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Trophic Level and Isotopic Composition of δ^{13}C and δ^{15}N of Pacific Angel Shark, *Squatina californica* (Ayres, 1859), in the Southern Gulf of California, Mexico

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ABSTRACT

δ^{13}C and δ^{15}N stable isotopes were used to determine trophic level and the assimilated food components of Pacific angel shark, *Squatina californica* and its variation between size, sexes and seasonally in the southern Gulf of California, Mexico. Muscle tissues were collected during 2001 to 2003. The quantification of δ^{13}C and δ^{15}N stable isotopes was realized by isotope ratio mass spectrometry. The values of stable isotope of carbon (δ^{13}C) ranged from -16.55 to -15.06‰ (average -15.94‰ ±0.34) and nitrogen (δ^{15}N) was between 18.30 to 19.52‰ (average 18.90‰ ±0.27), we found that as male as female feeds on same zones and not differences were found among years and seasons, therefore the angel shark present a same trophic level to feed on benthic prey in the coast zone. The trophic level was determined for *S. californica* with 4.5, therefore is considered a tertiary consumer in the southern Gulf of California. We concluded that Pacific angel shark present a same trophic pattern during migration to deep areas en the Gulf of California, feeding on benthic species with same trophic level.

Key words: *Squatina californica*, stable isotopes, Gulf of California, feeding habits, trophic level

INTRODUCTION

The Pacific angel shark, *Squatina californica* Ayres, 1859 is a demersal species that inhabit muddy and sandy bottoms of temperate and tropical waters. This shark is found in depth not more that 200 m, with preference between 3-45 m (Natanson and Cailliet, 1986). Their distribution is from southern Alaska to the Gulf of California and perhaps from Peru to southern Chile (Kato and Hernández-Carvallo, 1967; Compagno *et al.*, 1995). In the Gulf of California, the maximum size recorded is 95.5 cm (Escobar-Sánchez *et al.*, 2006) and show a seasonal presence within the Gulf of California, indicating a migration during summer associated to reproduction (Galvan-Magaña *et al.*, 1989).

In contrast to others sharks, *S. californica* is an ambush predator species associated with the marine bottom, where they remained to bury to capture its preys when swimming near to them. This specie is caught commercially and few biological information has been published to do a fisheries management in Mexico, because their populations have been decreased in the Gulf of California.

The trophic studies of *S. californica* have been done in the Pacific Ocean using observations *in situ* to examine the feeding behavior (Fouts and Nelson, 1999) and stomach content analysis (Pittenger, 1984; Galvan-Magaña *et al.*, 1989; Escobar-Sánchez *et al.*, 2006). The use of stomach contents in sharks (Estrada *et al.*, 2003) have been important to know the role of sharks associated to ecosystems; however, this method provide specific information on the recent feeding habits of sharks, while the stable isotope analysis give more information about the assimilated food and their origin (benthic or pelagic, oceanic and coastal prey) (Hesslein *et al.*, 1993; Harvey and Kitchel, 2000). The most important isotopes used in this kind of analysis are carbon and nitrogen (Schell *et al.*, 1998), which permit to know the energy flow and feeding relationships within aquatic food webs (Minagawa and Wada, 1984; Cabana and Rasmussen, 1994; Zanden *et al.*, 1997, 2000). The carbon isotopes are used to identify consumption from coastal or pelagic sources. While nitrogen isotopes are fractionated, leaving 3.4‰ $\delta^{15}N$ to be integrated into the consumer's tissue. This enrichment causes a stepwise increase in ^{15}N as the consumer's trophic level increases (Deniro and Epstein, 1981; Minagawa and Wada, 1984).

The accumulated information in muscle is approximately 3 months of isotopic signatures, which allows knowing the origin of assimilated prey by the predator. This technique is useful when there are a high percentage of empty stomachs with little information on consumed prey. Therefore, the goal of the present analysis was recognize the trophic level and the origins of food components by means of $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis by sex, size, season and locality in the southern Gulf of California.

MATERIALS AND METHODS

The samples were collected from February 2001 to March 2003 in the following fishing camps: El Pardito (PAR) (24°51' 42"N; 110°35' 22" W), El Portugués (PORT) (24°47' 38" N; 110°39' 39" W) and Ensenada de muertos (EMTOS) (23°58' 38" N; 109°49' 57" W), located in the southern Gulf of California, Mexico (Fig. 1). The sharks were caught using a multifilament gill net of mesh size

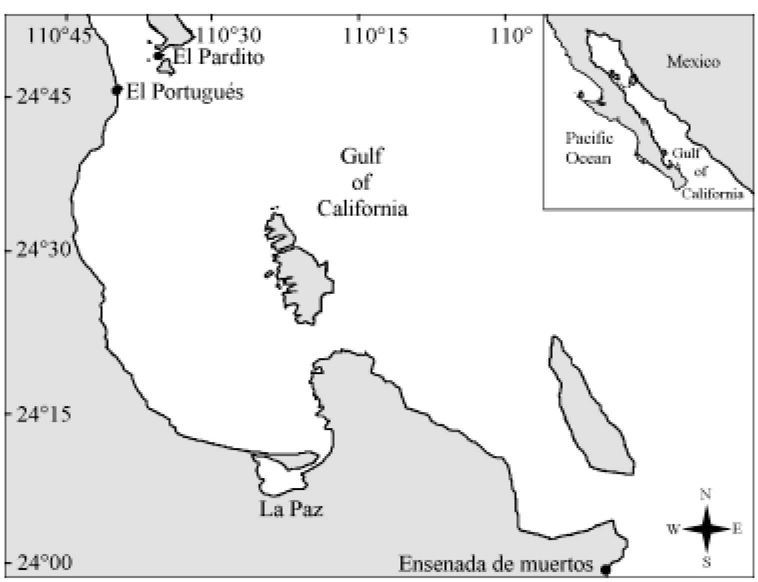

Fig. 1: Study area. Indicating the fishing camps in the southern Gulf of California

between 10 and 30 cm. The nets were set during the sunset and reviewed the following morning. In the field, Total Length (TL) and sex were recorded. The state of maturity of angel shark was determined following to Marqueda-Cava *et al.* (2003), it which considered to juveniles with a total length less than 80 cm, while the sharks with a TL more than 80 cm were considered like adults.

For isotopic analysis, the muscles from the anterior-dorsal region of 38 angel sharks were collected and preserved frozen. The muscles were lyophilized (LABCONCO) to -45°C and a pressure of $24\text{-}27\times10^{-3}$ MBAR during 24 h to extract humidity. The lipid extraction were realized in a microwave (Microwave Assisted Extraction-MAE model 1000 (CEM, Matthews, NL) with temperature and pressure controlled during 20 min. Each dried samples was ground and weighed (±0.001 g) in an analytical balance and put into tin capsules (8×5 mm). The isotope ratios of carbon and nitrogen were measured with a mass spectrometer (EMRI) (20-20 mass spectrometer, PDZ Europe, Scientific Sandbach, United Kingdom, UK) at the University of California in Davis (USA).

The δ values of stable isotopes were calculated according to the following equation:

$$\delta^{15}N \text{ or } \delta^{13}C\ (‰) = [(R_{sample}/R_{standar})-1] \times 1000$$

where, R_{sample} for $\delta^{15}N$ is the proportion between its isotopes $^{15}N/^{14}N$, while by $\delta^{13}C$ is the proportion of the isotopes of $^{13}C/^{12}C$. The standards used for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were Pee Dee Belemnite limestone (PDB) and atmospheric nitrogen (AIR), respectively.

The relative Trophic Level (TL) was determined using the following equation established by Post (2002):

$$TL = \lambda + \frac{\left(\delta^{15}N_{Predator} - \delta^{15}N_{Base}\right)}{\Delta_n}$$

where, λ is the trophic position of the specie used to estimate $\delta^{15}N_{Base}$, Δ_n is the enrichment in $\delta^{15}N$ per trophic level (the mean aquatic enrichment of 3.4‰ was assumed for all trophic estimations according to Minagawa and Wada (1984) and $\delta^{15}N_{Predator}$ is the direct signature of $\delta^{15}N$ for the predator to evaluate. According to Post (2002), to determine the $\delta^{15}N_{Base}$ it is necessary to use prey species that should share the same habitat as the predator to evaluate the isotopic signature of the food web. We used the fish *Diplectrum pacificum* as the $^{15}N_{Base}$, which is located in the Gulf of California and it was categorized as secondary consumer with a trophic level of 3.99.

The normality of isotope data was tested using the Kolmogorov-Smirnov (KS) ($p>0.05$). The data were normally distributed ($d = 0.091$ $p>0.05$ for $\delta^{15}N$; $d = 0.049$ $p>0.05$ for $\delta^{13}C$); therefore we used an Analysis of Variance (ANOVA) to determine the possible differences by categories (sex, location). Also we used a linear regression analysis to examine the possible relationship and trend between total length and the $\delta^{15}N$ signatures.

RESULTS

Stable isotopes signatures: The stable isotope analysis was applied to 38 angel sharks muscle that representing different sex, season, size, location and capture year. The stable isotopes of angel shark muscles showed $\delta^{13}C$ values of -16.55 to -15.06‰ (av. -15.94‰ ±0.34 sd); whereas the $\delta^{15}N$ values were 18.30 to 19.52‰ (av. 18.90‰ ±0.27) (Fig. 2).

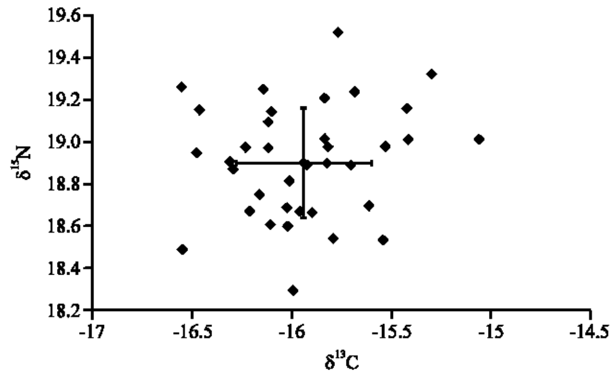

Fig. 2: Mean and standard deviation $\delta^{13}C$ and $\delta^{15}N$ stable isotope values of *Squatina californica* in the Southern Gulf of California

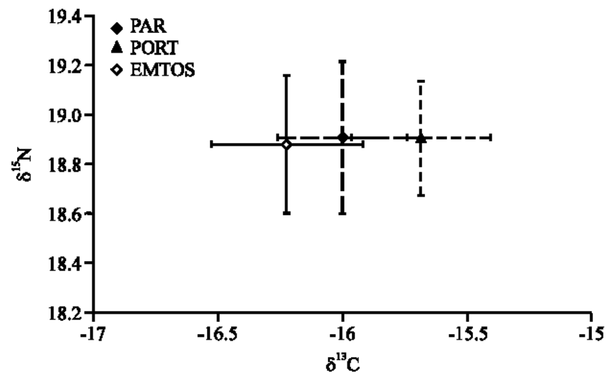

Fig. 3: $\delta^{13}C$ and $\delta^{15}N$ isotopic values of *Squatina californica* by location (PAR = El Pardito, PORT = El Portugués, EMTOS = Ensenada de muertos)

Table 1: Isotopic composition ($\delta^{13}C$ and $\delta^{15}N$ presented in ‰) of Pacific angel shark, *Squatina californica* in the southern Gulf of California

Sampling area	Sex	N	$\delta^{13}C$ average (SD)	$\delta^{15}N$ average (SD)
PAR	♀	8	-15.95±0.32	18.85±0.31
	♂	7	-16.06±0.17	18.96±0.32
PORT	♀	10	-15.67±0.31	18.95±0.22
	♂	4	-15.73±0.22	18.78±0.23
EMTOS	♀	6	-16.09±0.28	18.85±0.28
	♂	3	-16.50±0.05	18.94±0.32
GENERAL	♀	24	-15.87±0.34	18.89±0.26
	♂	14	-16.06±0.32	18.91±0.29

PAR: El Pardito, PORT: El Portugués, EMTOS: Ensenada de muertos

We did not found significant differences by location (ANOVA, $p>0.05$) (Fig. 3; Table 1), therefore, the isotopic data by different categories were analyzed in general data. The $\delta^{15}N$ values were similar in both locations (PAR and POR), where the means were of 18.91‰ ±0.31 and 18.91‰ ±0.23 respectively, while, in EMTOS the isotopic ratio was of 18.88‰ ±0.28; while the $\delta^{13}C$ by areas were –16.22‰ ±0.30 (EMTOS), –15.69‰ ±0.28 (POR) and –16.00‰ ±0.26 (PAR).

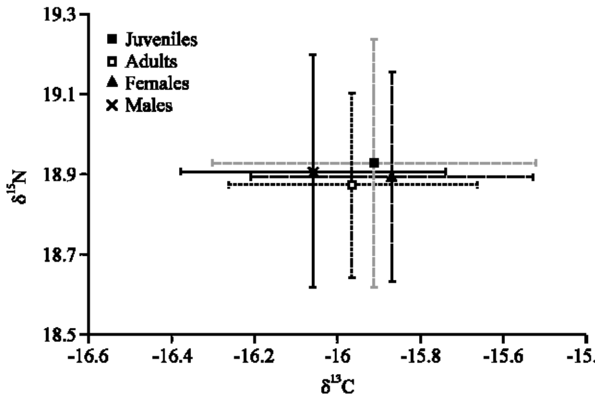

Fig. 4: $\delta^{13}C$ and $\delta^{15}N$ isotopic values of *Squatina californica* by sex and size (juveniles and adults)

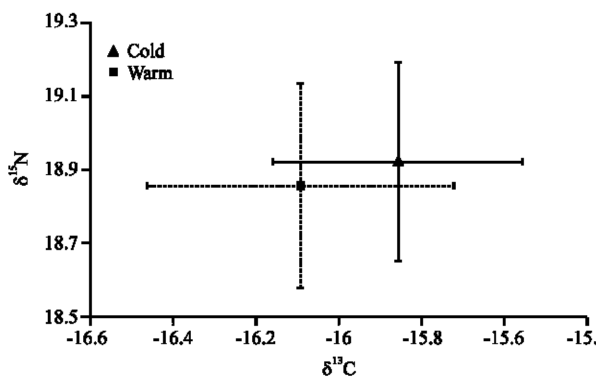

Fig. 5: $\delta^{13}C$ and $\delta^{15}N$ isotopic values of *Squatina californica* by season during the period 2001-2003 in the southern Gulf of California, Mexico

Stable isotope signatures by categories: Not significant differences in $\delta^{13}C$ and $\delta^{15}N$ were found between male ($\delta^{15}N$= 18.91‰ ±0.29; $\delta^{13}C$= -16.06‰ ±0.32) and female ($\delta^{15}N$= 18.89‰ ±0.26; $\delta^{13}C$ = -15.87‰ ±0.34) of angel sharks (ANOVA, $p>0.05$) (Fig. 4), but some males presented negative values of carbon and some females with positive values.

The stable carbon isotope ratios by size (juveniles and adults), were similar with means of –15.91‰ ±0.39 and –15.96‰ ±0.30, respectively (Fig. 4). Likewise, the nitrogen isotope ratios, the juveniles presented a mean of 18.93‰ ±0.31 and adults of 18.87‰ ±0.23.

The ratio of stable isotopes in warm season was –16.09‰ ±0.37 ($\delta^{13}C$) and 19.86±0.28 ($\delta^{15}N$); while in cold season the values had an overall mean of –15.86‰ ±0.30 ($\delta^{13}C$) and 18.92±0.27 ($\delta^{15}N$) (Fig. 5). When we compared the annual isotopic analysis, there were no significant differences among years (2001, 2002 and 2003) (Fig. 6).

In the Table 2 and Figure 7, we showed the isotopic values of $\delta^{13}C$ and $\delta^{15}N$ of different marine species located in the Gulf of California. Including others predators like sharks, marine mammals, rays and some prey of *S. californica*. We compared the isotopic values of this species with respect to isotopic values of Pacific angel shark. No significant relationship between $\delta^{15}N$ and total length of this shark was observed (R^2 = 0.014, N = 38, p = 0.47).

Table 2: Isotopic values of $\delta^{13}C$ and $\delta^{15}N$ of different marine species (*shark, **ray, +marine mammal), located in the Gulf of California (GC) and some important preys reported in stomach contents of *S. californica*. In marine mammal, the tissue analyzed was skin, while in the others species was muscle tissue

Specie	Area	$\delta^{15}N$ average (SD)	$\delta^{13}C$ average (SD)	Trophic level	References
*Sphyrna lewini** juvenil	La Paz Bay, GC	21.1 (0.46)	-16.3 (0.36)	4.21[a]	Aguilar-Castro (2003)
*Sphyrna lewini** adult	La Paz Bay, GC	19.1 (0.14)	-17.5	4.21[a]	Aguilar-Castro (2003)
*Zapterix exasperata***	Sonora, GC	19.2	-14.3	4.10	Blanco-Parra (2008)
*Rhizoprionodon longurio**	La Paz Bay, GC	20.5	-14.7 (0.5)	4.20	Conde-Moreno (2009)
*Rhincodon typus**	La Paz Bay, GC	12.9	-15.11	3.76[a]	Hacohen-Domene (2007)
Balaenoptera physalus+	La Paz Bay, GC	15.4	-17.53		Jaume-Schinkel (2004)
*Sphyrna zygaena**	La Paz Bay, GC	19.3 (0.66)	-15.3 (0.36)		Ochoa-Díaz (2009)
*Squatina californica**	Southern, GC	18.9 (0.27)	-15.9 (0.34)	4.50	Present study
Etrumeus teres [b]	GC	13.9	-17.9	3.40[a]	Gendron *et al.* (2001)
Diplectrum pacificum [b]	Mazatlan, GC	17.1	-16.0	3.99[a]	Torres-Rojas (2006)
Synodus spp. [b]	Mazatlan, GC	18.7	-13.9		Torres-Rojas (2006)

[a]: Taken of fishbase, [b]: Main prey of *S. californica* reported by Escobar-Sánchez *et al.* (2006)

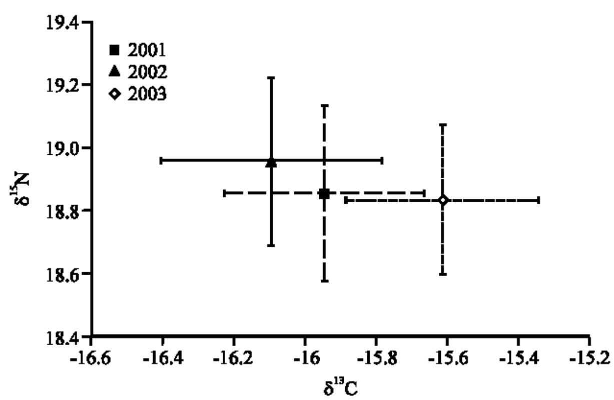

Fig. 6: $\delta^{13}C$ and $\delta^{15}N$ isotopic values by year in *Squatina californica* in the Southern Gulf of California, Mexico

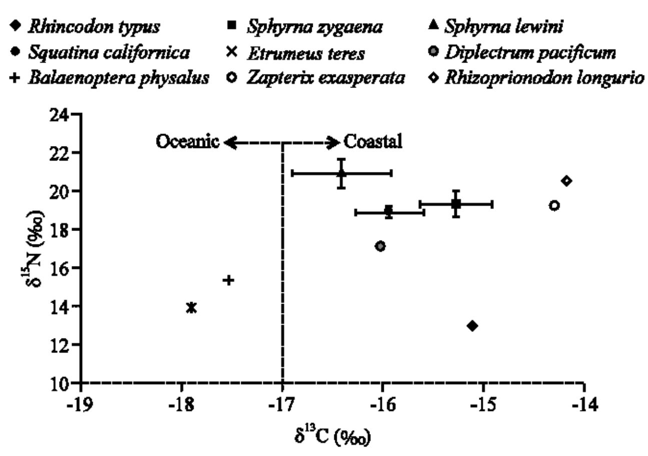

Fig. 7: Mean comparison of $\delta^{13}C$ and $\delta^{15}N$ isotopic values of different species caught in the Gulf of California

Trophic level: The trophic level of *S. californica* was 4.5±0.1 (range of 4.3-4.7), whereas by categories (juveniles-adults, males and females) the trophic level was the same (4.5 TL).

DISCUSSION

Considering that organisms associated to benthic food chain have an enrichment with ^{13}C (Jennings *et al.*, 1997), the observed isotopic values of carbon for angel shark (-15.94‰ ±0.34) are within the range of carnivorous fishes that feed on benthic prey (–18.0 to –15.9‰) reported by Jennings *et al.* (1997) for Mediterranean fishes. The accumulate information of ^{13}C of predator, indicated a similarity with the food content of angel shark, therefore, the angel shark´s diet is considered benthic-coastal.

Aguilar-Castro (2003) analyzed the hammerhead shark, *Sphyrna lewini* samples from the southern Gulf of California, recording a $\delta^{15}N$ isotopic ratio of 20.92‰ ±0.72, where juveniles had higher values of nitrogen than adults and therefore juveniles had a higher trophic level because they feed on carnivorous prey from coastal areas. Also Ochoa-Díaz (2009), found in smooth hammerhead shark *Sphyrna zygaena* from the Gulf of California that nitrogen isotope values indicated that this shark feeds mostly in deep waters, which is confirmed with the stomach contents analysis, where the main prey were oceanic cephalopods. In silky shark (*Carcharhinus falciformis*), Cabrera (2003), found that silky shark in the western coast of Baja California Sur, had a nitrogen ratio of 16.19‰ ±0.65, with a trophic position smaller than *S. lewini*. This author mentioned that silky shark feed in oceanic zone, with a short food chain, because their main prey (*Pleuroncodes planipes*) feeds on zooplankton or zooplanktophague prey.

The nitrogen values of angel shark (18.9‰ ±0.27), were between *S. lewini* and *C. falciformis* results. The main prey of *S. lewini* are benthic and pelagic prey; whereas *C. falciformis*, feed mainly on red crab (*Pleuroncodes planipes*), a common crustacean in the column water from the western coast of Baja California Sur. The other shark species recorded in the same area than *S. californica*, showed variations in the isotope values, which indicated the different feeding habits and distribution of each shark species in the marine ecosystems and therefore the different trophic level obtained by Pacific angel shark, using isotopes which is considered a tertiary consumer (TL = 4.5). Cortes (1999) also classified to *S. californica* as a tertiary consumer (TL= 4.1 average). Conde-Moreno (2009) determined the trophic level for sharpnose shark *Rhizoprionodon longurio* in the Gulf of California, which classified this shark species as a tertiary consumer or a high level carnivorous (TL> 4), because this shark feed mainly on carnivorous prey.

Two factors would contribute to the ^{15}N enrichment: the first is the shift of the preferred prey from species with low trophic level and low $\delta^{15}N$ (e.g., crustacean and planktivorous fishes), toward prey with high trophic level and high $\delta^{15}N$ values (e.g., cephalopods and benthic carnivorous fishes). The second factor is the increase in trophic position considering the predator size, as reported by Reñones *et al.* (2002). However, we do not found changes in isotopes values in angel shark, because the range of isotopic values of $\delta^{15}N$ and $\delta^{13}C$ were minimal (1.4 and 1.6‰, respectively).

The lack of differences in patterns of ^{15}N enrichment by areas and sizes, suggest that trophic niche is similar in angel sharks. This behavior was observed also in angel shark stomach contents (Escobar-Sánchez *et al.*, 2006), where the angel shark feed on prey with similar trophic level in different areas. Although we found few differences in $\delta^{13}C$ values, we think that angel shark fed on prey with different carbon origin because they move from different ecosystems (Rau *et al.*, 1992; Das *et al.*, 2000).

The variability in $\delta^{15}N$ and $\delta^{13}C$ was minimal, considering that enrichment of $\delta^{15}N$ in nature is 3-4‰ by trophic level and 1‰ in $\delta^{13}C$. We consider that angel sharks in the coastal area fed on the same trophic level (benthic prey), considering the $\delta^{15}N$ values of 18.3 to 19.5‰ (av.= 18.9‰ ±0.27 sd) and $\delta^{13}C$ of –16.5 to –15.06‰ (av.= –15.94‰ ±0.34 sd). These values changed few, which indicate that males, females, juveniles and adults feed on the same resources during years.

By other hand, the isotopic composition would change among organism with different size or seasonally in each area and this information would be used to know the migratory behavior of predators (Hesslein *et al*., 1993; Overman and Parrish, 2001), however little information exists on migration of angel sharks. Fishing information indicates that adults are found in deep waters during the warm season, following their reproductive cycle. Although, the nitrogen isotope values showed no differences in the trophic level, then we consider that despite the capture area of angel sharks, they feed in deep waters, where the prey have a constant trophic level. More biological information on angel sharks is necessary to know what happen during the warm season, because the artisanal fishermen do not catch angel shark in this season. Considering that fishermen put their nets at depths of 70-80 m, would indicate that angel sharks would be in deeper waters during this season.

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