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Stable isotopes provide evidence of a trophic shift in the lesser spotted dogfish *Scyliorhinus canicula* from the Central Tyrrhenian Sea

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Abstract

Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were analysed in vertebrae of *Scyliorhinus canicula* (*n*=40; 13 males, 27 females) from the central Tyrrhenian Sea to explore a potential trophic shift in relation to sex and maturity condition. Isotopic values were obtained from the centre and the periphery of the vertebra of each individual to test for the effect of maturity condition. The values of $\delta^{13}C$ and $\delta^{15}N$ showed significant differences between the immature and mature state of the individuals analysed (U'=1392, $P<0.0001$ for $\delta^{13}C$; U'=1385, $P<0.0001$ for $\delta^{15}N$). Differences in values of $\delta^{15}N$ demonstrated a trophic shift during maturation progress of *S. canicula.* The peripheral vertebral part, corresponding to the mature state of each individual, showed higher values of $\delta^{15}N$ (mean 5.41‰ SD±1.29‰), compared with the lower values of $\delta^{15}N$ (mean 2.78‰ SD±1.52‰) observed in the central vertebral part related to the immature state. In contrast, the isotopic signature of $\delta^{13}C$ was lower in the vertebral centra, and thus related to areas less enriched in C^{13} (mean -22.56‰ SD±1.26‰) in comparison with the vertebral periphery (mean -20.03‰ SD±1.34‰) linked to more productive environments. No significant differences in $\delta^{13}C$ or for $\delta^{15}N$ values were observed between sexes.

Keywords: Benthic shark; Mediterranean Sea; Scyliorhinidae; stable isotopes; vertebrae.

Introduction

The lesser spotted dogfish *Scyliorhinus canicula* (Linnaeus, 1758) is a common and widely distributed species in the Mediterranean Sea and in the north-eastern Atlantic Ocean, covering a bathymetric range from 10 to 780 m (Ebert & Dando, 2021; Micarelli *et al*., 2020). It can be found from the intertidal zone to the continental slope, with highest abundances reported at depths of 200-500 m (Olaso *et al*., 2005; Kousteni & Megalofonou, 2019; Leonetti *et al*., 2020). Considering that *S. canicula* is one of the most abundant demersal sharks in the Mediterranean Sea, and the one most resilient to the impact of human activities, the understanding of its ecology is fundamental to strengthen fishing management and conservation regulations in European countries such as Portugal, Spain, Greece, and Italy (Finotto *et al*., 2015; Serena *et al*., 2015; Ebert & Dando, 2021; Consales & Marsili, 2021).

As other dogfish species, *S. canicula* constitutes a mesopredator that plays a relevant role in the trophic web of demersal fish communities, representing a link between invertebrates and larger predatory fishes (Barría *et al*., 2018). It is an opportunistic and generalist scavenger/predator that feeds on crustaceans, cephalopods, and small fishes, with a diet that can vary considerably with respect to maturity, sex, and location (Martinho *et al*., 2012; Kousteni *et al*., 2017).

The diet of *S. canicula* has been studied through stable isotopes ($\delta^{13}C$; $\delta^{15}N$) and stomach contents analyses in different areas of the Atlantic Ocean (Lyle, 1983; Henderson & Dunne, 1999; Olaso *et al*., 1998; Martinho *et al*., 2012; Caut *et al*., 2013) and the Mediterranean Sea (Gravino *et al*., 2010; Karachle & Stergiou, 2010; Valls *et al*., 2011; Mnasri *et al*., 2012; Santic *et al*., 2012; Kousteni *et al*., 2017; 2018). In this regard, stable isotope analysis has proved to be a relevant tool for ecological studies, given that it provides data regarding the ecological niche of species by analysing trophic position ($\delta^{15}N$), feeding areas ($\delta^{13}C$), surrounding temperature ($\delta^{18}O$), pollution ($\delta^{202}Hg$), among other features (Newsome *et al*., 2007; Bevacqua *et al*., 2021; Le Croizier *et al*., 2020).

Stable isotope studies in the genus *Scyliorhinus* have been performed on soft tissues such as muscle, blood, kidney, and liver (Caut *et al*., 2013; Barría *et al*., 2018). However, there are no available studies regarding isotopic signature values in hard tissues such as vertebral cartilage. The use of vertebrae allows the analysis of stable isotopes throughout the ontogeny of sharks (Estrada *et al*., 2006; Bevacqua *et al*., 2021) given that this structure grows by concentric rings during the development of the individuals. Therefore, the central rings accumulate trophic information during the early stages of the individuals, while the marginal rings provide data regarding mature stages. The aim of the present study was to analyse stable isotopes ratios of N and C in the vertebrae of *S. canicula* from the central Tyrrhenian Sea to explore the trophic ecology of this species. The hypothesis tested was that differences in the isotopic values of the vertebrae of *S. canicula* in relation to sex and maturity state would be observed.

Materials and Methods

Sample collection

The collection of specimens was carried out at 12 nautical miles off Rocchette Punta Ala (Central Tyrrhenian Sea, Italy; Fig. 1) in collaboration with local fishers. These surveys were carried out in February 2017, August 2017, January 2018, and May 2018. A total of 40 lesser spotted dogfishes was collected by commercial bottom trawling at 150 m deep and then transferred to the Sharks Studies Center-Scientific Institute of Massa Marittima, Italy, where sex, total length (TL; cm) and total weight (TW; g) were recorded (Reinero *et al*., 2022). Maturity condition was assessed macroscopically by examining the state of the ovaries, oviducal glands, and uteri in females; as well as the condition of testes, vas deferens, claspers in males (Finotto *et al*., 2015; Kousteni *et al*., 2010; Kousteni & Megalofonou, 2019). Afterwards, five vertebrae were removed from the dorsal part near the origin of the first dorsal fin (Da Silva *et al*., 2021). Vertebrae were cleaned manually with a scalpel and stored frozen at -20°C prior to their transport to the Centro Interdisciplinario de Ciencias Marinas, Mexico, for stable isotopes analyses.

Stable isotopes analysis

All the specimens (n=40) were represented by vertebrae larger than 2 mm in diameter, which allowed the perforations in the centre and periphery to determine isotopic values from two different phases of the shark's life (mature vs immature state). Methods for sample preparation and isotopic analysis followed Estrada *et al*. (2006). The subsamples were obtained from each specimen's vertebrae by using a micro-drill (Cameron Micro Drill Presses) equipped with a drill pit (⌀=1 mm). Two perforations were made in each vertebra (2.5-3.0 mm in diameter): one close to the central part to obtain data on the isotopes relating to the immature state; and one from the distal part to obtain data relating to the mature state. All the collected powder subsamples were placed into 5 ml labelled tubes. Subsequently, subsamples (1 mg; ABT 220-5D analytical balance: 0.01 mg) underwent a preparation phase, in which ethylenediaminetetraacetic acid (EDTA) was inserted into each test tube to remove inorganic carbonates, for an accurate $\delta^{13}C$ assessment. The tubes containing the EDTA remained at rest for 24 h, after which they were placed in an oven at 45°C during 24 h allowing the acid to evaporate. Two consecutive rinses were carried out

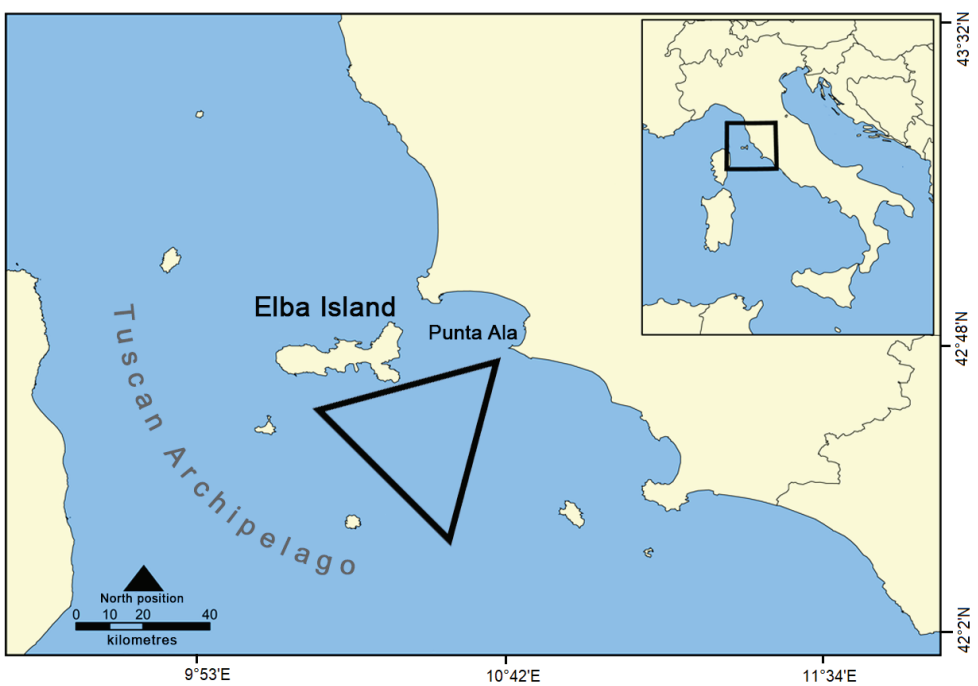

Fig. 1: Fishing area in the Tyrrhenian Sea (black triangle) where specimens of *S. canicula* were obtained during 2017-2018.

with distilled water, which was subsequently evaporated at 45°C for 24 h. Stable isotopes analyses were carried out by using a Delta V isotope ratio mass spectrometer (UV/VIS Spectrometer; Lambda 25, Perkin Elmer; Singapore). The isotopic ratio for N and C was estimated using the following equation:

$$\delta X = \frac{Rsample - Rstandard}{Rstandard} * 1000$$

Where *X*=difference in isotopic composition between sample and standard in parts per thousand (‰); and *R*=ratio of the heavy isotope to the light isotope. The standards used to calibrate the mass spectrometer were the glutamic acid and urea for $\delta^{15}N$ values, as well as sailfish and mangrove samples for $\delta^{13}C$ values (Estrada *et al*., 2006). The results on the stable isotopes were compared by sex and maturity state.

Statistical analysis

The normal distribution of the isotopic data obtained was determined by the Shapiro-Wilk's test, while Levene's test was used to verify if each group of the independent variable has the same variance; when Levene's statistic was significant, the null hypothesis that the groups have the same variance was rejected. The comparison between the median values of stable isotopes was performed using the Mann-Whitney test. All statistical analyses were performed by using the statistical programs R Studio 1.1.423 and Instat 3.0.

For the niche size, the recommendations of Newsome *et al*. (2007) were followed, based on which $\delta^{15}N$ variance values < 1 indicate a specialized diet, while values > 1 indicate a feeding behaviour of a generalist predator.

Results

All specimens included in the stable isotope analysis (n=40; 13 males, 27 females) were sexually mature. In males, the mean TL was 39±3.80 cm (range: 34-47 cm) and the mean TW was 220.31±63.51 g (range: 136-317.5 g). In females, the mean TL was 40.46±3.79 cm (range: 33-51 cm) and the mean TW was 257.26±85.79 g (range: 135-453 g; Table 1).

Stable isotopes

The Shapiro-Wilk's test showed a non-normal distribution, except for the $\delta^{13}C$ values in the mature state that showed a symmetrical distribution. The variances were uniform by exceeding the significance level of 5%, which leads to accepting the null hypothesis in the Levene's test. Individuals showed significantly higher $\delta^{13}C$ mean values in their mature state in comparison with their immature state (mean -20.03‰ SD±1.34‰ and mean -22.56‰ SD±1.26‰, respectively; U=168, U'=1392, $P<0.0001$). Similarly, the $\delta^{15}N$ showed significantly higher mean values in mature than immature state of the individuals (mean 5.41‰ SD±1.29‰; and mean 2.78‰ SD±1.52‰ respectively; U=175, U'=1385, $P<0.0001$; Table 1). When combined, the results on sex and maturity for both isotopes evidenced the lack of significant differences according to the Mann-Whitney test (mature and sex: U=141, U'=197, $P=0.41$ for $\delta^{13}C$; immature and sex: U=122.5, U'=228.5, $P=0.12$ for $\delta^{13}C$; mature and sex: U=168, U'=170, $P=0.9$ for $\delta^{15}N$; immature and sex: U=154, U'=196.5, $P=0.5$ for $\delta^{15}N$). Finally, the variances of $\delta^{15}N$ for all samples showed values > 1, which indicates a generalist and opportunistic diet for this species (Table 2).

Discussion

The Tyrrhenian Sea is one of the regions in the Mediterranean where the lesser spotted dogfish is currently exploited by fisheries for human consumption (Serena *et al*., 2015; Ebert & Dando, 2021; Leonetti *et al*., 2020). In this regard, this sea can be considered as a relevant study area for the monitoring and further research of this taxon and its capture trends (Leonetti *et al*., 2020). The present study is a first approach to explore the trophic ecology of *S. canicula* in this fishery area through the analysis of stable isotopes of C and N in vertebrae. The isotopic

Table 1. Data regarding the specimens of *Scyliorhinus canicula* (13 males, 27 females) used for stable isotope analysis of N and C in the Tyrrhenian Sea. Values of total length (TL) and total weight (TW) for each sex are presented as mean, standard deviation (SD), and range (minimum-maximum).

Date	Males			Females		
	n	TL (mm)	TW (g)	n	TL (mm)	TW (g)
Feb 2017	5	37.7 ± 3.2, 34-42	230.3 ± 69.2, 136-317.5	12	38.8 ± 3.2, 33-44.5	296.21 ± 91.3, 154-453
Aug 2017	2	38.5 ± 3.5, 36-41	167.5 ± 10.6, 160-175	2	39.2 ± 3.1, 37-41.5	155.00 ± 28.2, 135-175
Jan 2018	1	41.5	170	5	42.6 ± 1.7, 40-44.5	242.00 ± 37.5, 135-175
May 2018	5	40.0 ± 4.9, 34-47	241.4 ± 67.5, 136-317.5	8	41.8 ± 4.8, 35-51.0	233.95 ± 83.1, 154-390

Table 2. Mean values and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ obtained in the two vertebra perforations of *Scyliorhinus canicula* specimens (n=40 specimens; 80 subsamples; 26 male subsamples; 54 female subsamples) caught in the Tyrrhenian Sea during 2017-2018.

Category	Maturity stage	$\delta^{13}C$	$\delta^{15}N$
Males	Immature	-22.91(±0,97)	2.53(±1,31)
Females	Immature	-22.21(±1,55)	3.03(±1,74)
Males	Mature	-20.24(±1,20)	5.41(±1,17)
Females	Mature	-19.82(±1,49)	5.41(±1,42)

signature in the vertebral subsamples confirmed a trophic shift between immature and mature state of the individuals analysed, which has also been observed in previous diet studies (Kousteni *et al*., 2018; Barría *et al*., 2018).

Results of stable isotopes can be explained within the isoscape values reported for the western Mediterranean (-24‰ to -14‰ for $\delta^{13}C$) and are comparable with those of the sharks analysed (-23‰ to -19‰ for $\delta^{13}C$). This was consistent with *S. canicula* trophic position with the $\delta^{15}N$ baseline values for the area reported in regional prey (e.g., *Cotylorhiza tuberculata*, *Engraulis encrasicolus*, *Lampanyctus crocodilus*) and predator taxa (*Auxis rochei*, *Balaenoptera physalus*, *Prionace glauca*; Cardona *et al*., 2012). The present study can be used as a complementary baseline for future interdisciplinary research. In the light of diverse anthropogenic and climate change scenarios (Serena *et al*., 2015; Consales & Marsili, 2021), vertebrae is a feasible biological matrix to study present and previous conditions registered during the lifetime of an organism (Bevacqua *et al.*, 2021).

Trophic ecology studies performed on *S. canicula* from the Mediterranean Sea and the north-eastern Atlantic Ocean have defined this species as a medium-sized opportunistic predator that occupies a medium trophic level and feeds on a wide range of benthic crustaceans and demersal fish, whose diet can undergo a sexual and maturity variation (Olaso *et al*., 1998; Valls *et al*., 2011; Mnasri *et al*., 2012; Kousteni *et al*., 2017; 2018; Barría *et al*., 2018). In the Mediterranean, Mnasri *et al*. (2012) observed a sexual and not a maturity-related variation in the diet of the lesser spotted dogfish from the northern coast of Tunisia. Demersal fishes were preferentially consumed by males, while a preference for crustaceans was observed in females. When analysing the diet according to size, crustaceans were identified as the most abundant preys in all sharks (28.2 cm ≤ TL ≤ 53.1 cm). This was evidenced in the trophic level, as values did not show significant differences between size classes (28.2 cm ≤ TL ≤ 40 cm, and 40.1 cm ≤ TL ≤ 53.1 cm). In previous studies, Kousteni *et al*. (2018) reported a higher prey diversity in males and immatures of *S. canicula* when compared to the diet of females and mature individuals. In the western Mediterranean, Barría *et al*. (2018) observed that mature males show a higher predation on fishes than females, while juveniles of both sexes feeding mostly on preys of a lower trophic level such as euphausiids. In our research, analyses of C and N isotopes showed significant differences in the trophic ecology of *S. canicula* in relation to maturity state, with no differences between the sexes. The lack of significant differences between $\delta^{13}C$ and $\delta^{15}N$ values according to sex, demonstrated that both mature and immature sharks prey on taxa with a similar isotopic signature. In this regard, the previously reported sexual segregation of *S. canicula* could be linked to other biological functions such as reproduction (Finotto *et al*., 2015; Kousteni & Megalofonou, 2019).

Segregation in single-sex schools has been reported for *S. canicula*, in which mature sharks are usually found in deeper environments than immature individuals (Olaso *et al*., 2005; Ebert & Dando, 2021). Our results evidenced higher values of $\delta^{13}C$ and $\delta^{15}N$ in mature specimens, but with a lack of statistical differences regarding sex. Based on the evidence of sexual segregation in deep waters and the results obtained, it could be suggested that mature individuals of both sexes feed on prey with similar isotopic signatures, despite sexual segregation (Olaso *et al*., 2005; Gori *et al*., 2012). In comparison with immature sharks in shallower environments, mature *S. canicula* individuals would consume prey with isotopic values more enriched in C^{13} and N^{15} than juvenile preys, highlighting the relevance of the aggregation sites where this species is currently caught by fisheries (Barría *et al*., 2018; Ebert & Dando, 2021).

Obtained ^{15}N values confirm that *S. canicula* is an opportunistic predator that preys on a wide variety of demersal species. Nevertheless, the complete understanding of its trophic role in this changing environment will depend in future detailed descriptions with a statistically robust sample size for each maturity state with the consideration of environmental factors such as depth, temperature, productivity, etc. (Karachle *et al.,* 2010; Kousteni *et al.,* 2010; Bevacqua *et al.,* 2021). This can be achieved by implementing a regular scientific-based monitoring with the fishers from the Tyrrhenian Sea, who can provide specimens in a regular basis for the improve of shark ecological knowledge and therefore fisheries regulations under a Blue Economy approach (Cohen *et al*., 2019).

In terms of bottom trawl fisheries and pollution, the impact on these environments and the prey populations should be considered (Serena *et al*., 2015; Barragán-Méndez *et al*., 2019). The populations of *S. canicula* have shown an increase in European seas, due in part to their high survival rates during bycatch and reproductive features (Barragán-Méndez *et al*., 2019). However, future issues could arise if predators such as *S. canicula* are returned after capture, but prey species populations continue to be caught and depleted (Lotze *et al*., 2011): if the link between predators and prey is affected by an increase

in the number of predators and the reduction of prey, this practice without further regulations and research would generate an unbalance in deep ecosystems, which would promote future ecological and human affectations (Serena *et al*., 2015; Lotze *et al*., 2011).

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