Vertebral chemistry demonstrates movement and population structure of bronze whaler

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ABSTRACT: Analysing the elemental composition of calcified structures of fish, such as otoliths, can show movement patterns and population structure — essential data underlying effective fisheries management. Elasmobranch vertebrae provide an analogous, yet largely underexploited component to quantify patterns of population structure in cartilaginous fishes. We quantified multi-element vertebral profiles in bronze whaler sharks *Carcharhinus brachyurus* from 3 regions in South Australia using laser ablation-inductively coupled plasma mass spectrometry. We used multiple approaches to examine short- (months) and long- (lifetime, years) term perspectives of element incorporation. Boosted regression trees showed that variation in multi-element concentrations at the vertebral edge (representative of the time near capture) was explained primarily by spatial and shark length-based differences. An integrated lifetime elemental signal was similarly influenced by spatial differences and shark length. Cluster analyses of detrended elemental profiles across vertebrae indicated that movement patterns were highly individualistic, reflecting the mobile behaviour of this cosmopolitan species. Differentiation among sampling regions within South Australia suggests that current state-based management appears appropriate. However, when these findings are considered with existing telemetry and molecular genetic data on population structure for the species, it is likely that there is high connectivity throughout the species' range, and cooperation among management jurisdictions should be explored. More broadly, using multiple approaches to element analysis and the integration of methods (such as telemetry, genetics and chemistry) that vary in their representative temporal and spatial breadths will aid in quantifying the population structure and movement behaviour of sharks.

KEY WORDS: *Carcharhinus brachyurus* · LA-ICPMS · Fisheries management · Boosted regression tree analysis · Stock structure · Movement · Shark

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INTRODUCTION

Distributions of mobile marine fauna are governed by individual or social movement patterns, the distribution of important habitats, as well as the presence of biogeographic and/or oceanographic boundaries (Feldheim et al. 2001, Benavides et al. 2011). Quantifying distributions and patterns of movement facilitates the delineation of appropriate spatial scales of management for species of high commercial and conservation value (Walker 1998, Speed et al. 2010). The identification of spatially discrete units in elasmobranchs has been challenging, but is now more recently examined through developments in technol-

ogy and analytical methods in tracking and population genetic analyses (Chapman et al. 2015).

Tracking elasmobranchs (e.g. via telemetry and mark–recapture methods) is a valuable means for studying short-term movement patterns among habitats and/or populations (Kohler & Turner 2001, Sundström et al. 2001, Voegeli et al. 2001). With the advance of tagging technologies (e.g. smart position and pop-up satellite archival tags), a suite of data on various aspects of life history, physiology, movements and structuring of elasmobranch populations can be obtained. However, tagging large-bodied, highly mobile species is challenging, and the logistics and costs associated with the deployment of tags might not always be feasible (Voegeli et al. 2001, Speed et al. 2010). Furthermore, the requirement to apply many conventional tags, coupled with characteristically low recapture rates and/or high tag losses limit the use of conventional tagging to assess movements among regions (Kohler & Turner 2001, Bradshaw et al. 2013).

Measuring gene flow among spatially separated groups of elasmobranchs is widely used to assess population structure (Ovenden et al. 2009, Dudgeon et al. 2012). However, this approach has been hindered by low genetic differentiation among reproductively isolated populations (Dudgeon et al. 2012). Furthermore, the evolutionary time scales covered by molecular-genetic analyses might not be biologically appropriate for quantifying patterns of movement and habitat use (Ovenden et al. 2015), which for elasmobranchs might be influenced by environmental cues that occur on annual, seasonal or daily scales (e.g. Heupel et al. 2004, Ortega et al. 2009).

Elemental analyses of the calcified structures of teleosts (i.e. otoliths, scales and fin spines) yield important information on population structuring, movement and habitat use (e.g. Campana & Thorrold 2001, Elsdon et al. 2008, Tanner et al. 2016). This approach exploits the incorporation of naturally occurring trace and minor elements from the surrounding aquatic environment into the calcified structures of an organism (with secondary contributions from dietary sources) during development and growth, forming a geographic site-specific elemental signature in the structure (Campana 1999, Kerr & Campana 2014). The continual incorporation of elements into the calcified structures of aquatic organisms and the nature of growth-increment deposition allows patterns of habitat use and movement throughout the life history of an individual to be elucidated (Elsdon & Gillanders 2003). From a practical perspective, site-specific elemental signatures reflect phenotypic differences among populations on ecological time scales and are considered highly informative natural tags for identifying spatial management units (Begg & Waldman 1999, Campana et al. 2000). Hence, the application of otolith (and other hard-part) chemistry to determine teleost population structure has become common in fisheries science (Campana et al. 2000, Campana & Thorrold 2001, Kerr & Campana 2014).

Akin to teleost otoliths, the cartilaginous structures of elasmobranchs (e.g. vertebrae, fin spines and jaw cartilage) grow throughout the lives of individuals and remain metabolically and chemically stable once formed (Clement 1992, Dean & Summers 2006), thus making them amenable to answering ecological questions based on analyses of element composition (Smith et al. 2013). Generally, fisheries biologists have focused on the calcified cartilaginous structures of elasmobranchs to determine population age structure based on counts of growth increments (Cailliet & Goldman 2004). Edmonds et al. (1996) demonstrated that the elemental composition of the jaws of gummy sharks *Mustelus antarcticus* differed between broadly separated locations off the southwest coast of Western Australia, suggesting the use of calcified cartilage chemistry to identify sub-populations. More recently, analyses of the element composition of vertebrae have provided insights into the movement patterns and habitat use of coastal species of carcharhinid sharks (Tillett et al. 2011, Werry et al. 2011). However, this approach has not been widely applied in fully oceanic shark species.

The bronze whaler *Carcharhinus brachyurus* (Günther 1870) (Carcharhinidae) is a large-bodied (maximum total length ~3 m), temperate species that is highly mobile throughout its range and largely restricted to the coast and continental shelf (Last & Stevens 2009, Benavides et al. 2011). *C. brachyurus* is considered Near Threatened globally; however, the species is classified as Least Concern in Australia (Duffy & Gordon 2003) and is seasonally targeted by commercial and recreational fishers (Jones 2008). In Australia, *C. brachyurus* is caught predominantly in South Australia, where stock status is assessed using fishery-performance indicators (e.g. changes in catch and effort or high inter-annual variability in catch) against target reference points that help identify changes in the fishery (Fowler et al. 2014). Commercial catches have remained approximately stable since 1990 at around 80 t yr^{-1}, but several commercial fishery-performance indicators have been exceeded in the last 5 yr, including the highest catch in 2009/ 2010 (~160 t; Fowler et al. 2010). Given the *K*-selected life history of the species (i.e. slow growth and late age at maturity) (Walter & Ebert 1991, Drew et al.

2016), previous breaches of fishery-performance indicators, and the potential for fished stocks to cross state-based jurisdictional management boundaries (Huveneers et al. 2014, C. Junge unpubl. data), mean there is a need to acquire data to describe the population structure and movements of *C. brachyurus* to define units for management and conservation.

We measured multi-element profiles in the vertebrae of *C. brachyurus*, collected from 3 regions in South Australia, using laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS). We used multiple approaches, representing short- (months) and long- (lifetime, years) time scales of element incorporation. We expected that the degree of spatial separation among regions would decrease as the temporal breadth of the analyses increased, due to the species' capacity to move throughout the study area (Rogers et al. 2013a, Huveneers et al. 2014). Moreover, we explored the commonality in patterns of movement based on cluster analyses of multi-element profiles, and hypothesise that clustering reflects observed patterns of size- and/or sex-based segregation.

MATERIALS AND METHODS

Sample collection and preparation

Carcharhinus brachyurus were collected in the spring to autumn (September to May) of 2010/2011 and 2012/2013, with samples obtained from fisheries-dependent long-line catches and augmented with fisheries-independent sampling from Gulf St Vincent (South Australia) targeting the smaller size cohorts (for sampling details, refer to Drew et al. 2016). We also obtained samples from commercial catches collected from Gulf St Vincent and Spencer Gulf in South Australia (Fig. 1). Upon capture, observers recorded sex and measured total length (TL) and trunk length (TKL) to the nearest millimetre. When vertebrae were obtained from commercially processed specimens (i.e. trunks), we used TKL to estimate TL using an established linear regression (Drew et al. 2016).

We removed sections of vertebrae (n = 1 to 6) posterior to the cranium, and separated and cleaned them of excess tissue. We soaked individual vertebral centra in a 5 % hypochlorite solution for 15 to 120 min (depending on size), before triple rinsing in ultrapure water. We then air-dried the vertebrae before embedding them in a clear-setting epoxy resin (Struers Epofix), spiked with 40 ppm indium to discriminate the vertebrae and the epoxy during elemental analysis. We sectioned embedded vertebrae (~600 µm thickness) transversely through the vertebral focus using a low-speed saw (Buehler Isomet). We polished the sections on progressively finer grades of lapping film and cleaned them in ultrapure water, before mounting them onto microscope slides with a 100 ppm indium-spiked CrystalBond™ adhesive (Fig. 2).

Element analyses

We used multiple approaches independently to measure the multi-element composition of vertebrae. First, we analysed discrete spot ablations at the marginal edge (henceforth referred to as 'edge', Fig. 2a), which we assumed represented the time and location near capture (Gillanders & Kingsford 2003). Based on vertebral increment width measurements of 272 sharks from both sexes and a range of lengths (730 to 2810 mm TL), mean ± SE increment width irrespective of specimen age was 539.4 ± 12.0 µm (M. Drew unpubl. data). Assuming vertebral growth is linear through the year, *C. brachyurus* vertebrae grow 45.0 µm mo^{-1}; thus, a spot ablation size of 30 µm represents an integration time of <1 mo.
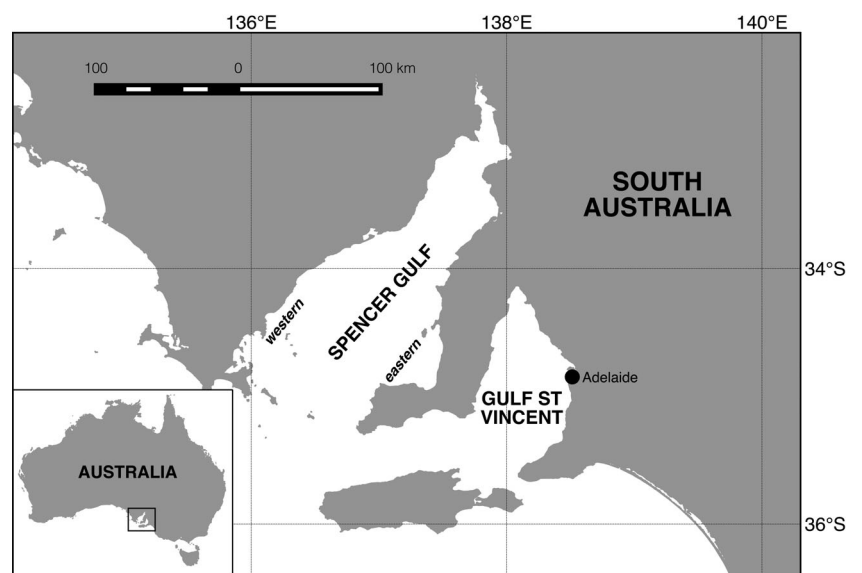

Fig. 1. Sampling regions (Gulf St Vincent and Spencer Gulf) for *Carcharhinus brachyurus* in South Australian gulfs

Fig. 2. Representative section of *Carcharhinus brachyurus* vertebra indicating the (a) marginal edge and (b) growth axis. The birthmark is indicated with the arrow. Scale bar = 250 µm

Second, we obtained element profiles along the growth axes of the vertebrae, from the birthmark to the marginal edge along the corpus calcareum (Fig. 2b). We assumed that these profiles represented the range of environments encountered throughout the lifetime of the organism. In addition, we calculated the mean elemental concentration of the profile (henceforth referred to as 'mean'), which we assumed represented an integrated-lifetime elemental sig-

nature. Elasmobranch vertebrae grow continuously throughout an individual's lifetime (Clement 1992, Dean & Summers 2006) and can be used to distinguish among individuals that have experienced differing environmental conditions. Hence, the mean integrated-lifetime elemental signature provides an elemental signal that is analogous to the analysis of whole otoliths (Campana et al. 2000).

We analysed vertebrae from 163 specimens of *C. brachyurus* via LA-ICPMS. We analysed all vertebral samples using both approaches, viz. marginal edge and profile analyses, and randomised samples prior to analysis.

We analysed the marginal edge and profile element analyses independently on different laser ablation systems coupled to quadrupole ICPMS housed at Adelaide Microscopy (University of Adelaide); see Table 1 for laser- and ICPMS-specific operating parameters. Elements comprised: ^{43}Ca, ^{138}Ba, ^{24}Mg, ^{88}Sr, ^{63}Cu, ^{55}Mn, ^{64}Zn and ^{115}In. For all data acquisition, irrespective of approach, we measured background concentrations of elements within the sample chamber for 30 s before each ablation to correct the sample concentrations. Prior to collecting the element profile data via transect analysis, we made a 40 µm diameter pre-ablation path. In a similar manner, for spot ablations at the marginal edge, we excluded the first 3 to 4 s of elemental signal (characterised by a sharp peak in the raw count data) during the signal-selection process, enabling the surface of

Table 1. Operating parameters for the laser ablation systems coupled to the quadrupole inductively coupled plasma mass spectrometers (ICPMS)

System	Spot (edge) ablations	Element profiles
Laser system	Resonetics M-50-LR 193 nm Excimer	NewWave NWR213-ESI
Wavelength (nm)	193	213
Mode	Q-switch	Q-switch
Laser frequency (Hz)	5	10
Spot/profile diameter (µm)	45	30
	(data selection excluded the first 3–4 s of signal)	(preceded by a 40 µm pre-ablation)
Profile scan rate (µm s^{-1})	–	10
Beam energy (J cm^{-2})	6–7	4–5
Carrier	Ar (0.80 l min^{-1})	Ar (0.92 l min^{-1})
ICPMS system	Agilent 7700s	Agilent 7500cx
Optional gas	He (700 ml min^{-1})	He (450 ml min^{-1})
Cone	Pt	Pt
Detection modes	Pulse and analogue	Pulse and analogue
Dwell times (in ms)	^{43}Ca (100), ^{138}Ba (200), ^{24}Mg (200), ^{88}Sr (100), ^{63}Cu (200), ^{55}Mn (200), ^{64}Zn (200), ^{115}In (50)	^{43}Ca (100), ^{138}Ba (100), ^{24}Mg (100), ^{88}Sr (100), ^{63}Cu (150), ^{55}Mn (150), ^{64}Zn (150), ^{115}In (50)
Mean recovery (%)	100.01	101.37
Mean precision (%)	1.99	2.08

the sample to be cleaned of any possible contaminants. We used periodic ablations on the standard glass National Institute of Standards (NIST) 612 and the US Geological Survey reference micro-analytical carbonate standard (MACS-3) to measure instrument drift, recovery and precision, which we deemed acceptable for all elements for both instruments (Table 1). We used ^{43}Ca as an internal standard (based on a constant value of 43.1 weight% for all *C. brachyurus* vertebral samples; C. Izzo unpubl. data). We converted raw counts to elemental concentrations (in ppm) using GLITTER software (www.glitter-gemoc.com) and expressed them as ratios to Ca (in mmol mol^{-1}) to account for fluctuations in the ablation yield (Thorrold et al. 1998).

Element profiles

We obtained multi-element profiles for all vertebral samples; however, due to the porosity of some vertebrae and the potential for ablation of resin, there were discontinuities in some profiles, requiring that 72 samples be excluded from analysis. Therefore, we included a sample of 91 specimens (45 females and 46 males) for the element profile analyses (Table 2). We excluded none of the spot ablations from the analysis.

We smoothed all element profile data with a 7-point running mean followed by a 7-point running median to reduce instrumental noise in the profile (Sinclair et al. 1998, Elsdon & Gillanders 2005) and to aid in identifying trends in the elemental signal among individuals (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m556p195_supp.pdf). Given the potential for age-related variation in the incorporation of individual elements into elasmobranch vertebrae (Hale et al. 2006, Smith et al. 2013, Kerr & Campana 2014), we detrended element profiles with a spline fit using the R *dplR* package (Bunn

Table 2. Geographic range and temporal collection span of *Carcharhinus brachyurus* vertebral samples. Sample sizes (n) of edge spot ablations are shown, with numbers in parentheses indicating the sample sizes of the element profile analyses. TL: total length in mm

Region	Sampling season n 2011/2012	Sampling season n 2012/2013	TL range
Gulf Saint Vincent	30 (23)	30 (10)	730–1789
Eastern Spencer Gulf	36 (18)	19 (18)	620–1605
Western Spencer Gulf	–	48 (22)	830–2810

2010). Detrending the profiles accounted for age-related patterns of elemental uptake, and aids in disentangling differences in mean elemental signatures due to size-based shifts in habitat use versus ontogenetic differences in physiology. We calculated mean integrated-lifetime elemental signatures from the detrended element profiles.

Statistical analysis

We included all element-to-calcium ratios in the analysis (with the exception of indium). We did all statistical analyses using R v3.2 (R Development Core Team 2015). We transformed the edge and mean multi-element data as $\log(x+1)$ and used principal component analysis to integrate the multi-element signals into a single, multi-dimensionally scaled variable (first principal component) using the *vegan* package (Oksanen et al. 2016). For the edge and mean multi-element data, the first principal component accounted for 83 and 65% of the variation, respectively (Fig. S2 in the Supplement).

Given that *C. brachyurus* likely displays length-based and sexually dimorphic differences in population structure and movement over a range of spatial scales (Duffy & Gordon 2003, Jones 2008), we used boosted regression trees (BRT) to assess the relative explanatory power of spatial (region near capture), temporal (season of capture: 2010/2011 or 2012/2013) and biological (specimen TL and sex) variables in vertebral chemistry (Elith et al. 2008). We applied BRT to the first principal component of the edge and mean element data independently with the packages *dismo* (Hijmans et al. 2016) and *gbm* (Ridgeway 2015). We fitted all BRT models with defined tolerances of 0.01, a learning rate of 0.001 and a bag-fraction of 0.75, ensuring a minimum of 1000 model fits (Elith et al. 2008). We examined partial dependency plots and regression trees using the *rpart* package (Therneau et al. 2015) to visualise and assess spatial, temporal and biological variation in the vertebral chemistry data.

We explored patterns of movement based on cluster analyses of the individual elemental profiles from a sample of 91 sharks (see above, Table 2). We decomposed individual element profiles into a set of 13 extracted statistical features, which describe the form of the elemental time series (Shima & Swearer 2009). Features included: serial correlation, nonlinearity, skewness, kurtosis, Hurst, Lyapunov stability, frequency, trend, seasonality, trend and seasonally adjusted (TSA) serial correlation, TSA nonlinearity, TSA skewness and TSA kurtosis (for more detail see

Wang et al. 2006). We analysed these 13 extracted features for each of the 6 elements (i.e. 78 features ind.$^{-1}$) in a hierarchical cluster analysis, with data fitted to a Euclidean distance resemblance matrix. This generated a dendrogram to assess the similarities among individuals based on a similarity profile (SIM-PROF) using the *sigclust* package (Huang et al. 2015). SIMPROF used 1000 permutations and p < 0.01 to define clusters (Shima & Swearer 2009). The incorporation of individual elements into elasmobranch vertebrae is likely affected by differing environmental conditions and physiological states (Hale et al. 2006, Smith et al. 2013, Kerr & Campana 2014), so this approach allows for the simultaneous analysis of multi-element profiles.

RESULTS

In total, we analysed 163 specimens (86 females and 77 males) of *Carcharhinus brachyurus* using both LA-ICPMS approaches (i.e. marginal edge and profile analysis). Sharks captured in western Spencer Gulf (mean ± SE: 1636 ± 69 mm TL) had the broadest length range (Table 2), with Gulf St Vincent (1043 ± 25 mm TL) and eastern Spencer Gulf (1112 ± 24 mm TL) having comparable length ranges (Fig. 3, Table 2).

Population structure

BRT indicated that variance in the edge elemental signal was primarily due to differences among sampling regions (82.2%: Fig. 4a). Partial dependency plots indicated that eastern and western Spencer Gulf showed minor differences, while Gulf St Vincent differed from the other regions. Specimen size also had a moderate influence on the edge vertebral chemistry (16%), and a visual inspection of the partial dependency plot indicated differences among sharks of TL < 2000 mm (Fig. 4a). Sample sex and season of capture had minimal influence on the data (<2%). Regression trees of the elemental composition at the vertebral edge indicated that shark length-based separation was evident (Fig. 5a). Overall, Gulf St Vincent formed a unique cluster comprising all available size classes, with sharks caught in eastern and western Spencer Gulf forming several shark length-based clusters comprising individuals from both regions.

BRT indicated that variation in the mean elemental signature was similarly influenced by spatial differences (49.3%) and shark length (47.2%; Fig. 4b).
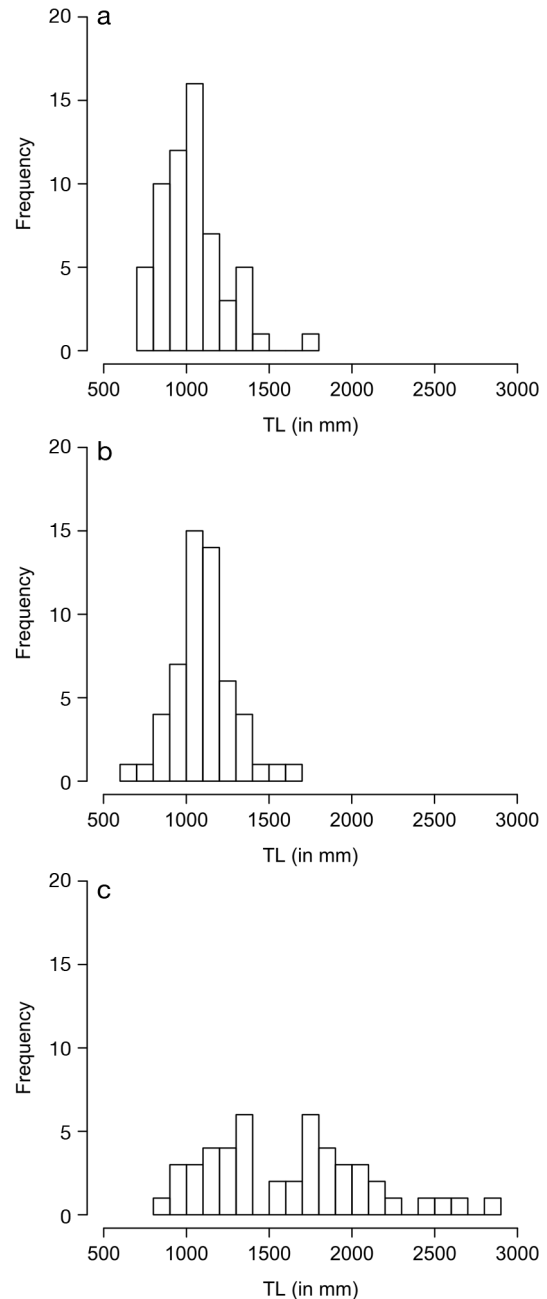

Fig. 3. Total length (TL, in mm) frequency plots of *Carcharhinus brachyurus* sampled from 3 regions in South Australian gulfs: (a) Gulf St Vincent; (b) eastern Spencer Gulf; and (c) western Spencer Gulf

Three nominal length cohorts were evident based on visual interpretation of the partial dependency plot for the mean elemental signatures: (1) TL < 1000 mm, (2) TL 1000 to 1500 mm and (3) TL > 1500 mm (Fig. 4b). Among the sampling regions, Gulf St Vincent and eastern Spencer Gulf were similar, but western Spencer Gulf was weakly differentiated from the other regions (Fig. 4b). Similar to the edge data, sex-
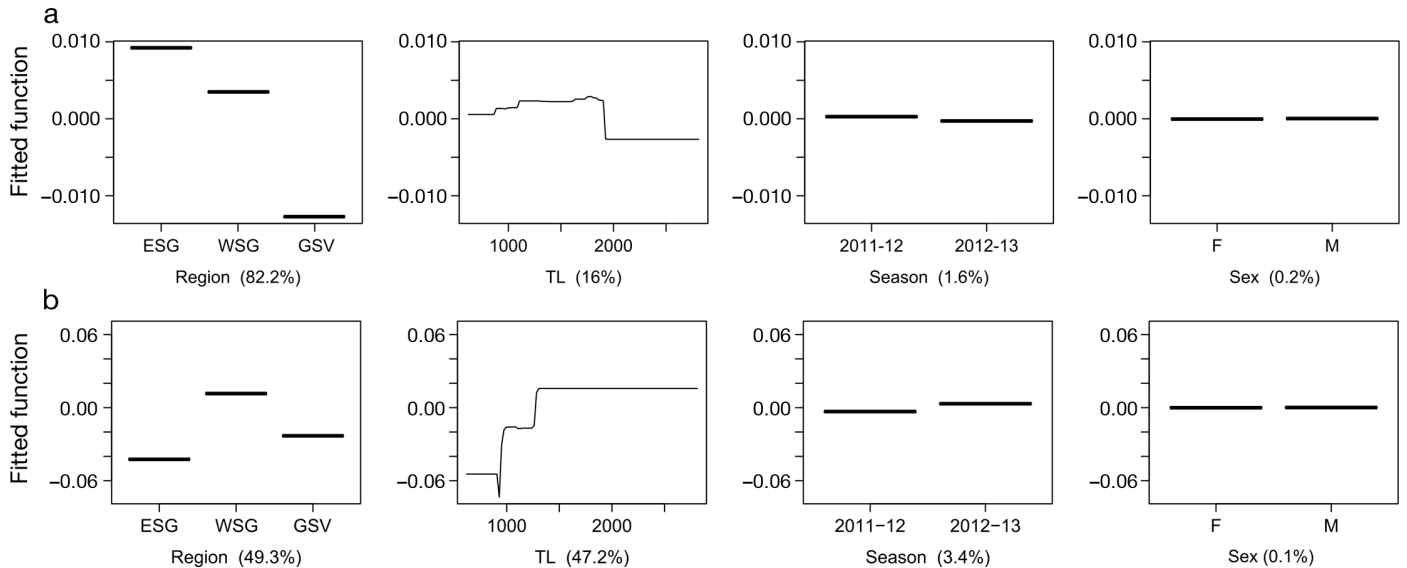
Fig. 4. Partial dependency plots for the models relating (a) vertebral edge and (b) the mean integrated elemental signal of *Carcharhinus brachyurus* from South Australian gulfs. Variables are sorted in order of decreasing relative contributions to the elemental data. GSV: Gulf St Vincent; ESG: eastern Spencer Gulf; WSG: western Spencer Gulf; TL: total length (mm)

ual dimorphism and season of capture explained only <4% of the variance. Regression trees exploring patterns of the shark length-based regional variation in the mean elemental signatures indicated that sharks caught in western Spencer Gulf formed a distinct group comprising all available sizes (Fig. 5b). Sharks caught in eastern Spencer Gulf and Gulf St Vincent formed multiple shark length-based clusters comprising individuals from both regions of capture (Fig. 5b).

Movement patterns

Hierarchical clustering identified 11 clusters of sharks (based on SIMPROF; p < 0.01: Fig. 6). Each cluster had between 1 and 31 individuals, with 4 clusters having ≤5 members (Table 3). Among individual clusters there was no obvious shark length- or sex-based segregation (Table 3).

DISCUSSION

Using multiple approaches to element analysis (marginal edge and profile analyses) that represent different time scales of element incorporation, we were able to measure short- (months) and long- (lifetime, years) term perspectives of shark population structure and infer movement patterns. Variation in the elemental composition at the marginal edge of the vertebrae arose primarily from spatial separation of *Carcharhinus brachyurus* among the sampling
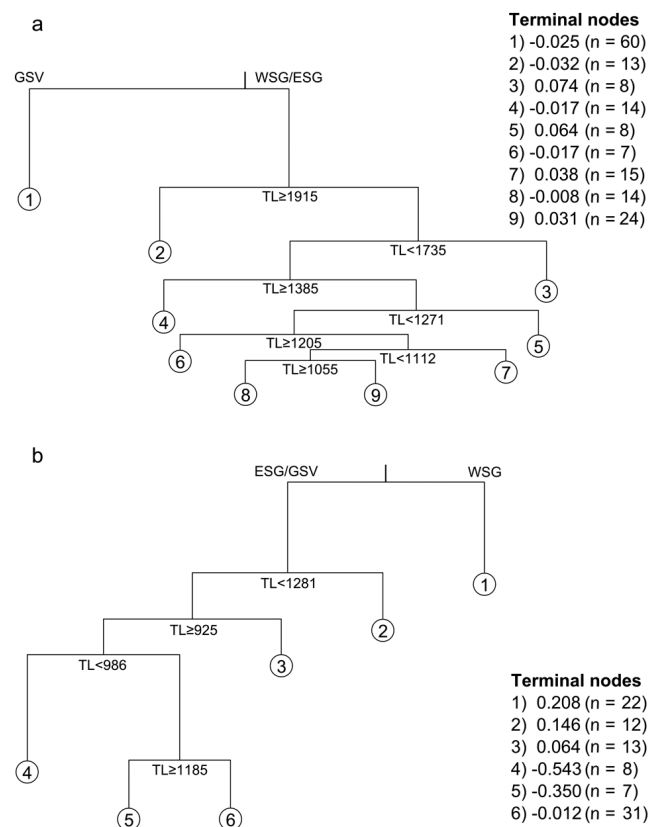

Fig. 5. Regression trees depicting separation among *Carcharhinus brachyurus* from South Australian gulfs based on the combined effects of total length (TL, in mm) and region of capture. Regression trees are based on (a) vertebral edge and (b) mean integrated elemental signal. The mean value and number of observations for each terminal node (numbered circles) are listed. Region codes as in Fig. 4
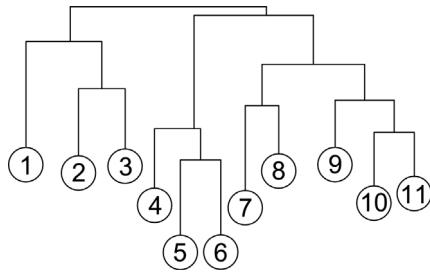
Fig. 6. Hierarchical cluster dendrogram based on multi-element profiles of *Carcharhinus brachyurus* from South Australian gulfs. Multi-element profiles are represented as a series of 13 statistical extracted features that describe the form of the elemental time series. Numbered circles represent supported clusters (SIMPROF p < 0.01). See Table 3 for summary of sharks within each cluster

regions. Given that the vertebral edge provides an elemental signal for the time near capture (Gillanders & Kingsford 2003), these data represent the spatial dynamics of the population during spring to autumn (September to May) and imply that the species uses the South Australian gulfs within that period. Considering that *C. brachyurus* mating and parturition occurs during the same period (Lucifora et al. 2005, Jones 2008, Drew et al. 2016), the South Australian gulfs might act as nurseries or pupping areas, consistent with tag-recapture results from South Australia that imply seasonal residency within the gulfs based on multiple recaptures near the tagging locations (Rogers et al. 2013a, Huveneers et al. 2014). The South Australian gulfs likely offer suitable nursery habitat for *C. brachyurus*, providing warmer water temperatures than the adjacent coastal areas

Table 3. Summary of clusters of *Carcharhinus brachyurus* from South Australian gulfs (SIMPROF p < 0.01). Hierarchical cluster analyses were based on 13 extracted statistical features that describe the form of the multi-element profiles (see Fig. 6). GSV: Gulf St Vincent; ESG: eastern Spencer Gulf; WSG: western Spencer Gulf; TL: total length in mm

| Cluster | Total n | Sex ratio (F:M) | Sampling region n | | | TL range |
			GSV	ESG	WSG	
1	31	15:16	14	14	3	730–1530
2	9	6:3	3	4	2	620–2460
3	10	2:3	2	5	3	830–2590
4	2	3:0	–	1	1	1240–1780
5	4	0:4	–	1	–	1040–1040
6	1	0:1	–	–	4	2050–2810
7	2	0:2	1	–	1	931–1050
8	9	3:6	2	4	3	919–1984
9	12	6:6	6	4	2	890–1262
10	5	4:1	4	–	1	1000–1360
11	6	3:3	1	3	2	1100–1540

(Nunes & Lennon 1986, de Silva Samarasinghe & Lennon 1987), potentially enhancing growth and providing sufficient prey availability for neonates and juveniles. Direct observation of nurseries is still required throughout the species' southern range in Australia. This can be assessed through the elemental analyses of the natal portion of the vertebrae of temporally matched samples (Elsdon et al. 2008, Smith et al. 2013, Lewis et al. 2016), to identify natal regions that act as sources (and sinks) of juvenile recruits to the adult cohort, and to provide an assessment of population connectivity (Gillanders & Kingsford 1996, Tillett et al. 2011). Depending on the duration of juvenile residency and status of the fishery, those natal regions that contribute juveniles to the adult cohort could be prioritised for reduced fishing pressure, such as seasonal closures of defined areas to increase the probability of long-term population persistence.

C. brachyurus is a highly mobile species, with individuals capable of travelling 10s to 100s of kilometres over a few days (Rogers et al. 2013a, Huveneers et al. 2014). Given that complete element incorporation in the vertebrae of bull sharks *C. leucas* occurs after 3 wk (Werry et al. 2011), the elemental composition at the marginal edge of the vertebrae might be representative of a range of habitats, thus impeding the delineation of spatial differences among individuals in the population. This might be further complicated by changes in the deposition rate of vertebral tissue in the oldest individuals (Andrews et al. 2011, Natanson et al. 2014), likely influencing element uptake (Hale et al. 2006). This could explain the moderate influence of specimen size on edge chemistry; however, strong spatial differences based on the edge element data suggest that sharks remain resident within the sampling regions over weeks. Other studies in the gulfs of South Australia have found elemental differences among the gulfs for teleost species based on otolith chemistry, e.g. snapper *Chrysophrys auratus* (Fowler et al. 2005) and southern garfish *Hyporhamphus melanochir* (Steer et al. 2009), indicating that the gulfs provide chemically heterogeneous environments. This inferred short-term residency is likely restricted within the gulfs, because there was a clear differentiation between sharks caught in Gulf St Vincent and Spencer Gulf. Vertebral edge chemistries indicated that eastern and western Spencer Gulf were similar, possibly because sharks caught in these regions were exposed to sim-

ilar environmental conditions in Spencer Gulf during the spring and autumn as well as having a similar diet (Smale 1991), both of which likely influence vertebral element composition (Mathews & Fisher 2009, Kerr & Campana 2014). Within Spencer Gulf, sharks of all length classes from the eastern and western regions appear to mix over monthly or seasonal scales; alternatively, water chemistries of these adjacent regions might not differ sufficiently to elicit differences in vertebral chemistry over a distance of 10s of kilometres.

Variation in the mean elemental signatures in vertebral chemical profiles over the lifetime of *C. brachyurus* were equally influenced by spatial differences and shark length. The mean elemental signature across the entire vertebral centra provided an integrated measure of the element chemistry throughout the entire life of individual sharks, an analogous elemental lifetime signal to that obtained from the dissolution of whole otoliths (Campana et al. 2000), enabling individuals to be grouped based on shared environmental exposures (Campana 2005). We expect that larger, older individuals would show greater differences in their environmental exposure than smaller, younger individuals. Partial dependency plots and partition trees indicated potential connectivity between the gulfs, with multiple shark length-based clusters comprising sharks from eastern Spencer Gulf and Gulf St Vincent possessing similar lifetime patterns of environmental exposure. In other parts of the species' range, *C. brachyurus* are reported to segregate based on size and sex (Smale 1991, Cliff & Dudley 1992), with juveniles inhabiting shallow waters (<30 m) year round and adults generally remaining in shelf waters, although adult females make inshore migrations in spring to pup (Lucifora et al. 2005). We identified no shark length- or sex-based clusters based on their multi-element profiles; in general, detecting multiple clusters consisting of few individuals likely reflects a lack of synchrony in movement behaviours and/or exposure histories among the sharks sampled. However, our samples only contained a few large juveniles and adults, hindering our power to identify size or sex segregation in large individuals.

Individual multi-element profiles provided a means of examining the combined influence of environmental and physiological conditions on element uptake. Given the potential for physiological regulation of vertebral chemistry, ontogeny likely influences rates of elemental uptake, either through ontogenetic shifts in habitat or diet (Walther et al. 2010) and/or through ontogenetic variation in physiology (Sturrock et al. 2014). We sought to overcome the potential confounding age-related effects on element incorporation by detrending element profiles prior to analysis, such that observed shark length-based differences in the element profile data might be attributed to shark length-based patterns of habitat use and not element uptake. Pre-treatment of the multi-element profiles was necessary given the inclusion of multiple transition metals (Mn, Cu and Zn) that are widely considered to be physiologically regulated in fish otoliths (Campana 1999, Sturrock et al. 2014) and elasmobranch vertebrae (Mathews & Fisher 2009, Smith et al. 2013). To date, profile analyses targeting shark vertebrae to explore movement patterns have focused primarily on euryhaline species, such as smalltooth sawfish *Pristis pectinata* (Scharer et al. 2012) and *C. leucas* (Tillett et al. 2011, Werry et al. 2011), with limited attention to fully marine species, likely due to the open marine environment being relatively physicochemically uniform and therefore demonstrating limited potential to infer shifts in habitat use (Sturrock et al. 2012). Applying elemental profile analyses in euryhaline species is attractive because shifts in the elemental time series are assumed to represent transitions between salinity environments with vastly different water chemistries.

When using the multi-elemental composition of calcified structures to examine population structuring, it is not necessary to determine how environmental variables influence the chemical composition of the structure; it is only necessary to compare the chemistries among different regions (Campana et al. 2000, Elsdon & Gillanders 2003). Accurately disentangling the patterns of elasmobranch movement, environmental exposure and habitat use based on element profiles depends on establishing relationships between vertebral chemistry and ambient environmental conditions such as temperature, salinity and water chemistry (Elsdon et al. 2008). Currently, understanding how these factors regulate element uptake into the cartilaginous structures of elasmobranches is limited (but see Smith et al. 2013), impeding the application of element profiles as a highly resolved tracer. As such, further investigation of the environmental and physiological controls on elasmobranch vertebral chemistry in controlled laboratory experiments is required. However, our approach demonstrates that it is a useful technique for exploring the shared traits of environmental histories. Coupled with tagging data (when available), we have the opportunity to reveal a comprehensive understanding of pelagic shark species' movement, habitat use and population structure (e.g. Werry et al. 2011).

The edge data had a strong spatial component representing the time near capture, suggesting that *C. brachyurus* populations are seasonally structured over spatial scales of 10s to 100s of kilometres. This contrasts with observed patterns of long-term connectivity for *C. brachyurus* in Australia based on molecular genetic data, which suggests generational-scale mixing throughout southwestern Australia and differentiation from the east coast (C. Junge unpubl. data). Acoustic and satellite telemetry similarly demonstrate movements of large individuals between South Australia, Victoria and Western Australia (Rogers et al. 2013a, Huveneers et al. 2014). Mean lifetime elemental signatures were influenced by spatial differences, yet suggest that the gulfs remain connected over extended time scales (i.e. years). However, we did not observe this with the multi-element profile cluster results. Similar discrepancies are evident in the sympatric dusky shark *C. obscurus*, which shows population structuring over 100s to 1000s of kilometres based on the elemental composition of jaw cartilage (Simpfendorfer et al. 1999). Despite observed movements between South Australia and Western Australia based on tagging data (Rogers et al. 2013a,b), these findings are analogous to the mean elemental signatures we discovered, and molecular genetic sub-division between the east and west coasts of Australia (Ovenden et al. 2009, Geraghty et al. 2014). Discrepancies between the 2 elemental datasets, as well as telemetry and molecular genetic results, likely reflect the differences in the spatial and temporal scope of these methods (Tanner et al. 2016). When combined, these data provide potential insights into the ecology of *C. brachyurus* such as natal philopatry, which is common in carcharhinid sharks (e.g. Tillett et al. 2012, Mourier et al. 2013). Observed genetic sub-divisions reflect long-term (i.e. generational) segregation habitats potential juvenile residency in nursery habitats based on tagging and edge data, despite individualistic movements of sharks throughout the species' distribution based on telemetry and cluster analysis.

Using our results to refine fisheries management depends on the respective spatial and temporal ranges represented. Marginal edge chemistry suggested that spatial differentiation between Gulf St Vincent and Spencer Gulf occurred at seasonal time scales. Combined with the residency observed through acoustic telemetry and if natal or regional philopatry occurs, spatially concentrated exploitation of *C. brachyurus* could be detrimental to local populations. However, current fishing pressure is lower (65 t in 2013/2014) than mean historic catches (~80 t;

Fowler et al. 2014), and not enough is currently known about the spatial extent of nursery areas and the degree of natal or regional philopatry (Chapman et al. 2015) within the South Australian gulfs to assess the likelihood of stock depletion. In contrast, corroboration among the elemental profiles, the observed large-scale movements and molecular genetic data for the species suggest that over an individual's lifetime there is high dispersion throughout the species' southern Australian range. This dispersion likely results in commercially targeted stocks straddling state-based management boundaries, such that cooperation among management jurisdictions should be explored for *C. brachyurus.*

We have shown that the element composition in the vertebrae of *C. brachyurus* from South Australia is a viable approach to provide information on movement over time scales of several months to years. A better understanding of controls on element incorporation into elasmobranch vertebrae is required to aid in the interpretation of these data. Vertebral chemistry provides a valuable tool for assessing population structure and inferring nursery areas, facilitating estimates of connectivity among juvenile and adult cohorts. When employed in combination with tagging and molecular genetic data, a greater body of evidence for understanding movements, habitat use and population structure throughout the entire life history is realised.

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