0	18.2	0.4	
Teleostei	60.0	27.3	0.9	32.0
Larvae	20.0	9.1	<0.1	
Unident. Teleost	40.0	18.2	0.9	
Partially digested material			44.8	

[Table 1c]

Prey Item	% O	% N	% W	%IRI
Crustacea	5.3	2.5	0.1	2.4

Unidentified crustacean	5.3	2.5	0.1	
Mollusca	78.9	60.0	79.1	68.0
Cephalopoda				
Teuthida	78.9	57.5	79.1	
Unidentified cephalopod	5.3	2.5	<0.1	
Thaliacea	5.3	2.5	0.0	2.4
Salpidae	5.3	2.5	<0.1	
Teleostei	36.8	22.5	20.1	24.5
<i>Diodon</i> spp.	10.5	12.5	0.5	
Exocoetidae	10.5	5.0	17.8	
Unidentified teleost	15.8	5.0	1.7	
Partially digested material			4.1	
Unidentified Animals	5.3	7.5	<0.1	
Macroalgae	5.3	2.5	1.1	2.7

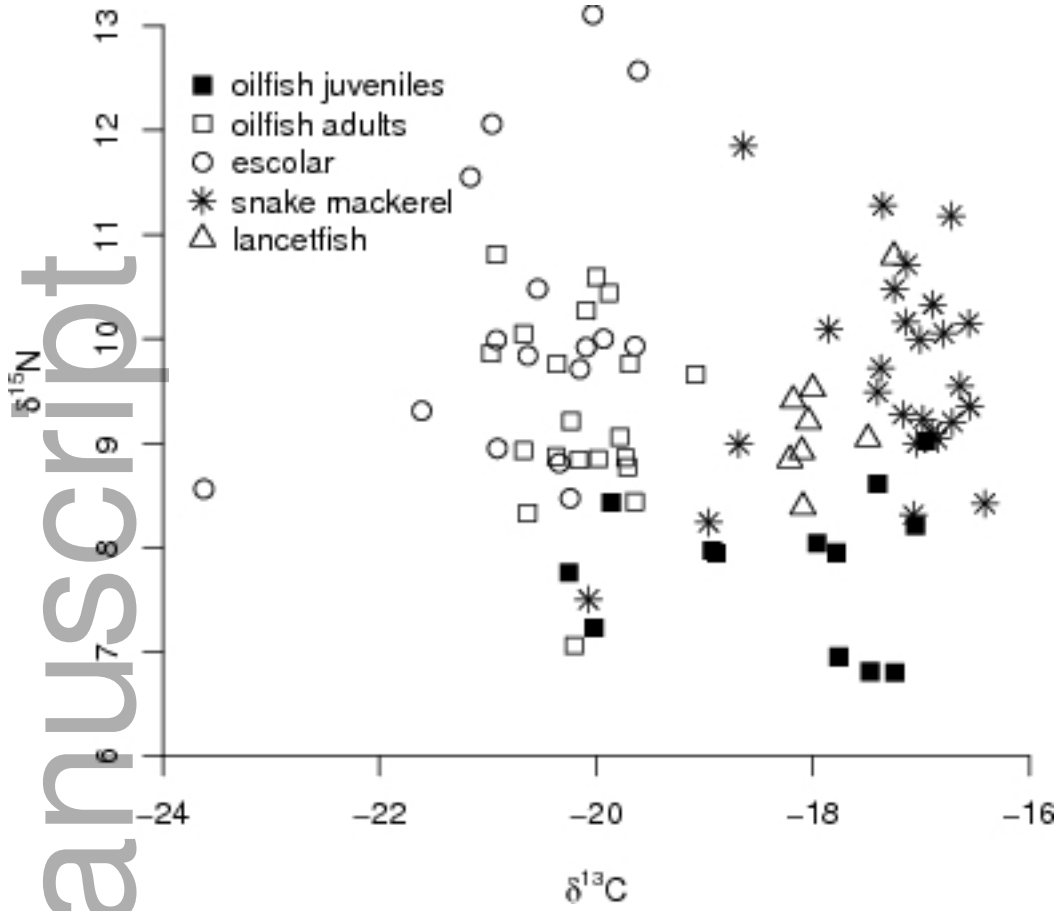
[Table 1d]

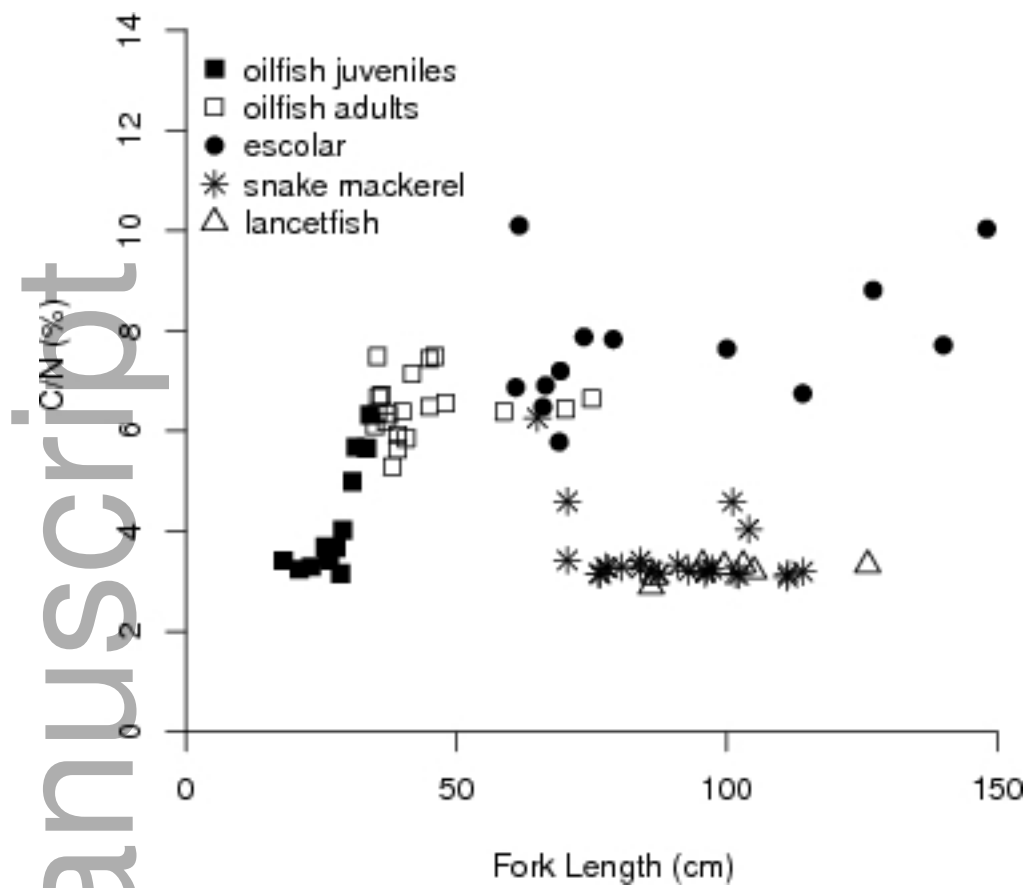
Prey Item	% O	% N	% W	%IRI
Crustacea	66.7	34.4	7.1	18.5
Hyperiidea	66.7	10.4	2.0	
Phronima	33.3	19.8	4.1	
Isopoda	33.3	4.2	1.0	
Annelida	33.3	10.4	3.0	8.0
Polychaeta	33.3	10.4	3.0	
Mollusca	100.0	15.6	14.8	22.3
Gymnosomata	66.7	12.5	11.3	
Cephalopoda	100.0	3.1	<.1	
<i>Argonauta</i>	33.3	1.0	0.5	
Teuthida	33.3	1.0	<.1	
Unidentified cephalopod	33.3	1.0	3.0	
Thaliacea	66.7	20.8	25.2	19.2
Salpidae	66.7	20.8	25.2	
Teleostei	66.7	13.5	25.7	18.1
Sternoptychidae	33.3	1.0	1.0	

<i>Alepisaurus</i> spp.	33.3	1.0	14.6	
Larvae	33.3	7.3	<.1	
Unidentified teleost	66.7	4.2	10.0	
Partially digested material			14.3	
Unidentified Animals	33.3	1.0	2.4	6.3
Marine pollution	33.3	1.0	10.6	7.7

[Table 2]

Species	n	Mean $\delta^{13}\text{C}$		$\delta^{13}\text{C}$ Range	Mean $\delta^{15}\text{N}$		$\delta^{15}\text{N}$ Range
		(‰)	SD	(‰)	(‰)	SD	(‰)
Oilfish	33	-19.40	1.23	-20.97 to -16.95	8.73	1.10	6.8 to 10.81
Juveniles	13	-18.27	1.18	-20.25 to -16.95	7.83	0.70	6.8 to 9.0
Adults	20	-20.14	0.48	-20.97 to -19.08	9.32	0.91	7.06 to 10.81
Escolar	16	-20.65	0.97	-23.62 to -19.61	10.20	1.41	8.47 to 13.1
Snake Mackerel	27	-17.31	0.86	-20.07 to -16.41	9.64	1.00	7.50 to 11.85
Lancetfish	7	-17.92	0.35	-18.21 to -17.25	9.27	0.71	8.39 to 10.79





maec12317-fig-0003.eps