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ORIGINAL PAPER

Sex influences elemental variation in the mineralized vertebrae cartilage of round stingray (*Urobatis halleri*)

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Abstract

The elemental composition of mineralized vertebral cartilage has proven useful for reconstructing the movement and environmental history of elasmobranchs. However, the effects of intrinsic and extrinsic factors on vertebral chemistry have not been well explored, making meaningful interpretations of results difficult. This study quantified trace element incorporation along the corpus calcareum of 116 Round Stingray (*Urobatis halleri*) vertebrae and explored the influence of intrinsic (i.e., sex) and extrinsic (i.e., location and year) factors on vertebral edge chemical variations. We quantified eight element/Ca ratios (Li/Ca, Mg/Ca, Mn/Ca, Co/Ca, Cu/Ca, Zn/Ca, Sr/Ca, Ba/Ca) by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Behavioral sexual segregation by Round Stingrays explained differences found in Mn/Ca, Zn/Ca, Sr/Ca, and Ba/Ca ratios. Higher vertebral Mn/Ca and Zn/Ca in females likely originate from the use of salt marsh habitats as a refuge as well as higher reproductive hormone concentrations during gestation, respectively. Female and male Round Stingrays were also found to have an inverse relationship between Sr/Ca and Ba/Ca, likely due to environmental differences (e.g., temperature) between their sexually segregated habitats. This study demonstrates how multielement signatures in vertebral cartilage may improve understanding of habitat use and migration throughout life and advances the development of biomineral usage as an environmental proxy in elasmobranchs.

Keywords Vertebrae Chemistry · Elasmobranch · LA−ICP−MS · Biomineralization · Behavioral Sexual Segregation

Introduction

The movement of individuals at different spatial and temporal scales influences the structure and dynamics of populations, communities, and entire ecosystems (Nathan et al. 2008). Elasmobranchs (sharks, skates, and rays), in particular, exhibit diverse patterns of spatial habitat use (Shipley et al. 2018), with movements that can range from hundreds of meters to thousands of kilometers, depending on the species (Papastamatiou and Lowe 2012). Ecologically, elasmobranchs influence energy transfer vertically through trophic levels as well as horizontally across various ecosystems owing to their movements and migrations (Dulvy et al. 2017). Temporal variations in the movements and behaviors of elasmobranchs can also influence a species' ecological role in a particular marine community, such as their trophic level within local food webs (Munroe et al. 2015). Biotelemetry and biologging offer effective means for monitoring behavior, spatial migration, and energetics (Guttridge et al. 2010). However, the duration of animal behavior monitored is limited to battery life, memory space, and/or duration of tag attachment, and sometimes requires physical retrieval of the tag to access data. By contrast, biomineralized hard parts that accrete during growth (notably, otoliths and vertebrae in fish) may encode chemical proxies of the habitats experienced throughout life (Campana and Thorrold 2001; Izzo et al. 2016a; Tzadik et al. 2017; Reis-Santos et al. 2022). Characterizing chemical variations in such structures obtained from captured specimens thus offers a retroactive means of interpreting behavior and habitat use.

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Mineralized cartilaginous structures of elasmobranchs (i.e., vertebral centra) grow throughout life and remain metabolically and chemically stable once formed (Izzo et al. 2016b). Vertebral centra are composed of calcified cartilage that forms through calcium phosphate (hydroxyapatite) mineralization of an extracellular protein matrix (Dean and Summers 2006; McMillan et al. 2017). In elasmobranchs, environmentally sourced elements can substitute for calcium or become trapped within the protein matrix of the vertebrae (Tillett et al. 2011). Accordingly, vertebrae elemental compositions may proxy water mass characteristics of the regions they inhabit (Smith et al. 2016), thereby allowing movement histories of individuals to be tracked across water masses. This approach has proven useful in determining ontogenetic habitat use (Tillett et al. 2011), migration patterns (Coiraton et al. 2020), and natal origins (Smith et al. 2016) in other elasmobranchs.

The reliability of elemental profiles as environmental tracers, however, also requires species-specific validation of how elements in ambient seawater are incorporated into mineralized, biological structures (i.e., vertebrae) (McMillan et al. 2017). Knowledge of intrinsic (i.e., biological) and extrinsic (i.e. environmental) factors that influence vertebrae chemistry is essential for a meaningful interpretation of results (Pistevos et al. 2019), but such factors have rarely been systematically studied in a laboratory setting for elasmobranchs (Smith et al. 2013; Pistevos et al. 2019). In a controlled study comparing water temperature and chemistry to elasmobranch vertebral growth and elemental uptake, using Round Stingray (*Urobatis halleri*) as a representative species, Smith et al. (2013) found that vertebral trace element variations can reliably distinguish individuals that experienced different environmental histories. In captive juvenile Round Stingray vertebrae, temperature had a positive (Mn/Ca) and negative (Mg/Ca and Ba/Ca) effect on element/Ca, but no such correlations were apparent for somatic growth or vertebral precipitation rate. Furthermore, ambient dissolved Ba/Ca was positively correlated with vertebral Ba/Ca. Ultimately, validation that mineralized structures can serve as natural environmental tracers requires assessment of whether intrinsic factors may outweigh or misconstrue environmental signals (Reis-Santos et al. 2018).

Owing to their use as a representative species in a variety of controlled studies (Hale et al. 2006; Hale and Lowe 2008; Smith et al. 2013), Round Stingrays are an ideal elasmobranch model to investigate the efficacy of vertebral chemistry for characterizing life histories in wild populations. The Round Stingray is an inshore, benthic elasmobranch that is native to the west coast of California, USA and Mexico (Babel 1967). Round Stingrays can undergo short migrations across shallow habitats (Plank et al. 2010), with some individuals traveling up to 30 km from tagging sites (Vaudo and Lowe 2006). However, depth is a barrier to movement for these animals. For example, two genetically distinct populations of Round Stingrays are found along the coast of mainland California and Santa Catalina Island, each separated from the other by a deep ravine (Plank et al. 2010). Within each location, Round Stingrays (similar to other elasmobranchs) are known to sexually segregate (Jirik and Lowe 2012). During gestation in the summer and fall, female stingrays occupy salt marshes while mature males remain in open coastal waters (Jirik and Lowe 2012; Lyons et al. 2018). Our study hypothesizes segregation by sex for part of the year coupled with the existence of two distinct populations leads to variation in vertebral signatures, reflective of these ecological differences.

For the present study, we consider chemical archives preserved in Round Stingray vertebral cartilage to investigate the effectiveness of biominerals as chemical environmental proxies in elasmobranchs. Using laser ablation inductively coupled plasma mass spectrometry (LA–ICP–MS), we document how vertebral signatures in wild Round Stingrays vary between sex and over spatiotemporal scales. We quantified multielement profiles along the radial edge of Round Stingray vertebrae to determine patterns of elemental incorporation associated with the most recently occupied habitats. We then apply permutational analysis to assess how sex, year, and capture location influence trace element signatures and investigate how vertebrae may be used to reconstruct environmental histories in elasmobranchs.

Materials and methods

Study area and sample collection

A total of 116 Round Stingray vertebrae samples were provided from previous research collections (Lyons et al. 2014; Sawyna et al. 2017; Lyons and Wynne-Edwards 2021). Specimens were captured opportunistically in 2010, 2011, 2014, and 2016 from three sites across Santa Catalina Island (Empire Landing, Two Harbors, and Cat Harbor), herein “Catalina”, and three sites along the southern coast of mainland California (Los Angeles (LA) Harbor, Seal Beach Wildlife Refuge, and Colorado Lagoon) (Fig. 1; Table 1). All localities receive negligible freshwater input. Males from Catalina Island were collected from sandy bottom substrates of open-coastline marine environments. By contrast, mature Catalina females were collected from sheltered, tidally

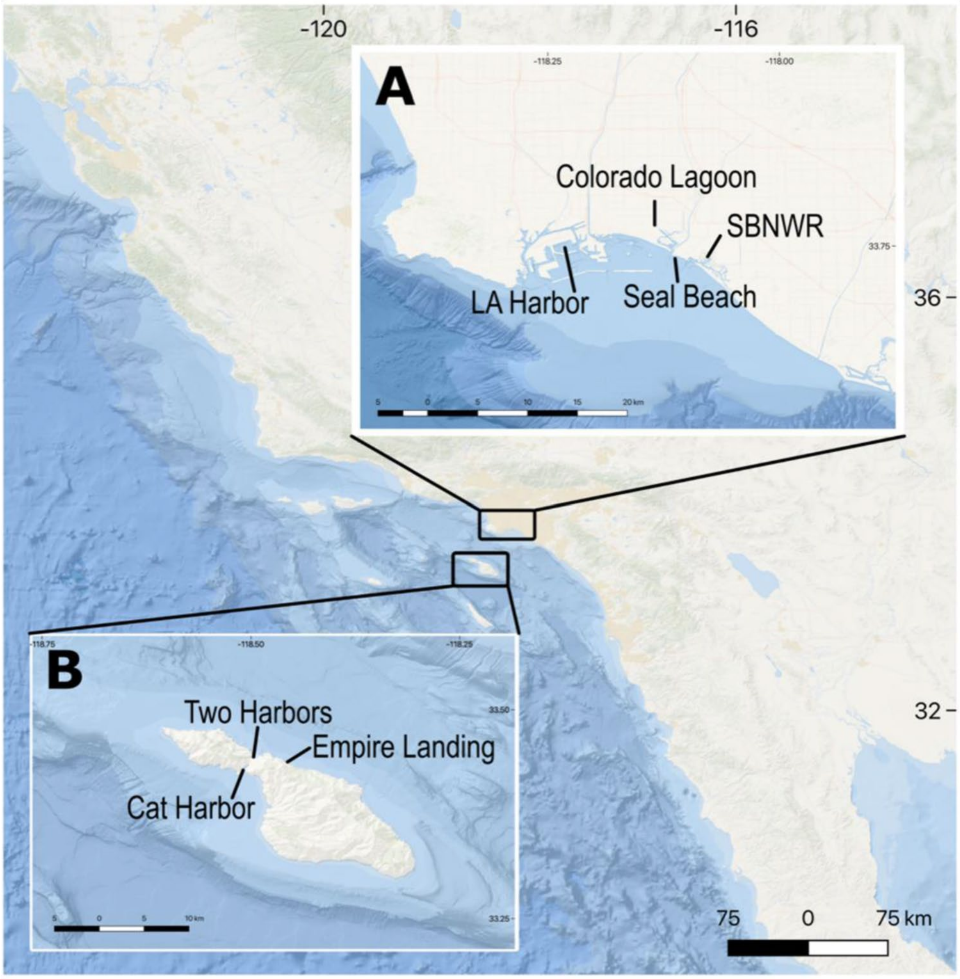

Fig. 1 Round Stingray (*Urobatis halleri*) were captured in 2010, 2011, 2014, and 2016 from **A** three sites along the southern coast of mainland California and from **B** three sites across Santa Catalina Island. The Catalina sampling locations consisted of Empire Landing, Two Harbors, and Cat Harbor. Mainland California sampling sites consisted of Los Angeles (LA) Harbor, Seal Beach National Wildlife Refuge (SBNWR), and Colorado Lagoon

Table 1 *Urobatis halleri* specimens ($n = 116$) collected from each location and subsampling site discriminated by sex and year of collection

Location	Sampling site	Sex	Year				Totals	
			2010	2011	2014	2016		
Catalina	Cat harbor	M	0	0	0	0	0	35
		F	2	6	27	0	35	
	Empire landing	M	0	0	18	0	18	22
		F	0	0	0	4	4	
	Two harbors	M	3	7	0	12	22	23
		F	0	0	0	1	1	
California	Colorado lagoon	M	0	0	0	0	0	2
		F	2	0	0	0	2	
	LA harbor	M	0	0	0	5	5	22
		F	0	0	0	17	17	
	Seal beach wildlife refuge	M	0	0	0	0	0	12
		F	1	11	0	0	12	
Total			8	24	45	39	116	

influenced, salt marshes—mainly from muddy substrates in the terminal back bay of Cat Harbor. Most mainland California females were collected from Seal Beach Wildlife Refuge, a federally restricted area with a highly anoxic silty substrate. Stingrays collected in 2016 were captured from sandy substrates of LA Harbor, a sheltered, tidally influenced bay.

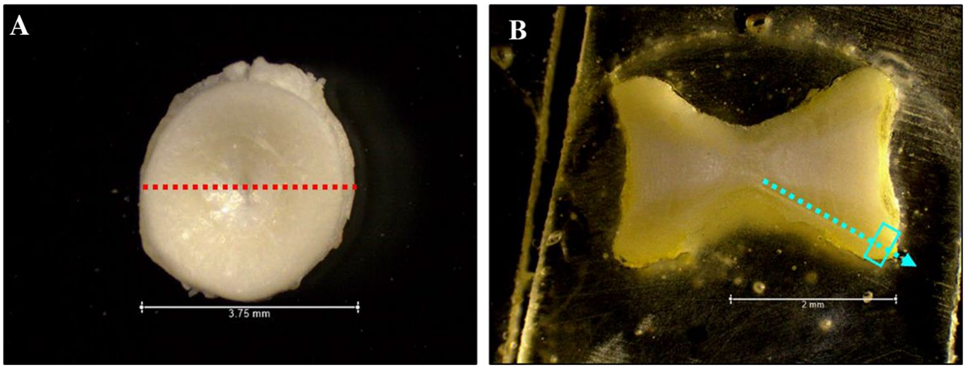

Fig. 2 **A** Whole vertebrae centrum from *Urobatis halleri.* The dotted red line represents the cross-section of the centrum that was sectioned. **B** *U. halleri* thin-sectioned (~ 1 mm) vertebral centrum. The dotted cyan arrow represents the laser ablation path on the corpus calcareum from the focus to the edge. The cyan box indicates the section of the vertebrae edge (40 µm) that was used to reflect the recent month before capture

Vertebral preparation and elemental analysis

Whole vertebrae centra were embedded in epoxy resin (Struers EpoFix, spiked with ~ 30 ppm Indium) and then sectioned using a low-speed saw (Buehler Isomet) to obtain longitudinal central cross-sections ~ 1 mm thick (Fig. 2a). Sections were mounted to glass slides using thermoplastic cement (Crystalbond) and lightly polished on 30 µm lapping film to obtain a smooth upper surface. Polished sections were then remounted on new microscope slides near other randomized specimen sections.

Elemental analysis of vertebrae sections was performed using laser ablation inductively coupled plasma mass spectrometry (LA–ICP–MS) at the University of Texas at Austin with an Elemental Scientific NWR193UC laser system with a large format sample cell, coupled to an Agilent 7500ce ICP-MS. Analytes included ^{7}Li, ^{24}Mg, ^{25}Mg, ^{43}Ca ^{44}Ca, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{115}In, ^{137}Ba, and ^{138}Ba. A series of test ablations were performed on representative vertebrae samples to optimize laser parameters. Vertebral transects started at the vertebrae focus and ran across the corpus calcareum to the outer radial edge (Fig. 2b). Certified reference standards (NIST 612, USGS MAPS-4 [synthetic bone], and USGS MACS-3 [pressed calcium carbonate]) were analyzed hourly in triplicate. To eliminate surface contamination before analysis, vertebrae and standard transects were pre-ablated using a 90 µm spot, 85% laser power (~ 4 J cm^{-2}), 20 Hz, and a scan rate of 75 µm s^{-1}. Ablation parameters included a 50 µm spot, 65% laser power, 10 Hz, and a scan rate of 5 µm s^{-1}. Laser energy densities over the analytical sessions averaged 3.07 ± 0.04 J cm^{-2} for line traverses. Baseline analyte intensity measurements were measured for 30 s with the laser before each analysis.

Analyte intensities were then converted to concentration (ppm) using Iolite software and assuming 35 weight % Ca as the internal standard with matrix-matched MAPS-4 as the primary calibration standard. Over the two days of LA-ICP-MS analysis, the overall average of secondary standard recovery fractions for all elements was 1.09 ± 0.15 for NIST 612 (n = 41) and 1.00 ± 0.06 for USGS MACS-3 (n = 40), relative to GeoREM preferred reference values (http://georem.mpch-mainz.gwdg.de). Elemental data was then converted to distance using the laser scan rate and smoothed by applying a 7-point median followed by a 7-point mean moving window. Indium intensities were only used to determine the position when the traverse first encountered epoxy resin, and thus the true vertebral "edge" position, as In is not naturally abundant in the environment (Chow and Snyder 1969).

Of the ten non-Ca analytes quantified, only eight were used for further analysis. Although ^{24}Mg and ^{138}Ba are potentially susceptible to overlaps (e.g., ^{12}C:^{12}Ca^{+}; ^{48}Ca^{2+}; ^{138}La, ^{138}Ce) compared to less abundant ^{25}Mg and ^{137}Ba, we utilized the former because no systematic differences were apparent in respective patterns for the less abundant isotopes.

Derived concentration (ppm) data were converted to mmol mol^{-1} (^{24}Mg, ^{88}Sr) and µmol mol^{-1} (^{7}Li, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{66}Zn, and ^{138}Ba) element/Ca ratios.

Transects provided continuous multielement profiles along the radial growth axes of the vertebrae, from the birthmark to the marginal edge, along the corpus calcareum, which should encompass lifetime growth through all experienced environments and physiological conditions. To assess variability in elemental signatures from known spatiotemporal references (i.e. points of capture), vertebral trace element profiles were only compared among stingrays for the last month of growth (Smith et al. 2016).

To determine how much vertebrae material is equivalent to a month of time, Round Stingray vertebrae precipitation rates needed to be calculated. Because vertebral precipitation rates are significantly affected by temperature for Round Stingray (Smith et al. 2013), we calculated an average precipitation rate over the various temperatures (15 °C, 18 °C, 24 °C) using rates determined by Smith et al. (2013). Hozo et al. (2005) found that the sample means and variances of a dataset can be described where not all the information is available. Using the methodology from Hozo et al. (2005), Table S1 from Smith et al. (2013) provided the ranges of precipitation rate for each temperature experiment while Fig. 6b provided mean vertebral precipitation rates for three replicate treatments during each temperature experiment. An estimated average precipitation rate of the replicate treatments from Fig. 6b was used as an estimated median value for this methodology. The approximate mean precipitation rate and standard deviation for each temperature were then calculated for each temperature and repeated to find an overall average precipitation rate, with the range being that of the entire temperature experiments and the median being substituted for the average of the temperature experiment means found previously. Through this calculation, the precipitation rate was 46.91 ± 27.81 μm month^{-1}. We then concluded that 40 μm would be a sufficiently conservative estimate for a month before an individual's capture and used this length to calculate last-month mean element/Ca ratios for each stingray. These were used to calculate overall average element/Ca ratios by sex, year, and location of capture (Table 2). A conservatively lower precipitation rate estimate was used to ensure a higher likelihood that individuals resided in the capture locality habitat for at least one month.

Statistical analysis

Even after logarithmic transformation, many element/Ca ratios lacked a normal distribution (Li, Mg, Co, Cu, Ba) and homogeneity of variance (Ba); therefore, nonparametric statistics were applied. A multi and single-element permutation multivariate analysis of variance (PERMANOVA) was run on all element/Ca ratios (Feitosa et al. 2020). Permutation analysis quantifies dissimilarity between each group of a population by converging on patterns through repeated randomized sampling from a distribution (Mantel 1967; Hope 1968; Anderson and Walsh 2013). We used a Euclidean distance resemblance matrix, 9999 permutations to generate pseudo-F and p values, and a significance level of 0.05 to evaluate multi and single-elemental factors that influence trace element signatures in Round Stingray vertebrae. Fixed categorical factors of year, location, and sex were tested separately to investigate variability in vertebral element/Ca. Multivariable tests with interactions were explored, but the low sample sizes available across combinations of year*location*sex limited the feasibility and complicated the interpretations of these tests. Finer-scale examination of female Stingray maturity status and pregnancy stage was also explored. Based on the resulting pseudo-F statistics and p values, a post hoc pairwise comparison was then used to determine which variables had a significant effect when considering the fixed factors. We used an alpha value of 0.05 for all statistical testing.

For significance discrimination, the null hypothesis in PERMANOVA is that there is no difference in the locations of groups in multivariate space (Anderson 2014). Therefore, the potential for Type 1 errors (finding a difference when none exists) depends on whether the dispersions of points in multivariate space are homogeneous. If this is not true, then the significance detected could be caused by differences in the dispersion of the centroids in multivariate space (Brooks et al. 2021), rather than an effect of the factor (location) being tested. Accordingly, a test for homogeneity of multivariate dispersions (PERMDISP) was also conducted in conjunction with the PERMANOVA to interpret the results. A PERMDISP compares within-group spread using the average value of the distances from individual observations to their group centroid (Anderson 2014). This can identify if the dispersion of points within a group is driving the significance, or if it is the group's centroid itself that is responsible for differences. All statistical analyses were performed in R (v4.0.3; R Foundation for Statistical Computing) using the *Vegan* package (Oksanen et al. 2022).

Results

Specimens in the sample collection were closely split between males and females (49% and 51%, respectively) (Table S2), with most specimens from Catalina (M: 70%; F: 68%) compared to the mainland (M: 30%; F: 32%). Catalina individuals were overwhelmingly mature, with 93% of males and 100% of females being sexually mature. Conversely, California mainland specimens were both mature and immature, with 47% of males and 84% of females being sexually mature. Of mature females, 80% of Catalina and 88% of California stingrays were in some stage of pregnancy. Across sampling sites and years, most specimens were collected in 2014 (39%), entirely from Catalina, while most mainland California Round Stingrays were collected in 2016 (85%) from LA Harbor, and in no other year were LA Harbor individuals collected.

In single factor analyses, all but three element/Ca ratios demonstrated homogeneous dispersion (Table 3). However, PERMANOVA results were not significant when investigating these element/Ca ratios with their respective factors (Table 4). Therefore, PERMANOVA and PERMDISP significance was not simultaneously observed for any element/Ca ratio within a single factor analysis. This suggests that all significant findings from the PERMANOVA were simply due to the dispersion of the data from their centroids. Therefore, even though homogeneous dispersion was rejected in three instances, Type 1 errors did not occur.

Table 2 Mean ± standard deviation (mmol mol^{-1} or μmol mol^{-1}) of element/Ca ratios discriminated by year, location, and sex for $n = 116$ *Urobatis halleri* vertebrae

Location	Year	Sex	n	Li/Ca (μmol mol^{-1})	Mg/Ca (mmol mol^{-1})	Mn/Ca (μmol mol^{-1})	Co/Ca (μmol mol^{-1})	Cu/Ca (μmol mol^{-1})	Zn/Ca (μmol mol^{-1})	Sr/Ca (mmol mol^{-1})	Ba/Ca (μmol mol^{-1})
Catalina	2010	M	3	20.2 ± 1.6	15.6 ± 2.6	72.0 ± 35.7	0.3 ± 0.1	20.7 ± 18.3	185.4 ± 47.4	3.1 ± 0.4	20.9 ± 10.4
		F	2	21.9 ± 1.6	16.3 ± 0.3	70.8 ± 3.1	0.2 ± 0.1	21.9 ± 24.3	194.3 ± 32.9	3.0 ± 0.1	17.6 ± 2.5
	2011	M	7	22.2 ± 7.0	16.6 ± 2.6	85.8 ± 64.0	0.3 ± 0.2	15.1 ± 22.2	205.5 ± 47.3	2.7 ± 0.2	11.9 ± 3.3
		F	6	24.0 ± 2.6	17.3 ± 1.9	167.8 ± 175.4	0.2 ± 0.1	6.7 ± 6.2	169.7 ± 52.8	3.1 ± 0.4	20.2 ± 16.0
	2014	M	18	16.7 ± 1.5	15.6 ± 1.1	80.3 ± 40.8	0.2 ± 0.1	8.5 ± 7.2	165.1 ± 42.6	2.8 ± 0.2	23.9 ± 13.2
		F	27	22.3 ± 8.2	15.7 ± 1.5	95.6 ± 58.7	0.4 ± 0.6	8.3 ± 5.4	203.9 ± 50.5	3.0 ± 0.2	11.9 ± 3.7
	2016	M	12	24.2 ± 5.0	16.0 ± 1.6	39.8 ± 19.8	0.2 ± 0.2	4.8 ± 2.5	190.0 ± 52.4	2.8 ± 0.2	13.3 ± 5.3
		F	5	24.0 ± 4.5	18.4 ± 1.0	68.0 ± 47.3	0.4 ± 0.2	11.2 ± 10.3	212.7 ± 57.8	3.0 ± 0.2	20.9 ± 12.2
California	2010	M	0	NA	NA	NA	NA	NA	NA	NA	NA
		F	3	21.4 ± 2.9	17.2 ± 2.6	40.5 ± 11.6	0.2 ± 0.1	21.4 ± 29.5	231.0 ± 63.6	2.8 ± 0.1	10.3 ± 4.1
	2011	M	0	NA	NA	NA	NA	NA	NA	NA	NA
		F	11	18.6 ± 3.4	15.3 ± 2.1	49.9 ± 41.4	0.2 ± 0.1	7.2 ± 6.5	226.8 ± 63.7	3.0 ± 0.3	10.9 ± 2.3
	2014	M	0	NA	NA	NA	NA	NA	NA	NA	NA
		F	0	NA	NA	NA	NA	NA	NA	NA	NA
	2016	M	17	21.0 ± 3.2	16.7 ± 2.0	14.3 ± 9.3	0.2 ± 0.1	15.7 ± 12.6	172.9 ± 59.2	3.0 ± 0.3	15.4 ± 4.9
		F	5	22.1 ± 4.6	18.8 ± 1.7	9.3 ± 2.2	0.2 ± 0.1	13.7 ± 15.0	152.7 ± 36.4	3.0 ± 0.4	15.5 ± 6.5

Untransformed element-to-calcium ratios are presented, but a $\log_{10}(x + 1)$ transformation was needed for statistical analysis

Table 3 Single-factor PERMDISP results with year, location, and sex as fixed factors

Element	Source	Sex			Location			Year		
		df	MS	*p*	df	MS	*p*	df	MS	*p*
Li/Ca	Model	1	0.0003	0.780	1	0.0217	**0.017**	3	0.0078	0.139
	Residuals	114	0.0040		114	0.0036		112	0.0041	
Mg/Ca	Model	1	0.0006	0.453	1	0.0012	0.288	3	0.0014	0.284
	Residuals	114	0.0011		114	0.0011		112	0.0010	
Mn/Ca	Model	1	0.0157	0.581	1	0.0680	0.138	3	0.0664	0.096
	Residuals	114	0.0534		114	0.0293		112	0.0302	
Co/Ca	Model	1	0.0091	0.150	1	0.0085	0.122	3	0.0027	0.553
	Residuals	114	0.0038		114	0.0037		112	0.0038	
Cu/Ca	Model	1	0.0070	0.690	1	0.0839	0.187	3	0.0797	0.132
	Residuals	114	0.0471		114	0.0440		112	0.0406	
Zn/Ca	Model	1	0.0000	0.900	1	0.0003	0.139	3	0.0000	0.943
	Residuals	114	0.0001		114	0.0001		112	0.0001	
Sr/Ca	Model	1	0.0000	0.735	1	0.0001	0.506	3	0.0009	**0.024**
	Residuals	114	0.0003		114	0.0003		112	0.0003	
Ba/Ca	Model	1	0.0523	0.059	1	0.0576	**0.037**	3	0.0244	0.221
	Residuals	114	0.0141		114	0.0145		112	0.0168	

Significant *p* values are bolded

Table 4 Single-factor PERMANOVA main-effects results with year, location, and sex as fixed factors with 9999 permutations

Element	Source	Sex			Location			Year		
		df	MS	*p*	df	MS	*p*	df	MS	*p*
Li/Ca	Model	1	0.0170	0.1917	1	0.0035	0.5489	3	0.0231	0.0598
	Residuals	114	0.0094		114	0.0095		112	0.0091	
Mg/Ca	Model	1	0.0011	0.5111	1	0.0037	0.2169	3	0.0072	**0.0283**
	Residuals	114	0.0024		114	0.0024		112	0.0023	
Mn/Ca	Model	1	0.9835	**0.0111**	1	7.1198	**0.0001**	3	2.3498	**0.0001**
	Residuals	114	0.1467		114	0.0928		112	0.0951	
Co/Ca	Model	1	0.0115	0.1283	1	0.0148	0.0713	3	0.0055	0.3186
	Residuals	114	0.0048		114	0.0047		112	0.0048	
Cu/Ca	Model	1	0.0515	0.4907	1	0.2985	0.0984	3	0.0000	0.1243
	Residuals	114	0.1085		114	0.1063		112	0.1089	
Zn/Ca	Model	1	0.0021	**0.0208**	1	0.000001	0.9592	3	0.0005	0.2766
	Residuals	114	0.0004		114	0.0004		112	0.0004	
Sr/Ca	Model	1	0.0047	**0.0151**	1	0.0026	0.0801	3	0.0003	0.7911
	Residuals	114	0.0008		114	0.0008		112	0.0009	
Ba/Ca	Model	1	0.2134	**0.0143**	1	0.0513	0.2255	3	0.0268	0.5134
	Residuals	114	0.0332		114	0.0346		112	0.0350	

Significant *p* values are bolded

Year

Only Mg/Ca and Mn/Ca showed significant differences among years (Table 4). A post hoc test for Mg/Ca revealed that there was only a significant difference between Mg/Ca in 2014 and 2016, whereas a post hoc test for Mn/Ca revealed that 2016 was significantly different when compared to all other years (Table S3). When comparing element/Ca ratios across years, 2016 had the highest Mg/Ca among all years (17.0 ± 2.0 mmol mol^{-1}), whereas 2014 had the lowest Mg/Ca among all years (15.6 ± 1.3 mmol mol^{-1}; Fig. 3; Table S4). Conversely, 2016 had the lowest Mn/Ca compared to all other sampling years (28.4 ± 27.7 µmol mol^{-1}).

Location

Between mainland California and Catalina sampling localities, Mn/Ca was the only vertebrae element/Ca ratio where location had a significant effect on concentration (Table 4). Catalina stingrays had significantly higher Mn/

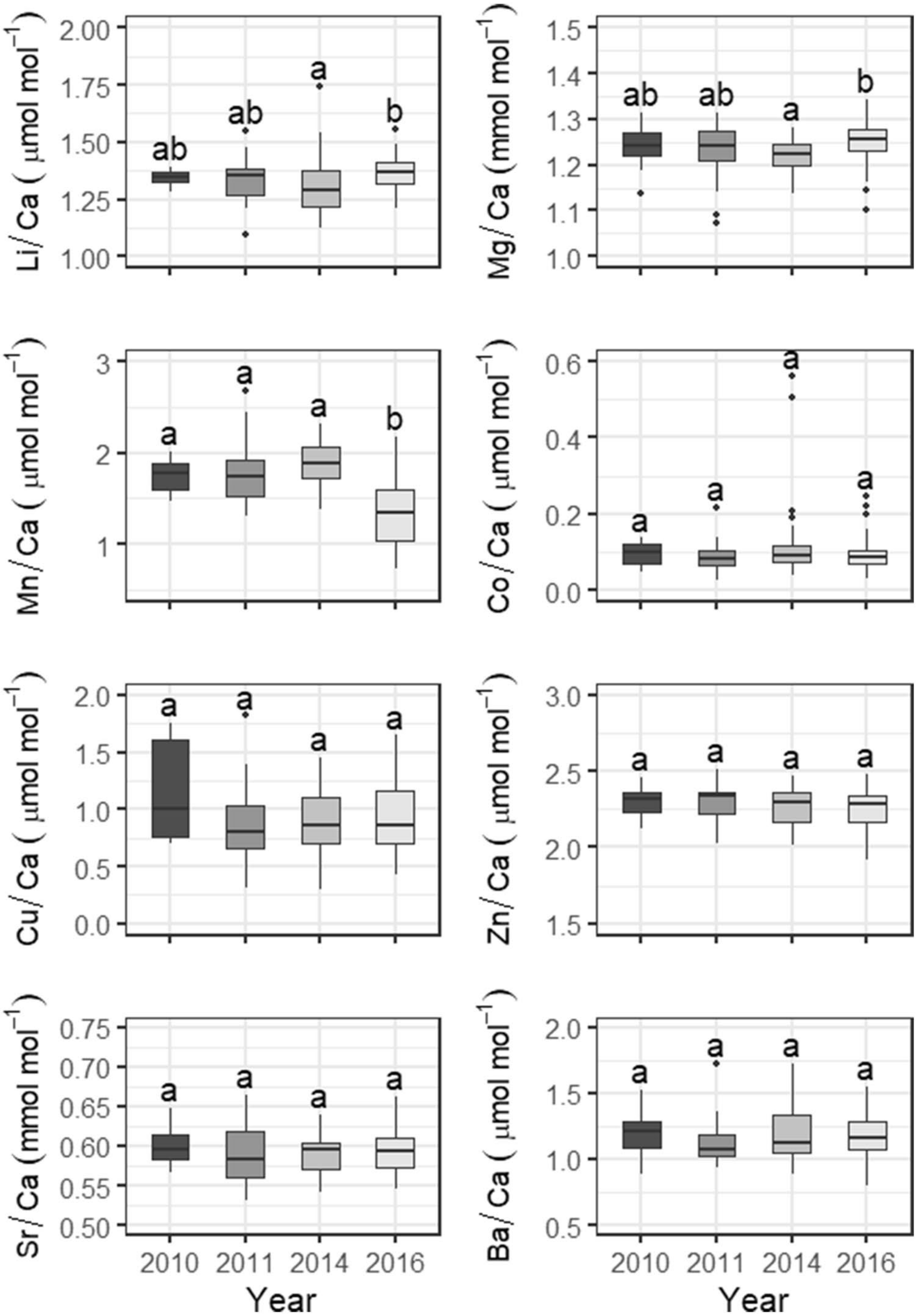

Fig. 3 Mean ± SD element ratios (mmol mol^{-1} or µmol mol^{-1}) in vertebrae edge from different years of collection. Different letters indicate statistically significant differences as seen in Table 4. The whiskers denote the upper and lower extremes and outliers are represented as dots outside of the whiskers. The median of the data is represented as the horizontal line within the plots. Element to Ca ratios were $\log_{10}(x+1)$ transformed

Ca (85.1 ± 69.7 µmol mol^{-1}) than mainland stingrays (26.7 ± 29.0 µmol mol^{-1}; Table S5). However, among mainland California sites LA Harbor, one of the busiest ports in the country, has a sandy substrate compared with muddy substrates at Seal Beach Wildlife Refuge and Colorado Lagoon. Therefore, a second PERMANOVA of location, reanalyzing all element/Ca ratios with LA Harbor as a third distinct location, was conducted. With LA Harbor separated, location had a significant effect on Mn/Ca, Zn/Ca, and Mg/Ca (Table 5). However, PERMDISP analysis was also significant for Mn/Ca, meaning that PERMANOVA significance may be due to the nonhomogeneous dispersion of group centroids. Ba/Ca was only marginally insignificant between sites ($p = 0.0505$).

A post hoc test for Mn/Ca revealed that all sampling locations were significantly different from each other (Table S6), with Catalina rays having the highest Mn/Ca (85.1 ± 69.7 µmol mol^{-1}) and LA Harbor the lowest (13.2 ± 8.4 µmol mol^{-1}; Table S7; Fig. 4). A post hoc test further revealed that vertebral Zn/Ca was significantly higher

Table 5 Single-factor PERMANOVA and PERMDISP results with location as a fixed factor with 9999 permutations

Element	Source	Location			PERMDISP
		df	MS	*p*	*p*
Li/Ca	Model	2	0.0098	0.3622	**0.044**
	Residuals	113	0.0094		
Mg/Ca	Model	2	0.0076	**0.0444**	0.471
	Residuals	113	0.0023		
Mn/Ca	Model	2	0.0077	**0.0001**	**0.006**
	Residuals	113	0.0005		
Co/Ca	Model	2	0.0090	0.1281	0.205
	Residuals	113	0.0047		
Cu/Ca	Model	2	0.0001	0.0765	0.233
	Residuals	113	2.22E-05		
Zn/Ca	Model	2	0.0020	**0.0068**	0.881
	Residuals	113	0.0004		
Sr/Ca	Model	2	0.0015	0.1695	0.893
	Residuals	113	0.0008		
Ba/Ca	Model	2	0.1021	0.0505	**0.018**
	Residuals	113	0.0335		

Location sites include mainland California (two subsites), Catalina Island (three subsites), and LA Harbor. Significant *p* values are bolded

in California Estuary stingrays compared to Catalina Island and LA Harbor rays, which exhibited significantly lower Zn/Ca. California Estuary rays had the highest Zn/Ca among all sites (227.7 ± 61.2 μmol mol^{-1}), whereas LA Harbor rays had the lowest Zn/Ca (168.3 ± 54.7 μmol mol^{-1}). A post hoc test revealed that the marginal insignificance for Ba/Ca was also most likely due to influence from the California Estuary rays, which were significantly lower (10.8 ± 2.6 μmol mol^{-1}) than Catalina and LA Harbor. Upon post hoc analysis, Mg/Ca was not significantly different between sites.

Sex

Maturity stage (immature/mature) was considered in the evaluation of sex as a significant factor in vertebral trace element concentrations. Only Zn/Ca showed significant differences between maturity stages; therefore, further Zn/Ca analysis compared individuals of the same maturity status. Among mature and immature individuals, Zn/Ca was not significantly different between sexes. However, mature males tended to have higher Zn/Ca (143.8 ± 45.0 μmol mol^{-1}) compared to immature males (134.3 ± 35.2 μmol mol^{-1}; Table S8). Conversely, mature females tended to have lower Zn/Ca (188.0 ± 48.8 μmol mol^{-1}) compared to immature females (205.8 ± 53.9 μmol mol^{-1}).

Sex was a significant factor for three element/Ca ratios when immature and mature individuals were pooled (Table 4). Pooled females tended to have significantly higher Mn/Ca (81.1 ± 79.1 μmol mol^{-1}) than pooled males (52.3 ± 44.5 μmol mol^{-1}; Table S9; Fig. 5). Average female Sr/Ca (3.0 ± 0.3 mmol mol^{-1}) was also significantly higher compared to males (2.9 ± 0.3 mmol mol^{-1}). Conversely, Ba/Ca was significantly higher in males (17.5 ± 9.6 μmol mol^{-1}) compared to females (13.7 ± 7.5 μmol mol^{-1}).

Among mature females, stage of pregnancy did not affect element/Ca ratios. However, interestingly, females that were in any stage of pregnancy had the highest average Zn/Ca (216.6 ± 55.0 μmol mol^{-1}), followed by females that were ovulating or close to ovulating (191.0 ± 53.0 μmol mol^{-1}), and lastly by individuals that were not pregnant (186.7 ± 46.2 μmol mol^{-1}).

Discussion

In this study, we explored how intrinsic (sex) and extrinsic (location and year) factors relate to Round Stingray vertebral element/Ca ratios. Vertebral Mn/Ca, Zn/Ca, Sr/Ca, and Ba/Ca all significantly differed between males and females. Capture location also contributed to variation in Mg/Ca, Mn/Ca, and Zn/Ca, especially after LA Harbor was assigned as a distinct sampling site. Sampling year contributed to significant variation in Mg/Ca and Mn/Ca, with 2016 heavily influencing this variation.

This data collection was retroactively created from multiple studies of opportunistically collected specimens. Therefore, while this study has a robust sample size often uncommon for elasmobranchs, uneven sample distribution among the investigated factors was a challenge beyond our control. For example, all 2016 mainland stingrays were caught in LA Harbor. This sampling could have inadvertently created collinearity between the year and location variables which may explain why 2016 element/Ca ratios appear to differ from other years with more even sampling among locations. Because of this, we do not believe there is enough evidence to conclude that interannual effects caused significant differences in vertebral signatures. Future studies from more representative, nonopportunistic specimen collections should facilitate a more robust multivariable analysis of factors influencing vertebral trace element signatures.

Despite uneven sampling efforts across years, our results suggest that the effect of both intrinsic (i.e., sex) and extrinsic (i.e., location) factors on vertebral signatures may be linked to the same behavioral aspect: sexual segregation. Mature, pregnant females have been observed to partially segregate from mature males for much of a year (Babel 1967; Jirik and Lowe 2012). Gestating females are typically found in dense aggregations within estuarine habitats during peak summer months when warm water temperatures likely speed embryonic development (Jirik

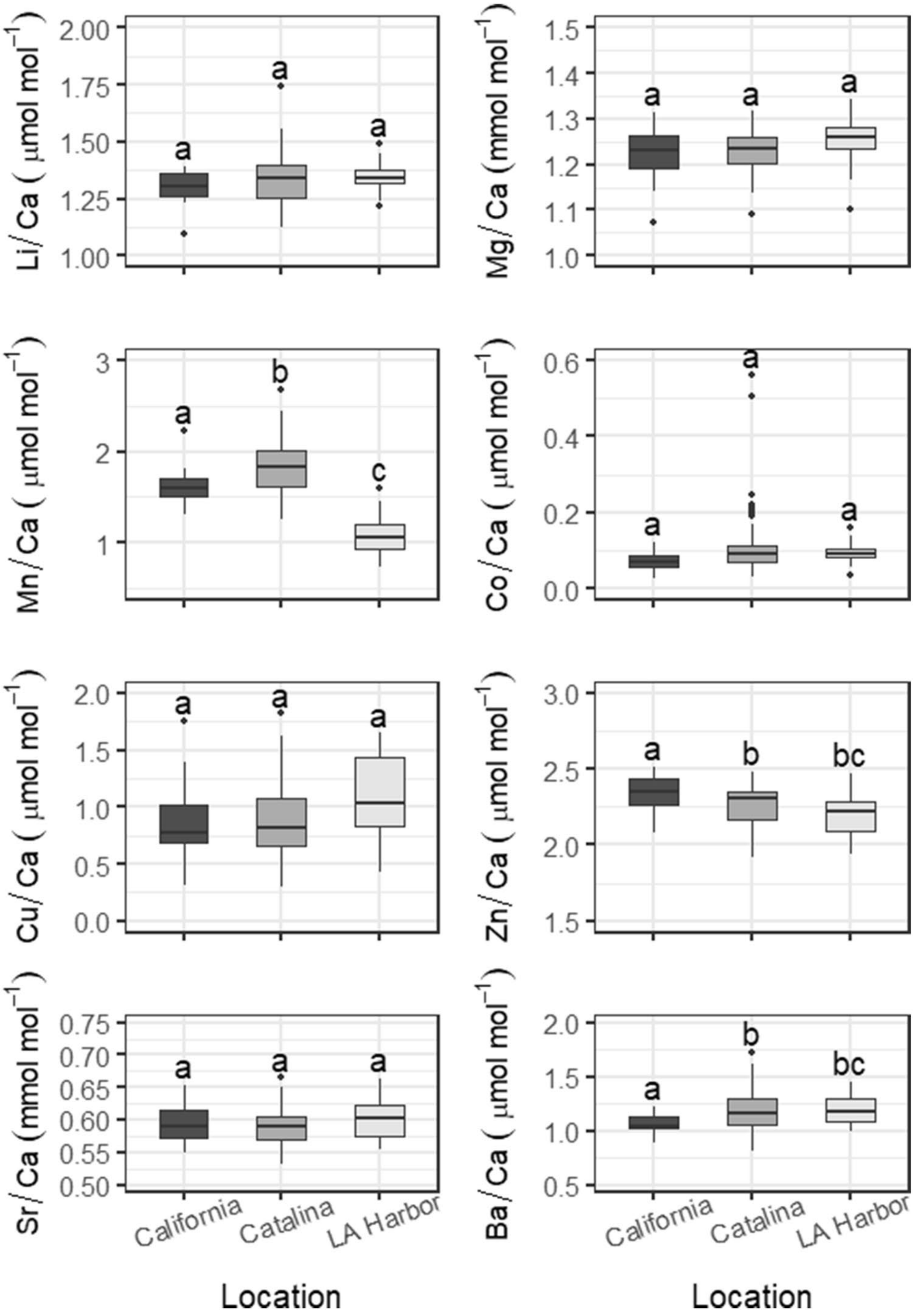

Fig. 4 Mean ± SD element ratios (mmol mol^{-1} or µmol mol^{-1}) in vertebrae edge from different sampling locations. Different letters indicate statistically significant differences as seen in Table 5. Element to Ca ratios were $\log_{10}(x+1)$ transformed

and Lowe 2012). Mature females in our study were almost entirely caught in Cat Harbor on Catalina (63%) and Seal Beach Wildlife Refuge on mainland California (21%). These sites are similarly sheltered from wave activity and possess environmental characteristics that are suitable for Round Stingray gestation, including isolated warm waters and correlated fine sedimentation (Straughan and Hadley 1980; Vaudo and Lowe 2006). By contrast, male Round Stingrays tend to remain in coastal, open waters (Jirik and Lowe 2012) with sampling sites such as Two Harbors (Straughan and Hadley 1980), Empire Landing (Hight and Lowe 2007; Schumann et al. 2012), and LA Harbor all possessing similar course, sandy bottom substrate.

Contrasts in habitat substrates and associated water mass chemistries may explain discrepancies in vertebral element/Ca ratios between specimen groupings (Smith et al. 2013). Below we assess the environmental proxy potential for Mn/Ca, Zn/Ca, Sr/Ca, and Ba/Ca, which showed the most significant variability among specimen groups.

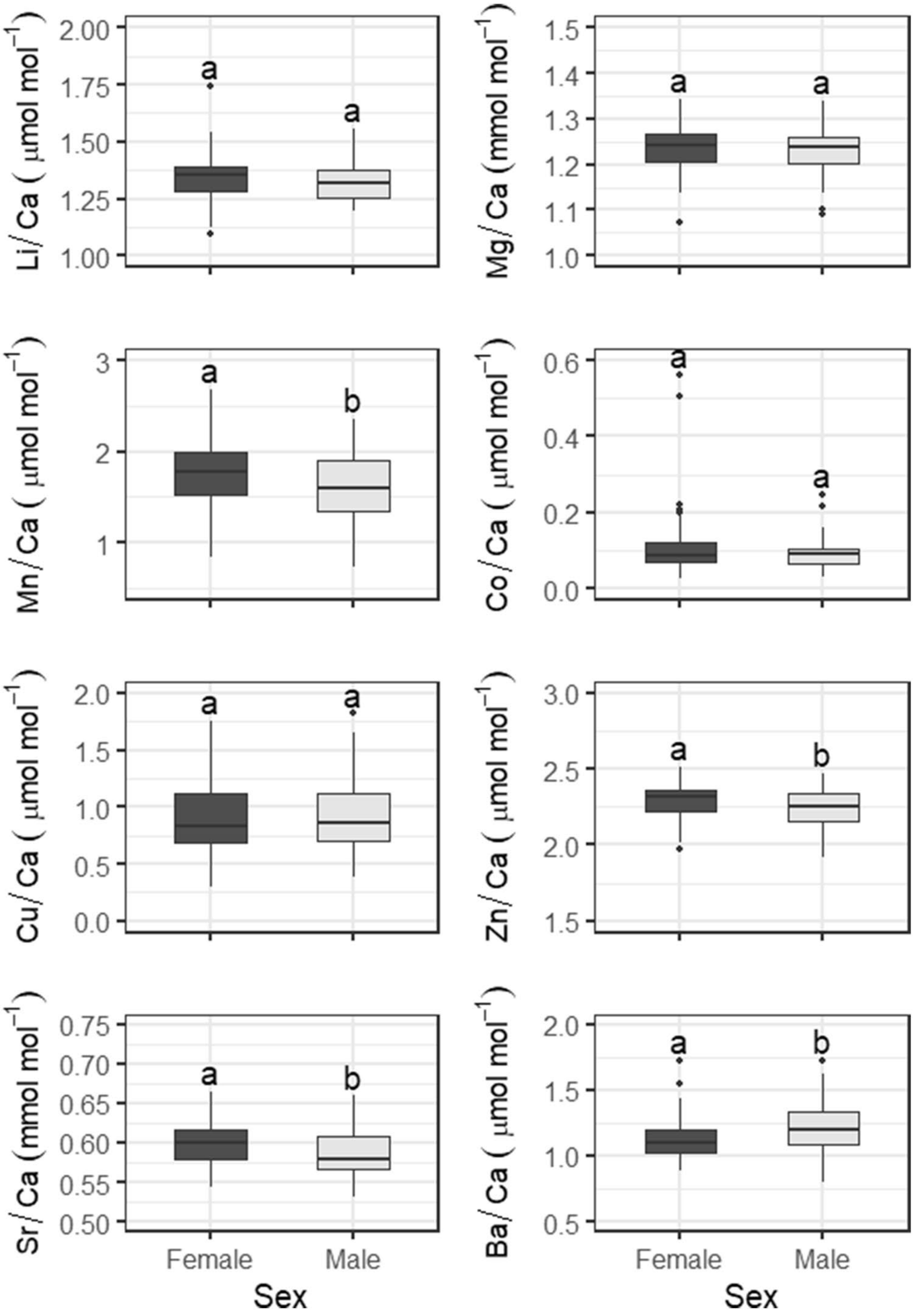

Fig. 5 Mean ± SD element ratios (mmol mol^{-1} or µmol mol^{-1}) in vertebrae edge from different sexes. Different letters indicate statistically significant differences as seen in Table 4. Element to Ca ratios were $\log_{10}(x+1)$ transformed

Mn/Ca

Vertebral Mn/Ca was the most consistent discriminator among sample groups. Sex and location were correlated with significant effects on vertebral Mn/Ca, with female rays and Catalina rays having 1.6x and 3.2x higher Mn/Ca than male rays and California rays, respectively. Manganese is an essential trace metal required for amino acid, lipid, protein, and carbohydrate metabolism and growth and development of bones and vertebrae in fish (Pancaldi et al. 2021). Manganese also plays a role in the activation of reproductive hormones (Feitosa et al. 2020). In this study, 84% of mature female Round Stingrays (and 74% of all females) were pregnant or reproductively active, and we found females to have 1.6x higher Mn/Ca than males. This suggests that female metabolic activity may require higher Mn/Ca during reproduction. Increased Mn has been shown to occur in other fish species during reproduction. For example, due to differing metabolic activities, female Redspot Emperor Fish (*Lethrinus lentjan*) had higher tissue metal concentrations than males (Al-Yousuf et al. 2000). Several other fish species that inhabit marine and freshwater habitats in temperate and tropical regions and use a variety of spawning methods have elevated Mn/Ca in otolith cores, possibly due to maternal offloading to the egg during reproduction (Brophy et al. 2004; Ruttenberg et al. 2005).

Differences in habitat along with reproductive biology may also contribute to Mn/Ca variation between males and females. Mn and Fe reduction in marine sediments play critical roles in biogeochemical cycles (Burdige 1993), and rare earth element concentrations are strongly enriched in pore water below the sediment–seawater interface relative to seawater (Elderfield and Sholkovits 1987). Fine-grained sediment deposits possess a high capacity to absorb or precipitate both oxidized and reduced metals, whereas sandy marine sediments usually contain less Mn available for biogeochemical transformation due to the coarse structure, high quartz, and low clay content (Kristiansen et al. 2002). For example, Skornyakova (1965) found that Mn concentrations in (Pacific) ocean sediments strongly correlate with sediment grain size, with concentrations increasing from sands to silt and mud. Substrate type could thus further explain why female Round Stingrays, which often reside in muddy salt marshes, had nearly 3.2x higher vertebral Mn/Ca compared to males, which often reside in coarse, sandy bottom habitats.

Temperature differences at sampling locations could also contribute to higher vertebral Mn/Ca values, as Mn incorporation is positively correlated with temperature in Round Stingray (Smith et al. 2013) as well as in Port Jackson Shark (*Heterodontus portusjacksoni*) (Pistevos et al. 2019) vertebrae. Females may have experienced higher temperatures in shallower sheltered estuaries, which aid gestation (Jirik and Lowe 2012), compared to deeper open coastline environments where males were sampled. Mull et al. (2008) states that Seal Beach Wildlife Refuge reaches water temperatures of 25 °C during the summer months, whereas the National Data Buoy Canter (NDBC) Long Beach Channel (buoy 46256) temperatures only approach an average of 20 °C in summers.

Diet has been experimentally determined to contribute > 90% of Mn accumulation in the soft tissue of Lesser Spotted Dogfish (*Scyliohinus canicular*) (Mathews and Fisher 2009), and may also contribute to Mn concentrations in other elasmobranch species (e.g., Pentreath 1973). Because dissolved Mn is scarce in oxic seawater, Smith et al. (2013) hypothesized that diet explains elevated Mn/Ca in Round Stingray vertebrae. Bivalves are the important dietary constituents of Round Stingrays, with shell fragments commonly found in the stomachs of large rays (Babel 1967). Because the Mn concentration in mollusk shells is thought to follow levels in ambient seawater (e.g., Barats et al. 2009), vertebral Mn/Ca may follow consumption rates of shelled prey items and/or vertebral incorporation rates of Mn. These factors could further vary by location. For example, invertebrate prey inhabiting anoxic sediments with high dissolved Mn^{2+} concentrations (such as Cat Harbor or Seal Beach Wildlife Refuge) could have higher tissue Mn concentrations compared to those inhabiting open coast sandy substrates. Higher Mn concentrations in prey items at one location over another, reinforced by sexual segregation and temperature, could explain why female Round Stingray vertebrae had significantly higher vertebral Mn/Ca compared to males.

Zn/Ca

Vertebral Zn/Ca differed substantially between sexes, with females being 1.1x higher than males, potentially due to physiological differences. Zinc is an essential trace element for a diverse array of biological and physiological processes including catalysis, hormonal mechanisms, cell regulation, and homeostasis, as well as playing a role in reproductive, gastrointestinal, neural, and endocrine systems (Williams 1984; Favier 1992; Vallee and Falchuk 1993; Stefanidou et al. 2006). Zinc is especially crucial for the embryonic development of multicellular organisms, and Zn deficiencies during pregnancy decrease embryo survival for fish, birds, and mammals, including humans (Vallee and Falchuk 1993). A reproductive performance study found that Nile Tilapia (*Oreochromis niloticus*) had a significantly larger spawn population per capita when taking Zn dietary supplements (Gammanpila et al. 2010).

Zn and other trace elements are critical for embryonic growth and development. Because Zn is an important cofactor of collagen proteins, including otolin-1, and is essential for yolk formation (vitellogenesis), Zn and Ca are the elements thought to most likely reveal potential reproductive cycles (Reis-Santos et al. 2022). The reproductive importance of Zn for females could explain the notably higher vertebral Zn/Ca levels over males in Round Stingray. An investigation of muscle and liver tissue of the Smalleye Smooth-Hound Shark (*Mustelus higmani*) found that Zn is offloaded at the highest concentrations from mother to offspring (de Souza-Araujo et al. 2020). Raoult et al. (2018) found high prebirth vertebral Zn/Ca in ten shark species, which may be related to high Zn concentrations in gestating mothers. Sturrock et al. (2015) found sex-specific differences in otolith element concentrations for Zn/Ca in European plaice (*Pleuronectes platessa* L.), which they attributed to processes regulating blood protein and Ca concentrations in marine fishes (such as gonad maturation) modifying Ca homologue concentrations, and in turn, their element/Ca ratios in tissues (Kalish 1991). This possibility is especially relevant for our study, as female rays were pregnant and reproductively active whereas males were collected in their quiescent phases (outside of their reproductive cycle). Our results show that female stingrays that were in any stage of pregnancy had the highest vertebral Zn/Ca values, followed by ovulating females. Vertebral Zn/Ca in females that were not reproductively active was 1.2x lower than in pregnant females. These findings support the hypothesis that vertebral Zn/Ca enrichment may partially follow physiological processes that occur during maturity, specifically reproduction.

Vertebral Zn/Ca also varied by location, with California estuary stingrays higher than Catalina (by 1.2x) and LA Harbor (by 1.4x) rays. The reason for this variation could be related to the spatial sex distribution of the specimen collections since most California estuary specimens were pregnant females and most LA Harbor rays were nonreproductive males. Temperature variations among the collection sites are another possible controlling factor since vertebral Zn/Ca has also been shown to follow temperature (Smith et al. 2013). The higher temperatures experienced by California estuary rays compared to their LA Harbor counterparts could thus potentially also account for enhanced Zn vertebral concentrations.

Although vertebral Zn/Ca could serve as a proxy of the ambient marine environment, specific pathways and mechanisms for Zn uptake in vertebral hydroxyapatite are uncertain and complicated (Smith et al. 2013). For example, Zn/Ca contrasts between sexes such as observed in our study are inconsistent across studies of other fish. For example, Barrera-García et al. (2013) found sex to be a significant factor in liver and kidney tissue Zn concentrations in Blue Sharks (*Prionace glauca*), but with males enriched compared to females. These differences were attributed to prey preferences between sexes and seasonal shifts in diet. Consistent with our findings, Al-Yousuf et al. (2000) found higher Zn concentrations in female liver and skin tissues compared to males for Redspot Emperor Fish (*Lethrinus lentjan*), which they attributed to hormones and the available number of active sites in the protein and cytochrome P-450 in female versus male fish. Finally, Endo et al. (2008) found no differences between sexes in tissue trace element concentrations (including Zn) among Tiger Sharks (*Galeocerdo cuvier*). The fact that vertebral Zn variations have been demonstrated within and among species suggests that physiological processes that drive vertebral development are highly varied (Raoult et al. 2018). This inconsistency complicates interpretations of how vertebral Zn/Ca levels in Round Stingrays relate to environmental and physiological processes.

Sr/Ca

Vertebral Sr/Ca differed significantly between sexes, despite being only 1.05x higher in female Round Stingrays. Unlike Mn/Ca and Zn/Ca, Sr/Ca has not been demonstrated to play a physiological role in fish (Chowdhury and Blust 2011; Smith et al. 2013). However, Sr may substitute for Ca during biomineralization (both are chemically similar Group II elements with similar ionic radii of 114 and 132 pm, respectively), in relation to its environmental availability (Lewis et al. 2016). The higher concentrations of Sr in seawater (~ 8 ppm) compared to freshwater (< < 1 ppm) environments (Musgrove 2021) facilitate its use as a salinity proxy for habitats experienced through life. For example, vertebral Sr/Ca has been used to document movement patterns of the euryhaline Bull Shark (*Carcharhinus leucas*) (Tillett et al. 2011). Although temperature has previously not been shown to affect vertebral Sr/Ca in Round Stingray (Smith et al. 2013), a positive relationship in the Port Jackson Shark (*Heterodontus portusjacksoni*) may indicate that vertebral Sr/Ca is sensitive to interspecific differences in physiological, metabolic, or kinetic factors between individuals (Pistevos et al. 2019).

Higher vertebral Sr/Ca in female Round Stingrays in our study may proxy more elevated temperature and salinity levels in sheltered salt marshes and estuaries. Seal Beach Wildlife Refuge, for example, experiences daily temperature fluctuations that can approach 10 °C in a single tidal cycle (Hoisington and Lowe 2005; Mull et al. 2008). Higher evaporation rates in this shallow marine habitat may be associated with higher salinity levels, leading to higher concentrations in vertebral Sr/Ca. Higher temperatures and associated increased growth rates could also explain females having higher vertebrae Sr/Ca compared to males, as Pistevos et al. (2019) found temperature may be an important control of elemental uptake in calcified tissue.

Ba/Ca

We found significant vertebral Ba/Ca differences by sex and location, with male stingrays 1.3x higher than females and LA Harbor and Catalina rays higher than California estuary rays by factors of 1.4 and 1.5, respectively. Like Sr, Ba (149 pm radii) can also substitute for Ca in calcified biomineral phases. Unlike Sr, however, Ba seawater concentrations are inversely related to salinity (Lewis et al. 2016). Smith et al. (2013) found Round Stingray vertebral Ba/Ca followed ambient seawater concentrations but also strongly decreased with temperature. High Ba, low-temperature environmental conditions have previously been linked with upwelling signatures in shark vertebrae (Mohan et al. 2018).

The low vertebral Ba/Ca levels in California estuary stingrays may follow from higher salinities and/or temperature ranges than occur in Catalina Island and LA Harbor. The higher vertebral Ba/Ca of male Round Stingrays inhabiting the coastal environment suggests that they experience lower temperatures and/or salinity levels, and possibly also upwelling-associated water masses (Mohan et al. 2018). Feitosa et al. (2020) similarly found relatively elevated Sr/Ca in females and Ba/Ca in males for Smalltail Shark (*Carcharhinus porosus*) vertebrae but could not attribute variations to environmental differences.

Conclusion

Round Stingray vertebrae compositions, standardized to the final month of growth, support that trace element signatures are likely influenced by sexual segregation, compared to location and year of capture. Differences in vertebral Mn/Ca, Zn/Ca, Sr/Ca, and Ba/Ca may be explained by physiological drivers of sexual segregation (i.e., pregnancy in females) leading to spatial separation of males and females within distinct habitats having differing water mass and sediment characteristics. Element/Ca differences among sex and location groups generally support that females experience higher temperatures and/or salinities. Certainly, further studies from more representative specimen collections should be conducted to pinpoint the extent to which physiological pathways and mechanisms influence vertebral uptake in elasmobranchs compared to environmental factors. Nonetheless, our study demonstrates the potential of vertebral element/Ca signatures to proxy habitats experienced and patterns of behavioral movement in elasmobranchs.

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Data availability The datasets generated during and/or analyzed during the current study are not publicly available due to further research being conducted, but are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed. All samples used for this study were obtained from dead specimens of Round Stingrays collected from other previous research studies.

References

Al-Yousuf MH, El-Shahawi MS, Al-Ghais SM (2000) Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. Sci Total Environ 256:87–94. https://doi.org/10.1016/S0048-9697(99)00363-0

Anderson MJ (2014) Permutational multivariate analysis of variance (PERMANOVA). Wiley StatsRef Stat Ref. https://doi.org/10.1002/9781118445112.stat07841

Anderson MJ, Walsh DCI (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecol Monogr 83:557–574. https://doi.org/10.1890/12-2010.1

Babel JS (1967) Reproduction, life history, and ecology of the round stingray Urolophus halleri. Toxicon. https://doi.org/10.1016/0041-0101(68)90079-2

Barats A, Amouroux D, Chauvaud L, Pécheyran C, Lorrain A, Thébault J, Church TM, Donard OFX (2009) High frequency barium profiles in shells of the great scallop *Pecten maximus*: a methodical long-term and multi-site survey in Western Europe. Biogeosciences 6:157–170. https://doi.org/10.5194/bg-6-157-2009

Barrera-García A, O'Hara T, Galván-Magaña F, Méndez-Rodríguez LC, Castellini JM, Zenteno-Savín T (2013) Trace elements and oxidative stress indicators in the liver and kidney of the blue shark (*Prionace glauca*). Comp Biochem Physiol Mol Integr Physiol 165:483–490. https://doi.org/10.1016/j.cbpa.2013.01.024

Brooks ML, Lovvorn JR, Behnke JH, Anderson EM (2021) Detecting silent stressors: Trace element effects on nutritional status of declining scoter ducks of Puget Sound, USA. Sci Total Environ 766:144247. https://doi.org/10.1016/j.scitotenv.2020.144247

Brophy D, Jeffries TE, Danilowicz BS (2004) Elevated manganese concentrations at the cores of clupeid otoliths: Possible environmental, physiological, or structural origins. Mar Biol 144:779–786. https://doi.org/10.1007/s00227-003-1240-3

Burdige DJ (1993) The biogeochemistry of manganese and iron reduction in marine sediments. Earth Sci Rev 35:249–284. https://doi.org/10.1016/0012-8252(93)90040-E

Campana SE, Thorrold SR (2001) Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can J Fish Aquat Sci 58:30–38. https://doi.org/10.1139/cjfas-58-1-30

Chow TJ, Snyder CB (1969) Indium content of sea water. Earth Planet Sci Lett 7:221–223. https://doi.org/10.1016/0012-821X(69)90054-5

Chowdhury MJ, Blust R (2011) Strontium. Fish Physiol 31:351–390. https://doi.org/10.1016/S1546-5098(11)31029-1

Coiraton C, Amezcua F, Ketchum JT (2020) New insights into the migration patterns of the scalloped hammerhead shark *Sphyrna lewini* based on vertebral microchemistry. Mar Biol 167:1–18. https://doi.org/10.1007/s00227-020-3668-0

de Souza-Araujo J, Andrades R, de Oliveira LM, Hussey NE, Giarrizzo T (2020) Maternal and embryonic trace element concentrations and stable isotope fractionation in the smalleye smooth-hound (*Mustelus higmani*). Chemosphere 257:127183. https://doi.org/10.1016/j.chemosphere.2020.127183

Dean MN, Summers AP (2006) Mineralized cartilage in the skeleton of chondrichthyan fishes. Zoology 109:164–168. https://doi.org/10.1016/j.zool.2006.03.002

Dulvy NK, Simpfendorfer CA, Davidson LNK, Fordham SV, Bräutigam A, Sant G, Welch DJ (2017) Challenges and priorities in shark and ray conservation. Curr Biol 27:R565–R572. https://doi.org/10.1016/j.cub.2017.04.038

Elderfield H, Sholkovits ER (1987) Rare earth elements in the pore waters of reducing nearshore sediments. Earth Planet Sci Lett 82:280–288. https://doi.org/10.1016/0012-821X(87)90202-0

Endo T, Hisamichi Y, Haraguchi K, Kato Y, Ohta C, Koga N (2008) Hg, Zn and Cu levels in the muscle and liver of tiger sharks (*Galeocerdo cuvier*) from the coast of Ishigaki Island, Japan: Relationship between metal concentrations and body length. Mar Pollut Bull 56:1774–1780. https://doi.org/10.1016/j.marpolbul.2008.06.003

Favier AE (1992) The role of zinc in reproduction-Hormonal mechanisms. Biol Trace Elem Res 32:363–382. https://doi.org/10.1007/BF02784623

Feitosa LM, Dressler V, Lessa RP (2020) Habitat use patterns and identification of essential habitat for an endangered coastal shark with vertebrae microchemistry : the case study of *Carcharhinus porosus*. Front Mar Sci 7:125. https://doi.org/10.3389/fmars.2020.00125

Gammanpila M, Yakupitiyage A, Bart A (2010) Evaluation of the effects of dietary vitamin C, E and Zinc supplementation on reproductive performance of Nile tilapia (*Oreochromis niloticus*). Sri Lanka J Aquat Sci 12:39. https://doi.org/10.4038/sljas.v12i0.2213

Guttridge TL, Gruber SH, Krause J, Sims DW (2010) Novel acoustic technology for studying free-ranging shark social behaviour by recording individuals' interactions. PLoS ONE 5:e9324. https://doi.org/10.1371/journal.pone.0009324

Hale LF, Lowe CG (2008) Age and growth of the round stingray *Urobatis halleri* at Seal Beach, California. J Fish Biol 73:510–523. https://doi.org/10.1111/j.1095-8649.2008.01940.x

Hale LF, Dudgeon JV, Mason AZ, Lowe CG (2006) Elemental signatures in the vertebral cartilage of the round stingray, Urobatis halleri, from Seal Beach, California. Environ Biol Fishes 77:317–325. https://doi.org/10.1007/s10641-006-9124-2

Hight BV, Lowe CG (2007) Elevated body temperatures of adult female leopard sharks, *Triakis semifasciata*, while aggregating in shallow nearshore embayments: Evidence for behavioral thermoregulation? J Exp Mar Bio Ecol 352:114–128. https://doi.org/10.1016/j.jembe.2007.07.021

Hoisington G IV, Lowe CG (2005) Abundance and distribution of the round stingray, *Urobatis halleri*, near a heated effluent outfall. Mar Environ Res 60:437–453. https://doi.org/10.1016/j.marenvres.2005.01.003

Hope ACA (1968) A simplified monte carlo significance test procedure. J R Stat Soc Ser B 30:582–598. https://doi.org/10.1111/j.2517-6161.1968.tb00759.x

Hozo SP, Djulbegovic B, Hozo I (2005) Estimating the mean and variance from the median, range, and the size of a sample. BMC Med Res Methodol 5:1–10. https://doi.org/10.1186/1471-2288-5-13

Izzo C, Doubleday ZA, Grammer GL, Gilmore KL, Alleway HK, Barnes TC, Disspain MCF, Giraldo AJ, Mazloumi N, Gillanders BM (2016a) Fish as proxies of ecological and environmental change. Rev Fish Biol Fish 26:265–286. https://doi.org/10.1007/s11160-016-9424-3

Izzo C, Huveneers C, Drew M, Bradshaw CJA, Donnellan SC, Gillanders BM (2016b) Vertebral chemistry demonstrates movement and population structure of bronze whaler. Mar Ecol Prog Ser 556:195–207. https://doi.org/10.3354/meps11840

Jirik KE, Lowe CG (2012) An elasmobranch maternity ward: female round stingrays *Urobatis halleri* use warm, restored estuarine habitat during gestation. J Fish Biol 80:1227–1245. https://doi.org/10.1111/j.1095-8649.2011.03208.x

Kalish JM (1991) Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus*. Mar Ecol Prog Ser 74:137–159. https://doi.org/10.3354/meps074137

Kristiansen KD, Kristensen E, Jensen EMH (2002) The influence of water column hypoxia on the behaviour of manganese and iron in sandy coastal marine sediment. Estuar Coast Shelf Sci 55:645–654. https://doi.org/10.1006/ecss.2001.0934

Lewis JP, Patterson WF, Carlson JK, McLachlin K (2016) Do vertebral chemical signatures distinguish juvenile blacktip shark (*Carcharhinus limbatus*) nursery regions in the northern Gulf of Mexico? Mar Freshw Res 67:1014–1022. https://doi.org/10.1071/MF15088

Lyons K, Wynne-Edwards KE (2021) Sublethal, sex-specific, osmotic, and metabolic impairments in embryonic and adult round stingrays from a location exposed to environmental contamination in southern California, USA. Environ Sci Pollut Res 28:27493–27510. https://doi.org/10.1007/s11356-021-12546-0

Lyons K, Lavado R, Schlenk D, Lowe CG (2014) Bioaccumulation of organochlorine contaminants and ethoxyresorufin-o-deethylase activity in southern california round stingrays (*Urobatis halleri*) exposed to planar aromatic compounds. Environ Toxicol Chem 33:1380–1390. https://doi.org/10.1002/etc.2564

Lyons K, Burket SR, Brooks BW (2018) Sex may influence environmental diphenhydramine accumulation in round Stingrays. Mar Pollut Bull 135:648–653. https://doi.org/10.1016/j.marpolbul.2018.07.042

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220. https://doi.org/10.1038/124844a0

Mathews T, Fisher NS (2009) Dominance of dietary intake of metals in marine elasmobranch and teleost fish. Sci Total Environ 407:5156–5161. https://doi.org/10.1016/j.scitotenv.2009.06.003

McMillan MN, Izzo C, Wade B, Gillanders BM (2017) Elements and elasmobranchs: hypotheses, assumptions and limitations of elemental analysis. J Fish Biol 90:559–594. https://doi.org/10.1111/jfb.13189

Mohan JA, Miller NR, Herzka SZ, Sosa-Nishizaki O, Kohin S, Dewar H, Kinney M, Snodgrass O, Wells RJD (2018) Elements of time and place: manganese and barium in shark vertebrae reflect age and upwelling histories. Proc R Soc B Biol Sci 285:20181760. https://doi.org/10.1098/rspb.2018.1760

Mull CG, Lowe CG, Young KA (2008) Photoperiod and water temperature regulation of seasonal reproduction in male round stingrays (*Urobatis halleri*). Comp Biochem Physiol Mol Integr Physiol 151:717–725. https://doi.org/10.1016/j.cbpa.2008.08.029

Munroe SEM, Heupel MR, Fisk AT, Simpfendorfer CA (2015) Geographic and temporal variation in the trophic ecology of a small-bodied shark: Evidence of resilience to environmental change. Can J Fish Aquat Sci 72:343–351. https://doi.org/10.1139/cjfas-2014-0230

Musgrove ML (2021) The occurrence and distribution of strontium in US groundwater. Appl Geochem 126:104867

Nathan R, Getz WM, Revilla E, Holyoak M, Kadmon R, Saltz D, Smouse PE (2008) A movement ecology paradigm for unifying organismal movement research Ran. Proc Natl Acad Sci 105:19052–19059

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2022) vegan: Community Ecology Package. R package version 2.6–4. https://CRAN.R-project.org/package=vegan

Pancaldi F, Páez-Osuna F, Soto-Jiménez MF, Whitehead DA, González-Armas R, Vázquez-Haikin A, Becerril-García EE, Galván-Magaña F (2021) Concentrations of silver, chrome, manganese and nickel in two stranded whale sharks (*rhincodon typus*) from the gulf of California. Bull Environ Contam Toxicol 107:827–832. https://doi.org/10.1007/s00128-021-03244-1

Papastamatiou YP, Lowe CG (2012) An analytical and hypothesis-driven approach to elasmobranch movement studies. J Fish Biol 80:1342–1360. https://doi.org/10.1111/j.1095-8649.2012.03232.x

Pentreath RJ (1973) The accumulation from sea water of 65Zn, 54Mn, 58Co and 59Fe by the thornback ray, *Raja clavata* L. J Exp Mar Biol Ecol 12:327–334. https://doi.org/10.1016/0022-0981(73)90062-2

Pistevos JCA, Reis-Santos P, Izzo C, Gillanders BM (2019) Element composition of shark vertebrae shows promise as a natural tag. Mar Freshw Res 70:1722–1733. https://doi.org/10.1071/MF18423

Plank SM, Lowe CG, Feldheim KA, Wilson RR, Brusslan JA (2010) Population genetic structure of the round stingray *Urobatis halleri* (Elasmobranchii: Rajiformes) in southern California and the Gulf of California. J Fish Biol 77:329–340. https://doi.org/10.1111/j.1095-8649.2010.02677.x

Raoult V, Howell N, Zahra D, Peddemors VM, Howard DL, de Jonge MD, Buchan BL, Williamson JE (2018) Localized zinc distribution in shark vertebrae suggests differential deposition during ontogeny and across vertebral structures. PLoS ONE 13:1–14. https://doi.org/10.1371/journal.pone.0190927

Reis-Santos P, Vasconcelos RP, Tanner SE, Fonseca VF, Cabral HN, Gillanders BM (2018) Extrinsic and intrinsic factors shape the ability of using otolith chemistry to characterize estuarine environmental histories. Mar Environ Res 140:332–341. https://doi.org/10.1016/j.marenvres.2018.06.002

Reis-Santos P, Gillanders BM, Sturrock AM, Izzo C, Oxman DS, Lueders-Dumont JA, Hüssy K, Tanner SE, Rogers T, Doubleday ZA, Andrews AH, Trueman C, Brophy D, Thiem JD, Baumgartner LJ, Willmes M, Chung MT, Johnson RC, Heimbrand Y, Limburg KE, Walther BD (2022) Reading the biomineralized book of life: expanding otolith biogeochemical research and applications for fisheries and ecosystem-based management. Rev Fish Biol Fish. https://doi.org/10.1007/s11160-022-09720-z

Ruttenberg BI, Hamilton SL, Hickford MJH, Paradis GL, Sheehy MS, Standish JD, Ben-Tzvi O, Warner RR (2005) Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. Mar Ecol Prog Ser 297:273–281. https://doi.org/10.3354/meps297273

Sawyna JM, Spivia WR, Radecki K, Fraser DA, Lowe CG (2017) Association between chronic organochlorine exposure and immunotoxicity in the round stingray (*Urobatis halleri*). Environ Pollut 223:42–50. https://doi.org/10.1016/j.envpol.2016.12.019

Schumann RR, Minor SA, Muhs DR, Groves LT, McGeehin JP (2012) Tectonic influences on the preservation of marine terraces: old and new evidence from Santa catalina island, California. Geomorphology 179:208–224. https://doi.org/10.1016/j.geomorph.2012.08.012

Shipley ON, Brownscombe JW, Danylchuk AJ, Cooke SJ, O'Shea OR, Brooks EJ (2018) Fine-scale movement and activity patterns of Caribbean reef sharks (*Carcharhinus perezi*) in the Bahamas. Environ Biol Fishes 101:1097–1104. https://doi.org/10.1007/s10641-017-0656-4

Skornyakova IS (1965) Dispersed iron and manganese in Pacific Ocean sediments. Int Geol Rev 7:2161–2174. https://doi.org/10.1080/00206816509474192

Smith WD, Miller JA, Heppell SS (2013) Elemental markers in elasmobranchs: effects of environmental history and growth on vertebral chemistry. PLoS ONE 8:e62423. https://doi.org/10.1371/journal.pone.0062423

Smith WD, Miller JA, Márquez-Farías JF, Heppell SS (2016) Elemental signatures reveal the geographic origins of a highly migratory shark: Prospects for measuring population connectivity. Mar Ecol Prog Ser 556:173–193. https://doi.org/10.3354/meps11844

Stefanidou M, Maravelias C, Dona A, Spiliopoulou C (2006) Zinc: A multipurpose trace element. Arch Toxicol 80:1–9. https://doi.org/10.1007/s00204-005-0009-5

Straughan D, Hadley D (1980) Ecology of Southern California island sandy beaches. The California Literature Records: Proceedings of a Multidisciplinary Symposium. Santa Barbara: Santa Barbara Museum of Natural History 369–393

Sturrock AM, Hunter E, Milton JA, Johnson RC, Waring CP, Trueman CN (2015) Quantifying physiological influences on otolith microchemistry. Meth Ecol Evol 6:806–816. https://doi.org/10.1111/2041-210X.12381

Tillett BJ, Meekan MG, Parry D, Munksgaard N, Field IC, Thorburn D, Bradshaw CJA (2011) Decoding fingerprints: elemental composition of vertebrae correlates to age-related habitat use in two morphologically similar sharks. Mar Ecol Prog Ser 434:133–142. https://doi.org/10.3354/meps09222

Tzadik OE, Curtis JS, Granneman JE, Kurth BN, Pusack TJ, Wallace AA, Hollander DJ, Peebles EB, Stallings CD (2017) Chemical archives in fishes beyond otoliths: a review on the use of other body parts as chronological recorders of microchemical constituents for expanding interpretations of environmental, ecological, and life-history changes. Limnol Oceanogr Methods 15:238–263. https://doi.org/10.1002/lom3.10153

Vallee BL, Falchuk KH (1993) The biochemical basis of zinc physiology. Physiol Rev 73:79–118. https://doi.org/10.1152/physrev.1993.73.1.79

Vaudo JJ, Lowe CG (2006) Movement patterns of the round stingray *Urobatis halleri* (Cooper) near a thermal outfall. J Fish Biol 68:1756–1766. https://doi.org/10.1111/j.0022-1112.2006.01054.x

Williams RJP (1984) Zinc: what is its role in biology? Endeavour 8:65–70. https://doi.org/10.1016/0160-9327(84)90040-1