

## ORIGINAL ARTICLE

# 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

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Escolar; lancetfish; oilfish; snake mackerel; stable isotope; stomach content.

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**Abstract**

Large, mesopelagic teleost fishes have a potentially keystone position in the ecology of the pelagic water column, yet remain relatively unstudied when compared with 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 seven lancetfish (*Alepisaurus* spp.) collected from pelagic longline gear 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 seven 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 to be 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 and 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 that may have contributed to the depletion in  $\delta^{13}\text{C}$  may include 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.

**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 differences 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 & Wada 1967; DeNiro & Epstein 1978, 1981; Owens 1987). Carbon stable isotope enrichment is relatively conservative and is used to trace carbon sources through a food web (DeNiro & Epstein 1978; Fry & Sherr 1984; Wada *et al.* 1993; France 1995; Hecky & Hesselein 1995). Stable nitrogen isotope enrichment is stepwise and is used to determine trophic level (DeNiro & Epstein 1978; Cabana & Rasmussen 1996; Van der Zanden & 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 & Fry 1987; Hobson & Welch 1992; Kling *et al.* 1992; Van der Zanden & 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 & Arnold 1982).

Oilfish, escolar and snake mackerel are members of Gempylidae (Collette *et al.* 1984; Nakamura & Parin 1993; Nelson, 2006), while lancetfishes are members of Alepisauridae (Robins *et al.* 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 mackerel and lancetfish (Robins *et al.* 1986; Nakamura 1991). Oilfish and anguilliform fishes like snake mackerel and lancetfish are thought to be ambush-type predators (Bone 1972; Nakamura & 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 & Parin 1993). Lancetfish have also been used for human consumption (Robins *et al.* 1986).

Oilfish, escolar, snake mackerel and lancetfish are known to feed on fishes, squids and crustaceans (Nakamura & Parin 1993; Potier *et al.* 2007; Vasilakopoulos *et al.* 2011). Lancetfish from the Indian Ocean diets, followed in occurrence by teleost fishes, with cephalopods rarely appearing. Diets varied geographically and Alepisauridae, including *Alepisaurus ferox*, was the main fish family in the gut contents measured by the mean proportion reconstituted weight, which indicated some level of cannibalism (Potier *et al.* 2007). The linkages between escolar, oilfish, snake mackerel, and lancetfish mesopelagic fishes and the apex predator commercial fishes have been examined with increasing detail (e.g. Romanov *et al.* 2008). However, descriptions of the diet and trophic position of the large mesopelagic fishes have not been carried out in a comprehensive manner, especially within the same geographic area. Using combined stomach content and stable isotope analyses, we describe the diets and trophic position characteristics of these four co-occurring mesopelagic fishes for the first time.

## Material and Methods

Samples were collected during commercial pelagic longline fishing operations targeting swordfish and tuna from January 2007 through January 2010 in US waters of the Western North Atlantic Ocean (between 28 and 36°N, west of 75°W). The hooks were predominantly baited with *Illex argentinus* squid ranging from 150 to 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 dealer. Although both longnose lancetfish *Alepisaurus ferox* and shortnose lancetfish *Alepisaurus brevirostris* are reported to occur in the geographic region of this study, all lancetfish individuals were combined into *Alepisaurus* spp. for analysis due to the difficulties in differentiation among partially damaged specimens obtained opportunistically from co-operating 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

3 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 (Crescent Dental Mfg. Co., Lyons, IL, USA) before weighing.

The extant literature does not include definitive values for size-at-maturity for these studied species. Simple gonadosomatic index (GSI) values were calculated as per Crim & 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 taxon. A 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 analysed 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 taxon.

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

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 & 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 & Hadley 1979):

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

The IRI was then converted to a percentage (as 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 & Epstein 1978, 1981). The formula for determining  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is as follows from DeNiro & Epstein (1978, 1981):

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

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

Tissue samples were analysed 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 (Thermo Fisher Scientific, Waltham, MA, USA). Dehydrated muscle tissue was weighed to achieve a final sample weight ranging from 0.6 to 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 and 29 May and the winter season median start date has been between 17 and 24 October (Beidinger & 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 Corp., version 19.0, Armonk, NY, USA) and R (R Foundation for Statistical Computing, Vienna, Austria) (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 seven 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 seven lancetfish, all of which were also sampled for stomach content analysis.

GSI values calculated during the study compared with season were consistent with spawning in mid- to late summer for oilfish (similar to Vasilakopoulos *et al.* 2011) and year-round spawning for longnose lancetfish (suggested in Nakamura & Parin 1993). Spawning seasons were not determined for escolar and snake mackerel from the GSI data in the present study, although Nakamura & 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 & 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 as 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 57% of seven 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 uniden-

tifiable, partially digested material also present with other identifiable prey items was low in oilfish (14%), escolar (17%) and lancetfish (17%), with a higher percentage in snake mackerel (36%). Stomach content data for each species are presented in Table 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 occurrence (50.0%), number (61.3%) and %IRI (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 in terms of occurrence (35.7%), number (16.1%), 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 occurrence (60%), number (72.7%), weight (54.3%) and %IRI (68.0). Teleosts had an equal occurrence (60%), and lower percent number (27.3%), 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 by occurrence (78.9%), number (60.0%), weight (79.1%) and %IRI (68). Teleosts were the second largest group in the diet by occurrence (36.8%), number (22.5%), weight (20.1%) and %IRI (24.5). Crustaceans and salps combined had a relatively low occurrence (5.3%), number (2.5%), 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 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 an occurrence of 66.7%, number of 13.5%, weight of 25.7% and %IRI of 18.1. Polychaetes were present with a occurrence of 33.3%, number of 10.4%, weight of 3.0% and %IRI of 8.0. A strip of a black plastic

**Table 1.** (a–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), (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.

(a)	prey item	%O	%N	%W	%IRI
Crustacea		50.0	61.3	9.2	40.6
Decapoda					
shrimps		35.7	54.8	6.0	
unidentified 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

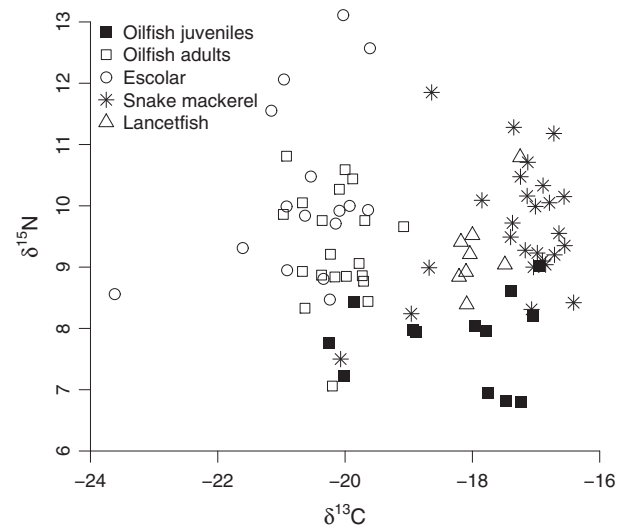
(b)	prey item	%O	%N	%W	%IRI
Mollusca		60.0	72.7	54.3	68.0
Cephalopoda					
Teuthida		60.0	54.5	53.9	
unidentified cephalopod		20.0	18.2	0.4	
Teleostei		60.0	27.3	0.9	32.0
larvae		20.0	9.1	<0.1	
unidentified teleost		40.0	18.2	0.9	
partially digested material			44.8		

(c)	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

(d)	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	

**Table 1.** Continued

(d)	prey item	%O	%N	%W	%IRI
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	<0.1	
<i>Argonauta</i>		33.3	1.0	0.5	
Teuthida		33.3	1.0	<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
Sternopterychiidae		33.3	1.0	1.0	
<i>Alepisaurus</i> spp.		33.3	1.0	14.6	
larvae		33.3	7.3	<0.1	
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



**Fig. 1.**  $\delta^{13}\text{C}$  versus  $\delta^{15}\text{N}$  of oilfish, escolar, snake mackerel and lancetfish white dorsal muscle from the Western North Atlantic Ocean, 2007–2010.

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 seven lancetfish that were also sampled for stomach contents. 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 juvenile and adult specimens. Significance was determined at  $\alpha \leq 0.05$  (Fig. 1, Table 2).

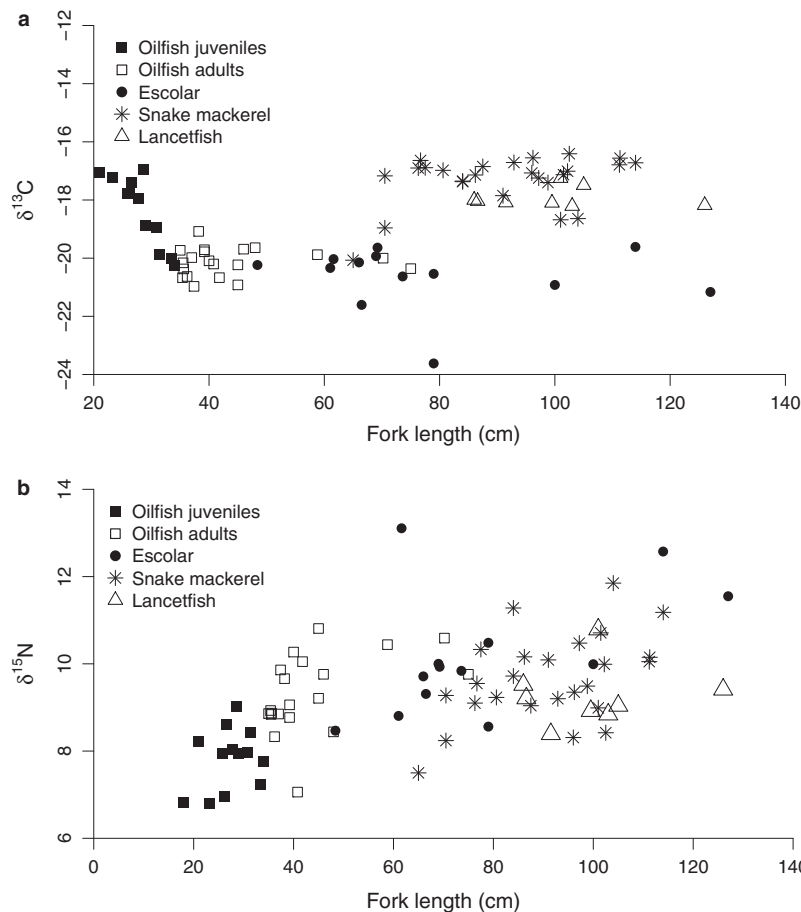
Kruskal–Wallis tests were performed on  $\delta^{13}\text{C}$  among species. Each species was isotopically segregated in  $\delta^{13}\text{C}$  ( $\chi^2 = 49.1$ ,  $df = 4$ ,  $P < 0.001$ ; Fig. 2). Mann–Whitney  $U$ -tests showed statistically equal  $\delta^{13}\text{C}$  distributions between genders for oilfish ( $W = 0$ ,  $P = 0.67$ ), escolar ( $W = 5$ ,  $P = 0.86$ ) and snake mackerel ( $W = 52$ ,  $P = 1.0$ ). Lancetfish were not analysed for gender differ-

ence 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 to be present due to the isotopic segregation in both  $\delta^{13}\text{C}$  ( $W = 28$ ,  $P < 0.001$ ; Fig. 3) and  $\delta^{15}\text{N}$  ( $W = 239$ ,  $P < 0.001$ ; Fig. 3).

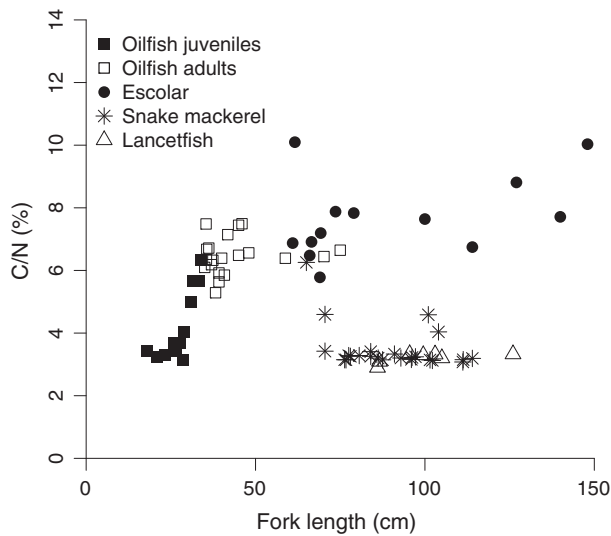
Kruskal–Wallis tests performed on  $\delta^{15}\text{N}$  indicated that each species was isotopically segregated ( $\chi^2 = 15.6$ ,

species	n	mean $\delta^{13}\text{C}$ (‰)	SD	$\delta^{13}\text{C}$ range (‰)	mean $\delta^{15}\text{N}$ (‰)	SD	$\delta^{15}\text{N}$ range (‰)
oilfish	33	-19.40	1.23	-20.97 to -16.95	8.73	1.10	6.8–10.81
juveniles	13	-18.27	1.18	-20.25 to -16.95	7.83	0.70	6.8–9.0
adults	20	-20.14	0.48	-20.97 to -19.08	9.32	0.91	7.06–10.81
escolar	16	-20.65	0.97	-23.62 to -19.61	10.20	1.41	8.47–13.1
snake mackerel	27	-17.31	0.86	-20.07 to -16.41	9.64	1.00	7.50–11.85
lancetfish	7	-17.92	0.35	-18.21 to -17.25	9.27	0.71	8.39–10.79

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



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



**Fig. 3.** C/N versus fork length of oilfish, escolar, snake mackerel and lancetfish white dorsal muscle from the Western North Atlantic Ocean, 2007–2010.

df = 4,  $P = 0.001$ ; Fig. 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 are hermaphroditic and thus were not examined for gender differences. 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 ( $W = 7$ ,  $P = 0.25$ ).

Owing to 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 change in fish with reproductive maturity, varying further 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 ( $S = 41$ ,  $\rho = 0.51$ ,  $P = 0.19$ ).

## Discussion

A total of 122 individuals of these four mesopelagic fish species was 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 & 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 presence of prey in the stomachs, this would indicate that fish predominantly forage in deeper waters during the day. At night, the time period 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. Wassersug & 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 over-represented 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 mack-

erel 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 that 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 (Fig. 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 (5.29–7.49) and escolar (5.78–10.10) when compared with juvenile oilfish (3.15–6.32), snake mackerel (3.07–6.26) and lancetfish (2.90–3.34) 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 & Parin 1993). Oilfish obtained in the present study showed a marked increase in C/N at 30–35 cm that was 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 with 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; Fig. 3).

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

Unfortunately, little stable isotope data exist for primary producers and consumers in this region of the wes-

tern North Atlantic Ocean. McClellan *et al.* (2011) 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 for the four mesopelagic fishes in this study would predominantly range from 5–8‰ for  $\delta^{15}\text{N}$  and –21 to –18‰ for  $\delta^{13}\text{C}$ . The values that we present here for these four predatory mesopelagic fishes would be supported by the oceanic teleost data reported by McClellan *et al.* (2011). Additional research at all trophic levels will provide further clarity regarding the ecological partitioning of 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 data exist: escolar, oilfish, snake mackerel, and lancetfish for which little trophic or life history data exist. Stomach contents provided a 'snapshot' into their diets, showing that they consumed crustaceans, annelids, salps, cephalopods and teleosts. Stable carbon and nitrogen isotope analyses showed statistical segregation among 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 analysing differences in ecosystem use among 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 this species. These data provide important information about the otherwise poorly known life histories of these four predatory fishes and suggest further research into ontogenetic behaviors in this mesopelagic complex.

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