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Elemental signatures reveal the geographic origins of a highly migratory shark: prospects for measuring population connectivity

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ABSTRACT: Distinguishing individual natal origins of highly dispersive species is essential for quantifying the extent of connectivity among spatially separated groups. Variation in the chemical composition of calcified structures has been used to determine natal origins of many organisms but the utility of this approach to sharks and rays has only recently been examined. We evaluated the ability to accurately classify young-of-the-year scalloped hammerhead sharks *Sphyrna lewini* to their putative natal origins using vertebral elemental signatures and assessed individual, temporal, and spatial variation in vertebral chemistry. Vertebrae were collected from sharks captured in artisanal fisheries along the Pacific coast of Mexico and Costa Rica in 2007 to 2009. Elemental signatures were measured using laser ablation inductively coupled plasma mass spectrometry. Elemental signatures were spatially distinct and served as reliable site-specific markers. Intra-annual variation in natal signatures was detected among and within sites. Natal signatures also differed across years within sites. Inter-annual variation was driven by a single year (2008) and site-specific signatures were similar for 2007 and 2009. Classifications to geographic origins exceeded chance expectations and discrimination among sites was achieved with 39 to 100 % success. Classification accuracy improved when data were analyzed at finer spatial and temporal resolution (i.e. year, season, month). Vertebral elemental signatures can successfully distinguish among sharks that have occupied different locations across broad spatial scales, from 10s to >1000 km. Identification of connectivity patterns and key areas of use through intrinsic natal signatures may propel more tractable, spatially explicit approaches to the conservation and management of elasmobranch populations.

KEY WORDS: Connectivity · Elemental signature · Intrinsic markers · Natal origin · Philopatry · Population structure · Elasmobranch

INTRODUCTION

Studies of shark and ray (elasmobranch) populations are often complicated by the high mobility and use of diverse habitats among species. Additionally, spatial distributions can be expansive and complex, frequently varying by season, sex, and life stage (Springer 1967, Speed et al. 2010). Despite these challenges, quantifying the extent of dispersal from and between spatially discrete but connected groups is essential to further our understanding of population dynamics (Hastings & Botsford 2006, Kerr et al.

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2010). Contributions from external sources can drive the stability and productivity of local populations (Pulliam 1988, Hilborn et al. 2003). Identifying the direction and strength of connectivity among geographically separated groups can reveal locations or components that are critical to the resilience and persistence of a population. Details on dispersal patterns and geographic linkages are essential for devising population- and ecosystem-based management strategies that reflect ecologically relevant spatial and temporal scales (Crowder & Norse 2008, Treml & Halpin 2012).

Declines in shark and ray populations have been increasingly reported throughout the world (Dulvy et al. 2014), yet most elasmobranch fisheries remain unregulated and are often unmonitored (Musick & Musick 2011). A general lack of biological and fisheries information for elasmobranchs, as well as their wide distribution and complex movement patterns, constrain efforts to carry out conventional population assessments and devise appropriate management strategies. Management actions that consider population connectivity have the potential to provide more effective conservation of migratory species, including elasmobranchs (Crowder & Norse 2008, Speed et al. 2010).

Distinguishing the natal origins of individuals is central to determining connectivity among populations and life stages (Cowen et al. 2007). A variety of intrinsic and extrinsic markers have been applied to respond to this challenge. In species with a high dispersal potential, genetic methods have generally found limited population structure and low genetic diversity (Hedgecock et al. 2007, Lowe & Allendorf 2010). Furthermore, molecular inferences into population structure summarize historical patterns of gene flow, providing information across generations at evolutionary rather than ecologically relevant time scales (Hedgecock et al. 2007). Tracing movements of individuals using external tags can provide vital details on movement patterns and connectivity on ecologically relevant time scales. However, these investigations are difficult to implement for wide-ranging marine species because of technological limitations and low recapture success, which results in low sample sizes (Kohler & Turner 2001, Musyl et al. 2011).

Analyses of elemental and isotopic signatures (also referred to as fingerprints, markers, or tags) in calcified structures (e.g. otoliths, shells) have invigorated studies of natal origins and population connectivity in marine and estuarine systems (Swearer et al. 1999, Ramos & González-Solís 2012). These techniques have been useful, in part, because elemental signatures are naturally acquired through respiration and feeding, present in all individuals, and may be retained for life (Campana & Thorrold 2001). Although physiological and kinetic factors influence elemental incorporation (see Campana 1999, Sturrock et al. 2015), the relative concentration of some elements within a calcified structure can be representative of the environmental conditions under which they were deposited (Campana 1999, Elsdon et al. 2008). Individuals or groups that occur within the same water mass therefore incorporate elements that are characteristic of the site or conditions, thus potentially producing distinctive elemental signatures that are retained within calcified structures even after the organisms disperse. When aligned with daily, seasonal, or annual growth bands, elemental analyses can reveal a time line of habitat use and movements by an individual during different stages of its life.

Elemental signatures have been successfully applied to distinguish natal origins for a diverse array of taxa, including mussels (Becker et al. 2005), crab (Carson et al. 2008), squid (Warner et al. 2009), marine fishes (Thorrold et al. 2001), and seabirds (Gómez-Díaz & González-Solís 2007). The use of this technique requires that the chemical composition of a calcified structure remains stable following deposition. Elasmobranchs (sharks, skates, and rays) are cartilaginous fishes that possess otoconia but lack otoliths within the inner ear, which deposit daily and annual increments and are typically used for age estimation and determination of elemental signatures in teleost fishes. However, elasmobranch vertebrae are useful ageing structures that record the growth of individuals throughout their lifetime (Cailliet & Goldman 2004). Unlike the vertebrae of teleost fishes (Campana & Thorrold 2001), metabolic activity within the mineralized cartilage of elasmobranchs appears to be minimal and shows no direct evidence of chemical remodeling or resorption (Clement 1992, Ashhurst 2004, Dean et al. 2015). Radiochemical age validation studies have provided further evidence of long-term chemical stability within elasmobranch vertebrae (Campana et al. 2002). Available evidence therefore supports the central requirement of chemical stability for the application of elemental analyses to elasmobranch vertebrae.

The use of discrete birthing and nursery areas is common to both viviparous and oviparous elasmobranchs (Heupel et al. 2007, Hoff 2010). Large coastal shark species, for example, tend to move inshore and give birth in shallow estuaries, embayments, or near-

shore habitats that are not otherwise occupied by adults (Springer 1967). Newborn sharks and rays may remain within a nursery area for the first weeks, months, or years of their lives before dispersing to other juvenile or adult habitats (Duncan & Holland 2006, Chapman et al. 2009). This extended period of occupancy within geographically separated nursery areas could facilitate the incorporation of distinctive, site-specific elemental signatures within elasmobranchs from which the natal sources of individuals could be revealed. Identification of natal origins through vertebral elemental analyses could provide a valuable, alternative approach to assessing the relative contribution of nursery areas, population connectivity, and population mixing. However, the applicability of this technique to elasmobranchs has only recently been explored (Tillett et al. 2011, Smith et al. 2013, Lewis et al. 2016). The spatial and temporal scales across which site-specific natal signatures can be used to discern natal origin requires critical evaluation before directed studies of population connectivity and inferences of nursery contributions can be pursued.

The objectives of this investigation were to evaluate the utility of vertebral elemental signatures as intrinsic spatial markers and assess the ability to distinguish site-specific natal origins from the signatures of a highly migratory shark, the scalloped hammerhead *Sphyrna lewini*. We examined the elemental composition of young-of-the-year (age 0) sharks captured within 6 nursery areas along >3000 km of the central eastern Pacific to evaluate multiple scales of variation in elemental signatures. First, we assessed the consistency of signatures within individual sharks. Next, we tested the utility of vertebral chemistry as a record of environmental transition by comparing in-utero and post-partum (natal) elemental signatures. We then evaluated the temporal consistency of elemental signatures for collection sites within and across 3 consecutive years. Finally, we quantified the potential to successfully assign individuals to natal sites using elemental signatures across multiple spatial scales (10s, 100s and >1000 km).

MATERIALS AND METHODS

Model species selection and sample collection

The scalloped hammerhead shark is a highly migratory coastal-pelagic species that occurs in warm temperate and tropical habitats throughout the world (Compagno et al. 2005). Like many marine fishes, *Sphyrna lewini* use relatively shallow nearshore habitats as birthing and nursery areas (Simpfendorfer & Milward 1993). Young-of-the-year scalloped hammerheads typically aggregate and may remain within nursery areas for 1 yr or more (Duncan & Holland 2006). Tag-recapture and tracking studies indicate that although young-of-the-year may disperse up to 5 km in a day, they consistently return to the same core area following more extensive nocturnal movements and do not exhibit ontogenetic shifts in habitat use during their residency in nursery areas (Holland et al. 1993, Duncan & Holland 2006). This pattern of aggregative behavior, restricted movement, and extended residence within nursery habitats make *S. lewini* a good candidate for incorporating distinctive natal elemental signatures. Additionally, young-of-the-year hammerhead sharks are a common seasonal component of small-scale fishery landings throughout much of the eastern Pacific (e.g. Bizzarro et al. 2009), providing a source of samples from a broad geographic area.

Although *S. lewini* are widely ditributed and possess the capacity for expansive movements, genetic evidence suggests that males undertake larger scale movements (including trans-oceanic) whereas female migrations are more restricted and may be modified by philopatry to specific reproductive regions or sites (Daly-Engel et al. 2012). The species is heavily exploited throughout its range and has been classified as 'Endangered' by the IUCN Red List of Threatened Species (Baum et al. 2007). The ability to reliably identify the natal origins of *S. lewini* from intrinsic elemental signatures would facilitate assessments of relative contributions of nursery grounds and provide improved insight into their complex population structure for improved, spatially explicit management.

We collected thoracic vertebrae of neonate and young-of-the-year *S. lewini* from artisanal fishery landings along the Pacific coast of Mexico and Costa Rica (Fig. 1a). In order to evaluate site-specific intra-annual variability in vertebral elemental signatures, 3 locations in Sinaloa, Mexico were sampled monthly between August and November, from 2007 to 2009 (Fig. 1b). We refer to these sites, which were separated by distances of ~66 to 120 km, as our 'primary' sample locations. Fishermen provided additional samples collected outside of this focal survey period in July 2008, May 2009, and June 2008 and 2009. Surveying a site did not guarantee that *S. lewini* would be present at that specific time and location and all sample collection was opportunistic. Ultimately, sufficient samples (≥7 individuals) were

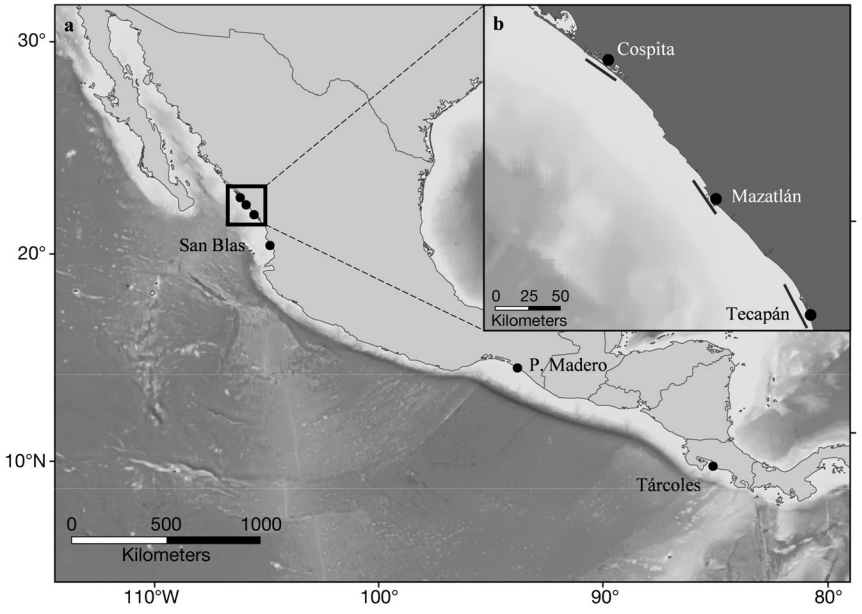

Fig. 1. (a) Sites where young-of-the-year scalloped hammerhead sharks *Sphyrna lewini* were collected in 2007 to 2009 to investigate the potential of vertebral elemental signatures to reveal their natal origins. (b) Location of the 3 primary study sites within the rectangle on the large-scale map where sampling was conducted on a monthly basis from August to November each year. The dark lines associated with each site indicate the approximate along-shore distance from which specimens were captured by artisanal fisheries

obtained from 6 sites for the period July to November from 2007 to 2009. Survey locations spanned >3000 km of coastline and encompassed areas of diverse geology and oceanographic circulation patterns (Castro et al. 2000, Tapia-Garcia et al. 2007). Vertebrae were excised from landed specimens within hours of capture and stored frozen. Whenever possible, the location of capture, sex, total length (cm), total weight (kg), and status of the umbilical scar (after Duncan & Holland 2006; open, partly healed, healed, well-healed) were recorded from landed *S. lewini*. Umbilical scars are typically open during the first week following birth and become closed (well-healed) by the end of the first year (Duncan & Holland 2006).

We collected vertebrae from 898 young-of-the-year *S. lewini* at the 6 locations surveyed. All sharks were captured in nearshore coastal habitats at depths of 15 to 35 m and ranged in size from 43.5 to 96.5 cm TL. Based on records of umbilical scar status and observations of thin-sectioned vertebrae, all sharks were <1 yr of age and ages ranged between 1 wk and ~7 mo. We restricted our analyses to samples collected from May through November of each year and between the 7th and 25th day of a given month. Locations with a minimum of 7 samples were used in this study and a maximum of 20 samples were prepared for a month and site (target = 15). Elemental analyses were completed using the vertebrae of 367 sharks that met these conditions.

Sample preparation

Cleaning and processing of *S. lewini* vertebrae followed procedures documented in Smith et al. (2013). Polished, thin-sectioned (~0.4 mm) centra were affixed to acid-washed petrographic slides (5 to 15 per slide). Sample arrangements and groupings were randomized to prevent systematic bias. Sample preparation and drying procedures were completed in a Class 100 laminar flow work station. We viewed mounted vertebral sections under a dissecting microscope and etched identifying marks in the resin adjacent to the birthmark to establish the transect position for elemental analysis (Fig. 2). Birthmarks, identified as the first band distal to the focus associated with a distinctive change in the angle of the vertebral centrum, are commonly representative of the transition from uterine to post-partum life history (Cailliet & Goldman 2004). Sample slides were rinsed with ultrapure 1% nitric acid, cleaned ultrasonically, triple rinsed, dried, and stored in plastic bags.

Elemental analysis

We quantified the elemental composition of young-of-the-year *S. lewini* vertebrae using laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS). Analyses were completed at Oregon State University's WM Keck Collaboratory for Plasma Spectrometry using a VG PQ ExCell ICPMS coupled with a DUV193 excimer laser (New Wave Research). The laser was set with an ablation spot size of 80 µm at a pulse rate of 5 Hz and translated across the sample at 5 µm s^{-1}. All transects were pre-ablated (100 µm spot size, 2 Hz, 100 µm s^{-1}) to reduce potential sample contamination. Laser transects were positioned across but entirely within the corpus calcareum and targeted the area of vertebral deposition following the birthmark to characterize natal ele-

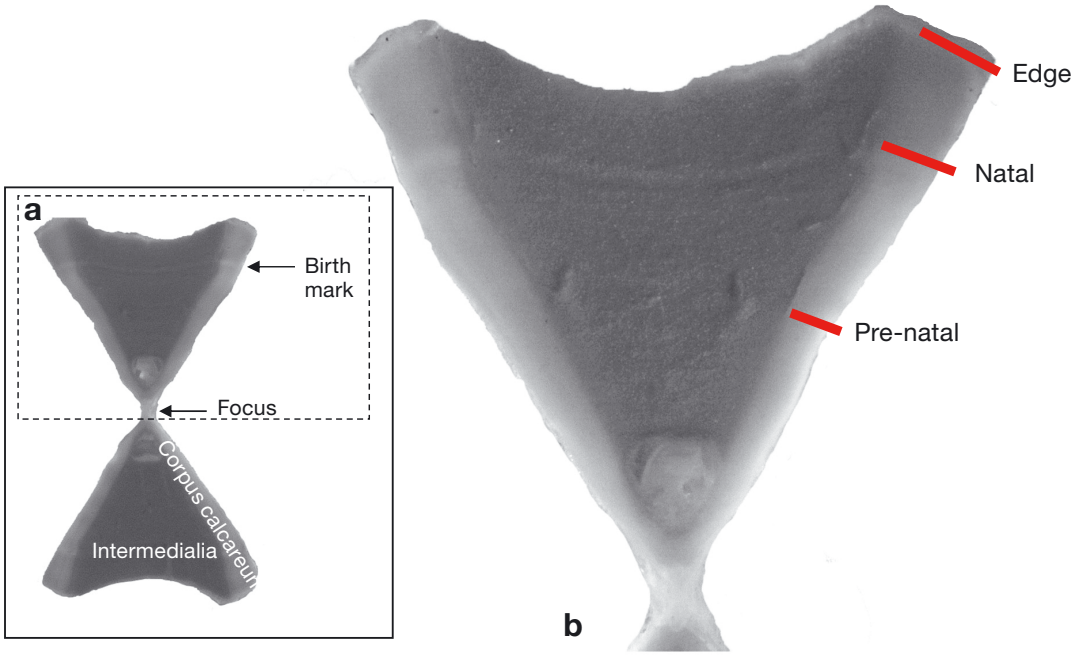

Fig. 2. Sagittal section of young-of-the-year scalloped hammerhead shark *Sphyrna lewini* vertebrae: (a) whole, thin-sectioned vertebra; (b) location of the regions selected for laser ablation in this study

mental signatures for all samples (Fig. 2), hereafter referred to as the 'natal signature'. Two additional laser transects were made within a subset of these vertebral samples to assess elemental composition at different periods in the life history of individual sharks: (1) in the area formed when in-utero ('pre-natal signature') and (2) at the outer-most edge, which is the most recently deposited material and represents the signature from the location at the time of capture ('edge signature') (Fig. 2b). Because edge signatures correspond to the time immediately prior to capture, they provide a known spatial and temporal reference from which the validity of elemental signatures as spatial markers can be evaluated (i.e. site of collection or geographic origins).

We collected data on 13 elements: lithium (Li), magnesium (Mg), calcium (Ca), vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), rubidium (Rb), strontium (Sr), cadmium (Cd), barium (Ba), lanthanum (La), and lead (Pb). Background levels of analyte isotopes were measured for 45 s prior to ablation and subtracted from those determined from counts obtained from the standard and vertebral samples. Analyte counts of vertebral transects were integrated and averaged using time-resolved software (PlasmaLab®). Cobalt (^{59}Co), cadmium (^{111}Cd), and lanthanum (^{139}La) counts were consistently below detection limits (defined as 3 × SD of the background level) and were therefore excluded from analyses. Subdetection level counts were occasionally obtained for V (n = 10, 3 % of samples) and Rb (n = 42, 13 %). These samples, however, were retained for analyses and assigned equivalent replacement values (run-specific detection limit/$\sqrt{2}$) to avoid reducing sample size or losing potentially relevant spatial and temporal information (Geffen et al. 2011). Thus, ^{7}Li, ^{25}Mg, ^{51}V, ^{52}Cr, ^{55}Mn, ^{85}Rb, ^{88}Sr, ^{138}Ba, and ^{208}Pb were examined. Count data were normalized by ^{43}Ca to adjust for variability in instrument sensitivity and the amount of ablated material and converted to elemental ratios (e.g. Ba/Ca) based on measurements of NIST 612 glass standard (Dove et al. 1996, Kent & Ungerer 2006). NIST 612 was used because an established calcium phosphate standard was not available. Elemental ratios are presented in mmol mol^{-1} (Mg, Sr) or µmol mol^{-1} (Li, V, Cr, Mn, Rb, Ba, Pb). Estimates of precision (percent relative standard deviation, %RSD) of these element-to-calcium ratios for the NIST 612 glass standard were: Li = 5.9 %, Mg = 13.8 %, V = 5.7 %, Cr = 5.1 %, Mn = 5.1 %, Rb = 6.2 %, Sr = 4.4 %, Ba = 5.0 %, and Pb = 8.5 % (n = 121 NIST runs).

Of the 367 individuals analyzed, 8 samples generated extremely low calcium counts and were excluded from analysis. Thirty-one samples were identified as outliers and removed, reducing the total number of samples to 328 young-of-the-year *S. lewini* used for temporal and spatial analyses (Table 1). Of those samples identified as outliers, 39 % (n = 12)

Table 1. Samples of vertebrae of young-of-the-year scalloped hammerhead sharks *Sphyrna lewini* included in elemental analyses. Sites where samples were collected along the Pacific coast of Mexico and Costa Rica (Fig. 1) are listed in order of location from north to south. Primary locations (P) were surveyed on a monthly basis and secondary (S) locations were sampled opportunistically. Distance from previous site indicates the approximate linear distance between the site and the one listed above. 'Size' is the average total length (TL) ± standard deviation (cm) of individuals included in the analysis. Size-at-birth is ~38 to 56 cm TL

Year	Site	Location type	Distance from previous site (km)	Month of collection							n	Size (cm)
				May	Jun	Jul	Aug	Sep	Oct	Nov		
2007	Cospita	P					×	×	×	×	33	65.6 ± 10.6
	Mazatlán	P	120				×	×	×	×	38	60.6 ± 10.3
	Tecapán	P	66				×	×	×		27	64.4 ± 8.1
2008	Cospita	P								×	15	65.0 ± 9.2
	Mazatlán	P	120		×	×	×		×	×	47	58.6 ± 8.7
	Tecapán	P	66				×		×	×	33	74.3 ± 11.9
	P. Madero	S	1643							×	10	61.5 ± 5.1
	Tárcoles	S	1268							×	11	74.0 ± 6.7
2009	Cospita	P					×	×	×	×	49	66.8 ± 9.8
	Mazatlán	P	120				×		×		10	62.4 ± 10.9
	Tecapán	P	66		×		×				24	55.0 ± 3.5
	San Blas	S	88	×							7	52.3 ± 2.9
	P. Madero	S	1302					×	×		24	53.8 ± 3.7

came from one of 3 sample slides suggesting that these may have been compromised during mounting or analytical procedures.

Data transformation and analysis

Elemental ratios were assessed for normality, and tested for homogeneity of variances/covariances, using univariate and multivariate techniques. Elemental ratios were log-transformed using a generalized procedure described by McCune & Grace (2002):

$$b_{ij} = \log(x_{ij} + d) - c \quad (1)$$

where, x_{ij} is the original value in row i and column j of the data matrix, b is the transformed value, x the original value, and c and d are order of magnitude and decimal constants, respectively. This transformation is particularly beneficial when working with low fractional values because it reduces the compression of data points that frequently result from log transformation while maintaining the original order of magnitudes (McCune & Grace 2002). Outliers (>3 SD of the mean average distance) were identified and removed following visual inspection of frequency distributions and Euclidean distances (McCune & Grace 2002). Transformation improved all distributional assumptions; however, univariate normality was not achieved for all elements (Shapiro-Wilk test) and multivariate distributions were negatively skewed in all 3 years (quantile-quantile plots). Variances were found to be equivalent for the majority of elemental ratios, with unequal variances detected among V/Ca and Mn/Ca in 2007, V/Ca in 2008, and Cr/Ca, Sr/Ca, and Pb/Ca in 2009 (Levene's test).

Individual variation

Consistency in natal signatures within individuals

For elemental signatures to serve as effective markers, elements should be deposited in a consistent manner (Campana & Thorrold 2001, Elsdon et al. 2008). Therefore, we first tested the hypothesis that natal elemental signatures are equivalent between vertebrae within individual sharks. Duplicate vertebral samples were prepared from 15 individuals. We applied a blocked variation of multi-response permutation procedures (MRPP) to test the null hypotheses of no difference in elemental composition between vertebrae within individuals (Hypothesis 1 in Table 2, Fig. 2). MRPP is a nonparametric test for differences between 2 or more groups (McCune & Grace 2002). It calculates the average multivariate distance within *a priori* groupings and determines whether the average within-group distance is significantly less than those obtained from randomly assigning the observed values to each group (McCune & Grace 2002, Mielke & Berry 2007). Like parametric paired or

Table 2. Summary of the primary hypotheses, scale of inquiry, and analytical approaches applied to investigate the potential of vertebral elemental signatures to reveal the natal origins of hammerhead sharks *Sphyrna lewini*. Signature(s) refers to the position along the vertebral centra from which samples were analyzed. MANOVA: multivariate analysis of variance; DFA: step-wise discriminant function analysis; MRPP: multi-response permutation procedure; MLE: maximum likelihood estimation

Hypothesis	Scale of inquiry	Analysis	Signature(s)	Secondary analysis	Signature(s)
1. Consistency in signatures between vertebrae	Individual	MRPP	Natal		
2. Differences between pre- and post-partum signatures	Individual	MRPP	Natal, pre-natal	Paired-*t*	Natal, pre-natal
3. Signatures consistent within sites each year	Intra-annual	MRPP	Natal, edge		
4. Signatures consistent within sites across years	Inter-annual	MRPP	Natal		
5. Characteristic signatures among sites	Spatial	MANOVA	Natal	DFA, MLE	Natal, edge

repeated measures tests, blocked MRPP is appropriate when samples are not independent but does not require the assumptions of equal variances and normal distributions (Mielke & Berry 2007). Separation between groups is characterized by the test statistic (T), with more negative values indicating greater separation between natal and edge signatures (groups). Similarity within groups is summarized by a chance-corrected measure of agreement (A). If signatures were perfectly identical within each group, A would be equal to 1.0. When within-group agreement equals expectation by chance, then $A = 0$. Test statistics were compared to a Pearson Type III distribution with mean, variance and skewness calculated from permuted datasets (McCune & Grace 2002).

Records of environmental transition

Though physiological and kinetic factors significantly affect elemental incorporation and modify relationships with the ambient environment, the chemical composition of calcified structures can provide records of the physical and chemical environment experienced by individuals (Elsdon et al. 2008, Kerr & Campana 2014, Sturrock et al. 2015). Vertebral chemistry should exhibit changes in composition in response to environmental transitions if elemental signatures are to serve as effective markers of natal origin and environmental history. We compared pre-natal and post-partum elemental composition to test the assumption that elemental signatures reflect known changes in the environment, as would be expected because adult females do not reside in nursery areas prior to giving birth (Hypothesis 2 in Table 2). A randomly selected subset of sharks (n = 47) was used to assess variation between pre-natal (in-utero) and natal (post-partum) elemental composition (Fig. 2b). A blocked MRPP was applied and followed with univariate paired *t*-tests on individual element-to-calcium ratios to determine which elements were associated with any observed variation between pre-natal and natal signatures (Zar 1996).

Temporal variation

To the extent that vertebral elemental signatures reflect ambient environmental conditions, temporal variation in water chemistry, salinity, or temperature could generate different natal signatures within a site, within and across years. Temporal variability in environmental conditions within a site can therefore obscure underlying patterns of variation in elemental signatures among sites (Hamer et al. 2003, Ruttenberg et al. 2008). Therefore, we evaluated intra- and inter-annual variation in elemental signatures from the primary sample locations before assessing spatial differences in natal and edge signatures.

Intra-annual variation

Our opportunistic surveys of fishery-derived specimens did not produce samples from each month to enable complete site-specific comparisons of intra-annual variation in natal signatures across the period of study. We therefore assessed the extent of variation in elemental signatures within sites and years by comparing natal signatures with that of the edge. Natal and edge signatures reflect the time of birth and the time of capture, respectively (Fig. 2b). Although there could be some differences in individual movement patterns since birth, similarity between natal and edge signatures suggest equivalent conditions and consistency in site-specific signatures between the 2 time periods. Assuming individuals have not dispersed from their natal sites, significant differences between natal and edge signatures would indicate change in environ-

mental and/or physiological conditions and thus a need to account for temporal instability in these spatial markers. We used a blocked MRPP to test the null hypothesis of no difference in elemental composition within sites between paired-sample natal and edge signatures by year and month of capture (Hypothesis 3 in Table 2). We calculated average Euclidean distances for natal and edge signatures by site and month as an additional measure of variability within each group. Lower mean Euclidean distances indicate greater similarity within the group. Data from our primary collection sites were used to evaluate the temporal stability of site-specific elemental signatures.

Inter-annual variation

Consistency in site-specific natal signatures across years was evaluated using standard (unblocked) MRPPs with data from our primary study sites. To determine which years and sites contributed to variation in natal signatures across years, we conducted pair-wise analyses of inter-annual differences in signatures by year and month (Hypothesis 4 in Table 2). Pair-wise comparisons of significance were identified using Bonferroni-corrected p-values. Blocked and standard MRPPs were run with PC-ORD (Version 6.13).

Spatial variation

The ability to distinguish the geographic origins of young-of-the-year *S. lewini* from natal and edge signatures was evaluated using multivariate analysis of variance (MANOVA) and linear discriminant function analysis (DFA) (Hypothesis 5 in Table 2). First, we applied single factor MANOVA to determine if differences could be detected in natal signatures among the primary sample locations. Pillai's trace was used as the test statistic because it is more robust in the case of small and unequal samples sizes (Tabachnick & Fidell 2007). Forward step-wise DFA was used to classify sharks and identify those elements that contribute most to group separation (McGarigal et al. 2000). Thus, each DFA considered all 9 elemental ratios but final classifications were based on a subset of these variables. Group classification accuracy was assessed using a leave-one-out jackknife procedure with the prior probabilities of group membership assumed to be uniform (Wilson White & Ruttenberg 2007). The reliability of group classifications was further evaluated by calculating a chance-corrected classification metric (*Tau*) to determine if group assignments predicted by DFA exceeded that of randomly assigning individuals to groups when prior probabilities are not assumed to be equal to group sample sizes (Klecka 1980). A maximum value for *Tau* of 1.0 signifies perfect agreement and 0 indicates no improvement over chance. DFA was conducted in SYSTAT (Version 12.0).

As a final evaluation of the utility of natal and edge signatures to correctly assign individuals to natal origins and sites of collection, we used maximum likelihood estimates (MLE) to calculate the error associated with group classification under simulated mixed stock conditions (Table 1). MLE have been found to provide improved discriminatory power in situations where individuals from different source populations are likely to mix (Wood et al. 1987, Wilson White & Ruttenberg 2007). Analyses were performed using the multipurpose simulation bootstrap analysis program (HISEA) developed by Millar (1990a, 1990b). MLEs were run for the same groupings of natal and edge signatures as applied in DFA and included all 9 elemental ratios. The program was run in simulation mode with baseline elemental data determined from the proportional contribution of sharks collected from each site. A hypothetical mixed stock was drawn from these baseline data and resampled with replacement over 1000 runs using a simulated mixture of 100 individuals per run to generate MLE of site-specific classifications.

RESULTS

Individual variation

Consistency in natal signatures within individuals

Analyses of natal signatures supported the assumption that elements are deposited consistently between vertebrae within individuals. The elemental composition of natal signatures did not differ between vertebrae within individual *S. lewini* (MRPP: $T = 0.37$, $p = 0.55$, $A = -0.01$, $n = 15$). However, the sample size for this comparison was low.

Records of environmental transition

Elemental signatures varied in response to known changes in the environment and life history. Paired comparisons between pre-natal and natal signatures

identified significant differences in elemental composition between the 2 corresponding vertebral regions (MRPP: $T = -8.67$, $p < 0.001$, $A = 0.013$, n = 47). Paired *t*-tests revealed that differences in V/Ca (paired *t*-test: $t = 3.37$, $p = 0.001$, n = 47), Mn/Ca (paired *t*-test: $t = 2.74$, $p = 0.013$, n = 47) and Sr/Ca ($t = 8.12$, $p < 0.001$, n = 47) were driving the observed variation. Each of these elemental ratios were elevated within areas of the vertebrae that were deposited following birth in comparison to the average elemental ratios measured within the pre-natal region of the same sample. Differences between pre-natal and natal signatures support the assumption that vertebral chemistry can record changes in the physical and chemical environments experienced by individuals.

Table 3. Blocked, multi-response permutation procedure (MRPP) tests of differences between paired samples of natal and edge signatures from vertebrae of young-of-the-year scalloped hammerhead shark within year, site and month (to test Hypothesis 3 in Table 2). Lower within-group differences (Euclidean distances) indicate greater similarity in elemental signatures. p-values in **bold** identify significant differences measured by the test statistic *T* between natal and edge signatures within a site. *A* represents a chance-corrected measure of agreement within groups

Year	Location	Month	n	Average within-group differences		*T*	p	*A*
				Natal	Edge			
2007	Cospita	August	10	1.92	1.41	−4.23	**0.005**	0.20
		November	13	0.82	0.83	−7.75	**<0.001**	0.52
	Mazatlán	August	8	1.76	1.67	−2.90	**0.016**	0.13
		September	5	1.58	1.63	−3.08	**0.015**	0.42
		October	6	0.90	1.82	−3.84	**0.008**	0.53
		November	9	1.24	1.15	−5.85	**0.001**	0.59
	Tecapán	August	6	1.32	1.42	−2.36	**0.031**	0.19
		September	8	1.02	1.20	−4.09	**0.006**	0.38
		October	9	1.24	1.28	−5.80	**0.002**	0.56
2008	Cospita	November	14	0.62	2.23	−9.44	**<0.001**	0.81
	Mazatlán	June	6	0.79	7.49	−1.13	0.129	0.14
		July	8	5.14	7.14	−1.00	0.159	0.06
		August	11	1.40	4.67	−6.71	**0.001**	0.76
		October	17	1.40	3.90	−11.51	**<0.001**	0.80
		November	5	1.36	0.87	−2.45	**0.029**	0.92
	Tecapán	August	12	3.16	5.04	−3.87	**0.008**	0.43
		November	12	1.14	0.95	−8.12	**<0.001**	0.93
2009	Cospita	August	6	2.31	1.72	−7.37	**<0.001**	0.70
		September	8	1.11	1.43	−13.07	**<0.001**	0.91
		October	7	1.03	2.02	−4.58	**0.004**	0.91
		November	14	0.97	0.90	−9.54	**<0.001**	0.93
	Mazatlán	August	11	4.64	4.83	−1.92	0.054	0.17
		October	7	1.10	1.03	−4.58	**0.004**	0.92
	Tecapán	August	10	2.95	2.05	−6.00	**0.001**	0.74

Intra-annual temporal variation

Site-specific natal signatures varied significantly within each year. Blocked MRPPs of paired natal and edge signatures revealed significant variation in elemental composition between the time following birth and the time of collection within all sites and most months (Table 3). Similarity between natal and edge signatures (as measured by *T*) generally decreased throughout the year. Among sharks landed in Mazatlán in 2008, for example, natal signatures were equivalent to edge signatures in June and July (MRPP: $T \leq 1.0$, $p \geq 0.13$, $A \geq 0.06$) but natal and edge signatures differed from one another in August, October, and November (MRPP: $T \leq -2.45$, $p \leq 0.03$, $A \geq 0.76$). This observed intra-annual variation in vertebral chemistry suggests that environmental or physiological changes occur between the time of birth (natal signature) and time of collection (edge signature) that alter chemical composition, that sharks may be arriving from other sites thereby increasing the variation between natal and edge signatures (i.e. introducing undocumented natal signatures), that sharks may be encountering different habitats within their nursery areas as a result of range expansions, or some combination thereof. Chance-corrected measures of within-group agreement (*A*) revealed an increase in similarity among signatures within groups across months (e.g. Mazatlán in 2008: *A* increases from 0.14 to 0.92). In many cases (Mazatlán, November 2008; Tecapán, November 2008; Cospita, September, October, and November, 2009; Mazatlán, October, 2009), within-group similarity in elemental signatures (i.e. signature type) was >90% better than chance, suggesting a low probability of mixing from other chemically distinct natal sources.

Inter-annual temporal variation

Natal signatures of *Sphyrna lewini* differed significantly across years within sites (Table 4). Li/Ca, Mn/Ca, Sr/Ca, Ba/Ca, and Pb/Ca displayed the greatest relative variation over the 3 yr

Table 4. Multi-response permutation procedure (MRPP) tests of difference in the elemental composition of site-specific natal signatures from vertebrae of young-of-the-year scalloped hammerhead shark by month and year (to test Hypothesis 4 in Table 2). p-values presented in **bold** identify significant differences measured by the test statistic *T* in site-specific natal signatures between years as within a site. *A* represents a chance-corrected measure of agreement within groups

Location	Month	n	Years	*T*	p	*A*
Cospita	August	22	2007 vs. 2009	0.29	0.524	–0.01
	November	42	2007 vs. 2008	–9.37	**<0.001**	0.16
			2007 vs. 2009	–2.38	0.030	0.04
			2008 vs. 2009	–8.36	**<0.001**	0.14
Mazatlán	August	35	2007 vs. 2008	–9.06	**<0.001**	0.22
			2007 vs. 2009	–2.30	0.036	0.06
			2008 vs. 2009	–9.17	**<0.001**	0.21
	October	34	2007 vs. 2008	–6.99	**<0.001**	0.09
			2007 vs. 2009	–2.94	0.080	0.01
			2008 vs. 2009	–7.44	**<0.001**	0.11
Tecapán	August	27	2007 vs. 2008	–5.07	**0.002**	0.15
			2007 vs. 2009	0.92	0.924	–0.02
			2008 vs. 2009	–5.00	**0.002**	0.11

study period (Fig. A1 in the Appendix). However, pair-wise MRPPs indicated that variation among years was driven by differences in the natal signatures from only one year, 2008 (Table 4). Natal signatures of the 2007 and 2009 cohorts within Cospita, Mazatlán, and Tecapán did not differ from one another between these years. Although inter-annual differences in natal signatures can be expected, equivalent signatures may also be produced within a location between years.

Spatial variation

Natal signatures of *S. lewini* differed significantly among the primary collection locations (MANOVA: Pillai's Trace = 0.26, $F_{2,275}$ = 34.96, p < 0.001). Although spatial differences in natal signatures were evident among sites based on MANOVA using samples pooled across years, results from our temporal analyses indicate that both intra- and inter-annual variation in natal signatures occurs within these sites that would likely confound our ability to successfully classify individual sharks to their natal origins (Tables 3 & 4). To more effectively evaluate the utility of vertebral elemental signatures as spatial markers, we removed the influence of inter-annual variability by focusing our analyses on individual years. We attempted to minimize the effects of within-year variation in elemental signatures on group classification in 2 ways. First, we classified individuals to their location of capture using edge signatures which are representative of conditions around the time of capture. The use of edge signatures therefore provides a common, known geographic reference (regardless of any variation in dispersal history or birth date) from which to evaluate the overall utility of elemental signatures as spatial markers. Next, we attempted to reduce temporal influences on variation in natal signatures by conducting analyses of sharks grouped by season of capture. Finally, we classified sharks to their putative natal origins using samples pooled across months within years to compare the success of assignments without adjusting for the influence of intra-annual variation on natal signatures.

Classifications were first performed using data collected from vertebral edges in order to determine the utility of elemental signatures as spatial markers from specimens of known geographic origins (i.e. location of capture). To narrow the temporal and spatial representation of edge signatures from each site, samples were restricted to only those *S. lewini* captured within 7 d of one another and from the same fishing area within a site. DFA of edge signatures validated the utility of vertebral elemental signatures as reliable spatial markers. Overall classification accuracy to collection sites using this refined data set ranged from 83 to 100%, which greatly exceeded chance expectations (*Tau* = 0.67 to 1.0) (Table 5, Figs. 3 & 4). Site-specific classification success rates varied among years from 50 to 100%. *S. lewini* collected from our primary sample sites were distinguished from those taken at the more distant location of Puerto Madero (~1600 km distance) with 100% accuracy in each month and year for which data were available. Ba/Ca, Sr/Ca, and Mg/Ca were most frequently identified as key discriminating variables among sites. V/Ca ratios ranked among the primary contributors for discerning groups in 2007. Li/Ca ratios were not assayed for a subset of the 2007 samples and were therefore not included in analyses for that year. However, Li/Ca ranked as the primary contributor to group distinction in October 2008 and the second most important contributor in the October 2009 classification based on step-wise DFA.

A second level of classification was examined using natal signatures from sharks that were col-

Table 5. Classification accuracy (%) of discriminant function analyses (DFA) and maximum likelihood (ML) estimates of young-of-the-year scalloped hammerhead sharks to collection locations using edge signatures, with samples grouped by month and site of collection (to test Hypothesis 5 in Table 2). *Tau* is a measure of improvement in classification accuracy over chance (1.0 = no errors in prediction, 0 = no improvement over random chance). 'Elemental ratios' are the element-to-calcium ratios that were retained in step-wise DFA to achieve optimal group separation, presented by rank (highest to lowest). Observed contributions are proportional to the number of sharks included in analyses from each site and time period. The ML contribution and standard deviation (SD) of sharks classified to each site were estimated from 1000 bootstrap simulations. Note that sample availability allowed us to compare the edge signatures of sharks captured at 2 different locations ~10 km apart within the Mazatlán fishery in October 2008 (Mazatlán A, Mazatlán B)

Year	Month	Overall (monthly) classification success (%)	*Tau*	Elemental ratios	Site	n	Classification accuracy (%)	Observed contribution	ML estimated contribution
2007	August	94	0.89	Ba, Rb, Sr	Cospita	11	91	0.65	0.71 ± 0.07
					Mazatlán	6	100	0.35	0.29 ± 0.07
	November	81	0.62	Rb, Cr, Pb	Cospita	13	85	0.62	0.69 ± 0.05
					Mazatlán	8	75	0.38	0.31 ± 0.05
2008	October	83	0.67	Li, Cr	Mazatlán A	7	86	0.39	0.32 ± 0.05
					Mazatlán B	11	82	0.61	0.68 ± 0.05
	November	86	0.68	Sr, Mg, Rb	Cospita	15	93	0.25	0.20 ± 0.06
					Tecapán	6	50	0.54	0.59 ± 0.07
					P. Madero	7	100	0.21	0.21 ± 0.04
2009	August	72	0.72	V, Sr, Ba	Cospita	13	64	0.42	0.48 ± 0.07
					Mazatlán	7	83	0.23	0.23 ± 0.05
					Tecapán	10	75	0.35	0.29 ± 0.06
	September	100	1.00	Mg, Sr, Mn, Ba	Tecapán	10	100	0.43	0.43 ± 0.05
					P. Madero	13	100	0.57	0.57 ± 0.05
	October	89	0.84	Mg, Sr, Li	Cospita	7	71	0.43	0.41 ± 0.06
					Mazatlán	5	100	0.24	0.26 ± 0.06
					P. Madero	7	100	0.33	0.33 ± 0.05

lected early (June to August) and those collected late (October to November) in each year. Classification success was mixed and generally moderate for early and late season cohorts within each site (Table 6). Among samples collected in 2007, classification accuracy ranged from 64 to 83% within sites. Mn/Ca ratios were the primary discriminator among sites in 2007. Classification accuracy was greater using early season (70%) versus late season (39%) designations in 2008. However, assignment of sharks to the most distant sites, Puerto Madero and Tárcoles, in 2008 was high to moderate, 80% and 64%, respectively. Ba/Ca ratios ranked as the most useful identifier of groups in 2008. Assignments to natal origins for 2009 were more successful among late season designations (71%). Contributions of individual elemental ratios to group discrimination also differed among early and late scenarios. Mg/Ca, Mn/Ca and Sr/Ca were the most important variables contributing to discrimination among early groups and Mn/Ca and Mg/Ca ranked as the principal variables used to discern source locations among individuals grouped within the late season.

The third evaluation of group assignment success was performed using natal signatures from samples pooled across months, within year. DFA of these pooled data generated an overall jackknifed classification success of 54% (Table 7). In 2007, assignment was most successful for the northernmost site, Cospita. Mn/Ca and Sr/Ca ratios accounted for 98% of the total variation among sites. Classifications using these discriminating variables achieved 42% fewer errors than would be expected by random assignment. Site-specific classification was highly variable in 2008, ranging from 39 to 80% with the lowest and highest classification rates derived from Tecapán and Tárcoles, respectively. Overall jackknifed classification success was 47% among the pooled 2008 samples which represented a significant improvement over random assignment to sites (*Tau* = 0.33). Stepwise selection of variables indicated that optimal group separation could be achieved using 4 of the 9 elemental ratios: Ba/Ca, V/Ca, Pb/Ca, and Mn/Ca. DFA of samples pooled across months in 2009 attained, overall, moderate classification success; 67% with site-specific values ranging from 50% (Teca-

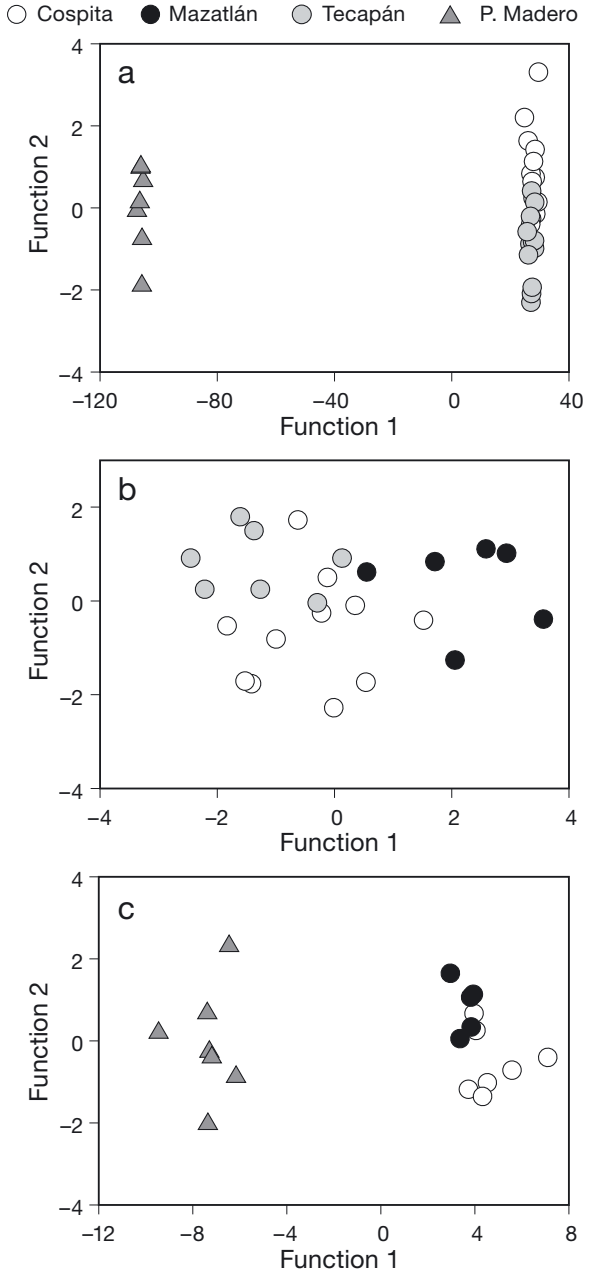

Fig. 3. Canonical function plots of discriminant function analyses (DFA) based on vertebral edge signatures of young-of-the-year scalloped hammerhead shark, grouped by site (Fig. 1), month, and year of collection: (a) Cospita, Tecapán, and Puerto Madero, November 2008; (b) Cospita, Mazatlán, and Tecapán, August 2009; (c) Cospita, Mazatlán, and Puerto Madero, October 2009

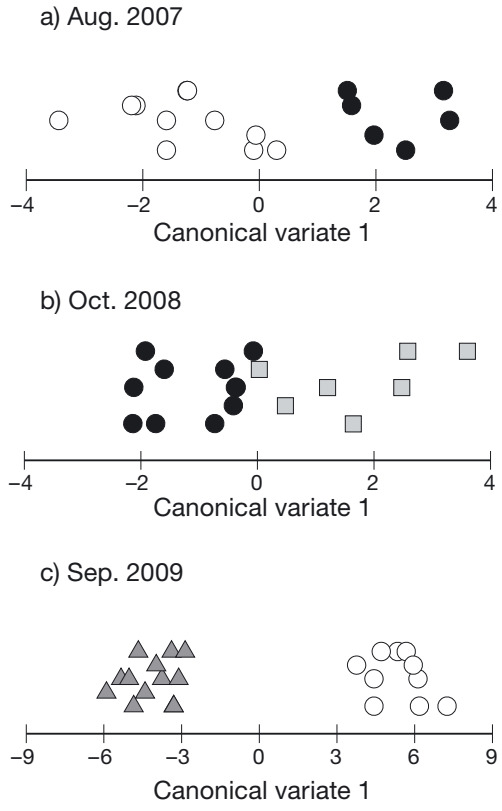

Fig. 4. Jittered dot density plots of canonical variate 1 resulting from discriminant function analyses (DFA) of vertebral edge signatures of young-of-the-year scalloped hammerhead shark, grouped by site, month, and year of capture: (a) Cospita vs. Mazatlán, August 2007; (b) comparison of 2 sites approximately 10 km apart in Mazatlán: Mazatlán A vs. Mazatlán B (grey squares), October 2008; (c) Cospita vs. Puerto Madero, September 2009. Other symbols as in Fig. 3

pán) to 76% (Cospita). Sr/Ca ratios provided by far the largest discriminatory power with Mg/Ca and Cr/Ca ranking as the second and third most important variables for group assignments based on F-to-remove statistics. V/Ca and Rb/Ca were the only elemental ratios excluded as discriminatory variables in this step-wise model. Group classification to collection locations demonstrated a significant improvement over random assignment (*Tau* = 0.58).

Maximum-likelihood simulations confirmed that the geographic origins of young-of-the-year *S. lewini* could be predicted with a high level of accuracy from elemental signatures (Tables 5–7). Estimated contributions were similar to known contributions and generated errors between 0.0–7.0% and <1.0–6.0% for edge and natal signatures, respectively, indicating distinctive site-specific signatures and a low probability of mixing among nursery areas. As observed within DFA, the accuracy of MLE group assignments also varied with the level of temporal resolution assigned to the data set. Standard deviations were lowest among samples grouped by month of capture (edge signatures: 4.1 to 6.9%) and greatest among samples pooled within year (natal signatures: 4.7 to 15.4%).

Table 6. Classification accuracy (%) of discriminant function analyses (DFA) and maximum likelihood (ML) estimates of young-of-the-year scalloped hammerhead sharks to locations of collection using natal signatures, with samples grouped by season (early, late), year and site of collection (Hypothesis 5 in Table 2). See Table 5 legend for further explanation of methods and results shown

Year	Overall (seasonal) classification success (%)	*Tau*	Elemental ratios	Site	n	Classification accuracy (%)	Observed contribution	ML estimated contribution
Early (July to August)								
2007	73	0.62	Mn, Sr, Pb, Ba, V, Mg	Cospita	11	64	0.33	0.28 ± 0.09
				Mazatlán	13	83	0.34	0.35 ± 0.07
				Tecapán	11	70	0.33	0.37 ± 0.10
2008	70	0.39	Ba, Pb, V, Mg, Mn, Li, Sr	Mazatlán	21	72	0.76	0.82 ± 0.08
				Tecapán	12	63	0.24	0.18 ± 0.08
2009	63	0.51	Mn, Mg, Sr	Cospita	31	72	0.37	0.34 ± 0.06
				Mazatlán	10	60	0.12	0.18 ± 0.05
				Tecapán	24	58	0.14	0.11 ± 0.04
				San Blas	7	45	0.08	0.08 ± 0.03
				P. Madero	12	71	0.29	0.29 ± 0.06
Late (October to November)								
2007	63	0.48	Mn, Cr, V, Rb	Cospita	17	71	0.34	0.37 ± 0.09
				Mazatlán	22	59	0.46	0.43 ± 0.08
				Tecapán	10	60	0.20	0.21 ± 0.07
2008	39	0.24	Ba	Cospita	15	27	0.18	0.20 ± 0.11
				Mazatlán	23	13	0.27	0.22 ± 0.14
				Tecapán	25	39	0.30	0.34 ± 0.11
				P. Madero	10	80	0.12	0.11 ± 0.05
				Tárcoles	11	64	0.13	0.14 ± 0.05
2009	71	0.49	Mg, Mn, V, Ba, Sr, Pb Li	Cospita	21	71	0.49	0.49 ± 0.07
				Mazatlán	7	43	0.16	0.15 ± 0.07
				P. Madero	15	85	0.35	0.36 ± 0.05

Table 7. Classification accuracy (%) of discriminant function analyses (DFA) and maximum likelihood (ML) estimates of young-of-the-year scalloped hammerhead sharks to locations of collection using natal signatures, with samples grouped by site and year of collection (Hypothesis 5 in Table 2). See Table 5 legend for further explanation of methods and results shown

Year	Overall (annual) classification success (%)	*Tau*	Elemental ratios	Site	n	Classification accuracy (%)	Observed contribution	ML estimated contribution
2007	54	0.37	Mn, Sr	Cospita	33	70	0.34	0.33 ± 0.13
				Mazatlán	38	61	0.39	0.36 ± 0.15
				Tecapán	27	26	0.28	0.32 ± 0.10
2008	47	0.33	Ba, V, Pb, Mn	Cospita	15	47	0.13	0.13 ± 0.10
				Mazatlán	47	43	0.41	0.39 ± 0.11
				Tecapán	33	39	0.28	0.32 ± 0.12
				P. Madero	10	67	0.09	0.07 ± 0.05
				Tárcoles	11	80	0.09	0.10 ± 0.05
2009	67	0.58	Sr, Mg, Cr, Ba, Mn	Cospita	49	76	0.37	0.37 ± 0.06
				Mazatlán	10	60	0.12	0.13 ± 0.07
				Tecapán	24	50	0.14	0.12 ± 0.04
				San Blas	7	57	0.08	0.08 ± 0.03
				P. Madero	24	71	0.29	0.30 ± 0.07

DISCUSSION

Our analyses of *Sphyrna lewini* vertebrae revealed significant variation in elemental signatures across a range of spatial scales (e.g. tens of kilometers, Mazatlán to Tecapán; >1000 km, Cospita to Puerto Madero) that can be used to distinguish the natal origins of young-of-the-year sharks. Classification success improved (to 100% within some sites) when data were partitioned with increasing temporal resolution. Paired-sample comparisons indicated that elemental signatures were deposited consistently within indi-

viduals. The elemental composition of pre-natal signatures differed significantly from natal signatures, providing further evidence that vertebral chemistry can record environmental transitions experienced by individual elasmobranchs. Together, these results indicate that vertebral elemental signatures can be effective spatial markers in an elasmobranch. Additionally, the site-specific variation observed between natal and edge signatures within and across months suggests that vertebral chemistry may also be useful for assessing the environmental history and habitat use of elasmobranchs, although additional environmental data would be needed to more fully evaluate this possibility. Our findings are consistent with a growing body of evidence that support the application of elemental signatures as a viable tool not only for distinguishing natal origins and connectivity patterns but also for assessing the environmental history and habitat use of elasmobranchs over their lifetime (Tillett et al. 2011, Werry et al. 2011, Smith et al. 2013, Lewis et al. 2016).

Classification accuracies of *S. lewini* to putative natal origins were similar to or exceeded those obtained for marine fishes (Rooker et al. 2003, Patterson et al. 2004, Brown 2006) and seabirds (Gómez-Díaz & González-Solís 2007). Discrimination accuracies of market squid paralarvae (43 to 80%; Warner et al. 2009) and sessile coastal mussels (56 to 90%; Becker et al. 2005) from elemental signatures produced similar overall group classification success as in our study. Regional nursery areas of a large coastal shark, *Carcharhinus limbatus*, were distinguished with similar success among 2 cohorts (80 and 90%; Lewis et al. 2016). Though direct comparisons of DFA classification accuracy are complicated by different spatial and temporal scales of the studies and different life histories and physiologies of species, our results confirm that elemental signatures within elasmobranch vertebrae can be applied to discern natal origins as effectively as has been achieved with other calcified structures (i.e. otoliths, statoliths, shells).

Intra-annual temporal variation

Intra-annual variation in natal signatures has been documented on the order of weeks, months, and season in marine fishes (Gillanders 2002, Hamer et al. 2003, Cook 2011). We observed significant differences in elemental signatures within sites across months and seasons. The birthing period of *S. lewini* is reported to occur from late July to October in the eastern Pacific (Madrid et al. 1997). However, we encountered neonates as early as May (open umbilical scars). We would not expect the physical and chemical properties of this coastal environment to be static across a pupping period of 6 or more months. Many physical and biological processes including discharge from rivers, oceanographic circulation, local geology, biogeochemical cycles, wind, and anthropogenic input of pollutants influence the elemental composition of seawater (Bruland & Lohan 2003). As a result, sharks born earlier in the parturition period are likely to experience an environment that differs physically and chemically from that experienced by those born a few months later. Within our primary study sites, the parturition period is punctuated by the region's maximum rainfall, typically between July and September (60 to 80% of the total annual precipitation; Stensrud et al. 1995). Cyclones, tropical storms, and increased freshwater input into coastal areas during these months would alter salinity, temperature, and water chemistry, thereby modifying ambient environmental conditions and influencing physiological processes that could modify the composition of elemental signatures within sites (Elsdon & Gillanders 2006, Walther et al. 2010, Smith et al. 2013). Although we were unable to monitor water temperature and chemistry within our sample locations due to logistical constraints, MRPP analyses indicated greater variability within both natal and edge signatures (as measured by average within-group Euclidean distances, Table 3) during this period of elevated precipitation (July and August) than was found in the preceding months. DFA provided further evidence of changes within sites based on increased classification accuracies using seasonal designations versus samples pooled across the entire year. However, the potential for range expansion and mixing among sites restricts our interpretations of the mechanisms responsible for site-specific variation in natal signatures. Integration of elemental composition using laser transects taken along rather than across the corpus calcareum (see Smith et al. 2013) or across the corpus calcareum with a larger spot size (e.g. 100 to 120 µm) could reduce some of the variability in natal signatures by incorporating slightly longer depositional periods, thereby establishing site-specific signatures that may be less influenced by more ephemeral events.

Assessments of population connectivity and nursery ground contributions for elasmobranchs will require an understanding of species life history and site-specific intra-annual variation in natal signatures across the entire pupping season. Intra-annual variation in natal signatures may otherwise obscure

underlying patterns of spatial variation. Extended parturition periods are not representative of elasmobranchs in general. Other highly mobile sharks and rays, including spiny dogfish *Squalus suckleyi* (Tribuzio et al. 2005), blacktip sharks *Carharhinus limbatus* (Castro 1996), and cownose rays *Rhinoptera bonasus* (Poulakis 2013) exhibit more discrete parturition periods (1 to 2 mo). Efforts to collect samples that reflect the variation in natal signatures across the pupping season within a site may be somewhat simplified when working species with comparatively brief birthing periods.

Smith et al. (2013) established that the incorporation of some elements into elasmobranch vertebrae is dependent on water temperature and relative to concentrations in the ambient environment. Numerous factors have a direct and potentially interactive influence on the elemental composition of calcified structures that may have contributed to the temporal variation in signatures that was observed in this study (Campana 1999, Walther & Thorrold 2009, Sturrock et al. 2015). However, it is important to separate water chemistry from temperature, which we consider a component of the environment. The incorporation of many elements across a broad range of taxa is related to temperature variation (Zumholz et al. 2007, Miller 2009, Smith et al. 2013). While changes in individual physiology may be the ultimate mechanism for certain changes in vertebral chemistry, if those physiological changes are temperature-dependent (which is often the case for ectotherms) then the vertebral chemistry is reflective of the environment. Yet, the mechanisms regulating elemental incorporation are poorly studied for many elements (e.g. Li, Rb, V). Other elements that are frequently found to be valuable for distinguishing among groups, such as manganese, may be primarily derived from dietary sources (Mathews & Fisher 2009). We chose to exclude zinc as a constituent of vertebral elemental signatures because its vital physiological role and pathway of incorporation imply limited utility as a spatial marker (Miller et al. 2006, Smith et al. 2013). To eliminate the potential influence of ontogenetic effects on elemental incorporation (Walther et al. 2010), we have included only individuals of the same age and life stage in our study. Smith et al. (2013) concluded that individual variation in somatic growth and vertebral precipitation rates did not influence vertebral elemental composition in round stingrays *Urobatis halleri*. However, growth effects may differ among species, life stage, or geographic locations and this may influence elemental composition (Elsdon et al. 2008, Walther et al. 2010). Additional research on the regulating mechanisms and pathways of elemental incorporation into elasmobranch vertebrae is needed to clarify why elemental signatures may differ and which elements may be most relevant for spatial discrimination. Despite a limited knowledge of the mechanisms that influence elemental uptake and incorporation, the ability to identify spatially distinct signatures presents a viable tool for distinguishing natal origins and assessing population structure and connectivity in and of itself (Kerr & Campana 2014).

Inter-annual temporal variation

Inter-annual variation in natal signatures has frequently been documented among fishes (e.g. Gillanders 2002, Ruttenberg et al. 2008). Improvements in group classification accuracy have been reported when cohorts are examined by birth years, even where variation across years has been shown to be minimal (Brown 2006). Our observations of inter-annual differences in natal signatures of *S. lewini* confirm the importance of assessing potential variation across years, the benefit of cohort-specific analyses, and necessity of developing annual reference atlases to improve classification accuracy and advance studies of natal origin, habitat use, and population connectivity using elemental signatures (Gillanders 2002, Miller et al. 2013). Analyses of samples pooled across years and age classes may diminish the ability to detect spatial differences in natal signatures among nursery areas. However, Lewis et al. (2016) found no loss in overall DFA classification success of juvenile *C. limbatus* to regional nursery areas when 2 year classes were combined (84%). Where temporal variation in elemental composition is less than the observed spatial difference, pooling of samples across years may be accomplished without reducing group classification accuracy (Elsdon et al. 2008). Though not a proxy for vertebral chemical composition (Warner et al. 2005), collection and analysis of water samples from sites of interest would provide a useful reference for the range of environmental variation within sites to inform interpretations of observed spatial differences among elemental signatures.

Spatial variation

Our results confirm that spatial differences in vertebral chemistry can provide intrinsic, site-specific

markers of natal origin in an elasmobranch. DFA classification accuracy to putative natal origins ranged from low (all months pooled: 47 to 67%) to moderate (early vs. late season: 39 to 73%), depending on the degree of spatial and temporal refinement of the data set. MLE of group contributions similarly improved when data were analyzed with finer temporal resolution. Intra-annual variation in elemental signatures significantly influenced spatial discrimination and classification success. Edge signatures discriminated *S. lewini* by location of capture with high overall classification success (72 to 100%), providing direct evidence that vertebral elemental signatures can provide site-specific records of origin in elasmobranchs occupying coastal marine environments. By extension, if vertebral chemistry remains stable following deposition, natal signatures should provide permanent records of geographic origins following the dispersal of individuals from their nursery grounds. Movement patterns and natal source contributions could therefore be determined by developing atlases of natal signatures to match the signatures of individuals to their putative natal origins. Natal atlases should include broad spatial representation and characterize intra- as well as inter-annual variation in elemental signatures (Gillanders 2002, Miller et al. 2013).

Multivariate analyses revealed significant differences in the elemental signatures of young-of-the-year *S. lewini* at a variety of spatial scales. Natal origins were distinguished between sites in Mexico and Costa Rica from samples pooled across months within year with 80% accuracy (>2500 km). Edge signatures discriminated between hammerhead sharks captured in the Gulf of Tehuantepec (Puerto Madero) and those from the entrance to the Gulf of California off Sinaloa with 100% accuracy (>1600 km). This level of spatial resolution is well-aligned with the dispersal capacity of *S. lewini* (>1900 km; Bessudo et al. 2011). Elemental signatures of *S. lewini* also produced discernible markers at finer, local spatial scales. Samples from Mazatlán in 2008 successfully distinguished between sharks separated by ~10 km with 83% accuracy. Though comparison at this scale was opportunistic and the sample size was small, significant variation at this fine scale suggests the potential to reveal individual or sex-specific differences in habitat use within sites of particular interest (i.e. embayments, estuaries) using elemental signatures. Dorval et al. (2005) used otolith chemistry to discern the use of specific seagrass beds by juvenile spotted seatrout *Cynoscion nebulosus* at similarly fine spatial scales of 15 km. Analyses of vertebral elemental signatures may provide an alternative and complementary tool for studying elasmobranch movements and habitat use at regional and local scales.

The use of intrinsic elemental signatures relies on the assumption that there are characteristic and reproducible markers that, to some extent, represent the environment an individual has occupied (Campana 1999, Elsdon et al. 2008). Elemental signatures, therefore, can reliably distinguish among groups that have inhabited distinct environments but cannot differentiate those individuals that have occupied similar conditions despite potential geographic separation (Kerr & Campana 2014). Though we evaluated the likelihood of individuals from undocumented sources mixing within nursery areas in our MLE, accounting for similar signatures among sources is also an important consideration for distinguishing natal origins. Concerted effort should be made during sample collection to document not only those sources which are distinctive but also those that may be similar. We did not attempt to sample all potential source populations and it is not likely to be feasible to do so in broadly distributed or often rare species such as elasmobranchs. Given the likelihood of incomplete source characterization among highly mobile species, alternative classification techniques are a critical consideration. DFA, MRPP, and MLE were sufficient for evaluating temporal variation and the utility of elemental signatures as spatial markers in this exploratory study. However, these procedures rely on the assumption that all potential source populations are represented within a data set (McCune & Grace 2002, Wilson White & Ruttenberg 2007). Alternatively, a variety of statistical techniques are available for separating and evaluating the quality of groups in the absence of *a priori* designations. Two-step cluster analyses, for example, have been applied in fisheries research to define appropriate management units and could be modified to identify natural groups from elemental signatures (Cope & Punt 2009). Emerging geospatial statistical approaches go beyond the identification of unique groups to estimate continuous patterns of elemental signatures based on samples available from a subset of a species' range (Simmonds et al. 2014). Bayesian analyses can be used to distinguish the number of groups and the uncertainty associated with classifications from mixed and unknown sources (Munch & Clarke 2008, Neubauer et al. 2013). Alternative statistical approaches such as these will be essential to account for multiple sources of uncertainty in directed, population-level analyses using elemental signatures.

Conclusions and applications

Movement patterns of wide-ranging sharks and rays exhibit much more than the capacity for long-distance dispersal. The migration patterns that are now emerging are similar to those of many seabirds, sea turtles, and marine mammals: individuals commonly show fidelity to core feeding, breeding, birthing, and nursery areas that are linked by directed (and often long-distance) movements between geographically discrete locations (Jorgensen et al. 2010, Mourier & Planes 2013, Lea et al. 2015). Connectivity and exchange among core locations facilitates population persistence and resilience and influences critical ecological functions within these habitats and ecosystems (Hastings & Botsford 2006, McCauley et al. 2012). If geochemical and physical gradients differ among the core areas that sharks and rays reside in or transit through, elemental signatures have the potential to generate insights into the spatial and temporal dynamics of elasmobranch populations.

How much time is required for a site-specific natal signature to be recorded in the vertebrae of an elasmobranch? Daily growth increments, evident in otoliths during the early life history of fishes, do not appear to be present in elasmobranch vertebrae (Campana and Thorrold 2001, Cailliet and Goldman 2004) and restrict the chronological record from which environmental history can be inferred. Elasmobranchs exhibit a high degree of maternal investment in their offspring, producing comparatively fewer but well-developed and larger young than other fishes and with often protracted gestation periods. As a result, maternal provisioning could influence vertebral chemical composition following birth. Female elasmobranchs may also offload chemical contaminants to their young that could further influence vertebral chemistry and obscure site-specific signatures (Lyons & Lowe 2013). Changes in vertebral chemistry in response to external changes in the ambient environment were reported to occur within weeks in captive juvenile bull sharks *Carcharhinus leucas* (Werry et al. 2011). However, the sample size used in the Werry et al. (2011) study was low and focused on a sharp gradients between marine and riverine systems. Elemental incorporation studies directed toward understanding potential maternal lag effects and the response times required for elemental composition to converge on site- or habitat-specific elemental signals are needed to inform and guide future studies.

Recent studies have confirmed that adult females of many elasmobranch species return exclusively to the location of their birth to reproduce (Feldheim et al. 2014, Chapman et al. 2015). Fidelity to natal origins (natal philopatry), whether returning to exact or regional birth locations, can generate finer-scale, local population structure that is critical to identify for effective management practices (Hueter et al. 2005, Crowder & Norse 2008). Natal signatures could be used to identify those nursery areas (or regions) that contribute the greatest proportions to overall population productivity in an effort to prioritize and direct conservation and management efforts (Gillanders 2002). Gravid female sharks and rays are often targeted by fisheries as they aggregate in nursery areas (Smith et al. 2009), reducing the reproductive potential of a population as it is briefly centralized in these discrete locations. Assessing the relative source contributions from different nursery areas and extent of natal philopatry provides options for designating protected areas or establishing spatiotemporal management strategies (i.e. gear restrictions, temporary area closures) that reduce fishing mortality. Protected areas and spatiotemporal restrictions may be more feasible to enact in areas that lack infrastructure for directed monitoring or fishing assessment.

As studies of vertebral elemental composition in elasmobranchs are extended from exploratory research to directed investigations of spatial ecology, researchers have the benefit of guidance from decades of work on otolith chemistry. Vertebral elemental signatures may offer a valuable tool to distinguish population connectivity, natal origins, migratory pathways, resident versus transient populations, site fidelity, population structure, segregation, and habitat use (Campana & Thorrold 2001, Elsdon et al. 2008) in elasmobranch populations. These intrinsic markers could be used to inform spatial models and direct management efforts toward key areas of congregation and focal use. We anticipate that the integrated use of elemental signatures with genetic (Miller et al. 2005, Rundel et al. 2013), stable isotopic (Thorrold et al. 2001, Carlisle et al. 2012), essential amino acids (McMahon et al. 2011), or electronic tracking (Ceriani et al. 2012) techniques are likely to generate greater resolution of spatial dynamics and environmental history than could be obtained using any single method alone. The use of vertebral elemental analysis has the potential to propel more tractable, spatially explicit approaches to the conservation and management of vulnerable shark and ray populations.

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Appendix

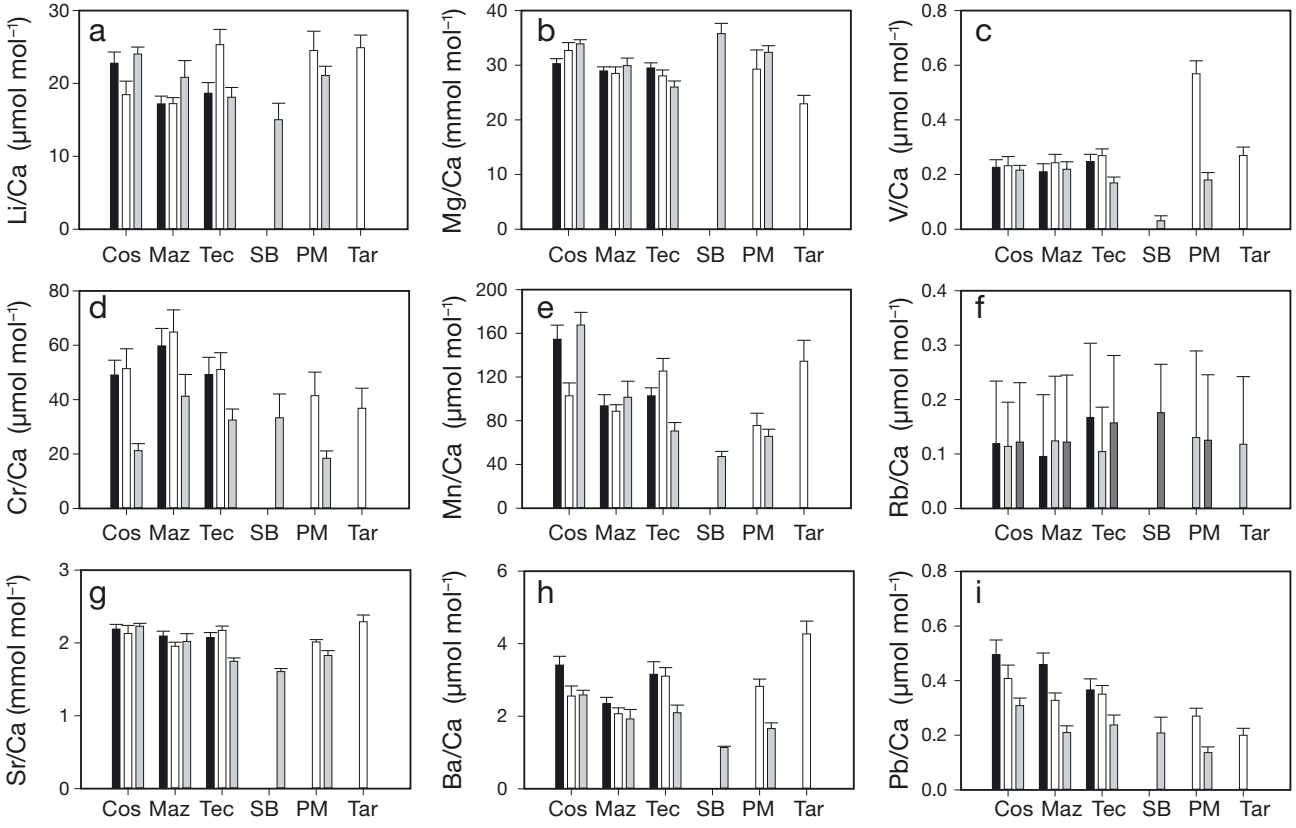

Fig. A1. Temporal variation in the chemical composition of natal signatures of scalloped hammerhead sharks *Sphyrna lewini* (Table 2; Hypotheses 3, 4). Mean (± SE) element-to-calcium ratios of (a) lithium, (b) magnesium, (c) vanadium, (d) chromium, (e) manganese, (f) rubidium, (g) strontium, (h) barium, and (i) lead measured from natal signatures within sites by year. Sites are arranged from north to south and include the primary study locations of Cospita (Cos), Mazatlán (Maz), and Tecapán (Tec) and secondary sites of San Blas (SB), Puerto Madero (PM), and Tárcoles (Tar). Bar shade indicates sample year: black = 2007, white = 2008, grey = 2009. Note that measurements of Li were only available from a subset of samples in 2007

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