

Elemental analysis of vertebrae discerns diadromous movements of threatened non-marine elasmobranchs

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Abstract

River sharks (*Glyphis* spp.) and some sawfishes (Pristidae) inhabit riverine environments, although their long-term habitat use patterns are poorly known. We investigated the diadromous movements of the northern river shark (*Glyphis garricki*), spartooth shark (*Glyphis glyphis*), narrow sawfish (*Anoxypristis cuspidata*), and largetooth sawfish (*Pristis pristis*) using in situ laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) on vertebrae to recover elemental ratios over each individual's lifetime. We also measured elemental ratios for the bull shark (*Carcharhinus leucas*) and a range of inshore and offshore stenohaline marine species to assist in interpretation of results. Barium (Ba) was found to be an effective indicator of freshwater use, whereas lithium (Li) and strontium (Sr) were effective indicators of marine water use. The relationships between Ba and Li and Ba and Sr were negatively correlated, whereas the relationship between Li and Sr was positively correlated. Both river shark species had elemental signatures indicative of prolonged use of upper-estuarine environments, whereas adults appear to mainly use lower-estuarine environments rather than marine environments.

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Decreases in Li:Ba and Sr:Ba at the end of the prenatal growth zone of *P. pristis* samples indicated that parturition likely occurs in fresh water. There was limited evidence of prolonged riverine habitat use for *A. cuspidata*. The results of this study support elemental–environment relationships observed in teleost otoliths and indicate that in situ LA-ICP-MS elemental characterization is applicable to a wide range of elasmobranch species as a discriminator for use and movement across salinity gradients. A greater understanding of processes that lead to element incorporation in vertebrae, and relative concentrations in vertebrae with respect to the ambient environment, will improve the applicability of elemental analysis to understand movements across the life history of elasmobranchs into the future.

KEYWORDS

habitat use, laser ablation inductively coupled plasma mass spectrometry, life history, microchemical analysis, river sharks, sawfish

1 | INTRODUCTION

Riverine environments (fresh-water and estuarine) are critical to the life cycle completion of non-marine elasmobranch species (sharks and rays) (Grant et al., 2019). Riverine environments are spatially restricted and strongly influenced by seasonal changes in temperature and rainfall, whereas lower riverine environments are additionally influenced by saltwater intrusion from tides. There are 10 euryhaline and 19 estuarine generalist elasmobranch species that use riverine habitats during their life histories (non-marine species categories and environmental salinity ranges: fresh water ≤ 5 ppt; estuarine > 5 to ≤ 30 ppt; and marine > 30 ppt, per Grant et al., 2019). Globally, humans have had drastic impacts on riverine environments through a prolonged history of resource extraction (e.g., fisheries and irrigation), waste disposal, and environmental modification (e.g., construction of barriers to water flow and land clearing within catchments for agriculture and development purposes) (Tickner et al., 2020). Furthermore, climate change is forecasted to be a further compounding factor of these threats into the future (Gillanders et al., 2022; Lennox et al., 2019). These human pressures have led to widespread declines in biodiversity and deteriorating populations for many riverine species (e.g., He et al., 2019), although declines in elasmobranch populations remain poorly understood (Compagno & Cook, 1995; Grant et al., 2019). Presently, most euryhaline and estuarine generalist elasmobranch species are listed in threatened categories (critically endangered, endangered, or vulnerable) on the IUCN Red List of Threatened Species (Dulvy et al., 2021; Grant et al., 2019). However, for many of these species there is a need to gain a greater understanding of their life history and habitat use to inform temporal vulnerabilities to riverine pressures for use in future conservation planning.

In the Indo-Pacific, river sharks (*Glyphis* spp.) and sawfishes (Pristidae) underline the imperiled conservation state of non-marine

elasmobranchs, with viable populations now largely restricted to regions of low human population density (e.g., Grant, White, Amepou, Appleyard et al., 2021; Morgan et al., 2011). Presently, our limited understanding of life-history-specific habitat use patterns of river sharks and sawfishes may be impeding their effective conservation management (Dulvy et al., 2016; Stevens et al., 2008; White et al., 2015). However, drastic declines in their historical range coupled with reduced populations (Li et al., 2015; Yan et al., 2021), mean that obtaining new information on aspects of their life history and movement for use in conservation management is challenging. Species protections often preclude the possibility of lethal sampling (e.g., Heupel & Simpfendorfer, 2010), whereas declines in abundance can make conventional tagging approaches time consuming and costly to achieve an effective sample size. Furthermore, sampling efforts are hampered by a lack of knowledge of broadscale habitat use patterns, creating difficulties in locating animals in data-poor size classes. However, an increased understanding of habitat use throughout the life history of river sharks and sawfishes is needed to provide better management advice for conservation efforts.

Elemental (including isotopic) analysis of chondrichthyan (sharks, rays, and chimaeras) hard parts (mostly vertebrae but also dorsal spines and caudal thorns) is a technique that has been used to investigate life history, habitat use, and movement (McMillan et al., 2017). Chondrichthyan vertebrae are composed of calcified cartilage (hydroxyapatite) within an extracellular matrix of proteins (proteoglycan and collagen) (Dean & Summers, 2006). These materials accrete concentrically in a distal orientation from the vertebrae's focus as the animal grows, and once deposited they are metabolically inert with no remodeling or reabsorption (Clement, 1992). Accretion is heterogeneous over time, and it is generally accepted that seasonal differences in environmental factors such as temperature cause a “banding pattern” within accretions (Cailliet & Goldman, 2004). The seasonal accretion of “band pairs” with differing density properties is the basis of ageing chondrichthyan vertebrae (Cailliet & Goldman, 2004).

Although this ageing technique is a widely used and valuable tool, recent studies have questioned its validity as being broadly applicable in vertebrae across all chondrichthyan species (e.g., Burke et al., 2020; James, 2020; James & Natanson, 2020) or in larger size classes (Harry, 2018). However, several studies have successfully validated or verified annual band pair depositions for size classes approaching, and close to, sexual maturity, when somatic growth rates are highest (e.g., Hall et al., 2012; Simpfendorfer et al., 2002). Although some discrepancies persist regarding the temporal periodicity of band pair depositions in vertebrae, including at different life-history stages or across taxonomic groups, the concentric manner of accretion and inert properties of accreted material is not challenged. Therefore, examining the elemental composition of vertebrae holds potential to be biologically informative, as vertebrae effectively preserve a continuous record of the animals' life history (McMillan et al., 2017).

Elemental analyses of vertebrae for habitat use investigation focus on “environmental trace elements” (e.g., Li, Cu, Sr, and Ba) rather than elements absorbed predominantly via dietary pathways (e.g., Mn and Zn), which do not reflect environmental element concentrations (Smith et al., 2013). Generally, the relative concentration of particular elements within vertebrae can be used to infer their concentration in the local environment that the animal was inhabiting at the time of accretion (see McMillan et al., 2017, for discussion on this assumption). Elements enter the blood primarily through diffusion across gills and are accreted in vertebrae mainly through direct substitution with Ca in calcium phosphate hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ or may less commonly be “trapped” within the protein matrix (e.g., Zn is incorporated only in this way) (McMillan et al., 2017). The most common application of elemental analysis of vertebrae to elasmobranchs has been to investigate stock structure (McMillan et al., 2017), such as identifying the relative contribution of juveniles from different regions to the adult population (Feitosa et al., 2020). Other applications include using elemental concentrations to assist in ageing or for environmental reconstructions and movement inferences (Mohan et al., 2018; Pistevo et al., 2019). However, the use of elemental analysis to discern life-history movements of diadromous species between low- and high-salinity environments has been only sparsely applied to elasmobranchs (e.g., Otake et al., 2005; Peverell, 2009; Scharer et al., 2012; Tillett et al., 2011), despite wide application to teleost fishes (Elsdon et al., 2008). Therefore, it is not presently clear if elemental concentrations in vertebrae are effective for reconstructing ecological life histories across salinity environments for a range of species.

This study used elemental analysis of vertebrae to examine concentrations of a range of salinity-indicating elements used in the teleost literature. Diadromous environmental use patterns were explored for two river sharks (the northern river shark *Glyphis garricki* and the spartooth shark *Glyphis glyphis*) and two species of sawfish (largetooth sawfish *Pristis pristis* and narrow sawfish *Anoxypristis cuspidata*). Neonates and juveniles of both river shark species are known to occur in large macro-tidal rivers of northern

Australia (Dwyer et al., 2020; Lyon et al., 2017; Pillans et al., 2009) and Papua New Guinea (PNG, Grant, White, Amepou, Appleyard et al., 2021). However, it is unclear how long they remain in riverine environments and whether these species have segregated or communal nurseries in rivers they co-occur in. Moreover, information is lacking on habitat use patterns of subadult and adult size classes. Habitat use patterns of *P. pristis* are better understood (e.g., Lear et al., 2019; Morgan et al., 2011), although it remains unclear if pupping occurs directly in low-salinity estuarine and freshwater nursery environments or in inshore marine environments requiring juveniles to migrate upstream through the estuary. Habitat use of *A. cuspidata* is presumed to be largely estuarine and inshore based on capture locations (e.g., Thorburn et al., 2003; White et al., 2017), although limited information is available on long-term habitat use patterns within estuarine and marine environments during their life history.

The overall aim of this study was to (1) determine the effectiveness of elemental analysis of elasmobranch vertebrae for reconstructing diadromous life histories and (2) provide information on the temporal use of riverine environments for these river shark and sawfish species across their life histories to aid future conservation planning.

2 | METHODS

2.1 | Sample collection

Samples were collected from three broad regions: northern PNG (sampling locations: Sepik River and Bismarck Sea), southern PNG (Gulf of Papua, Kikori River, and South Fly Coast), and northern Australia (Adelaide, Daly, and West Alligator Rivers) (Figure 1). Vertebral samples of *G. garricki*, *G. glyphis*, *A. cuspidata*, and *P. pristis* were collected opportunistically during small-scale fishery observations in PNG from 2017 to 2020 (Grant, White, Amepou, Appleyard et al., 2021). River shark samples from the South Fly Coast were opportunistically collected in 2014, also from observations of small-scale fisheries (White et al., 2015). Vertebral samples of *P. pristis* in Australia were opportunistically collected from deceased animals found in evaporated floodplain waterholes of the Daly River by members of Charles Darwin University (CDU) in collaboration with the Malak Malak Ranger Group. An additional *P. pristis* was an unexpected mortality of another project on the Adelaide River and was donated to CDU. Australian *G. glyphis* samples from the Adelaide River were collected during a vertebral ageing study (Kyne et al., 2022). One *G. glyphis* from the West Alligator River was an unexpected mortality of historic fishery surveys and was opportunistically located in a storage freezer at CDU.

A number of additional species were also included in the analysis to allow environmental elemental signatures to be interpreted relative to other species. The bull shark *Carcharhinus leucas* was included as a well-studied euryhaline generalist species that is known to use riverine environments, including fresh water, for its first few years of life, before moving to marine environments (e.g., Heupel et al., 2010).

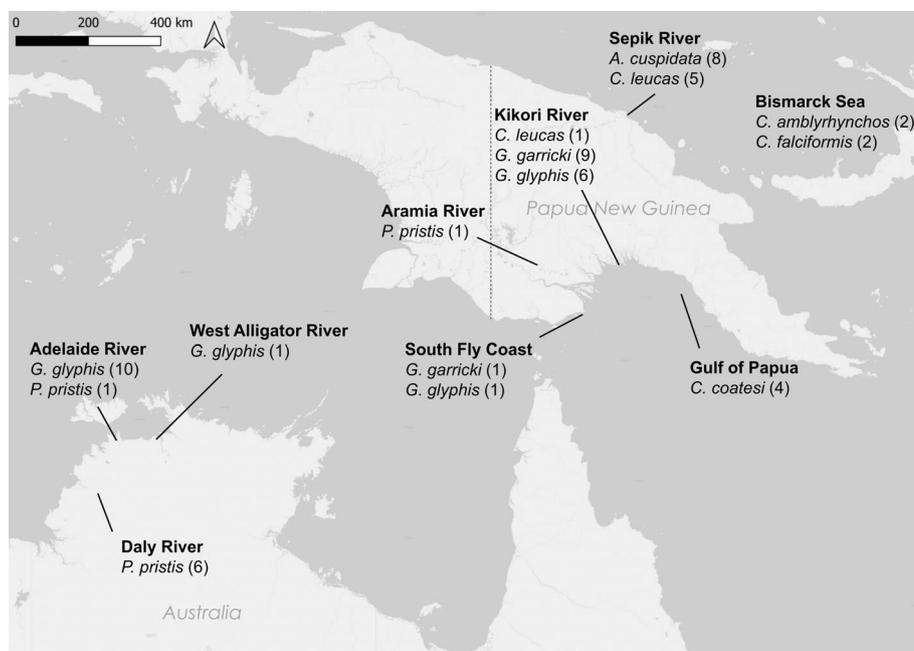


FIGURE 1 Location and sample size of vertebral samples for each species used in elemental analysis.

C. leucas vertebral samples were collected opportunistically during the same small-scale fishery observations in PNG that the *A. cuspidata* and *Glyphis* spp. samples were collected. Several stenohaline-marine inshore and offshore species were also included to provide a reference for a range of marine environment elemental signatures. Silky shark (*Carcharhinus falciformis*) and gray reef shark (*Carcharhinus amblyrhynchos*) samples were obtained from commercial longline vessels in 2014 by observers from the National Fisheries Authority (NFA) of PNG (Grant et al., 2018; Smart et al., 2016). Samples of Australian blackspot shark (*Carcharhinus coatesi*) were obtained from the Gulf of Papua Prawn Trawl Fishery by observers from the NFA in 2014–2015 (Baje et al., 2019).

All specimens had total length and sex recorded at the time of observation. All samples from Australia and some samples from PNG additionally had maturity status assessed following the method prescribed by White et al. (2001). Due to the nature of small-scale fishery observations in PNG, it was not always possible to perform dissections to assess maturity status in females (see Grant, White, Amepou, Appleyard et al., 2021). Measurements of salinity were taken at the time of collection for all Australian *G. glyphis* and one *P. pristis* sample, whereas salinity measurements were not able to be taken for other Australian *P. pristis* or any PNG samples (Appendix S1, Tables S1 and S2). At least 10 thoracic vertebrae were removed from each animal at the time of specimen collection. Residual tissue was removed, and vertebrae were stored frozen until preparation for analysis at James Cook University or CDU.

2.2 | Sample preparation

Protocols for preparing vertebrae for ageing and elemental analyses followed Cailliet et al. (2006). Vertebrae were thawed, and remaining

tissues, including neural and hemal arches, were removed using a scalpel. Vertebrae were then carefully divided into individual centra. Specimens of *C. amblyrhynchos*, *C. falciformis*, and *C. coatesi* from PNG, and *G. glyphis* and *P. pristis* from Australia, were soaked in a 4%–5% sodium hypochlorite solution for 5–30 min, pending size of centra, to remove excess tissue. Samples of *G. garricki*, *G. glyphis*, *A. cuspidata*, *P. pristis*, and *C. leucas* from PNG were not soaked in sodium hypochlorite as an a priori precaution to elemental analysis. It should be noted that there is little evidence that brief exposure to sodium hypochlorite (or “bleaching” generally) affects the internal elemental composition of vertebrae for elements analysed in this study (Mohan et al., 2017). All samples were rinsed with Milli-Q water and placed in a drying oven at 60°C for 24 h.

One centra from each individual was randomly selected for sectioning. Centra were mounted into the chuck of a low-speed circular saw (Beuhler, Illinois, USA), and a section of approximately 400–600 μm was cut on a sagittal axis using two parallel-mounted diamond-encrusted wafering blades. Between sectioning of each individual centra, the saw apparatus, including blades and water reservoir, was cleaned in an ultrasonic bath with Milli-Q water to prevent cross-sample contamination. Vertebral sections were mounted onto glass slides using Crystal Bond resin (I509) (SPI Supplies, Pennsylvania, USA). Vertebral sections were then rinsed again in an ultrasonic cleaner using Milli-Q water and placed in a drying oven at 60°C for c. 1 h. Samples were then stored in a sealed slide box until ageing and elemental analyses.

2.3 | Age estimation

Mounted vertebral sections were examined through a dissecting microscope using transmitted light. Ages were estimated by counting

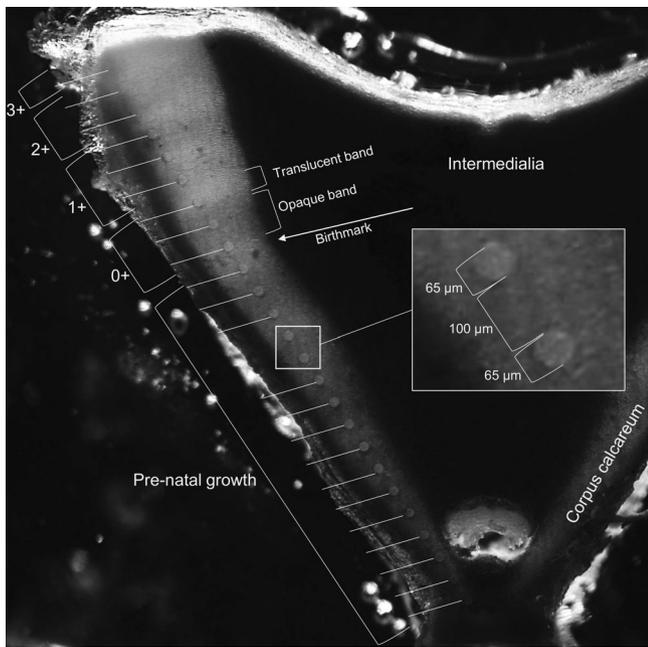


FIGURE 2 Ablated vertebral section of an immature *Glyphis garricki* (79.2 cm TL [total length]) from Papua New Guinea estimated to be 3 years old. Brackets indicate the age class each ablation spot was allocated to. The location of the birthmark and first opaque and translucent band pair (collectively c. 1 year of growth) are indicated.

the number of translucent and opaque bands (collectively called “band pairs”) in the corpus calcareum (Figure 2). The birthmark was identified by a clear acute angle change on the inner margin of the corpus calcareum, which often coincided with a thin translucent band. These features indicate the shift from prenatal to postnatal growth (Cailliet & Goldman, 2004). Each preceding band pair was considered to represent a year of growth (Figure 2). Only *C. leucas* has had annual band pair depositions verified (Hoarau et al., 2021), although most carcharhinids generally appear to deposit growth bands annually, at least in younger age classes (e.g., Chin et al., 2013; Hall et al., 2012; Harry et al., 2013). No age validation studies have been conducted for sawfish species included in this study, although annual band pair deposition has been validated for age 0–3 smalltooth sawfish *Pristis pectinata* through analysis of seasonal salinity trends and Sr:Ca ratios (Scharer et al., 2012).

An age estimate for each individual was achieved by having two “readers” independently estimate ages without knowledge of the individuals’ total length. Reader 1 (M.I.G.) conducted two reads to allow measurements of ageing bias and precision within reader, whereas reader 2 (S.M.) conducted one read to allow measurements of ageing bias and precision between readers. Age bias analysis was conducted only on PNG river shark specimens as other samples had already been aged in previous studies or had low sample sizes that precluded statistical age bias analysis. Some systematic bias was evident in estimated

TABLE 1 Length range and estimated ages for each species, from each sample region.

Region	Species (n)	Length range (cm TL)	Estimated age classes present (years+)
Northern Papua New Guinea			
Bismarck Sea	<i>Carcharhinus amblyrhynchos</i> (2)	143.0–144.0	9, 11
	<i>Carcharhinus falciformis</i> (2)	130.8–147.6	7, 9
Sepik River	<i>Anoxypristis cuspidata</i> (8)	103.4–222.8	
	<i>Carcharhinus leucas</i> (5)	76.0–157.7	0, 5, 11
Southern Papua New Guinea			
Aramia River	<i>Pristis pristis</i> (1)	90.0	0
Gulf of Papua	<i>Carcharhinus coatesi</i> (4)	73.0–77.0	9, 10, 11
Kikori River	<i>C. leucas</i> (1)		2
	<i>Glyphis garricki</i> (9)	74.0–93.4	2, 3, 4, 5
	<i>Glyphis glyphis</i> (6)	59.3–122.0	0, 1, 2, 4
South Fly Coast	<i>G. garricki</i> (1)	113.0	6
	<i>G. glyphis</i> (1)	228.0	22
Northern Australia			
Adelaide River	<i>G. glyphis</i> (10)	59.8–189.0	0, 1, 4, 6, 8, 10, 11
	<i>P. pristis</i> (1)	106.0	0
Daly River	<i>P. pristis</i> (6)	102.0–121.0	0, 1
West Alligator River	<i>G. glyphis</i> (1)	85.0	2

Note: Locations within each region are indicated in Figure 1. Further information on each individual's size, age, and characteristics of the environment at the time of capture is provided in Appendix S1 (Tables S1 and S2).

Abbreviations: n, sample size; TL, total length.

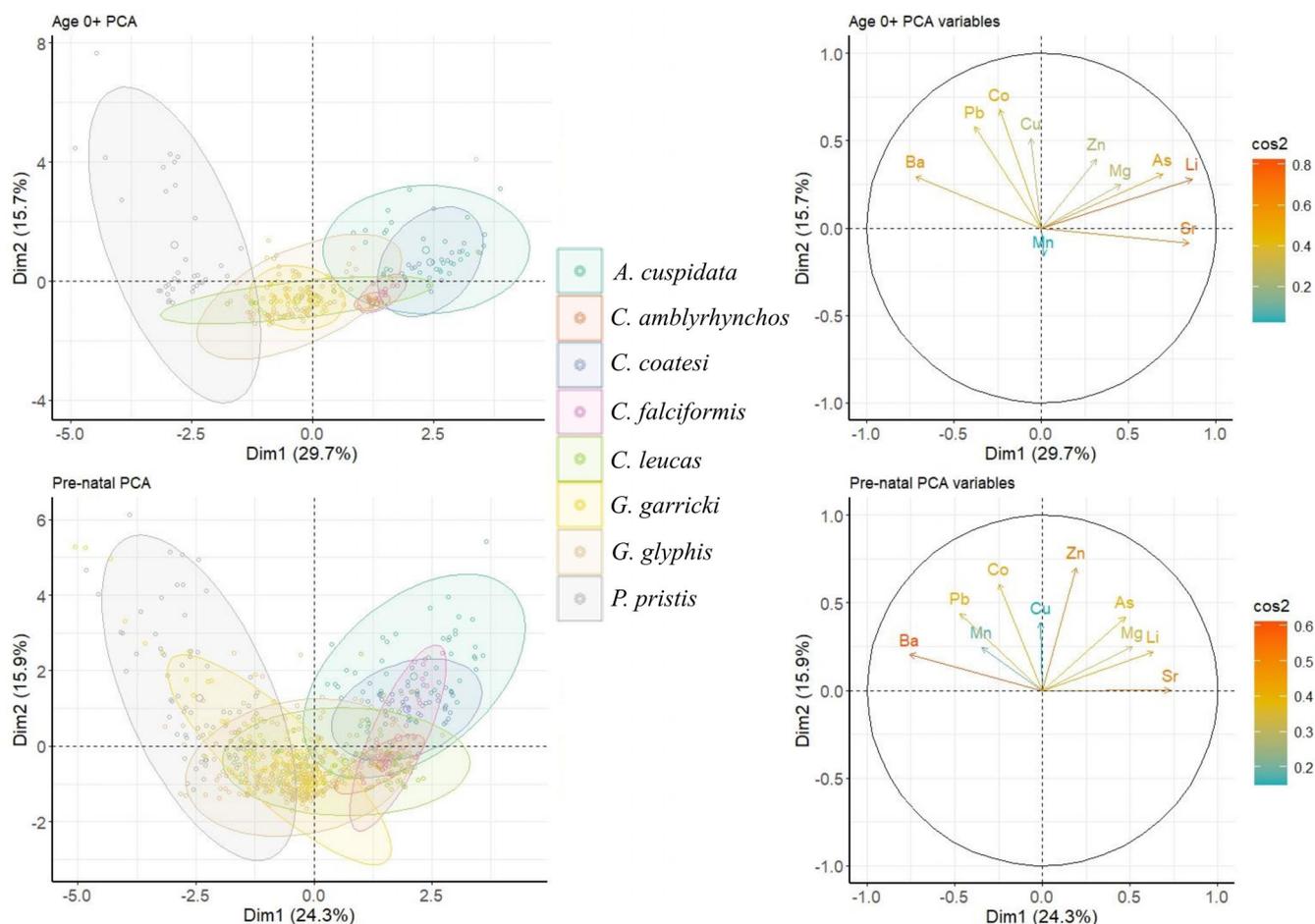


FIGURE 3 Left: principal component analysis (PCA) of LA-ICP-MS (laser ablation inductively coupled plasma mass spectrometry) results in the age 0+ and prenatal vertebral zone for each species. Right: contributing variables to each principal dimension. \cos^2 values closer to 1 indicate a larger contribution. Region-specific PCAs are provided in Appendix S1.

ages within and between readers for the PNG river shark specimens, although average percentage agreement ± 1 year was high (Appendix S1, Figure S1). Any issues with differing age estimates between readers were overcome by conducting consensus reads (between M.I.G. and S.M.). Difficulties were encountered in estimating ages for *A. cuspidata* due to extreme variability in banding pattern and large inconsistencies between suspected band pairs present and specimen length. Consensus reads involving a third reader (C.A.S.) were conducted, although no age estimates could be agreed, so *A. cuspidata* samples were not aged. Band pairs from specimens aged in previous studies (*C. amblyrhynchos*, Smart et al., 2016; *C. coatesi*, Baje et al., 2019; *C. falciformis*, Grant et al., 2018; and Australian *G. glyphis*, Kyne et al., 2022) were recounted by M.I.G. and S.M.

2.4 | Laser ablation using laser ablation inductively coupled plasma mass spectrometry

Elemental analysis of vertebrae was conducted at the Advanced Analytical Centre of James Cook University (Townsville campus),

using a Teledyne Analyte G2 193-nm ArF Excimer laser ablation system connected to a Thermo iCAP-RQ. The ablation cell HelEx II was connected to the iCAP-RQ via Tygon tubing and a three-way mixing bulb (volume: c. 5 cm³). This system provides both a very stable time-resolved signal and a rapid signal washout.

All instrument tuning was performed on NIST 610 glass standard using a 5-Hz repetition rate, 50- μ m beam aperture, and 3-J/cm² energy density. Under these conditions, the ablation rate for NIST 610 was c. 0.1 μ m per laser pulse. Tuning was achieved by iteratively adjusting the He carrier gas, Ar sampling gas, RF power, and voltages of extraction lens and other lenses to achieve the best sensitivity while keeping a ²³⁸U/²³²Th ratio of c. 1 and ThO/Th <1% (typically 0.5%) for NIST 610. For sample analysis, the total measurement time was set at 60 s. The first 30 s was for gas blank measurement (laser firing but with the shutter closed after 20 s), with the shutter opened to allow sample ablation for the final 30 s. NIST 610 standard was used as the calibration standard, and NIST 612 was analysed as a secondary standard for quality control purposes. Both these NIST glasses have been found to provide a reasonable standard for biological calcium phosphate (e.g., Tillett et al., 2011). Standard bracketing was used throughout the study to correct for remaining elemental fractionation and mass bias by conducting duplicate

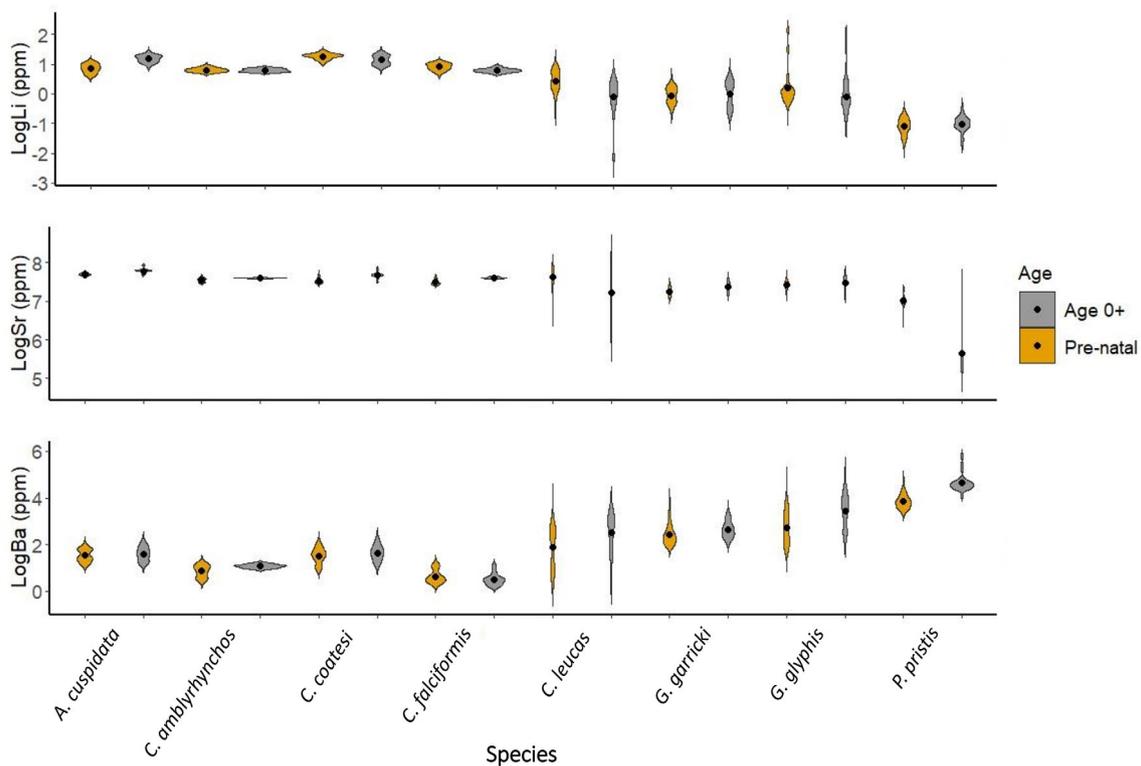


FIGURE 4 Violin plots for log-transformed concentrations of Li, Sr, and Ba for each species (all regions considered together). The black dot represents the mean value within the distribution. Region-specific violin plots are available in Appendix S1.

analyses of both NIST 610 and NIST 612 before and after every three sample transects.

A range of elements commonly used in environmental reconstructions, Li, Mg, P, Mn, Co, Cu, Zn, As, Sr, Ba, and Pb (McMillan et al., 2017), were measured in all vertebral samples, and concentrations were normalized to Ca. Samples were ablated along a transect line, at 165- μm intervals within the corpus calcareum, starting at the vertebral focus and ending at the distal edge (Figure 2). All data were processed using lolite software and extracted in parts per million (ppm). To remove potential surface contaminants on vertebral sections, material ablated in the first second of each spot was removed from all data.

Elemental analysis data outputs generated using lolite software were organized in Microsoft Excel, and the corresponding ablation spot data were allocated to either the prenatal zone or an annual band pair (i.e., age) along the growth axis for each specimen. This was achieved by photographing each ablated vertebral section using a camera attached to a dissecting microscope and overlaying markers on band pairs digitally (Figure 2). This additionally allowed the position of ablation spots to be checked and erroneous ablations overlapping residual tissue to be removed.

2.5 | Statistical analysis

Multivariate principal component analysis (PCA) was used to first investigate broad environmental habitat use patterns between each

species across life stages through simultaneously plotting all element concentrations (ppm). PCAs were used to identify differences in trace element abundance across all species in age class 0 (hereafter age 0+) and prenatal growth (as a proxy of pregnant female habitat use). PCAs were conducted only on these two life-history phases as all samples could be included, whereas available sample size would decrease substantially with comparisons of age classes greater than 0+.

Preliminary results from the PVA indicated that Ba concentration was higher in species known to occur in freshwater and lower-salinity parts of estuaries (e.g., *P. pristis*), and was an effective indicator of fresh water, whereas Li and Sr were effective indicators of marine water due to their high concentration in species known to occur in marine environments. To investigate this further, a Spearman's rank test was used to examine pair-wise correlations between Li, Sr, and Ba. Data were log transformed to meet assumptions of normality. This log-transformed data was also used to plot the distribution and mean values of Li, Sr, and Ba for each species. When the northern PNG data were analysed discreetly, Li was not log transformed to maintain a linear relationship and fit the assumptions of the Spearman's rank test. Analyses were conducted in the R environment (RStudio Team, 2020), and PCAs were constructed using Factoextra (Kassambara & Mundt, 2020) and FactoMineR (Le et al., 2008) packages, following Kassambara (2017).

To investigate changes in elemental ratios across the growth transect of each individual, mean values (\pm standard error) of Ba:Ca, Li:Ca, and Sr:Ca, and Li:Ba and Sr:Ba, were calculated for each age class.

Due to ageing difficulties in *A. cuspidata*, only mean elemental ratios were calculated for the prenatal growth zone, whereas elemental ratios for each subsequent ablation spot along the postnatal growth axis were presented individually. To investigate parturition in *P. pristis*, Li:Ba and Sr:Ba ratios were analysed for each ablation spot from the vertebral focus through the distal edge, to capture changes in these element ratios leading up to and beyond the birthmark.

3 | RESULTS

3.1 | Age estimates

The size of individuals, along with their estimated ages, indicated that most were juveniles and subadults (Table 1). Only one specimen of each of *A. cuspidata* (228.8 cm male) and *G. glyphis* (228.0 cm male, 22 years) were mature. All Australian *G. glyphis* (estimated age: 0–11 years) were immature (Kyne et al., 2022). A lack of size and age classes between 11 and 22 years in this study precludes any further interpretations of size and age at maturity for this species.

3.2 | Elemental signatures

Differences among species (all regions considered together) were observed across elemental signatures in the age 0+ and prenatal vertebral zones (Figure 3). In both the age 0+ and prenatal PCAs, Li, Sr, and Ba had the largest percentage contributions to dimension 1 (Appendix S1, Figures S2 and S3). Species known to use lower-salinity environments (*P. pristis* and *Glyphis* spp.) were clustered toward the region mainly driven by high Ba. These species also generally had lower Li and Sr (Figure 4). Species known to use higher-salinity environments (*Carcharhinus* spp. apart from *C. leucas*) were clustered toward the region mainly driven by Li and Sr. These species also had lower Ba. *A. cuspidata* was clustered with these marine species, with similar concentrations of Li, Sr, and Ba. *C. leucas*, both *Glyphis* species, and *P. pristis* had a broader distribution of concentrations for Li, Sr, and Ba, with *P. pristis* showing the highest Ba and lowest Li and Sr concentrations (Figure 4). Across all species and regions considered together, and species within each region considered separately, there was a significant negative correlation between Ba–Li and Ba–Sr; meanwhile, Li–Sr had a significant positive correlation (Table 2). The only exception was Ba–Sr and Li–Sr in the prenatal vertebral zone of individuals from the northern PNG region where an apparent lack of individuals using lower-salinity environments in this life stage likely precluded the ability to detect a pattern for these elements in this life stage for this region.

PCAs and elemental distribution plots (Li, Sr, and Ba) were also produced for each of the northern PNG, southern PNG, and northern Australian regions separately (Appendix S1). Each regional PCA had similar patterns to those observed for the PCA that considered all species.

TABLE 2 Correlation and significance values demonstrating the relationships between Li, Sr, and Ba across regions in the age 0+ and prenatal vertebral zones.

Region	Age	Elements	R ²	p
All regions	Age 0+	Ba–Li	–0.84	<0.005
		Ba–Sr	–0.70	<0.005
		Li–Sr	0.83	<0.005
	Prenatal	Ba–Li	–0.78	<0.005
		Ba–Sr	–0.56	<0.005
		Li–Sr	0.65	<0.005
Northern Papua New Guinea	Age 0+	Ba–Li	–0.25	<0.05
		Ba–Sr	–0.32	<0.05
		Li–Sr	0.58	<0.005
	Prenatal	Ba–Li	–0.26	<0.005
		Ba–Sr	–0.10	0.12
		Li–Sr	0.07	0.29
Southern Papua New Guinea	Age 0+	Ba–Li	–0.48	<0.005
		Ba–Sr	–0.40	<0.005
		Li–Sr	0.73	<0.005
	Prenatal	Ba–Li	–0.57	<0.005
		Ba–Sr	–0.50	<0.005
		Li–Sr	0.61	<0.005
Northern Australia	Age 0+	Ba–Li	–0.79	<0.005
		Ba–Sr	–0.42	<0.005
		Li–Sr	0.87	<0.005
	Prenatal	Ba–Li	–0.69	<0.005
		Ba–Sr	–0.53	<0.005
		Li–Sr	0.75	<0.005

Note: Bold indicates significant values.

3.3 | Environmental salinity at age

In northern PNG, the Li:Ba and Sr:Ba ratios indicated differing environmental habitat use patterns between species analysed (Figure 5). The prenatal Sr:Ba values of *C. leucas* were similar to values observed across *C. amblyrhynchos* age classes, whereas Li:Ba of prenatal *C. leucas* was slightly lower than *C. amblyrhynchos*. After parturition, age 0+ and 1+ of *C. leucas* showed a distinct decrease in values indicating use of lower-salinity environments in subsequent age classes, likely reflecting prolonged freshwater use within the Sepik River (Figure 5). For both Li:Ba and Sr:Ba, *C. falciformis* had higher values than other species. Individuals of *A. cuspidata* each had varied and fluctuating values across the ablation transect. Li:Ba and Sr:Ba values were more similar to *C. amblyrhynchos* than *C. leucas*, implying use of marine habitats primarily, though some individuals appeared to use a lower-salinity environment for prolonged periods.

In southern PNG, *C. coatesi* was clearly separated from the euryhaline generalists (all other species) through higher Li:Ba and Sr:Ba values (Figure 5). *P. pristis* was also clearly separated from other euryhaline generalists through lower Li:Ba and Sr:Ba values. This indicates the use of

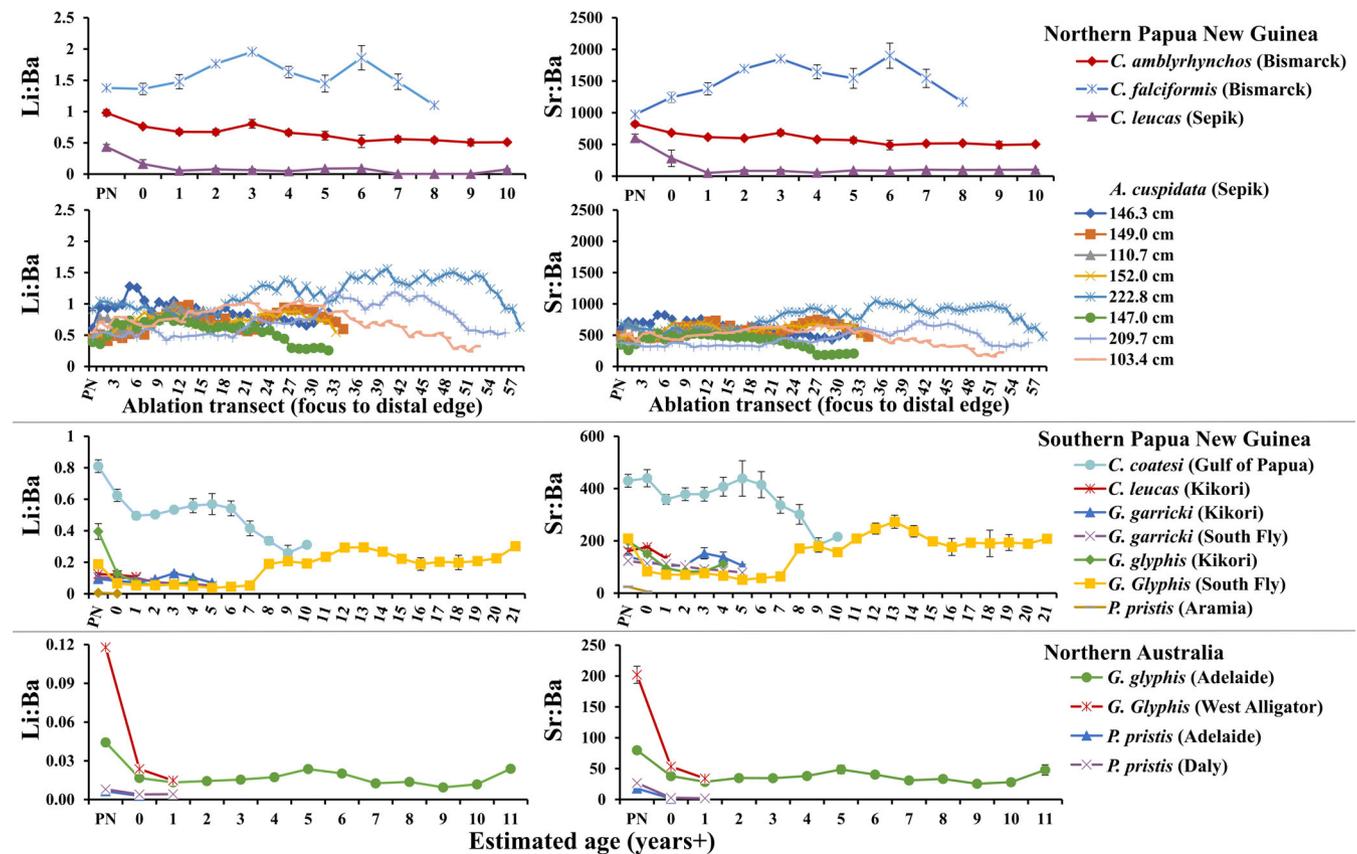


FIGURE 5 Mean Li:Ba and Sr:Ba values in each age class for species from each region. Error bars indicate standard error. No age classes were assigned to *Anoxypristis cuspidata* vertebrae due to difficulties in band pair interpretation, so ablation spot values preceding the birthmark are plotted, extending toward the distal edge of the vertebrae. PN, prenatal vertebral zone.

lower-salinity environments (likely fresh water) by *P. pristis* in life stages analysed, whereas the river sharks and *C. leucas* appear to be using estuarine environments. These river shark and *C. leucas* individuals had similar element ratios across prenatal and natal growth, indicating no major differences in environmental salinity habitats used between species. A notable shift in both element ratios was observed at age 8+ in the South Fly Coast *G. glyphis* individual, indicating a distinct change to a higher-salinity environment at this age class. This change corresponded to this individual's prenatal values of Li:Ba and Sr:Ba, and showed some overlap with *C. coatesi* age classes 9+ and 10+.

In northern Australia, prenatal Li:Ba and Sr:Ba ratios for *P. pristis* were slightly lower than ratios observed for postnatal age classes of *G. glyphis*, indicating use of a similar or slightly lower-salinity environment. Meanwhile, lower Li:Ba and Sr:Ba ratios in ages 0–1+ indicated that *P. pristis* juveniles occurred at lower environmental salinities than *G. glyphis* (Figure 5). Element ratios did not change substantially across Adelaide River *G. glyphis* age classes (0–11 years) analysed. A large decrease in Li:Ba and Sr:Ba was observed for the individual *G. glyphis* from the West Alligator River between prenatal and age 0+.

Between pre- and postnatal growth for *P. pristis*, Sr:Ba values were higher in the prenatal growth zone for all individuals in each of the Adelaide, Daly, and Aramia Rivers (Figure 6). A sharp decrease in Sr:Ba values was observed between the last two prenatal ablation

points for all individuals. Only two individuals, one in each of the Daly and Aramia Rivers, continued to show a slight decrease in Sr:Ba values in ablation spots preceding the birthmark (i.e., postnatal growth). Sr:Ba values were low and remained stable in the postnatal zone of vertebrae, indicating the use of a freshwater environment with no marine influence. This same general pattern was also observed for Li:Ba, although it was less pronounced compared to Sr.

4 | DISCUSSION

The present study asserts that relationships known to exist between environmental elements used as “salinity” indicators (i.e., Ba, fresh water; Li and Sr, marine) and their relative concentration in diadromous teleost otoliths (e.g., Gillanders, 2005; Walther & Thorrold, 2006) are reflected in the vertebrae of multiple elasmobranch species. Ba was the main indicator of freshwater environments (evidenced by high levels in juvenile *P. pristis*), whereas Sr and Li were the main indicators of marine environments (indicated by high levels in a range of marine species analysed). Furthermore, PCAs and correlation analysis showed that these assertions were supported by consistently strong inverse relationships between concentrations of Ba and those of Sr and Li.

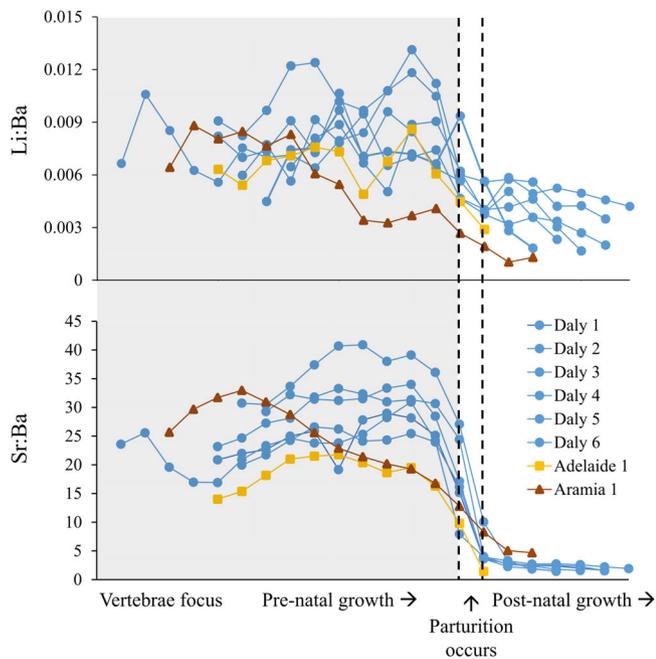


FIGURE 6 Li:Ba and Sr:Ba values for *Pristis pristis* individuals from the Daly and Adelaide Rivers in northern Australia and Aramia River in southern Papua New Guinea. For each individual, Li:Ba and Sr:Ba values are plotted from the vertebral focus to the distal edge. The black dotted lines represent the period in between the last ablation spot falling in the prenatal zone and the first ablation spot falling in the postnatal zone along the growth transect. Differences in the number of ablation points in the prenatal zone were due to differences in the size of vertebral centra between individuals.

The present study indicates that analysis of Li:Ba and Sr:Ba ratios provides more reliable insights into diadromous life-history movements of elasmobranchs compared to element:Ca ratios. Although corresponding changes in salinity-indicating element ratios were generally observed along the growth transect of vertebrae, in some instances Sr:Ca remained stable despite fluctuations in Ba:Ca and Li:Ca (Appendix S1, Figures S12 and S19). For example, there was a lack of a freshwater signal from Sr:Ca for *P. pristis* in the Aramia River and less pronounced variation in Sr:Ca for *A. cuspidata* despite fluctuations in Ba:Ca at the Sepik River. Sr:Ca also remained stable throughout the life of the adult *G. glyphis*, despite other element ratios shifting at the eighth band pair. To our knowledge, no other studies investigating diadromous movements of elasmobranchs through elemental analysis have presented data on changes in each of Ba:Ca, Li:Ca, and Sr:Ca. All studies have presented data for only one (Sr:Ca, Otake et al., 2005; Peverell, 2009; Scharer et al., 2012) or two (Ba:Ca and Sr:Ca, Feitosa et al., 2021). In the teleost literature, the relationship between ambient salinity and Sr:Ca is generally positive (Elsdon et al., 2008), although there are studies that show a negative or no apparent relationship (see Gillanders, 2005, for further discussion on Sr:Ca relationships).

Differing geological characteristics between river basins that specimens were collected from may explain the noncorresponding Sr:Ca values. The Aramia River *P. pristis* and South Fly Coast *G. glyphis*

individuals were collected from habitats highly influenced by the Fly River basin. It is possible that the Fly River basin has ambient freshwater Sr concentrations that are similar to marine environments. Ambient marine Sr concentrations are relatively stable globally, whereas freshwater Sr concentrations can vary substantially across temporal and spatial scales (Elsdon et al., 2008). The lack of Sr:Ca fluctuation for the Sepik River *A. cuspidata* is more complex, as corresponding fluctuations between Sr:Ca and other salinity-indicating elements were observed for *C. leucas* samples also from the Sepik River. The discrepancy in *A. cuspidata* Sr:Ca ratios may be attributed to their predominant use of very shallow inshore marine environments (see Section 4.2), which may be influenced by direct terrigenous rainfall runoff from the shoreline rather than outflow from the Sepik River basin. However, there may also be underlying physiological differences in the incorporation of elements into vertebrae between these species. Collectively, this highlights our present lack of understanding of factors influencing incorporation of different elements and their relative concentration into vertebrae. We suggest consideration of other salinity-indicating elements (i.e., Ba and Li) in addition to Sr in future investigations of diadromous movements of elasmobranchs until influencing factors are better understood. Given the consistently inverse relationship between Ba and Li and between Ba and Sr across the various species and sample locations in the present study, it appears that analyzing the ratio of a freshwater indicator to a marine water indicator (i.e., Ba:Li or Ba:Sr in the present study) is an effective way to navigate a lack of variation in a particular element, which may be due to unknown geological or physiological factors.

With investigations into diadromous movements of elasmobranchs using elemental analysis techniques being very limited (e.g., Feitosa et al., 2021; Otake et al., 2005; Scharer et al., 2012; Tillett et al., 2011), this study also demonstrates that a frame of reference is useful in making inferences about elemental concentrations in vertebrae and how they relate to the ambient environment. In the present study, investigations into the use of non-marine environments were performed by analyzing elemental ratios of multiple species with known association with freshwater (juvenile *P. pristis*) and marine (*C. amblyrhynchos*, *C. coatesi*, and *C. falciformis*) environments, and comparing to species with less-understood environmental use patterns (i.e., river sharks and *A. cuspidata*). Alternative approaches could analyse elemental composition of water across a salinity spectrum of local environments or incorporate time series measurements of local physical water parameters over the corresponding temporal period of animal growth (e.g., use of temporal salinity data in Scharer et al., 2012). However, physiological processes leading to element incorporation in elasmobranch vertebrae require further research, as do relative elemental concentrations between vertebrae and the ambient environment (Pistevos et al., 2019). Furthermore, elemental analysis of vertebrae from historic collections may create logistical difficulties in collection of water samples or relevant data on temporal physical environmental parameters. Until further information is available on these factors, the present study demonstrates that comparisons of species from different environments provide a suitable basis for inferring environmental use patterns for less-understood species.

An assumption relied upon in the present study is that physiological processes leading to incorporation of environmental elements into vertebrae are reasonably consistent across species and family groups. The “all-species” PCA also assumed that environmental element concentrations are relatively similar among river basins and between Australia and PNG. This is unlikely to be true due to differing geological characteristics across the sample locations (e.g., Martin & Meybeck, 1979), and therefore, data were also presented in three broad regions (northern PNG, southern PNG, and northern Australia; see Appendix S1), where large differences in environmental element concentrations due to geological characteristics would likely be less pronounced. Fittingly, the two main principal components accounted for slightly more variance in the regional PCAs (Appendix S1, Figures S6, S13, and S20) compared to the “all-species” PCA, although it is not possible to determine whether this was due to finer spatial resolution or differences in the number of species being concurrently analysed.

Finally, we also assume that incorporation of Ba, Li, and Sr in the prenatal zone of vertebrae is reflective of the ambient environment of pregnant females and that there is no differential absorption due to physiological factors during gestation. For elasmobranchs, this remains an untested assumption. Raoult et al. (2018) found some evidence that Zn accumulates in the prenatal zone; however, zinc is absorbed mainly via dietary pathways (Smith et al., 2013). For Br, Li, and Sr that are absorbed across exposed body surfaces (e.g., branchial absorption) (McMillan et al., 2017), prenatal values of these elements indicated habitat use patterns that could be reasonably expected for gestating females with limited evidence to suggest an accumulation or deficiency in Br, Li, or Sr in the prenatal zone due to unknown physiological processes. For example, high Ba and low Li and Sr in the prenatal zone indicated estuarine habitat use for pregnant *P. pristis*. This species has a short gestation period of 5 months (Thorson, 1976), and it is sensible to suggest that they remain close to freshwater outflows during the wet season while they are gestating (see Section 4.2). Freshwater input during wet season increases riverine productivity (Lear et al., 2020), and the estuary may provide a rich foraging area for gestating females, with the added benefit of being well positioned to pup opportunistically during large freshwater flows in the late wet season, which is the behavior observed from capture of pregnant females (Peverell, 2009) and the timing of neonate presence (Lear et al., 2019). The interpretation of the ablation transects from *C. leucas* individuals in the Sepik River, northern PNG, indicates that they remain in fresh water up to 10 years, and there was no evidence of a “return” to environments in the prenatal zone. *C. leucas* have been demonstrated to leave freshwater and estuarine habitats at c. 2–4 years; however, these studies have been conducted in smaller macro-tidal rivers, with significantly less, and mainly seasonal freshwater flow (e.g., Dwyer et al., 2020; Heupel et al., 2010; Tillett et al., 2011), or in temperate systems with large marine influences with more sporadic and often smaller freshwater flow regimes (e.g., Niella et al., 2022). The Sepik River by contrast is an extremely large system with year-round high-volume freshwater flow, and it is not unreasonable for such large systems to accommodate the

ecological requirements of subadult *C. leucas* as the present data suggest. In the Kikori River in southern PNG, the prenatal zone of the *C. leucas* sample suggested a similar environment use between the prenatal zone and age classes 0–1. The northwest Gulf of Papua has an expansive inshore estuarine zone, with several adjacent major rivers draining into the region, and it is again reasonable that this estuarine zone could support the ecological requirements of a gestating female. Nonetheless, physiological factors that may affect the incorporation of elements into the prenatal vertebral zone remain an outstanding knowledge gap.

4.1 | River shark life-history environmental habitat use patterns

Elemental signatures indicated that immature individuals of both river shark species used predominantly estuarine environments, with no substantial overlap in elemental values with freshwater (juvenile *P. pristis*) or marine species. The salinity-at-age analysis indicated that *G. garricki* in PNG remain within low-salinity environments for at least 5 years, whereas *G. glyphis* appeared to remain within low-salinity environments for 8 years in southern PNG and for at least 11 years in the Adelaide River. During this period of prolonged lower-salinity environment use, there was little fluctuation in salinity-indicating elements. This is consistent with movement studies, where *G. glyphis* demonstrated long-term movements to remain within particular salinity ranges in response to seasonal rainfall (Dwyer et al., 2020; Lyon et al., 2017), whereas short-term movements of *G. glyphis* appear to be tidally assisted over small spatial ranges (c. 12–14 km), which may also be linked to environmental preferences, including salinity (Dwyer et al., 2020; Pillans et al., 2009). The results from the present study add to findings from these previous studies and further indicate that in comparison to *C. leucas*, which can be observed in environments ranging from fresh water to marine in juvenile life stages (Heupel et al., 2010; Thorburn et al., 2003), river sharks seem to be more restricted to a narrower estuarine salinity range.

There is limited information available on whether there are inter-specific differences in riverine habitat use between *G. garricki* and *G. glyphis*. The salinity-at-age analysis suggests a general overlap in the Kikori River and Fly River. This is consistent with catch data in northern Australia, where *G. garricki* juveniles have been observed in salinities ranging from 2 to 36 ppt (Pillans et al., 2009; Thorburn & Morgan, 2004) and *G. glyphis* juveniles have been observed in salinities ranging from 0.4 to 28 ppt (Dwyer et al., 2020; Pillans et al., 2009). However, in King Sound, Western Australia, where rainfall is extremely limited and highly seasonal, most observations of *G. garricki* (Morgan et al., 2011) are in marine salinities (>30 ppt). It appears that differing local factors (e.g., environmental availability, interspecific competition, and predation risk) influence juvenile habitat use patterns across different river systems. In South Alligator River, northern Australia, Every et al. (2019) found that *G. garricki* were consuming more fresh water-associated prey and less estuarine-associated prey than *G. glyphis*, although differences were quite minor.

There are clearer distinctions available for differences in habitat use between river sharks and *C. leucas*. Dwyer et al. (2020) found inter-specific habitat segregation between juvenile *G. glyphis* (lower estuary, mean salinity: 19.22 ppt) and *C. leucas* (upper estuary, mean salinity: 1.99 ppt) in a communal estuary in the Wenlock River, northern Australia. Habitat segregation between river sharks and *C. leucas* also appears to occur in southern PNG rivers, as catch in lower-estuarine delta environments is dominated by river sharks, with only a few *C. leucas* being concurrently caught (Grant, White, Amepou, Appleyard et al., 2021; Grant et al., 2022). It is likely that a combination of methods used by Dwyer et al. (2020) and Every et al. (2019) would be effective in further understanding the ecological and spatial overlap of *G. garricki* and *G. glyphis* in communal rivers.

This study also provides some insights into the poorly understood environment use patterns of adult river sharks. Individuals of both river shark species had prenatal elemental signatures that were partially overlapping *C. coatesi* in southern PNG, although the higher concentration of Ba in the river sharks suggests greater overlap with estuarine environments. Meanwhile, the prenatal elemental signature of *C. leucas* indicated substantial overlap with both river sharks, but also substantial overlap with *A. cuspidata*, *C. coatesi*, *C. amblyrhynchos*, and *C. falciformis*. Adult *C. leucas* are observed in a range of riverine, inshore, and offshore environments (e.g., Espinoza et al., 2021; White et al., 2020), and this diversity in adult environment use is reflected in the broad prenatal elemental signature across individuals. Conversely, the environment shift observed in the adult male *G. glyphis* from southern PNG appeared to indicate prolonged use of lower-estuarine environments rather than marine environments. From the *G. glyphis* samples in northern Australia, the prenatal zone had lower Li:Ba and Sr:Ba values in the Adelaide River compared to the individual from West Alligator River. Although it is difficult to put these values in context to those that would be expected in the inshore marine environments of Van Diemen Gulf, the prenatal values in the West Alligator River are similar to the highest river shark Li:Ba and Sr:Ba values observed in southern PNG, though we acknowledge the aforementioned geographic influences that may confound this comparison. The present indication from these results is that adult river sharks primarily using lower estuaries and river outflow-influenced inshore environments and not venturing into non-river-influenced inshore or offshore environments for prolonged periods like *C. leucas*, and this may explain their limited catch in commercial inshore fisheries within the Northern Territory (Field et al., 2013). However, recent genetic population structure studies on *G. garricki* (Feutry et al., 2020) and *G. glyphis* (Kyne et al., 2021) indicate that some dispersal does occur for both species between river systems that would require transit through marine environments. Moreover, there is a single observation of an adult *G. garricki* in the Wessel Islands, Northern Territory, further substantiating that use of marine environments does occur, at least for the purpose of transiting between river systems (Pillans et al., 2009).

The apparent environmental restriction of river sharks to estuaries suggests that populations would be inherently small. Small river shark population sizes are supported for *G. garricki* and *G. glyphis*

based on close-kin mark-recapture methods (Bravington et al., 2019; Patterson et al., 2022), and furthermore, all river shark species are presently listed as threatened on the IUCN Red List based on small mature population sizes (Kyne, Rigby, Cheok et al., 2021; Kyne, Rigby, Darwall et al., 2021; Rigby et al., 2021). This places increased necessity on protection for river shark populations and their habitats, as they appear to be susceptible to rapid depletion from fisheries due to their reliance on spatially restricted estuarine environments. This life strategy of river sharks appears to contrast that of *C. leucas*, which use a range of environments as both juveniles and adults.

4.2 | Sawfish life-history environmental habitat use patterns

It is well established that juvenile *P. pristis* utilize freshwater environments within Australia and PNG (e.g., Grant, White, Amepou, Appleyard et al., 2021; Thorburn et al., 2003), and results from elemental analysis reflected this. The Li:Ba and Sr:Ba ratios indicated that juveniles were using environments with substantially higher Ba concentrations than all other species except for some overlap with *C. leucas* (e.g., Figures 3 and 4).

Elemental ratios for the prenatal zone of *P. pristis* suggest no overlap with *C. leucas* or river sharks. This may indicate that pregnant *P. pristis* are primarily using upper estuaries and encroaching into fresh water. Peverell (2009) found a similar pattern for *P. pristis* in Queensland, Australia, where Sr:Ca ratios during prenatal growth were only slightly higher than juvenile Sr:Ca values, though substantially lower than adult life-stage Sr:Ca values. The present results suggest that *P. pristis* are pupping in fresh water or at least at the uppermost freshwater margin of the estuary. Sr:Ba values in the last ablation spot in the prenatal growth zone showed a clear decrease in all individuals. A further decrease in similar magnitude was observed in the first postnatal growth ablation spot, where subsequent postnatal growth values remained constant with no marine influence evident. Although the same general pattern was observed for Li:Ba, it was less pronounced. This may be due to ambient Li concentrations being very low in the fresh water of the river basins analysed, limiting the ability to detect changes in the upper estuary. These differences between Li:Ba and Sr:Ba further indicate the value of looking at multiple element ratios in the absence of information on the local geological characteristics and biophysiological processes of element incorporation into vertebrae.

Although it is known that *P. pristis* pupping occurs during the wet season (e.g., Lear et al., 2019; Peverell, 2005), it is still unclear how far *P. pristis* may penetrate into rivers to give birth. The present results indicate two possibilities regarding the location of pupping: (1) parturition may occur around the river mouth and be triggered by large rainfall events when downstream estuarine environments become fresh (e.g., Peverell, 2005), or (2) pregnant individuals may migrate upstream into freshwater environments to give birth. The former is most likely in spatially constrained systems, whereas the latter is additionally possible in large systems such as the Fly River. The individual from the Aramia River may provide some evidence for this, as Ba:Li and Ba:Sr values decreased in the prenatal zone approaching parturition. Local

fishers in the Aramia River report that *P. pristis* enters the Aramia River from the Fly or Strickland Rivers during wet season, when these three rivers are connected by an expansive floodplain (Grant, White, Amepou, Baje et al., 2021).

For *A. cuspidata*, the elemental analysis indicated the use of a predominately inshore marine environment. At the individual level there was substantial variation in Li:Ba and Sr:Ba ratios. Two individuals appeared to utilize an environment with increased freshwater influence for a prolonged period (indicated by multiple adjacent ablation spots with lower Li:Ba and Sr:Ba values), although most individuals had element signatures fluctuating around values more indicative of marine environments, similar to *C. amblyrhynchos*. In particular, the only adult *A. cuspidata* individual appeared to be using a marine environment with limited freshwater influence in its latter life-history stages (possibly further offshore). Based on fisheries interactions in northern Australia, juvenile *A. cuspidata* are known to use shallow inshore environments as juveniles, whereas adults are more commonly captured further offshore (Field et al., 2013; Peverell, 2005). In east Queensland, Australia, *A. cuspidata* juveniles occupy extremely shallow nearshore habitats (Adkins et al., 2016), which is likely a predator-avoidance behavior. Coastal regions of the East Sepik Province receive substantial rainfall, and the fluctuations in Li:Ba and Sr:Ba may indicate that juvenile *A. cuspidata* remain in nearshore habitats during periods of high rainfall. This contrasts other inshore species such as pignore sharks (*Carcharhinus amboinensis*) that temporarily move away from areas of decreased salinity during rainfall periods (Knip et al., 2011).

Despite *A. cuspidata* being the most commonly caught sawfish species in northern Australian fisheries, there is a lack of research on this species' ecology. Across Australia and PNG there is very limited evidence that *A. cuspidata* penetrates into estuaries within rivers (e.g., lack of riverine records in study areas of Grant, White, Amepou, Appleyard et al., 2021; Field et al., 2013; Morgan et al., 2011; Peverell, 2005; Thorburn et al., 2003). All *A. cuspidata* specimens used in the present study were obtained from a fishery operating mainly within, and closely adjacent to the mouth of, the Sepik River (Grant, White, Amepou, Appleyard et al., 2021). However, the salinity-indicating elements suggest that these individuals were generally not using these low-salinity environments at the river mouth for prolonged periods, with the exception of two individuals (it should be noted that the Sepik River lacks a defined estuarine zone within the river, with water being practically fresh at the mouth; Coates, 1987). The results of this study indicate that *A. cuspidata* likely does have an increased physiological tolerance to lower salinities (per its inclusion as an estuarine generalist by Grant et al., 2019), although it seems that this species does not occur within the confined waters of riverine environments regularly during its life history.

5 | CONCLUSION

The use of elemental analysis of vertebrae in this study has provided further support for the application of this technique to

elasmobranchs for (1) discerning broad habitat use patterns across a range of species and (2) identifying temporal habitat use patterns in diadromous species. The congruence in concentrations of environmental salinity-indicating elements when compared to life stages of species with well-understood temporal diadromous habitat use patterns (e.g., *C. leucas* and *P. pristis*) also supports the inertness of vertebral band pair depositions throughout an individual's life history. This study has indicated that Ba and Li and Sr are effective indicators of freshwater and marine environments, respectively, and therefore useful indicators for diadromous movements of elasmobranch species.

For the primary species analysed, the results of this study suggest that river sharks have prolonged use of estuarine environments throughout their life history, with limited indication of prolonged use of freshwater or marine environment. This study has also provided evidence that parturition occurs in fresh water for *P. pristis* and that pregnant females primarily occur in upper-estuarine environments encroaching on fresh water. Meanwhile, there was no substantial indication that *A. cuspidata* use riverine environments during their life history, though some evidence for prolonged tolerance of lower-salinity environments was observed. Further research on the underlying physiological processes that lead to environmental element incorporation in vertebrae, and relative concentrations of elements between the ambient environment and vertebrae would assist in future applications of elemental analysis for elasmobranchs. Until such information is available, we suggest that future studies consider analysis of multiple species to aid interpretation of data or incorporate measurements of physical or chemical water parameters over a broad range of differing salinity environments.

AUTHOR CONTRIBUTIONS

Michael I. Grant, Peter M. Kyne, Julie James, Yi Hu, Andrew Chin, Christopher Wurster, and Colin A. Simpfendorfer conceived the study; Michael I. Grant, Peter M. Kyne, Yolarnie Amepou, Leontine Baje, Grant Johnson, and William T. White contributed samples; Michael I. Grant, Julie James, Yi Hu, and Sushmita Mukherji conducted the analysis; Michael I. Grant, Peter M. Kyne, Julie James, Yi Hu, Andrew Chin, Tegan Lee, Brandon Mahan, Christopher Wurster, William T. White, and Colin A. Simpfendorfer interpreted the data; all authors contributed to the writing and editing of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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