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Exploring diet shifts and ecology in modern sharks using calcium isotopes and trace metal records of their teeth

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Abstract

Sharks occupy all living environments of the marine realm as well as some freshwater systems. They display varied and flexible feeding behaviours, but understanding their diet remains challenging due to their elusive ecology and the invasiveness of stomach content analyses in regard of their threatened status. As a potential alternative, we discuss the variability in $\delta^{44/42}\text{Ca}$ values recorded in the tooth enamel of size-graded individuals belonging to three species of large sharks with distinct diets (*Isurus oxyrinchus*, *Hexanchus griseus* and *Carcharodon carcharias*). The preliminary results highlight shifts in diet linked to ontogeny (*I. oxyrinchus* and *H. griseus*) and spatial distribution (*C. carcharias*) characterizing feeding behaviour in these species at individual and population level. These outcomes agree with the results of traditional stomach analyses supporting that nontraditional stable isotopes thus represent new perspectives for the study of modern and extinct shark ecology. In addition, for the first time, the Sr/Ca elemental ratios measured in *H. griseus* reflect sexual differences that could be interpreted in terms of spatial segregation or physiological heterogeneities.

KEYWORDS

alkaline metals, calcium, ecology, elasmobranchs, ontogeny, trophic level

1 | INTRODUCTION

As marine apex predators, large sharks exert strong top-down control on the food web (Ferretti *et al.*, 2010; Hussey *et al.*, 2014). They are principally opportunistic predators displaying behaviours such as active predation or scavenging (Domeier, 2009). Understanding their feeding behaviours is a key to estimating the trophic interactions in both extinct and present marine ecosystems and an opportunity to increase conservation efforts as well. However, the feeding ecology of numerous shark species remains poorly known due to their scarcity in the wild, their threatened conservation status and their elusive behaviour during their life spans (Hussey *et al.*, 2012). Feeding preferences in sharks have

been estimated for years on the basis of stomach contents from captured or dead specimens (Cortés, 1999; Ebert, 1994; Simpfendorfer *et al.*, 2001a). This method offers a snapshot of the short-term diet of individuals that allows the last meal of a specimen to be characterized (Baker *et al.*, 2014).

In recent decades, the use of stable isotopic analyses (SIA) on soft and hard tissues using nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotopes has been proposed to investigate the ecology and feeding behaviours of shark species (Barría *et al.*, 2018; Cree *et al.*, 1999; Estrada *et al.*, 2006; Kast *et al.*, 2016, 2022; Tierney *et al.*, 2008; Vennemann *et al.*, 2001). These analyses offer the opportunity to estimate the diet over periods of time from months to years to complement previous data from

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stomach content analyses (Reum *et al.*, 2020). However, the nitrogen isotopic composition of seawater, and by extension in the food-web baseline, is subject to strong geographical variability due to the spatial variations of nutrient supply and terrigenous inputs (Holmden *et al.*, 1997; Matich *et al.*, 2021; Schmitt *et al.*, 2001) that complicate the comparison of specimens from distant areas (Jennings & van der Molen, 2015; Pethybridge *et al.*, 2018), which is problematic for highly mobile organisms such as sharks. Furthermore, the carbon isotopic composition of biological tissues is also subject to variations linked to photosynthetic pathways in the trophic baseline (Matich *et al.*, 2021), allometry or tissue-dependent fractionation processes (Angilletta *et al.*, 2004). Alternatively, even if biopurification of trace elements contained in mineralized tissues (*e.g.*, strontium or barium concentrations normalized to calcium) allow to infer trophic levels in terrestrial ecosystems (Balter, 2004; Peek & Clementz, 2012; Tacail *et al.*, 2020), trace element behaviour in marine ecosystems appears to follow different processes that do not reflect trophic ecology (Peek & Clementz, 2012).

Calcium isotopes (expressed below as $\delta^{44/42}\text{Ca}$), measured in mineralized tissues (*e.g.*, tooth bioapatite), represent a novel addition to the SIA toolkit for reconstructing trophic organization in marine ecosystems (Akhtar *et al.*, 2020; Clementz *et al.*, 2003; Martin *et al.*, 2015; Skulan & DePaolo, 1999). Among bony and cartilaginous fishes, the circulation of water through the gills is considered to act as a stable buffer of calcium,

with observed variations in $\delta^{44/42}\text{Ca}$ values reflecting dietary inputs (Clementz *et al.*, 2003; Martin *et al.*, 2015; Tacail *et al.*, 2020). As a general rule, the observed decrease in $\delta^{44/42}\text{Ca}$ values across the marine food web has been hypothesized to arise from a depletion of heavy calcium (^{44}Ca) in mineralized tissues from the bottom to the top of the food web (Figure 1) (Martin *et al.*, 2015; Skulan & DePaolo, 1999). This is estimated to result from the partition of lighter against heavier isotopes from the feeding bowl (or dietary source) during physiological assimilation of the Ca (*e.g.*, during bone mineralization and/or urine excretion). Current knowledge on the physiological processes behind Ca isotopic fractionation is limited to experiments and box models conducted on mammals (*e.g.*, Morgan *et al.*, 2012; Eisenhauer *et al.*, 2019; Tacail *et al.*, 2020; Hassler *et al.*, 2021), while no experimental studies on elasmobranchs have been published yet. Nevertheless, teeth from elasmobranchs with filter-feeding behaviours have the highest $\delta^{44/42}\text{Ca}$ values (~ -0.1 to -0.3‰) (Akhtar *et al.*, 2020; Martin *et al.*, 2015; Skulan & DePaolo, 1999), whereas top predators ingesting the mineralized tissue of smaller predators (by swallowing other elasmobranchs and/or bony fishes) have the lowest $\delta^{44/42}\text{Ca}$ values (~ -0.7 to -1‰). However, because most sharks are opportunistic predators, feeding differently on both live food and carrion during their life span, the intraspecies range of $\delta^{44/42}\text{Ca}$ values can be wide (Figure 1), which blurs the delimitation between the groups. Since the publication of Martin *et*

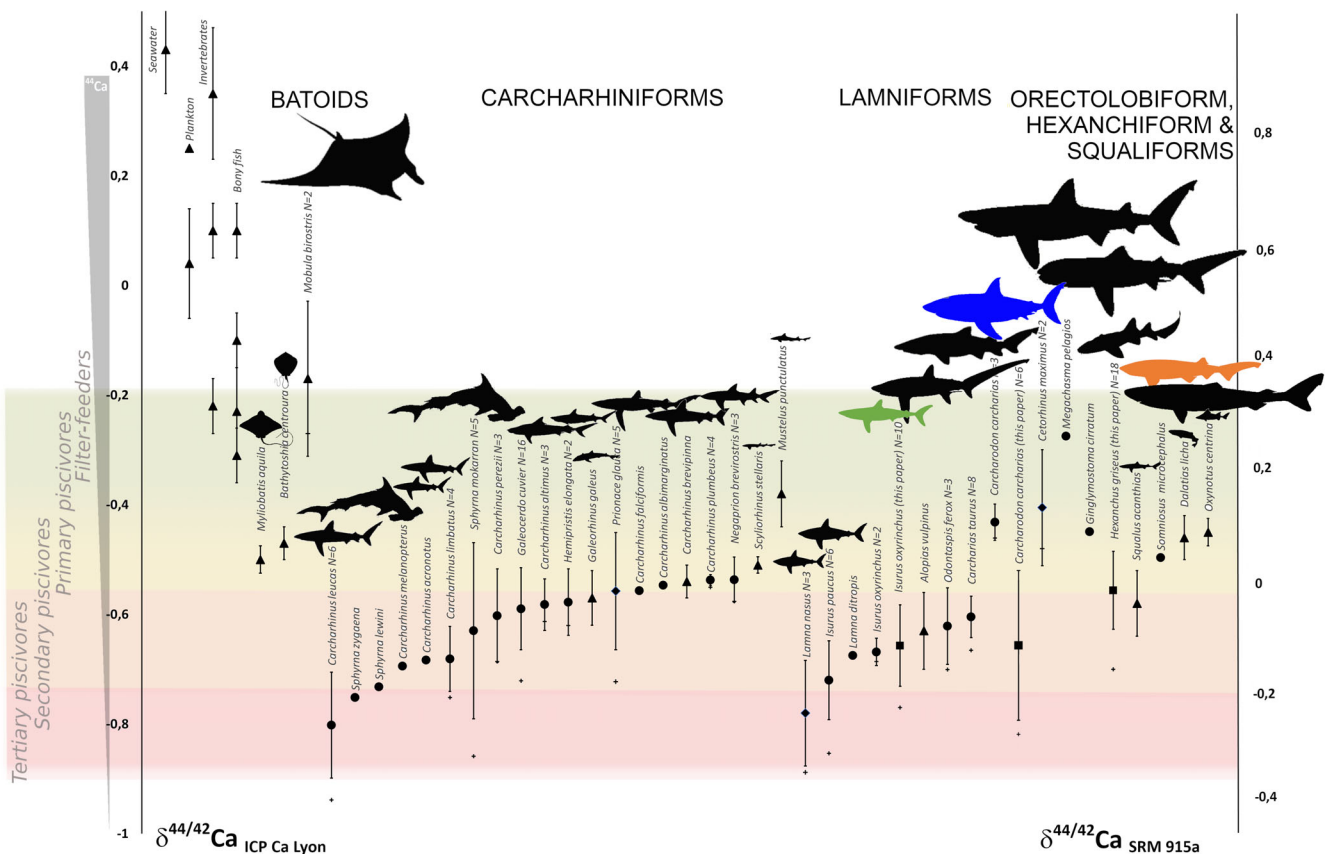


FIGURE 1 Global trophic level of elasmobranchs species using $\delta^{44/42}\text{Ca}$ isotopes (see Supporting Information Table S1) from the literature (Akhtar *et al.*, 2020; Martin *et al.*, 2015). Relative size between species and their global shapes are provided. Boxes represent trophic levels based on Martin *et al.* (2015). Squares, this paper; triangles, data from Martin *et al.* (2015); circles, data from Akhtar *et al.* (2020); rhombuses, combined data from Martin *et al.* (2015) and Akhtar *et al.* (2020)

al. (2015), a large range of elasmobranchs Ca stable isotopes is now available (Akhtar *et al.*, 2020) to enrich the comprehension of elasmobranch ecology (Figure 1). Here, we can observe that even if the completion of the trophic spectrum has smoothed the distinction between the four groups of consumers based on prey type that are present in the literature (*i.e.*, plankton-feeders, primary, secondary and tertiary consumers), these groups have been recognized through $\delta^{44/42}\text{Ca}$ analyses of elasmobranch bioapatite. Elasmobranchs display a wide range of trophic position at the species level regardless to the order showing that the trophic signal highlighted by calcium SIA does not rely on phylogeny (Figure 1). Moreover, physiological differences between fishes and mammals (*e.g.*, involving tooth replacement, seawater consumption or lactation, respectively) prevent a direct comparison of $\delta^{44/42}\text{Ca}$ values between these two groups and trophic food-web inferences should be discussed considering these physiological differences (Martin *et al.*, 2015). With these assets and limitations in mind, the replacement and loss of elasmobranch teeth represent an opportunity for analysing calcium isotopes, allowing a more comprehensive understanding of shark ecology based on a larger sampling from freshly captured specimens and those from public collections (including fossil material).

As an overview, Figure 1 allows the comparison of the trophic positions of numerous species of elasmobranchs. It shows that the trophic signal retrieved from calcium SIA does not rely on phylogeny, as each elasmobranch order displays a wide range of trophic position at the species level. Well-known plankton feeders such as the Giant manta ray *Mobula birostris* and the Megamouth shark *Megachasma pelagios* are characterized by particularly high $\delta^{44/42}\text{Ca}$ values. It is noticeable that the filter-feeder basking shark (*Cetorhinus maximus*) displays variations in $\delta^{44/42}\text{Ca}$ values that fall in the lower part of the plankton-feeders spectrum. This could be explained by the nonselective consumption of small fish induced by its foraging behaviour, which drives Ca isotopic compositions to more negative values. The tiger shark (*Galeocerdo cuvier*) is known to be a top predator in its environment, displaying a wide range of prey and behaviours from active predation on small fish to mammal scavenging (Simpfendorfer *et al.*, 2001b). The $\delta^{44/42}\text{Ca}$ values presented in the literature for this species are high for an apex predator. The regurgitation of marine mammal bones by tiger sharks after digestion of mammal soft tissues (a common behaviour in sharks; Brunnschweiler *et al.*, 2005; Randall & Cea, 2011) can limit the contribution of mammals to the sum of dietary Ca intakes, which could drive average diet $\delta^{44/42}\text{Ca}$ values closer to the isotopic composition of the fish pole. The opportunistic aspect of its feeding behaviour coupled with a wide range of prey, including mammal flesh, could explain its medium Ca isotope values. A broader analysis of *G. cuvier* calcium stable isotopes could assess more precisely its feeding behaviour and trophic position. The wide range of trophic position exhibited by the great hammerhead shark (*Sphyrna mokarran*) could highlight shifts in trophic position linked to ontogeny as sharks from the *Sphyrna* genus hunt in shallow waters, which allows access to a large range of prey. Even if adult hammerhead sharks mainly feed on elasmobranchs, this should be supported by low tooth $\delta^{44/42}\text{Ca}$ values. Juveniles seem to incorporate a high percentage of invertebrates (Torres-Rojas *et al.*, 2010), which could significantly drive the Ca isotope values toward ^{44}Ca -depleted values. However,

the lack of size information for literature specimens has precluded most precise inferences on the variations in *Sphyrna mokarran* $\delta^{44/42}\text{Ca}$ values so far. Akhtar *et al.* (2020) discussed the low Ca isotope values in the bull shark *Carcharhinus leucas* and assigned its high trophic level to an overestimation linked to its incursions in freshwater. This behaviour could generate an environmental bias by inducing a depletion in heavy calcium within the organism caused by the more ^{44}Ca -depleted isotopic compositions of riverine waters passing through the gills (Holmden *et al.*, 2012).

For many shark species, ontogeny may impact the way they behave and feed, influencing the size of prey they can catch and the distribution of habitat they occupy (Ebert, 2002a; Ellis & Musick, 2007; Lucifora *et al.*, 2009; Vögler *et al.*, 2009). This change in predatory behaviour is often marked by an increase in the trophic position as testified by analyses from stomach contents (Vögler *et al.*, 2009). The diets of *Hexanchus griseus*, *Isurus oxyrinchus* and *Carcharodon carcharias* have been studied for years for ecological purposes and for this reason they represent key taxa for isotopic analyses. Their feeding behaviours have been principally estimated on the basis of stomach contents (Cortés, 1999; Ebert, 1994; Simpfendorfer *et al.*, 2001a). Based on this evidence, the shortfin mako shark (*I. oxyrinchus*) is estimated to be a highly piscivorous species from pelagic habitats, preying mainly on teleosts and elasmobranchs (Cliff *et al.*, 1990; Maia *et al.*, 2006; Stevens, 2008). The bluntnose sixgill shark lives in deep waters and displays a feeding behaviour that is known to change with growth (Celona *et al.*, 2005; Ebert, 1994). While small specimens <120 cm of total length (TL) feed mainly on primary consumers like cephalopods and small bony fishes, larger specimens start to incorporate bigger prey like large osteichthyes or other elasmobranchs as well. Specimens over 2 m TL present another shift in diet as they start to incorporate mammals in their diet. The great white shark is known as one of the major apex predators of marine ecosystems. It displays a wide range of prey linked to an opportunistic feeding behaviour. However, prey distribution impacts diet preferences among populations of great white sharks. South African great white sharks prey preferentially on fur seal colonies (Hammerschlag *et al.*, 2006; Martin *et al.*, 2005). However, as the Mediterranean Sea no longer presents pinniped colonies, nowadays Mediterranean great white sharks mainly predate on large bony fishes like tuna, with which it shares migratory behaviours (Kabasakal, 2016).

Here we refine our understanding of Ca isotope variability in elasmobranchs and investigate the relationship between body size, ecology and tooth $\delta^{44/42}\text{Ca}$ values and trace metal concentrations (Sr, Ba and Mg) among three species of large sharks presenting distinctive ecologies and feeding behaviours: the shortfin mako shark *I. oxyrinchus*, the great white shark *C. carcharias* and the bluntnose sixgill shark *H. griseus*.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Tooth fragments were sampled from 34 complete tooth crowns of 34 small to large individuals belonging to three large shark species:

TABLE 1 Calcium isotope values of teeth coupled with biometric and ecological data from elasmobranchs analysed in this study

Collection name	Lab sample	Taxon	Tooth CH (mm)	TL (cm)	Weight (kg)	Weight estimated ^a (kg)	Sex	Provenance	Sample d44/42Ca		d43/42 (‰)	Replicates	d44/42Ca (% ^o P15a)	Ca (%)	P (%)	Sr (ppm)	Ba (ppm)	Mg (ppm)	Sr/Ca (mMol/mol)	Ba/Ca (mMol/mol)	Mg/Ca (mMol/mol)	References		
									25D ICP	25D ICP Ca Lyon														
n ^o 1 Kabasakal, & Gedikoglu, S.O., 2008	aa43	<i>Carcharodon carcharias</i>	-	125	-	21.66	M	MED	1.09	-0.82	0.17	-0.39	0.07	4	18.0	7.8	1392.4	169.8	623.1	3.55	0.276	5.720	This study	
ERB0932	aa46	<i>Carcharodon carcharias</i>	-	212	55	108.48	F	SW IND	0.97	-0.51	0.05	-0.28	0.08	4	0.01	14.9	6.7	615.0	26.6	468.2	1.89	0.052	5.176	This study
ERB0495	aa44	<i>Carcharodon carcharias</i>	-	266	133	216.72	M	SW IND	1.19	-0.54	0.12	-0.27	0.09	3	-0.02	14.5	7.6	638.4	1.6	292.9	2.02	0.003	3.338	This study
ERB0496	aa45	<i>Carcharodon carcharias</i>	-	343.8	280	473.97	F	SW IND	1.27	-0.55	0.11	-0.26	0.05	3	-0.03	14.3	7.5	713.5	1.0	757.6	2.29	0.002	8.746	This study
REC 0610 M	-	<i>Carcharodon carcharias</i>	-	300	-	312.78	-	MED	-	-0.74	0.07	-0.38	0.08	3	-0.22	-	-	-	-	-	-	-	-	Martin et al., 2015
REC 0812 M	-	<i>Carcharodon carcharias</i>	-	400	-	752.14	-	MED	-	-0.78	0.12	-0.45	0.06	4	-0.26	-	-	-	-	-	-	-	-	Martin et al., 2015
REC567M	aa58	<i>Isurus oxyrinchus</i>	9.38	109.9 ^a	-	9.63	-	MED	0.89	-0.57	0.1	-0.29	0.04	4	-0.05	24.1	12.6	984.9	-	831.7	1.87	-	5.686	This study
REC564M	aa51	<i>Isurus oxyrinchus</i>	10.51	121.2 ^a	-	13.22	-	MED	1.19	-0.55	0.15	-0.28	0.09	4	-0.03	15.4	8.2	728.1	0.6	1329.3	2.16	0.001	14.196	This study
REC182M	aa53	<i>Isurus oxyrinchus</i>	16.85	184.5 ^a	-	47.06	-	MED	1.06	-0.58	0.13	-0.30	0.07	4	-0.06	17.4	9.1	621.7	0.7	516.6	1.64	0.001	4.904	This study
REC565M	aa54	<i>Isurus oxyrinchus</i>	19.04	206.4 ^a	-	66.26	-	MED	0.94	-0.7	0.13	-0.32	0.08	3	-0.18	18.9	9.8	744.5	8.3	575.2	1.80	0.013	5.019	This study
REC563M	aa59	<i>Isurus oxyrinchus</i>	20.74	223.3 ^a	-	84.26	-	MED	1.25	-0.77	0.07	-0.37	0.04	4	-0.25	18.1	9.3	709.2	2.0	461.5	1.79	0.003	4.202	This study
REC10-11 M	aa56	<i>Isurus oxyrinchus</i>	21.69	232.8 ^a	-	93.75	-	MED	0.92	-0.64	0.08	-0.34	0.01	3	-0.12	17.6	9.2	817.3	-	501.7	2.13	-	4.705	This study
REC160M	aa50	<i>Isurus oxyrinchus</i>	24.25	258.4 ^a	-	131.05	-	MED	1.15	-0.68	0.07	-0.34	0.06	3	-0.16	17.0	8.5	1333.4	6.8	599.4	3.59	0.012	5.821	This study
REC1330M	aa52	<i>Isurus oxyrinchus</i>	25.6	271.9 ^a	-	153.81	-	MED	1.03	-0.75	0.13	-0.37	0.08	4	-0.23	14.3	7.6	648.6	0.3	878.3	2.08	0.001	10.153	This study
REC566M	aa57	<i>Isurus oxyrinchus</i>	26.75	283.4 ^a	-	173.44	-	MED	0.91	-0.65	0.06	-0.34	0.08	4	-0.13	13.0	7.6	674.4	1.4	3595.3	2.37	0.003	45.510	This study
REC381M	aa55	<i>Isurus oxyrinchus</i>	29.4	309.8 ^a	-	228.59	-	MED	1.2	-0.68	0.09	-0.36	0.03	4	-0.16	17.6	9.1	919.4	2.7	515.4	2.39	0.004	4.825	This study
REC201 M	201 M	<i>Hexanchus griseus</i>	-	58	-	1.03	-	MED	2.89	-0.50	0.15	-0.22	0.08	3	0.02	13.5	8.0	945.0	1.0	1865.0	3.21	0.002	22.840	This study
REC201 M	201 Mb	<i>Hexanchus griseus</i>	-	58	-	1.03	-	MED	1.27	-0.54	0.06	-0.25	0.09	3	-0.02	23.0	13.7	3593.0	1286.0	4549.0	7.16	1.635	32.680	This study
REC201 M	-	<i>Hexanchus griseus</i>	-	58	-	1.03	-	MED	-	-0.66	0.06	-0.37	0.08	3	-0.14	-	-	-	-	-	-	-	-	Martin et al., 2015
REC172M	172	<i>Hexanchus griseus</i>	-	72	-	2.03	-	MED	0.68	-0.62	0.10	-0.32	0.10	4	-0.10	24.0	13.0	1180.0	3.0	2191.0	2.25	0.0037	15.080	This study
REC173M	173	<i>Hexanchus griseus</i>	-	75	-	2.30	F	MED	0.69	-0.57	0.03	-0.27	0.05	3	-0.05	8.9	5.2	1091.0	3.0	2068.0	5.59	0.0098	38.170	This study
REC174M	174	<i>Hexanchus griseus</i>	-	80	-	2.81	M	MED	1.56	-0.54	0.18	-0.27	0.10	3	-0.02	22.7	13.0	1290.0	4.0	2995.0	2.60	0.0051	21.780	This study

TABLE 1 (Continued)

Collection name	Lab sample	Taxon	Tooth CH (mm)	TL (cm)	Weight (kg)	Weight estimated ^b (kg)	Sex	Provenance	Sample weight (mg)	d44/42Ca		d43/42 (%)	2SD	ICP Ca Lyon	2SD	ICP Ca Lyon	d44/42Ca (%) ^a	Ca (%)	P (%)	Sr (ppm)	Ba (ppm)	Mg (ppm)	Sr/Ca (mMol/mol)	Ba/Ca (mMol/mol)	Mg/Ca (mMol/mol)	References	
										2SD	ICP Ca Lyon																
REC166M	166	<i>Hexanchus griseus</i>	-	117	-	9.17	F	MED	0.72	-0.58	0.02	-0.28	0.01	3	0.02	3475.0	26.0	14.7	3475.0	6.10	0.0056	5.0	3081.0	6.10	0.0056	19.510	This study
REC171M	171	<i>Hexanchus griseus</i>	-	120	-	9.92	M	MED	1.55	-0.52	0.09	-0.21	0.11	3	0.09	2120.0	34.1	19.0	2120.0	2.84	0.0026	3.0	2743.0	2.84	0.0026	13.260	This study
REC1236	1236	<i>Hexanchus griseus</i>	-	126	-	11.54	-	MED	1.43	-0.56	0.10	-0.26	0.08	3	0.10	1278.0	21.6	12.1	1278.0	2.71	0.0054	4.0	2355.0	2.71	0.0054	17.960	This study
REC164M	164	<i>Hexanchus griseus</i>	-	160	-	24.27	M	MED	1.64	-0.54	0.04	-0.26	0.06	3	0.04	1386.0	24.9	13.9	1386.0	2.55	0.0023	2.0	1885.0	2.55	0.0023	12.490	This study
REC1478	1478	<i>Hexanchus griseus</i>	-	190	-	41.41	F	MED	2.47	-0.56	0.07	-0.26	0.18	3	0.07	1417.0	11.4	6.6	1417.0	5.69	0.0077	3.0	1206.0	5.69	0.0077	17.470	This study
REC163M	163	<i>Hexanchus griseus</i>	-	191	-	42.09	F	MED	1.22	-0.62	0.07	-0.30	0.06	3	0.07	2372.0	17.4	10.0	2372.0	6.22	0.0033	2.0	4222.0	6.22	0.0033	39.940	This study
REC175M	175	<i>Hexanchus griseus</i>	-	195	-	44.90	M	NE ATL	0.69	-0.60	0.03	-0.29	0.04	2	0.03	1572.0	26.8	14.6	1572.0	2.68	0.0022	2.0	1588.0	2.68	0.0022	9.760	This study
REC203M	-	<i>Hexanchus griseus</i>	-	200	-	48.57	-	MED	-	-0.68	0.13	-0.33	0.11	3	0.13	-	-	-	-	-	-	-	-	-	-	-	Martin et al., 2015
REC162M	162	<i>Hexanchus griseus</i>	-	228	-	73.01	M	MED	1.27	-0.50	0.04	-0.23	0.04	3	0.04	1368.0	23.4	13.0	1368.0	2.68	0.0025	2.0	1975.0	2.68	0.0025	13.930	This study
REC1332M	1332	<i>Hexanchus griseus</i>	-	300	-	171.41	M	MED	2.61	-0.60	0.05	-0.28	0.06	2	0.05	1602.0	26.9	14.9	1602.0	2.72	0.0033	3.0	1443.0	2.72	0.0033	8.840	This study
REC204M	204	<i>Hexanchus griseus</i>	-	320	-	209.52	-	MED	2.87	-0.42	0.13	-0.16	0.12	2	0.13	1091.0	17.9	10.2	1091.0	2.79	0.0098	6.0	1451.0	2.79	0.0098	13.370	This study
REC758M	758	<i>Hexanchus griseus</i>	-	463	-	660.93	F	NE ATL	1.85	-0.59	0.04	-0.29	0.04	3	0.04	3404.0	25.9	14.1	3404.0	6.02	0.009	8.0	1906.0	6.02	0.009	12.160	This study
-	-	SRM1486 Standard	-	-	-	-	-	-	-	-1.01	0.11	-0.49	0.12	28	0.11	-	-	-	-	-	-	-	-	-	-	-	This study
-	-	IAPSO Standard	-	-	-	-	-	-	-	0.37	0.13	0.25	0.04	6	0.13	-	-	-	-	-	-	-	-	-	-	-	This study

Abbreviations: M, male; F, female.

^aTotal length estimation following Shimada (2002).

^bWeight estimation following Froese et al. (2014).

18 teeth/18 individuals were collected for *H. griseus* (bluntnose sixgill shark), 10/10 for *I. oxyrinchus* (shortfin mako shark) and 6/6 for *C. carcharias* (great white shark). Specimens were collected within the collections of the University of Montpellier, the University of Lyon, Elasmobranch Research Belgium (F.H.M., personal collection) and the Hakan Kabasakal collection. Among *C. carcharias* specimens, three specimens came from the Mediterranean Sea (two from Lion Gulf, southern France; one from Turkey; Kabasakal, & Gedikoğlu, 2008) and three from the Indian Ocean (South Africa). Two specimens of *H. griseus* came from the Atlantic Ocean (western France) while 16 specimens came from the Mediterranean Sea (Lion Gulf, southern France). *I. oxyrinchus* material consisted of 10 lower lateral teeth belonging to the historical collections of the University of Montpellier and fished from the Mediterranean Sea, although no precise geographical information of which area was available. The total length of every specimen of *H. griseus* and *C. carcharias* was measured on the fresh body. Sizes of *I. oxyrinchus* specimens were estimated using crown-high (CH)-based regression [$TL = 16.205 + 9.987CH$ (anterior); Shimada, 2002]. Except for the three South African *C. carcharias* for which the specimen's body mass was known, the weight of other individuals was estimated using Bayesian approach (Froese *et al.*, 2014) for estimating length-weight relationships available for each species in FishBase (Froese & Pauly, 2000): *Isurus*: $W = TL^{3.03} \times 0.00646 \times 10^{-3}$; *Carcharodon*: $W = TL^{3.05} \times 0.00871 \times 10^{-3}$; *Hexanchus*: $W = TL^{3.11} \times 0.00339 \times 10^{-3}$ ($R^2 > 0.5$).

Enameloid fragments weighing about 1–3 mg were collected using a sharp blade under a binocular microscope. Dentine was removed manually to keep only enameloid, as calcium incorporation is known to differ between these tissues (Akhtar *et al.*, 2020; Martin *et al.*, 2015). Sample information is recorded in Table 1. Specimens from Akhtar *et al.* (2020) and Martin *et al.* (2015) were used to perform a global estimation of the trophic position of elasmobranch species (Figure 1) and data from Akhtar *et al.* (2020) were converted from 915a to ICP Lyon as ($X_{ICP\text{Lyon}} = X_{915a} - 0.52$).

2.2 | Chemical analyses

Chemical purifications and analyses were conducted on the facilities at Laboratoire de Géologie de Lyon, France. Each sample was dissolved in 1 ml of concentrated HNO_3 and heated until complete dissolution and then evaporated.

All samples were retaken in 1 ml of 0.05 N HNO_3 for concentration analyses for Ca, P, Sr, Ba and Mg, which were measured from one aliquot of 500 μl for each sample. The concentration analysis was conducted on an iCAP Pro ICP-AES inductively coupled plasma atomic emission spectrometer (ICPAES; Thermo Fischer). Blanks were regularly monitored and bone powder standard NIST SRM 1486 bone meal was treated as a sample and measured three times, yielding an average value within one standard deviation of 247 ± 1.3 ppm of Sr, $30 \pm 0.3\%$ of Ca, $14.9 \pm 0.4\%$ of P and 4987 ± 178 ppm of Mg in agreement with the certified value.

Calcium purification followed Tacail *et al.* (2014). Two steps of elution were performed using nitric and hydrochloric acids. The first elution was performed through a cationic resin AG50W-X12 to remove matrix elements that could interfere with the calcium isotopic measurements. The second elution step was performed using a Sr-specific resin (Sr-spec Eichrom) that allowed strontium to be separated from calcium. Procedural blanks (0.1 μg) and standard samples with known concentrations of calcium were added to the analyses to assess the congruence of the results.

Ca isotopes from the purified fraction of calcium were measured using a Thermo Neptune Plus Multiple Collector-Inductively Coupled Plasma-Mass Spectrometre (MC-ICP-MS), Laboratoire de Géologie de Lyon equipped with an Aridus for sample introduction (see Tacail *et al.*, 2014 for details). All samples were diluted in 0.05 M HNO_3 before uptake to a concentration of 1.5 ppm. Measurements were conducted at medium or high resolution, with each analysis consisting of 40 measurements of 4.2 s integrations on $^{42}\text{Ca}^+$, $^{43}\text{Ca}^+$ and $^{44}\text{Ca}^+$ ions in static mode. Signals at 43.5 m/z , corresponding to $^{87}\text{Sr}^{2+}$, were measured for correction of Sr double charge interferences on Ca isotopes ($^{88}\text{Sr}^{2+}$ on $^{44}\text{Ca}^+$, $^{86}\text{Sr}^{2+}$ on $^{43}\text{Ca}^+$ and $^{84}\text{Sr}^{2+}$ on $^{42}\text{Ca}^+$). Each analysis was preceded by a washout pumping in 0.5 M HNO_3 . A second washout pumping was carried out in 0.05 M HNO_3 and measured as the blank. Delta values were obtained using the standard bracketing method with the Lyon ICP Ca standard, referred to as ICP Ca Lyon (Tacail *et al.*, 2014), and issued from a Specpure calcium plasma standard solution (Alfa Aesar). Standard reference material (SRM) 1486 and International association for the physical sciences of the ocean (IAPSO, Ocean scientific international ltd, OSIL) solutions were used as secondary standards. For each analytical session of samples, SRM 1486 was measured in turn. This sequence was repeated at least twice to check that a given sample could be replicated.

The analysis of the blanks revealed that they present about 150 ng of Ca which is far less than the less concentrate samples that display values around 200 μg of Ca. This difference in the concentration of samples versus blanks allows us to consider that Ca pollution in the blanks is negligible. The standard values are $\delta^{44/42}\text{Ca}_{ICP-Ca\text{ Lyon}} = -1.01$ with a two times standard deviation (2SD) of $\pm 0.11\%$ ($n = 28$) for SRM1486 and $\delta^{44/42}\text{Ca}_{ICP-Ca\text{ Lyon}} = +0.37 \pm 0.14\%$ (2sd, $n = 6$) for IAPSO seawater. These values are congruent with the standard values given by Tacail *et al.* (2014) and Martin *et al.* (2015). The calculated linear regression for mass-dependent fractionation is given in Supporting Information Figure S1. The regression slope is 0.5464, which falls in the range of general agreement with the 0.5067 slope predicted by the linear approximation of exponential mass-dependent fractionation.

3 | RESULTS

3.1 | Ca isotope composition of elasmobranch enameloid

Size and weight in the three species of elasmobranchs covariate in the same proportions (Kendall test: $T = 0.925$, $z = 7.774$, $P = 7.55 \times 10^{-15}$).

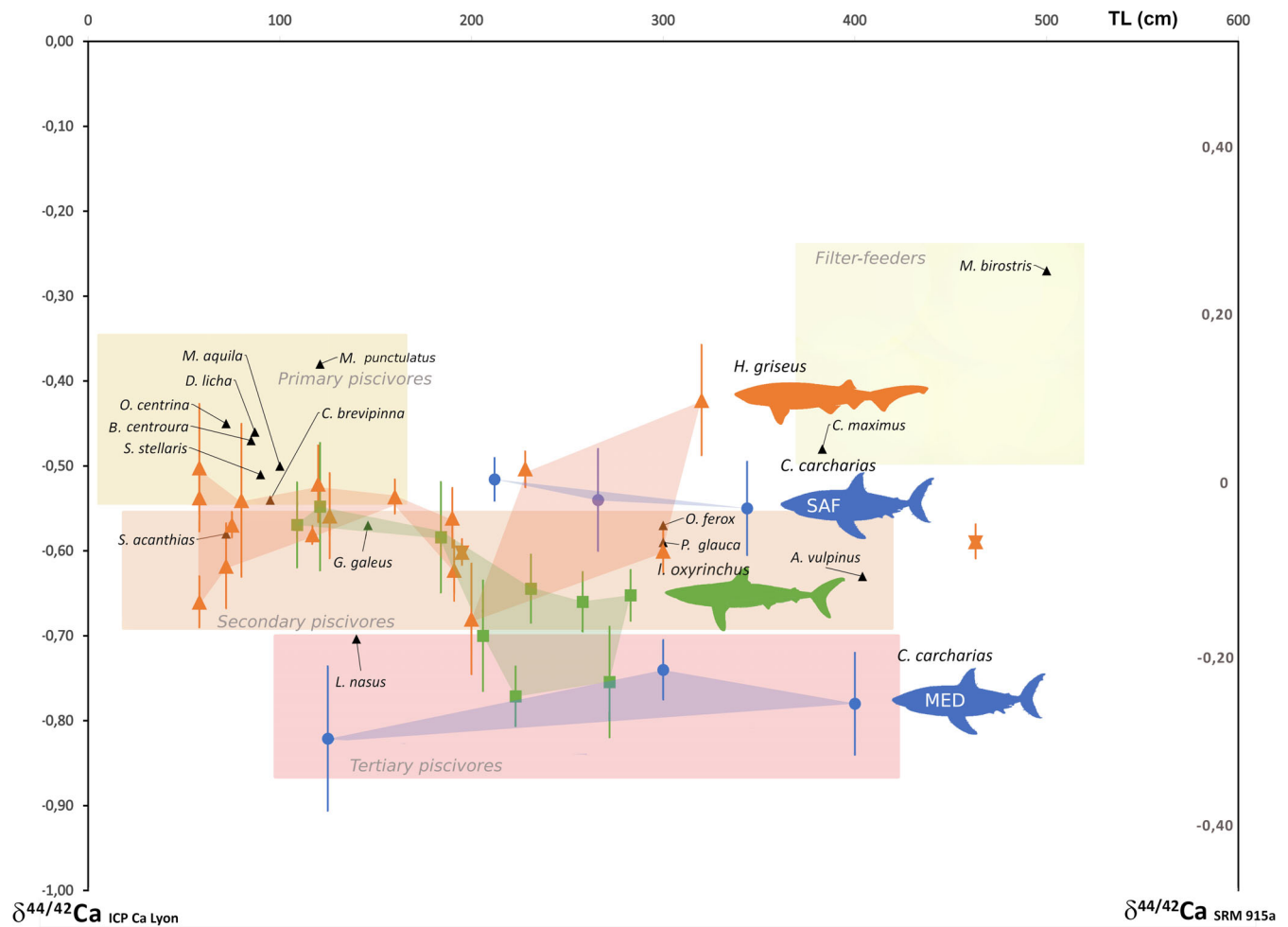


FIGURE 2 Trends of $\delta^{44/42}\text{Ca}$ values with size (total length [TL]) and ecology among three species of large elasmobranchs. Blue, *Carcharodon carcharias*; MED, Mediterranean Sea; SAF, South Africa; orange, *Hexanchus griseus*; triangles, Mediterranean; polygon, Atlantic; green, *Isurus oxyrinchus*. Vertical traits display two times standard deviation (2SD). Comparative species (black points) are derived from Martin et al. (2015). Boxes represent trophic levels based on Martin et al. (2015)

As body length and weight evolve the same way, $\delta^{44/42}\text{Ca}$ values and trace element concentrations will be expressed below against body length. Here we express the $\delta^{44/42}\text{Ca}$ values relative to the ICP-Ca Lyon standard (Martin et al., 2015, 2017) and all values are converted relative to SRM915a (Table 1). The three species analysed in this paper present $\delta^{44/42}\text{Ca}$ values ranging from -0.42 to -0.84‰ (Figure 2).

Specimens belonging to the shortfin mako shark *I. oxyrinchus* displayed a decrease in $\delta^{44/42}\text{Ca}$ values from -0.54 to -0.77‰ coinciding with an increase in body length and mass in this species. Specimens with total length between 109 and 200 cm presented a median $\delta^{44/42}\text{Ca}$ value of $-0.57 \pm 0.04\text{‰}$ (2sd, $n = 3$) while specimens over 200 cm presented a median value of $-0.72 \pm 0.1\text{‰}$ (2sd, $n = 7$).

In the Mediterranean bluntnose sixgill shark *H. griseus*, we observed variation ranging from -0.34 to -0.68‰ in relation to total body length (TL) (Figure 2). The smallest *Hexanchus* specimens (<1.2 m TL) presented a median $\delta^{44/42}\text{Ca}$ value of $-0.54 \pm 0.11\text{‰}$ (2sd, $n = 8$). The specimens between 1.2 and 2 m TL presented a median $\delta^{44/42}\text{Ca}$ value of $-0.56 \pm 0.07\text{‰}$ (2sd, $n = 5$) while specimens over 2 m TL displayed a median $\delta^{44/42}\text{Ca}$ value of $-0.50 \pm 0.10\text{‰}$ (2sd,

$n = 3$). The two specimens from the Atlantic Ocean that measured 1.95 and 4.63 m presented values of about -0.60‰ .

The great white shark *C. carcharias* $\delta^{44/42}\text{Ca}$ values presented large differences between specimens from the Mediterranean Sea and specimens from the Indian Ocean. For similar length, oceanic-dwelling specimens presented values that were 0.20–0.30‰ lower than Mediterranean specimens. All the South African specimens presented very high values, ranging from -0.51 to $-0.55\text{‰} \pm 0.03$ (2sd, $n = 3$), compared to the Mediterranean specimen values, which ranged from -0.74 to $-0.82\text{‰} \pm 0.09$ (2sd, $n = 3$).

3.2 | Alkaline earth metal concentrations in elasmobranch enameloid

Measurements of Sr/Ca, Mg/Ca and Ba/Ca elemental ratios in our analysis present fluctuations between and among the species ($n = 28$). The graphs of co-evolution of Sr/Ca, Mg/Ca and Ba/Ca are given in Supporting Information Figure S2. The Sr/Ca ratio appears to be correlated to Mg/Ca

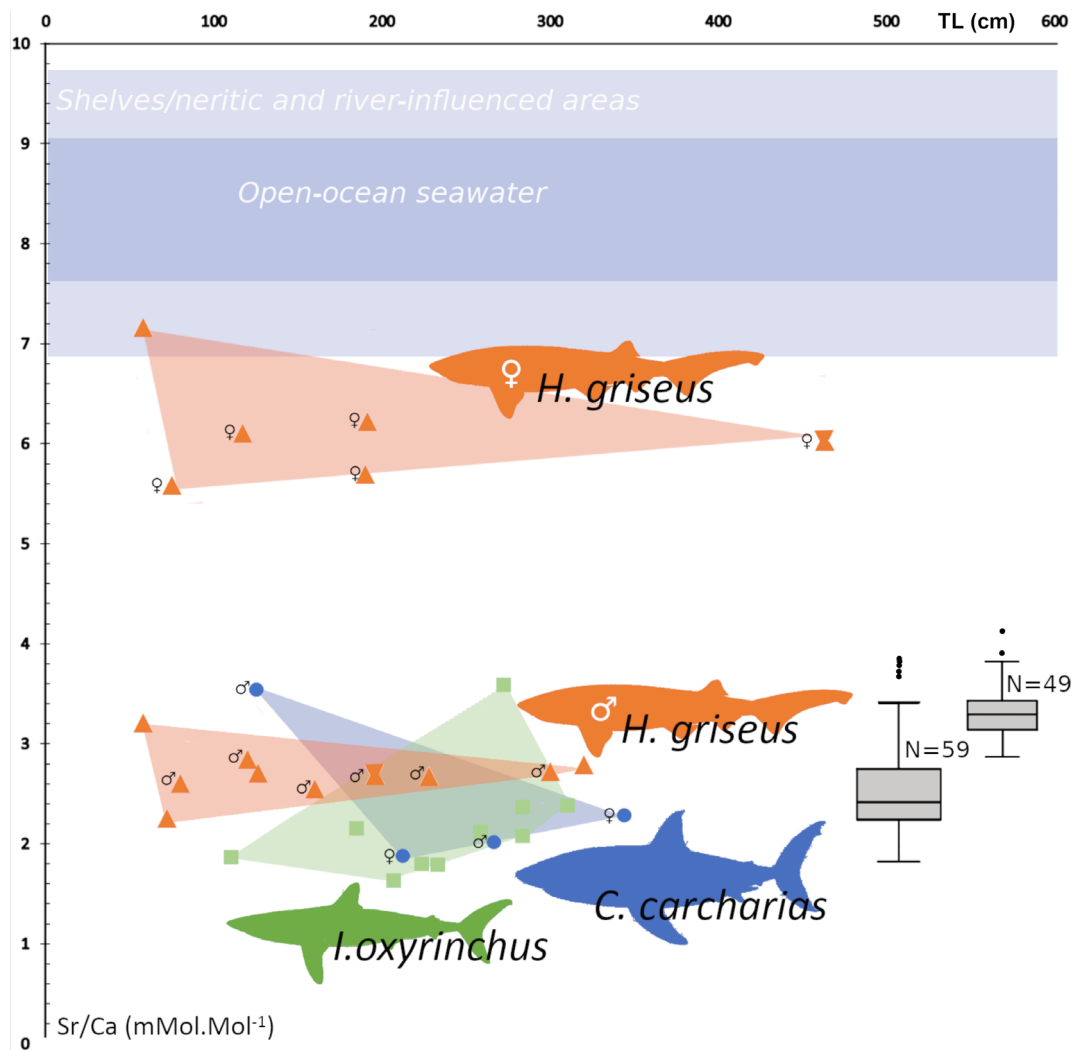


FIGURE 3 Evolution of Sr/Ca (mMol.Mol⁻¹) with size (total length [TL]) among three analysed species of large elasmobranchs (blue, *Carcharodon carcharias*; orange, *Hexanchus griseus*; green, *Isurus oxyrinchus*) with a focus on sexual segregation of *H. griseus* specimens (triangle, Mediterranean specimens; hourglass, Atlantic specimens). The right grey boxplot represents a literature compilation of 59 other sharks (e.g., *Carcharhinus*, *Galeocerdo*, *Sphyrna*, *Lamna*, *Carcharias*) and 49 individuals of *Carcharhinus leucas* (encountered at the Fiji Shark Reef Marine Reserve) for which Sr/Ca ratios from teeth enameloid are available in the literature (Akhtar *et al.*, 2020 and Kocsis *et al.*, 2015, respectively). Data from the literature are presented in Supporting Information Table S1). Blue banner, global open-ocean seawater Sr/Ca range; light blue, global shelves neritic and river-influenced areas Sr/Ca range, according to Lebrato *et al.* (2020)

(Kendall $T = 0.46$, P value = 0.0002) and slightly correlated with Ba/Ca ($T = 0.31$, P value = 0.019). However, Mg/Ca and Ba/Ca appear not to be correlated ($T = 0.04$, P value = 0.79). These variations are not related to the size of the specimen or to its diet or location, as Mediterranean specimens and Atlantic ones display similar values for these ratios (Supporting Information Table S1). Strontium and magnesium concentrations appear to be higher in *H. griseus* specimens than in *C. carcharias* and *I. oxyrinchus*. We also observed significant differences among Sr/Ca ratios between males ($n = 6$) and females ($n = 5$) in *H. griseus* (Wilcoxon $t = -25.116$, P value = 2.16×10^{-06}) (Figure 3). However, these differences are not clearly observed in Mg/Ca and Ba/Ca ratios. The Sr/Ca ratios displayed by females of *H. griseus* appear to be dramatically higher than those of every other elasmobranchs analysed. The Sr/Ca values measured in *H. griseus* females are about twice those of the *H. griseus* males as well as all the

specimens of *I. oxyrinchus* and *C. carcharias* as well as *Carcharhinus leucas* from Kocsis *et al.*, 2015 ($n = 49$) and several elasmobranch genera ($n = 59$, e.g., *Carcharhinus*, *Galeocerdo*, *Sphyrna*, *Lamna*, *Carcharias*, *Hemipristis*, *Negaprion*, *Prionace*, *Somniosus*) from Akhtar *et al.*, 2020 (Figure 3 and Supporting Information Table S1).

4 | DISCUSSION

4.1 | Intraspecific variation of calcium isotopes in three elasmobranchs

The three species of large sharks *H. griseus*, *I. oxyrinchus* and *C. carcharias* are very elusive and threatened in the oceans. The development of SIA based on nontraditional elements could bring new

clues to the understanding of their ecology. Even if the statistical power of such a constrained assemblage of specimens remains low, the $\delta^{44/42}\text{Ca}$ analyses permit to underline the contribution of calcium isotopes to diet reconstruction of elasmobranchs. Contrary to carbon and nitrogen isotopes, the calcium isotopic composition of seawater is homogeneous, which allows potential geographical bias to be avoided (Fantle & Tipper, 2014; Gussone *et al.*, 2020). Moreover, Ca isotopic fractionation in elasmobranch bioapatite is largely controlled by dietary input (Akhtar *et al.*, 2020; Martin *et al.*, 2015).

As expected from stomach contents, the shortfin mako (*I. oxyrinchus*) $\delta^{44/42}\text{Ca}$ values tend to decrease when body length increases, inducing a rise in trophic level with growth (Figure 2). The adult shortfin mako sharks are known to feed on cephalopods and pelagic bony fish populations (Maia *et al.*, 2006; Preti *et al.*, 2012; Stillwell & Kohler, 1982). The $\delta^{44/42}\text{Ca}$ values for this species (≈ -0.55 to -0.75%) match values for secondary to tertiary consumers (Martin *et al.*, 2015), which is congruent with their stomach content (Stillwell & Kohler, 1982). The decreasing $\delta^{44/42}\text{Ca}$ values with ontogeny present fluctuations but follow a progressive increase in the trophic chain for the biggest specimens. This could be explained by the ability to catch bigger fishes when mako sharks increase in size (Lowe *et al.*, 1996).

The food consumption of bluntnose sixgill sharks (*H. griseus*) during their life span is well known, at least for some populations (Andrews *et al.*, 2009; Ebert, 1994). Stomach contents are known to change from cephalopods and bony fishes for the smallest individuals to a majority of bony fishes and elasmobranchs for 2 m long specimens, while the largest specimens tend to feed mainly on large marine mammals. This supposes a size dependence of the eaten prey related to the size of the shark, but also an effect of the available feeding resources and thus spatially related diet. According to carbon and nitrogen isotope-based analyses on a population of subadult sixgill sharks, this species was interpreted as a generalist feeder with very small differences between individuals (Reum *et al.*, 2020), which contrasts with stomach contents that suggest a size-based diet (Ebert, 1994). The calcium isotope values reported here for the sixgill sharks indicate fluctuations that could be linked with the progressive incorporation of new prey items in its diet during growth (Figure 2). As expected, specimens under 2 m TL present $\delta^{44/42}\text{Ca}$ values (-0.56%) that correspond to a primary to secondary piscivorous consumer. This is consistent with the ingestion of cephalopods, bony and cartilaginous prey documented from stomach contents. Such diversity of prey could explain the fluctuations of $\delta^{44/42}\text{Ca}$ values observed among these specimens. The largest specimens of sixgill sharks (TL > 2 m) present slightly higher $\delta^{44/42}\text{Ca}$ values (-0.50%), indicating a similar to lower trophic level than younger individuals, which is surprising regarding their size and their expected rise in trophic position with growth. For these large individuals, strong fluctuations in $\delta^{44/42}\text{Ca}$ values are also reported (from -0.42 to -0.68%). In this size range, sixgill sharks are known to incorporate a larger percentage of marine mammals in their diet. We hypothesize that the relatively high $\delta^{44/42}\text{Ca}$ values (relevant to their size and stomach contents) associated with fluctuations of these values in such large specimens could

be linked to the scavenging habits of some specimens as well as the regurgitation of indigestible parts like bone, a common behaviour in sharks (Brunnschweiler *et al.*, 2005; Randall & Cea, 2011). Because they do not ingest mammal bones that are ^{44}Ca depleted compared to soft tissues, their enamel $\delta^{44/42}\text{Ca}$ values tend to be higher. Alternatively, a mammal-based diet associated with bone regurgitation could decrease Ca dietary intakes overall when compared to a fish-based diet, the latter involving the digestion of scales and other mineralized tissues. This could lead to proportionally increased Ca body intakes from ^{44}Ca -enriched sea water, resulting in higher shark enamel $\delta^{44/42}\text{Ca}$ values. Supporting these two nonexclusive hypotheses, a large part of the material reported from the large sixgill shark's stomach from the Mediterranean Sea consisted of dolphins (Kabasakal, 2006) and an unidentifiable mishmash of digested tissues (Celona *et al.*, 2005), which could coincide with the ingestion of marine mammal soft tissues only in large individuals of *H. griseus*.

The analysis of the great white shark *C. carcharias* specimens shows that they present two very distinct patterns regarding the localities they come from rather than variations linked to ontogeny. As *C. carcharias* are known to dominate the marine trophic chain, it is not surprising that they display a strong variability in the type of prey they feed on, incorporating all kinds, from cephalopods to whales (Grainger *et al.*, 2020; Tricas & McCosker, 1984). However, we observed that $\delta^{44/42}\text{Ca}$ values were different when considering their geographic position. Individuals from the western Mediterranean Sea displayed the lowest $\delta^{44/42}\text{Ca}$ median value of the analysis (-0.78% , $n = 3$), placing them in the tertiary piscivorous group (Figure 2). In contrast, specimens of the same species from the Indian Ocean (caught off South Africa) displayed particularly high $\delta^{44/42}\text{Ca}$ values (-0.53% , $n = 3$) (Figure 2). This indicates the existence of noteworthy differences in calcium sources for both these populations that could reflect differences in feeding behaviour between South African and Mediterranean populations of great white sharks that seem independent of their body sizes (Figure 2). Changes in feeding behaviour could rely on the variations of prey availability between both environments and/or population prey preferences. The very low $\delta^{44/42}\text{Ca}$ values displayed by Mediterranean specimens correspond to a high trophic level that could be linked to a preferential predation on bony fishes and other large elasmobranchs. This is supported by the occurrences and migration synchronicity of great white sharks in the Mediterranean area with large populations of tunas that they preferentially predate (De Maddalena & Heim, 2012; Galaz & De Maddalena, 2004; Kabasakal, 2016).

Comparatively, the higher $\delta^{44/42}\text{Ca}$ values measured in all South African great white shark individuals coincide with large *H. griseus* isotopic values and could be interpreted as a prevailing consumption of mammals. Large South African great white sharks are known to predate mainly on colonial mammals like fur seals (Martin *et al.*, 2005). As for sixgill sharks, the increased mammal consumption (active predation or scavenging) (Long & Jones, 1996; Dudley *et al.*, 2000; Fallows *et al.*, 2013) could drive their $\delta^{44/42}\text{Ca}$ values toward ^{44}Ca -enriched values compared to Mediterranean populations. Moreover, great white sharks are known to regurgitate the bones and

indigestible parts after eating mammals (Martin *et al.*, 2005), which could result in more ^{44}Ca -enriched Ca intakes. These results are congruent with the $\delta^{44/42}\text{Ca}$ values (-0.43% , $2\text{sd} = 0.06\%$, $n = 3$) reported for other South African *Carcharodon* specimens by Akhtar *et al.* (2020).

These behaviours highlight the plasticity of the great white shark's diet regarding the availability of prey in its environment and/or the habits of distinct populations. Prey availability, prey density and prey profitability are sometimes identified as factors influencing the diet of some large sharks (Simpfendorfer *et al.*, 2001a) and seem to explain the geographical differences in $\delta^{44/42}\text{Ca}$ values for the great white shark. Moreover, great white sharks are known to present demographically isolated populations due to their high degree of site fidelity (Jorgensen *et al.*, 2009).

Recently, a study reconstructing seawater Ca isotopic composition through geological time using fossil shark teeth concluded that fluctuations of $\delta^{44/42}\text{Ca}$ values in elasmobranch enamel must be the result of numerous factors (Akhtar *et al.*, 2020). Our present results suggest that the main factors that control the isotopic composition of calcium are linked to the dietary behaviour of the considered species. The differences in the isotopic trajectories between *Isurus* and *Hexanchus* specimens with growth, not only allow to consider trophic position but also permit to consider the preferential type of prey they feed on.

4.2 | Alkaline earth metal concentrations as a proxy for ecological studies of elasmobranchs

Trace metal elemental ratios (expressed against Ca concentrations), such as Sr/Ca, Mg/Ca and Ba/Ca ratios, have been investigated in marine vertebrates for their relevance in dietary inferences (Peek & Clementz, 2012). However, contrary to calcium isotopes, such elemental ratios show that they seem not to be representative of any dietary pattern across our samples, but rather inform about environmental distribution. Concentrations of elements such as magnesium (Mg), strontium (Sr) and barium (Ba) reflect the dynamic of exchanges between earth, oceans and atmosphere. In terrestrial food webs, these elements represent biogeochemical indicators to study trophic levels as measured from mammal apatite (Balter, 2004; Peek & Clementz, 2012). However, in the marine realm, the contribution of these elements to diet is not well understood due to the buffering effect of seawater. The ratios of these elements expressed against calcium concentration (Mg/Ca, Sr/Ca and Ba/Ca) are known to present some spatial variations in seawater linked to vertical fluxes in the water column or terrigenous inputs in estuaries and coastal areas (Akhtar *et al.*, 2020; Lebrato *et al.*, 2020).

The prevalence of Sr and Mg in *H. griseus* relative to *Isurus* and *Carcharodon* could be explained by its differential occupation of the water column. Unlike the two lamniforms, *H. griseus* spends more time in deep water and in benthic habitats down to 1500 m depth, which are typically rich in some trace metals (Lebrato *et al.*, 2020; Rodriguez-Cabello *et al.*, 2018). For example, Mg and Sr concentrations are

known to increase with depth in the oceans (De Villiers, 1999; Jaquet *et al.*, 2016; Peek & Clementz, 2012), which could explain the high concentration observed in benthic *H. griseus* compared to the pelagic species and the increase in Sr/Ca ratio with growth in *Isurus* (Figure 3) (Sepulveda *et al.*, 2004). Moreover, the unexpected sexual distinction revealed in *H. griseus* Sr/Ca ratios (Figure 3) could reflect differences in the spatial distribution between sexes, as known for other deep water shark species (Coelho & Erzini, 2010). However, estimating the occupation of the water column by males and females of *H. griseus* remains poorly documented and misunderstood (Ebert, 2002b; Grainger *et al.*, 2020; Mili *et al.*, 2021; Moura *et al.*, 2014; Sims, 2006). These sexual differences in Sr/Ca values could also reflect physiological differences between males and females specific to this species of *Hexanchus*. This hypothesis is supported by a lack of difference in sexual variations in other shark species, including our whole dataset of *Isurus* and *Carcharodon* as well as the previously published dataset of Akhtar *et al.* (2020) (e.g., *Carcharhinus*, *Galeocerdo*, *Sphyrna*, *Lamna*, *Carcharias*, *Hemipristis*, *Negaprion*, *Prionace*, *Somniosus*) (Akhtar *et al.*, 2020), which all fall within the Sr/Ca range of *Hexanchus* males. It is noticeable that specimens from *H. griseus* whom sex is unknown and whom Sr/Ca was analysed ($n = 5$), present Sr/Ca values consistent with the two groups based on sex information (Figure 3). The latter ratios in *H. griseus* females show a Sr/Ca ratio of 5.93 ($n = 5$) significantly higher than that of males (2.68, $n = 6$) (Wilcoxon P value = 0.004). Even if the process remains unclear, it could result from physiological as well as spatial differences, and could allow us to determine *a posteriori* the sex of undocumented material in historical collections using Sr/Ca ratios. Considering this, among the specimens that do not display sex information in our dataset we speculate that these specimens represent one female and four males. In this paper, Sr/Ca and Mg/Ca ratios are reported to be significantly higher in *H. griseus* than in *I. oxyrinchus* and *C. carcharias* (Figure 3). However, sexual variations of the Mg/Ca ratio appear not to be as clear as reported for Sr/Ca ratios. Concerning the Ba/Ca ratio, even if Ba concentration in seawater is known to increase with depth (Hsieh & Henderson, 2017; Neff, 2002), the absence of a difference in the values we observe between shortfin mako sharks and bluntnose sixgill sharks seems not to segregate pelagic species from more benthic ones. This result indicates that in marine food webs barium is not fractionated from prey by predators (Neff, 2002).

5 | CONCLUSIONS

We conclude that Ca isotope analyses seem promising as a noninvasive tool for diet reconstruction in sharks, offering possibilities for the understanding of changes in the type of prey linked with ontogeny and/or population distribution in bluntnose sixgill sharks and great white sharks. Moreover, SIA and trace metal concentrations analyses open up new perspectives in a large range of biological, ecological and evolutionary disciplines and will allow a complementary understanding of marine vertebrate ecology. These proxies coupled with the high preservation of elasmobranch teeth in the fossil record offer another

opportunity to investigate the diet of extinct elasmobranch species (Kast *et al.*, 2022; McCormack *et al.*, 2022).

CONTRIBUTIORS

A.A., J.E.M. and S.A. carried out the analyses and edited the manuscript. A.A., J.E.M., K.B. and F.A.G. performed the data acquisition. All authors drafted the manuscript and gave final approval for publication.

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CONFLICT OF INTEREST

We declare we have no competing interests.

DATA AVAILABILITY STATEMENT

All data used in this study are available from Table 1 and supporting information files Table S1.

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