Trace elements and isotopes analyses on historical samples of white sharks from the Mediterranean Sea

L. BEVACQUA 1, F. R. REINERO 1,2, E. E. BECERRIL-GARCÍA 3, F. R. ELORRIAGA-VERPLANCKEN 2, D. JUARISTI-VIDEGARAY 2, P. MICARELLI 2, F. GALVÁN-MAGAÑA 3, P. CURIEL-GODOY 3, G. GIGLIO 1, S. TRIPEPI 1, D. BARCA 1, & E. SPERONE 1

1Università della Calabria, Rende (CS), Italy, 2Centro Studi Squali-Sharks Studies Center, Massa Marittima (GR), Italy, and 3Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, La Paz (BCS), Mexico

(Received 25 June 2020; accepted 12 November 2020)

Abstract
The white shark Carcharodon carcharias has been present in the Mediterranean Sea since 3.2 million years ago. Nevertheless, the current population shows a low genetic variability suggesting an endangered small population, on which there is scarce information regarding ecotoxocology or trophic ecology. Given that white shark’s sightings are rare in the Mediterranean and the possibility of obtaining samples is highly limited, the aim of this research was to provide general information regarding the concentration of trace elements and stable isotopes (δ¹⁵N and δ¹³C). Laboratory analyses were performed on 18 and 12 subsamples from two different white sharks’ vertebrae obtained from two adult specimens caught in 1987, in Favignana Island, Italy. Perforations were made along the vertebrae to describe both trace elements and stable isotopes at different life stages. A total of 38 trace elements were analysed, in which the highest concentrations were found in Fe, Sr, U, Pb, and Zn. The fluctuations of these elements during the ontogeny of both individuals could have been related to changes in diet and environment, although the specific origin remains unknown. Regarding stable isotopes, the vertebrae from the male showed an isotopic range from 9.6%o to 10.8%o (δ¹⁵N) and from −16.5‰ to −13.0‰ (δ¹³C) with a mean ± SD value of 10.3 ± 0.4% for δ¹⁵N and −14.6 ± 1.3% for δ¹³C; whereas the female vertebrae had an isotopic range from 9.8% to 11.1% (δ¹⁵N) and from −16.9% to −15.0% (δ¹³C), with a mean ± SD value of 10.8 ± 0.6% for δ¹⁵N and −15.8 ± 0.8% for δ¹³C. There were no significant δ¹⁵N differences (U = 6, p = 0.07346) between the two individuals. However, there were just significant differences in δ¹³C (t = −1.8, p = 0.049256), which could suggest sexual segregation in terms of habitat use and feeding habits.

Keywords: Carcharodon carcharias, ecotoxicology, stable isotopes, trace elements, threatened species

Introduction
The white shark Carcharodon carcharias (Linnaeus, 1758), is a lamniform species that has an essential role in the maintenance and function of coastal and pelagic ecosystems (Huveneers et al. 2018). As with other top predators, a reduction in their numbers could result in relevant affectations to the health of marine ecosystems by causing ecological cascade effects with rather marked environmental consequences (Ferretti et al. 2010).

The white shark is a protected species in countries such as South Africa, Australia, New Zealand, Mexico, and the United States and it is globally listed as Vulnerable since 1996 (Fergusson et al. 2009). In the Mediterranean Sea, the white shark has been present since the Pliocene (3.2 million years ago), with a population that originated from the dispersion of the Pacific-Australian paleo-populations (Leone et al. 2020). However, the current white shark population of the Mediterranean shows a significantly low rate of genetic variability that could suggest a small number of individuals in danger of extinction. Accordingly, the white shark population from the Mediterranean is listed as critically endangered by demonstrating a decline of 52–96% of its population (Moró et al. 2019).
The causes of a drastic decrease in the white shark population of the Mediterranean are related to the increasingly invasive anthropogenic activities, over-exploitation, marine pollution, climate change, and habitat destruction, along with the intrinsic characteristics of the species such as a slow growth rate, low fecundity and late sexual maturity (Huveneers et al. 2018; Moro et al. 2019). In addition, marine top predators like the white sharks are strongly affected by changes in the quality of the environment in which they inhabit and, given that elasmobranchs integrate the toxic effects of trace elements by means of a complex trophic network, they can be considered as bioindicators of environmental pollution (Pethybridge et al. 2010; Mathews & Fisher 2009; Barrera-Garcia et al. 2012).

Given that white shark’s sightings are rare in the Mediterranean and the possibility of finding samples is scarce, the present study provides novel data regarding trace elements and stable isotopes of two white shark specimens accidentally caught in 1987 in the Sicily Channel, Italy. In this regard, our aim is to contribute to the understanding of the bioaccumulation of trace elements in vertebrae of white sharks and describe the spatial and trophic niche of these specimens through the study of stable isotopes of δ¹⁵N and δ¹³C.

Materials and methods

Collection of samples

Analyses of trace elements were performed on 18 vertebrae subsamples while stable isotopes (δ¹⁵N and δ¹³C) were analyzed in 12 vertebrae subsamples belonging to a pair of white shark specimens accidentally caught in a tuna trap off Favignana Island, Italy, in early September 1987. Vertebrae were obtained from the caudal area and preserved for museum and scientific purposes. The female shark (sample V1) measured 5.40 m total length (TL) and a weight of 2.35 tons (Figure 1(a,b)), while the male (sample V2) measured 4.50 m TL and weighed 1.80 tons. In regard to stomach contents, a total of 20 Atlantic bluefin tuna (Thunnus thynnus) and an entire 200 kg dolphin (probably bottlenose dolphin, Tursiops truncatus) were found inside the female white shark, while stomach of the male specimen was empty.

Trace elements analyses

Trace elements in ppm (parts per million or mg/kg) were analyzed in the vertebrae of the two specimens through the Laser Ablation associated with Inductively Coupled Plasma Mass Spectrometry.
(LA-ICP-MS; De Donato et al. 2017; Barca et al. 2019). In addition to the LA-ICP-MS, the electron probe micro-analysyser with an energy-dispersive X-ray spectrometry (EPMA-EDS) were also used for the analysis of the major elements in order to detect the presence of elements with concentrations higher than 0.2 wt % (Shoval 2018). The instrument used for this study was the result of the combination of a New Wave Research UP213 Nd-YAG Laser (213 nm) and an Elan DRCe Inductively Coupled Plasma Mass Spectrometer (Perkin Elmer/SCIEX). The ICP-MS was controlled through the ELAN management software provided by Perkin Elmer, while data were filtered through the GLITTER software of Gemoc Macquarie University.

The NIST SRM 612–50 ppm (nominal concentration) glass, produced by the National Institute of Standards and Technologies and the reference glass BCR-2 G (Basalt Columbia River) supplied by the USGS (United States Geological Survey), both used as not standard samples (Pearce et al. 1997), were implemented as external calibration standards. Calcium ($^{43}\text{Ca}$), determined through EMP (Electron Microprobe) analysis, was used as internal standard with a concentration of 50% (Fryer et al. 1995). Once the instrument was calibrated, the vertebrae fixed on a slide with their respective codes were inserted inside the ablation chamber to carry out, under vacuum, the nebulisation of samples with the laser through the perforations. Ten perforations and eight perforations were made, respectively, on the vertebrae of the female (V1) and male (V2); in both cases, the perforations were carried out following a transect that extended from the central part to the periphery, in order to pick up vertebral material that had gradually accumulated during the ontogeny of each shark (Figure 1(c,d)).

Further analyses were performed with the use of the EPMA-EDS to detect major elements such as Na$_2$O, MgO, SO$_3$, CaO, P$_2$O$_5$, and Cl. The EPMA-EDS was a JEOL-JXA 8230 equipped with an EDS–JEOL EX-94310FaL1Q spectrometer. The EPMA-EDS conditions were those used in the previous studies (Barca et al. 2019). For this type of analysis, samples were partially metallised in order to make the surface conductive and to reduce the effects related to electrostatic charges. To metallise the sample, the conductive carbon sputter coating was deposited on the sample for a thickness of 200 angstroms (Å) by means of vacuum thermal vapourisation (Sweatman & Long 1969). In this case, three analyses were carried out on each sample, always starting from the centre of the vertebrae towards the periphery. Production of the tables on the results of trace elements and the graphs relating to the concentrations of iron (Fe), strontium (Sr), uranium (U), and lead (Pb) were carried out using the Excel 16.0.12 program.

**Stable isotopes analysis**

Subsamples were obtained at each millimetre from the centre to the periphery of each vertebrae (Figure 1(c,d)) using a Proxxon Micromet 50/E micro drill with a 0.8 mm diameter drill tip and the sample stored in Eppendorf tubes. From each subsample, a total of 1 mg was placed in a silver capsule (3.2 x 4 mm) for the removal of inorganic carbon through an acidic steam bath. In this process, the subsamples were exposed to HCl Ultrex steam 37% in a sealed container for 24 h (Hedges & Stern 1984). Stable isotope analyses were carried out in the Laboratory of Environmental Biochemistry of the Interdisciplinary Center of Marine Sciences (CICIMAR-IPN) using a Delta V isotope ratio mass spectrometer. Isotopic ratio for δ$^{18}$O and δ$^{13}$C were estimated using the next equation:

$$
\delta X = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000
$$

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 standard for δ$^{13}$C was Pee Dee Belemnite (PDB), while the standard for δ$^{15}$N was atmospheric nitrogen. The obtained values of δ$^{13}$C and δ$^{15}$N were compared using a Student’s t-test for data with a normal distribution and a Mann-Whitney U test for data with a non-normal distribution.

**Results**

**Trace elements**

The concentration of elements in the ten selected points along the female vertebral surface, starting from the central part of the vertebra (V1-A) to the outermost one (V1-L) is shown in Table I. Regarding the male V2 vertebra sample, the concentration of elements in the eight selected points along the vertebral surface (from V2-A to V2-H) is shown in Table II. From these results and due to their importance, the concentrations of nickel (Ni), barium (Ba), and cadmium (Cd) were analysed in detail, since these elements have been related to
Table I. Concentration of elements in 10 selected points along the vertebral surface, starting from the central part of the vertebra (V1-A) to the outermost one (V1-L).

| Element | V1-A | V1-B | V1-C | V1-D | V1-E | V1-F | V1-G | V1-H | V1-I | V1-L |
|---------|------|------|------|------|------|------|------|------|------|------|
| S       | 0.31 |      | 0.4  |      | 0.2  | 0.84 | 0.34 |      |      |      |
| Ti      | 55.45| 132.33| 91.16| 102.17| 45.12| 44.84| 29.51| 43.57| 15.4| 12.13|
| V       | 2.01 | 4.51 | 2.57 | 5.09 | 1.34 | 1.35 | 2.47 | 1.87 | 1.4 | 0.48 |
| Cr      | 2.61 | 4.63 | 5.68 | 3.32 | 2.64 |      |      |      | 3.84| 1.95 |
| Mn      | 20.56| 39.63| 28.74| 28.68| 21.54| 20.52| 36.49| 26.86| 16.3| 13.05|
| Fe      | 223  | 1477 | 689  | 1045 | 498  | 239  | 472  | 711  | 624 | 290 |
| Co      | 0.58 | 0.97 | 0.87 | 1.37 | 0.35 | 0.31 | 1.28 | 0.72 | 0.42| 0.35 |
| Cu      | 5.26 | 5.51 | 5.58 | 6.61 | 2.54 | 3.4  | 4.36 | 5.72 | 3.49| 2.95 |
| Ni      | 2.07 | 2.88 | 2.09 | 3.33 | 1.91 | 2.38 | 2.13 | 1.66 | 1.29| 0.62 |
| Zn      | 194  | 210  | 251  | 262  | 139  | 140  | 239  | 181  | 79  | 87  |
| As      | 1.46 | 1.94 | 0.36 | 0.66 | 0.89 | 0.69 | 1.1  | 1.8  | 0.86| 0.77|
| Rb      | 1.14 | 3.55 | 1.83 | 3.65 | 1.35 | 0.94 | 2.27 | 3.18 | 0.86| 0.77|
| Sr      | 1257 | 1268 | 1312 | 1363 | 1346 | 1246 | 1344 | 1257 | 1262| 1418|
| Zr      | 0.45 | 0.74 | 0.43 | 0.86 | 0.13 | 0.24 | 0.56 | 0.4  | 0.18| 0.05|
| Nb      | 0.1  | 0.31 | 0.22 | 0.11 | 0.1  | 0.29 | 0.16 | 0.27 | 0.08| 0.03|
| Mo      | 0.11 | 0.12 | 0.22 | 0.15 | 0.1  | 0.29 | 0.16 | 0.24 | 0.35| 0.13|
| Cd      | 0.27 | 0.13 | 0.2  | 0.54 | 0.19 | 0.21 | 0.56 | 0.17 | 0.23| |
| Sn      | 0.44 | 0.89 | 0.94 | 1.01 | 0.59 | 0.44 | 1.28 | 0.84 | 0.27| 0.31|
| Sb      | 5.16 | 0.76 | 0.36 | 0.96 | 0.56 | 0.66 | 51.01| 0.66 | 0.23| 0.25|
| Cs      | 0.19 | 0.39 | 0.18 | 0.26 | 0.19 | 0.09 | 0.22 | 0.15 | 0.12| 0.02|
| Ba      | 24.79| 26.97| 63.52| 67.01| 21.15| 18.03| 24.54| 206  | 14.33| 14.61|
| La      | 0.19 | 0.42 | 0.28 | 0.45 | 0.13 | 0.32 | 0.22 | 0.24 | 0.07| 0.09|
| Ce      | 0.67 | 1.64 | 1.29 | 1.55 | 1.23 | 0.72 | 0.52 | 1.5  | 0.37| 0.31|
| Pr      | 0.04 | 0.23 | 0.1  | 0.7  | 0.07 | 0.06 | 0.07 | 0.13 | 0.03| 0.03|
| Nd      | 0.49 | 0.42 | 0.24 | 0.37 | 0.13 | 0.19 | 0.08 | 0.24 | 0.03| 0.07|
| Sm      | 0.08 | 0.11 | 0.06 |      | 0.08 | 0.14 |      | 0.02 | 0.26| |
| Eu      | 0.03 | 0.04 |      |      | 0.05 | 0.01 |      |      |      |      |
| Gd      | 0.49 | 0.47 | 0.1  | 0.07 | 0.05 | 0.07 |      |      |      |      |
| Tb      | 0.01 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.03 | 0.01 |      |      |
| Dy      | 0.03 |      |      | 0.05 | 0.04 | 0.05 |      |      |      |      |
| Ho      | 0.01 | 0.01 | 0.01 |      | 0.01 | 0.03 |      |      |      |      |
| Er      | 0.08 |      |      | 0.07 |      |      |      |      |      |      |
| Tm      | 0.02 |      |      |      | 0.01 |      |      |      |      |      |
| Yb      | 0.04 |      |      |      |      | 0.08 | 0.03 | 0.06 |      |      |
| Lu      | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 |      |      |      |
| Pb      | 194  | 190  | 213  | 229  | 57.8 | 66.74| 98   | 93   | 60.77| 27.61|
| Th      | 0.04 | 0.37 | 0.01 | 0.07 | 0.01 | 0.03 | 0.05 | 0.03 | 0.04| 0.04|
| U       | 0.18 | 0.42 | 0.37 | 0.44 | 0.23 | 0.41 | 0.29 | 0.41 | 0.28| 0.17|

The different physicochemical characteristics of the surrounding environment and the diet of white sharks in terms of bioaccumulation and biomagnification. Analyses of major elements such as sodium (Na), calcium (Ca), manganese (Mg), sulphur (S), chlorine (Cl), and phosphorus (P) indicate the presence of a correlation with the growth of each animal. Ca, the most concentrated element (V1 = 26.15 ppm and V2 = 34.14 ppm on average from the three analysed subsamples) increased in the V1 female sample from the centre of the vertebra (V1_03) to the outermost part (V1_01). However, in the V2 male sample there was a fluctuation of elements, mainly for Cl, Ca, and Na.

The second element regarding its concentration was P (V1 = 17.98 ppm and V2 = 22.01 ppm on average from the three subsamples), where an increase from the juvenile (V1_03) to the adult phase (V1_01) was evident in the female V1 sample, with an oscillation in the male V2 sample. Data relating to the percentages of major elements are reported in Tables III and Tables IV. The concentration (ppm) of some of the trace elements present during different stages of the sharks' biological cycle are reported according to Fe (Figure 2(a)), Sr (Figure 2(b)), U (Figure 2(c)), and Pb (Figure 2(d)).

In order to validate the use of element profiles within shark vertebrae, we established whether the vertebral centre matrix and its associated elements were stable. The null hypothesis is that has been no exchange of Ca and other elements through ontogenesis (Doyle 1968). Results of Tables I, Tables II,
and Tables III provide evidence that the reworking of elements within vertebrae was minimal. In fact, the two sharks’ element profiles were different and did not follow any trend during growth, which could evidence the effect of environmental conditions.

Stable isotopes

The two white shark vertebrae showed different isotopic values ($\delta^{15}N$ and $\delta^{13}C$). The male individual (seven growth layers) presented an isotopic range from 9.6‰ to 10.8‰ ($\delta^{15}N$) and from −16.5‰ to −13.0‰ ($\delta^{13}C$); these showed a mean ± SD value of 10.3 ± 0.4‰ for $\delta^{15}N$ and −14.6 ± 1.3‰ for $\delta^{13}C$. The female individual (five growth layers) presented an isotopic range from 9.8‰ to 11.1‰ ($\delta^{15}N$) and from −16.9‰ to −15.0‰ ($\delta^{13}C$), displaying a mean ± SD value of 10.8 ± 0.6‰ for $\delta^{15}N$ and −15.8 ± 0.8‰ for $\delta^{13}C$ (Table V). Overall there were no significant differences ($U = 6$, $p = 0.07346$) between the two individuals in terms of $\delta^{15}N$ mean values; however, there were significant differences ($t = −1.8$, $p = 0.049256$) in the case of $\delta^{13}C$; both at a significance level of 0.05. Nevertheless, a higher $\delta^{15}N$ value was observed in the female vertebra when compared to the male values, especially, near the centre of the vertebrae. In contrast, values of $\delta^{13}C$ were higher in all vertebrae samples of the male (Figure 3).

Discussion

The present study constitutes the first stable isotope and trace element observations made in the critically
Table III. Concentration of major elements in the three selected points along the white shark vertebrae V1.

| Sample  | NA2O | MGO | SO3 | CAO | P2O5 | CL | Total  |
|---------|------|-----|-----|-----|------|----|--------|
| V1_01   | 1.12 | 0.75| 1.12| 29.23| 20.65| 0.08| 52.93  |
| V1_02   | 1.44 | 0.67| 1.63| 27.35| 20.58| 0.09| 51.74  |
| V1_03   | 1.76 | 0.89| 1.53| 21.88| 12.69| 0.17| 38.88  |
| Minimum | 1.12 | 0.67| 1.12| 21.88| 12.69| 0.08| 38.88  |
| Maximum | 1.76 | 0.89| 1.63| 29.23| 20.65| 0.17| 52.93  |
| Mean    | 1.44 | 0.77| 1.43| 26.15| 17.98| 0.11| 47.85  |
| Dev. Std| 0.32 | 0.11| 0.27| 3.82 | 4.58 | 0.05| 7.79   |

Table IV. Concentration of major elements in the three selected points along the white shark vertebrae V2.

| Sample  | NA2O | MGO | SO3 | CAO | P2O5 | CL | Total  |
|---------|------|-----|-----|-----|------|----|--------|
| V2_01   | 1.70 | 0.78| 0.71| 34.54| 14.42| 0.06| 52.19  |
| V2_02   | 1.71 | 0.63| 0.85| 36.28| 26.78| 0.16| 66.37  |
| V2_03   | 0.87 | 0.37| 0.30| 31.61| 24.82| 0.06| 58.02  |
| Minimum | 0.87 | 0.37| 0.30| 31.61| 14.42| 0.06| 52.19  |
| Maximum | 1.71 | 0.78| 0.85| 36.28| 26.78| 0.16| 66.37  |
| Mean    | 1.42 | 0.59| 0.62| 34.14| 22.01| 0.09| 58.86  |
| Dev. Std| 0.48 | 0.21| 0.28| 2.36 | 6.64 | 0.06| 7.13   |

Endangered population of white sharks in the Mediterranean. Although these observations come from only two specimens, the use of both historical samples provides a faithful perspective of the accumulation of potentially toxic elements and the trophic ecology of the white shark in the 1980s. In this regard, this research can be considered as a baseline for future studies in this population, which will allow an appropriate evaluation in further studies regarding the ecotoxicology and ecology of the white shark in this highly exploited sea.

Trace elements

Analyses with LA-ICP-MS indicate a high accumulation of specific elements in both sharks. Different concentrations of metals and non-metals in the specimens seem to have been influenced by various factors, such as belonging to a particular trophic level, diet, and ecology (Turoczy et al. 2001; Pethybridge et al. 2010). Lamnid sharks, that occupy high trophic levels (Cortes 1999), usually evidenced high concentrations of metals and non-metals which in turn are associated with a diet consisting of large teleost fish, other elasmobranchs, and marine mammals (Capelli et al. 2008; Barrera-Garcia et al. 2012).

White sharks from the Mediterranean showed significant high concentrations of Fe and Zn. In particular, Fe concentration can vary seasonally, being lower in winter and spring and higher in summer and autumn because its iron could increase along with salinity values (Mance & Campbell 1988). In this regard, both vertebrae showed the increase and decrease of Fe concentration which followed a trend, probably related to the seasonal changes in the environment (Mance & Campbell 1988). In addition, in the sample V1 it was possible to notice a peak in the increase of Fe; while the sample V2 evidenced levels of Fe lower than V1 which could be related to a different diet between both sharks.

Figure 2. Fe (a), Sr (b), U (c), Pb (d) concentrations according to the ten (from A to L) and eight (from A to H) perforations selected along the vertebral surface of the female V1 (red triangle) and male V2 (blue circle) samples.
Zn is an essential element that is easily bioaccumulated in marine species, although its accumulation process is interrupted when concentrations highly increase. For this reason, even in the presence of high concentrations of Zn, it is difficult to explain its toxicological significance (Mathews & Fisher 2009). Individuals investigated in this study were mature and included large bony fishes and marine mammals in their diet that have a greater number of proteins capable of binding to Zn, which could be a partial explanation of the observed concentrations (Wang & Zhang 2006).

Previous studies in vertebrae from South African white sharks have evidenced the presence of trace elements in this population (Christiansen 2011). In the case of Sr, the average calculated by Christiansen (2011) was 1100 mg/kg, similar to the 1,307.3 mg/kg found in the present study. However, it is necessary to consider the relationship between temperature and accumulation rates of Sr in biomineralized structures (Has-Schon et al. 2006), along with the fact that white sharks can spend more time in oceanic areas after subadult stages (Bonfil et al. 2005; Weng et al. 2007) and are able to dive in deep waters according to an increase in body size (Bonfil et al. 2005; Weng et al. 2007). Although individuals caught in this study were adults, a decrease in Sr concentrations was not observed according to age, suggesting that both sharks likely occupied areas with homogeneous temperatures.

Regarding the trend of uranium (U), high quantities were found in the Mediterranean white shark samples, with an average of 0.32 mg/kg between the two individuals, while in sharks considered by Christiansen (2011) the average was 0.066 mg/kg. A consistent pattern of increase in the amplitude of the oscillations of U along the two analyzed vertebrae was observed. High concentrations of U were already present in the first years of life of the white sharks and remained preserved during their growth. This increase in the amplitude of oscillations with the shark growth could indicate that individuals changed habitats once they reached a certain size (>200 cm), using a specific habitat on a seasonal basis where U was present in higher concentrations. In addition, it has been suggested that white sharks can begin to feed on marine mammals when they reach 200 cm TL (Hussey et al. 2012) and the increase in U may indicate that the change in habitat was related to a change in feeding habits.

As for lead (Pb) concentrations, an average of 0.40 mg/kg was calculated for South African white sharks related to a variable intake of Pb during growth (Christiansen 2011). Within Mediterranean samples, a different pattern was observed, in which the female specimen (V1) showed an average intake of Pb of 123 mg/kg with a minimum of 27.61 mg/kg and a maximum of 229 mg/kg. The greatest quantities of Pb were found all within the early stages of life, reaching the maximum peaks and then reducing in the intermediate stages, and then getting the minimum values before death. A different trend was found for the male, with an average of 19 mg/kg, maintaining these values throughout its life. The difference would indicate that the first specimen, the female, underwent a not-constant intake of Pb.

Table V. $\delta^{15}$N and $\delta^{13}$C values in the samples of the male and female C. carcharias samples of vertebrae from the Mediterranean. Location in vertebrae from the centre to the periphery.

| Sample | Vertebral location | $\delta^{15}$N | $\delta^{13}$C |
|--------|-------------------|----------------|----------------|
| Male1  | 0                 | 10.14          | −15.29         |
| Male1  | 1                 | 9.63           | −16.52         |
| Male1  | 2                 | 9.83           | −15.95         |
| Male1  | 3                 | 10.20          | −14.12         |
| Male1  | 4                 | 10.75          | −13.92         |
| Male1  | 5                 | 10.64          | −13.03         |
| Male1  | 6                 | 10.68          | −13.52         |
| Female1| 1                 | 9.78           | −15.73         |
| Female1| 2                 | 10.86          | −16.91         |
| Female1| 3                 | 11.11          | −16.38         |
| Female1| 4                 | 10.99          | −15.12         |
| Female1| 5                 | 11.07          | −15.03         |

Figure 3. Values of $\delta^{15}$N (a) and $\delta^{13}$C (b) from the male (blue circle) and female (red triangle) white shark vertebrae from the Mediterranean Sea ($n = 12$ samples).
probably related to its diet, in which it could have fed on prey with higher concentrations of lead such as cephalopods or small fishes, and then changed its diet by feeding on prey with lower concentrations of lead, such as large fishes or marine mammals (Barrera-García et al. 2012). In this regard, the male evidenced a constant trend, explained by the fact that this specimen could have fed on the same prey or on prey with a constant level of lead.

**Stable isotopes**

Differences in $\delta^{15}$N values could be related to potential sexual segregation of these white sharks during immature stages (Carlisle et al. 2012). In terms of foraging habits, it is possible that the female white shark fed on different prey at high trophic levels during sexual maturation, since $\delta^{15}$N values from both male and female were similar at the end of their lives. This sexual segregation could be evidenced by the significant differences observed in $\delta^{13}$C, where the male white shark showed values related to more coastal areas in comparison to the pelagic areas where female could have fed, as it has been suggested for other populations of this same species in the Eastern and South Pacific Ocean (Strong et al. 1996; Carlisle et al. 2012; Kim et al. 2012).

The trophic ecology of white sharks in the Mediterranean Sea could be similar to other top predators that occur in the Mediterranean Sea. Other marine top consumers, such as Risso’s dolphins *Grampus griseus* and Cuvier’s beaked whales *Ziphius cavirostris*, have shown similar $\delta^{15}$N values compared to the white sharks of the present study (Capelli et al. 2008). This suggests that the white sharks in the Mediterranean Sea feed on prey with similar $\delta^{15}$N (trophic position) that those observed in those cetaceans, such as the European hake *Merluccius merluccius* (Sinopoli et al. 2012), the Mediterranean scadfish *Amoglossus laterna* (Fanelli et al. 2009), and blue fin tunas *Thunnus thynnus*, or similar prey that could be considered ecological equivalents in terms of their position in the trophic web (Sarà & Sarà 2007). This could evidence that the white sharks in the Mediterranean Sea could feed on mostly fishes and cephalopods rather than marine mammals.

We acknowledge that our results are based on multiple samples from only two individuals; future studies should assess larger sample sizes, consider trophic variations between individuals, as well as assess stomach contents (Carlisle et al. 2012; Kim et al. 2012). This study provides important knowledge into the ecology of the white shark from the Mediterranean Sea, regarding intrinsic (e.g. foraging) and extrinsic (e.g. pollutants) aspects that should be taken into consideration, when the overall conservation status and vulnerability of this population are assessed.

**Acknowledgements**

Authors are very grateful to Marco Minervino, Cristian Marchio and Francesco Luigi Leonetti for the assistance during the laboratory activities. Special thanks go to Clemente Ventrone and Gerlando Spagnolo given that they contributed to the recovery of the white shark samples from Favignana.

**Funding**

This work was supported by the Associazione Isole, under Grant 49/2019. FREV and FGM thank Instituto Politécnico Nacional received support through the Contracting Excellence Program and Fellowship by EDI and COFAA from IPN; EEBG, and DJV received a scholarship provided by CONACyT.

**Disclosure statement**

The authors report that there is no conflict of interests.

**ORCID**

L. Bevacqua [http://orcid.org/0000-0001-5226-2684]
F. R. Reiner [http://orcid.org/0000-0001-8400-1227]
E. E. Becerril-García [http://orcid.org/0000-0002-1173-2309]
F. R. Elorriaga-Verplancken [http://orcid.org/0000-0002-7360-8039]
D. Juaristí-Videgaray [http://orcid.org/0000-0002-1277-9926]
P. Micareli [http://orcid.org/0000-0003-0582-3770]
F. Galván-Magaña [http://orcid.org/0000-0002-7613-4617]
P. Curiel-Godoy [http://orcid.org/0000-0003-0909-4210]
G. Giglio [http://orcid.org/0000-0002-9603-6678]
S. Tripepi [http://orcid.org/0000-0002-4158-911X]
D. Barca [http://orcid.org/0000-0002-3087-8418]
E. Sperone [http://orcid.org/0000-0002-3180-7611]

**References**

Barca D, Fiorenza E, D’Andrea M, Le Pera E, Musella M, Sudano F, Taliano Grasso A. 2019. Chemical and petrographic characterization of stone and glass tesserae in the Nereid and Geometric Mosaics from the S. Aloe Quarter in
Vibo Valentia–Calabria, Italy. Minerals 9(12):729. DOI: 10.3390/min9120729.

Barrera-Garcia A, O’Hara T, Galván-Magaña F, Méndez-Rodriguez LC, castellini JM, Zenteno-Savin T. 2012. Oxidative stress indicators and trace elements in the blue shark (Prionace glauca) off the east coast of the Mexican Pacific Ocean. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 156(2):59–66.

Bonfil R, Meyer M, Scholl MC, Johnson R, O’Brien S, Oosthuizen H, Swanson S, Kotze D, Paterson M. 2005. Transoceanic migration, spatial dynamics, and population linkages of white sharks. Science 310:100–103. DOI: 10.1126/science.114898.

Capelli R, Das K, De Pellegrini R, Drava G, Lepoint G, Miglio C, Poggi R. 2008. Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios. Science of the Total Environment 390 (2–3):569–578. DOI: 10.1016/j.scitotenv.2007.10.036.

Carlisle AB, Kim SL, Semmens BX, Madigan DJ, Jorgensen SJ, Perle CR, Block BA. 2012. Using stable isotope analysis to understand the migration and trophic ecology of northeastern Pacific white sharks (Carcharodon carcharias). PLoS One 7(2): e30492. DOI: 10.1371/journal.pone.0030492.

Christiansen H. 2011. Developing and applying elemental composition of shark vertebrae as a tool for quantifying life history characteristics over ontogeny. Windsor, Canada: University of Windsor.

Cortes E. 1999. Standardized diet compositions and trophic levels of sharks. ICES Journal of Marine Science 56 (5):707–717. DOI: 10.1006/jmsc.1999.0489.

De Donato C, Barca D, milazzo C, Santoro R, Giglio G, Tripepi S, Sperone E. 2017. Is trace element concentration correlated to parasite abundance? A case study in a population of the green frog Pelophylax sylvil. hicianus from the Neto River (Calabria, southern Italy). Parasitology Research 116 (6):1745–1753. DOI: 10.1007/s00436-017-5453-7.

Doyle J. 1968. The Lorenzen sulphates: A comparative study. Comparative Biochemistry and Physiology 24:479–485. DOI: 10.1016/0010-406X(68)90099-7.

Fanelli E, Badalamenti F, D’Anna G, Pipitone C. 2009. Diet and trophic level of sc镔lfish Anguilla anguilla in the southern Tyrrhenian Sea (western Mediterranean): Contrasting trolled versus Untrawled areas. Journal of the Marine Biological Association of the United Kingdom 89 (4):817–828. DOI: 10.1017/S0025315409000137.

Ferguson I, Compagno LJV, Marks M. 2009. Carcharodon carcharias. The IUCN Red List of Threatened Species 2009.e.T3855A10138372.

Ferretti F, Worm B, Britten GL, Heithaus MR, Lotze HK. 2010. Patterns and ecosystem consequences of shark declines in the ocean. Ecological Letters 13(8):1055–1071.

Fryer BJ, Jackson SE, Longrich HP. 1995. The design, operation and role of the laser ablation microprobe coupled with an inductively coupled plasma mass spectrometer (LAM-ICP-MS) in the earth sciences. Canadian Mineralogist 33:302–312.

Hos–Schon E, Bogut I, Strelec I. 2006. Heavy metal profile in five fish species included in human diet, domiciled in the end fl ow of river Neretva (Croatia). Archives of Environmental Contamination and Toxicology 50:545–551. DOI: 10.1007/s00244-005-0047-2.

Hedges JJ, Stern JH. 1984. Carbon and nitrogen determinations of carbonate-containing solids. Limnology and Oceanography 29(3):657–663. DOI: 10.4319/lo.1984.29.3.0657.

Hussey NE, Mccan HM, Cliff G, Dudley SFJ, Wintner SP, Fisk AT. 2012. Size-based analysis of diet and trophic position of the white shark (Carcharodon carcharias) in South African waters. Page (in press). In: Domeier M, editor. Global perspectives on the biology and life history of white shark. Boca Raton, FL: CRC Press. pp. 27–49.

Huveneers C, Apps K, Beceril-Garcia EE, Bruce B, Werry JM. 2018. Future research directions on the ‘elusive’ white shark. Frontiers in Marine Science 5:455. DOI: 10.3389/fmars.2018.00455.

Kim SL, Tinker MT, Estes JA, Koch PL. 2012. Ontogenetic and among-individual variation in foraging strategies of northeast Pacific white sharks based on stable isotope analysis. PLoS One 7(9). DOI: 10.1371/journal.pone.0045068.

Leone A, Puncher GN, Ferretti F, Sperone E, Tripepi S, Micarelli P, Gambarelli A, Sarà M, Arculeo M, Doria G, Garibaldi F, Bressi N, Dall’Asta A, Minelli D, Cilli E, Vanni S, Serena F, Diaz-Aimes P, Baele G, Cariani A, Tinti F. 2020. Pliocene colonization of the Mediterranean by Great White Shark inferred from fossil records, historical jaws, phyleogeographic and divergence time analyses. Journal of Biogeography 47:1119–1129. DOI: 10.1111/jbi.13794.

Mance G, Campbell JA. 1988. Proposed environmental quality standards for list II substances in water. Iron. Medenham, UK: Water Research Centre.

Mathews T, Fisher NS. 2009. Dominance of dietary intake of metals in marine elasmobranch and teleost fish. Science of the Total Environment 407:5156–5161. DOI: 10.1016/j.scitotenv.2009.06.003.

Moro S, Jona-Lasinio G, Block B, Micheli F, De Leo G, Serena F, Bottaro M, Scacco U, Ferretti F. 2019. Abundance and distribution of the white shark in the Mediterranean Sea. Fish and Fisheries 21(2):338–349. DOI: 10.1111/faf.12432.

Pearce NJC, Perkins WT, Westgate JA, Gorton MP, Jackson SE. 1997. A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass reference materials. Geostandards Newsletter 21:115–144. DOI: 10.1111/j.1751-908X.1997.tb00538.x.

Pethybridge H, Cossa D, Butler ECV. 2010. Mercury in 16 demersal sharks from southeast Australia: Biotic and abiotic sources of variation and consumer health implications. Marine Environmental Research 69(1):18–26. DOI: 10.1016/j.marenvres.2009.07.006.

Sara G, Sara R. 2007. Feeding habits and trophic levels of bluefin tuna Thunnus thynnus of different size classes in the Mediterranean Sea. Journal of Applied Ichthyology 23 (2):122–127. DOI: 10.1111/j.1439-0426.2006.00829.x.

Shoval S. 2018. The application of LA-ICP-MS, EPMA and Raman micro-spectroscopy methods in the study of Iron Age Phoenician Bichrome pottery at Tel Dor. Journal of Archeological Science. Reports 21:938–951. DOI: 10.1016/j.jasrep.2017.03.040.

Sinopoli M, Fanelli E, D’Anna G, Badalamenti F, Pipitone C. 2012. Assessing the effects of a trawling ban on diet and trophic level of hake, Merluccius merluccius, in the southern Tyrrhenian Sea. Scientia Marina 76 (4):677–690.

Strong WR, Bruce BD, Nelson DR, Murphy RD. 1996. Population dynamics of white sharks in Spencer Gulf, South Australia. In: Klimley AP, Ainley DG, editors. Great white sharks: The biology of Carcharodon carcharias. San Diego: Academic. pp. 401–414.
Sweatman TR, Long JVP. 1969. Quantitative electron-probe microanalysis of rock-forming minerals. Journal of Petrology 10(2):332–379. DOI: 10.1093/petrology/10.2.332.

Turoczy NJ, Mitchell BD, Levings H, Rajendram VS. 2001. Cadmium, copper, mercury, and zinc concentrations in tissues of the king crab (Pseudocarcinus gigas) from southeast Australian waters. Environmental International 27(4):327–334. DOI: 10.1016/S0160-4120(01)00064-2.

Wang G, Zhang Q. 2006. Adsorption behavior of the bamboo-charcoal for Zn (2+). Biomass Chemical Engineering 3: 17–20.

Weng KC, O’Sullivan JB, Lowe CG, Winkler CE, Dewar H, Block BA. 2007. Movements, behavior and habitat preferences of juvenile white sharks Carcharodon carcharias in the eastern Pacific. Marine Ecology Progress Series 338:211–224. DOI: 10.3354/meps338211.