

# Natural tags identify nursery origin of a coastal elasmobranch *Carcharhinus leucas*

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## Abstract

1. Traditional methods for evaluating the importance of elasmobranch nursery habitats have focused on estimating densities or abundances of juveniles within individual nurseries; however, rates of juvenile mortality may vary among nurseries. Thus, abundance may not reflect contribution to adult populations, and contribution estimates require methods to identify the nursery origin of adults.
2. We evaluated the use of natural tags, vertebral chemistry (stable isotope analysis, elemental analysis) and genetic markers, to identify bull shark *Carcharhinus leucas* nursery origin along the Texas coast (northwestern Gulf of Mexico) at multiple spatial scales.
3. Sharks were most accurately assigned (89% accuracy) to their regional origin (Northern vs. Southern Texas) using a combination of vertebral chemistry and genetic markers. Accuracy decreased when incorporating only one type of natural tag (vertebral chemistry: 58%–84%; genetics: 30%–58%), or when assigning sharks among nurseries grouped into three or four regions (30%–71%; ~100 km spatial scale).
4. *Synthesis and applications.* We describe a novel method integrating multiple natural tags to identify the nursery origin of a highly migratory coastal elasmobranch, over smaller spatial scales than previously investigated. This framework provides a powerful tool to estimate the relative source (nursery) contributions to adult populations of highly mobile species, which represent production estimates sought in the designation of essential fish habitat and ultimately incorporated into marine spatial planning practices.

## KEYWORDS

bull shark, genetics, LA-ICP-MS, next-generation sequencing, philopatry, stable isotope analysis, vertebral chemistry

## 1 | INTRODUCTION

Elasmobranchs (sharks, skates and rays) play an important role in maintaining the function and biodiversity of ecosystems. They may influence the population dynamics and distribution of organisms in

ecosystems by exerting top-down control through a series of density- (direct) or trait-mediated (indirect) interactions with members of lower trophic levels (Preisser, Bolnick, & Benard, 2005; Werner & Peacor, 2003). Thus, declining abundances of elasmobranchs may result in trophic cascades with profound consequences for the

function and stability of marine ecosystems (Heithaus, Wirsing, & Dill, 2012). Worldwide, one-quarter of chondrichthyan fishes are listed by the International Union for Conservation of Nature as threatened (Dulvy et al., 2014). In the Gulf of Mexico (GoM), elasmobranch populations have experienced precipitous declines (Baum & Myers, 2004), largely due to anthropogenic activities, including overfishing and habitat loss or degradation.

Elasmobranchs differ from teleosts as they have comparatively low fecundities and high ages at maturity. Therefore, population growth rates of elasmobranchs depend on low rates of juvenile mortality (Ferretti, Worm, Britten, Heithaus, & Lotze, 2010). Many elasmobranchs spend their early life inhabiting bays or estuaries, which provide refuge from predators and abundant prey resources (Branstetter, 1990; Castro, 1993). Elasmobranch nurseries are defined as those habitats containing elevated densities of juveniles exhibiting residency over extended periods, and temporal stability in use over multiple years (Froeschke, Stunz, Sterba-Boatwright, & Wildhaber, 2010; Heupel, Carlson, & Simpfendorfer, 2007). Unfortunately, these habitats may be subject to the greatest degree of anthropogenic disturbance because they often overlap with areas of human habitation and use. Therefore, sustainable management requires both identifying shark nurseries and determining their proportional contributions to recruitment into the adult population.

Elasmobranch vertebrae form as a series of concentric layers comprising hydroxyapatite in a collagen matrix, and the chemical composition of these layers is influenced by ambient environmental conditions. Elasmobranch vertebrae have been shown to be resistant to metabolic reworking after deposition, unlike other biogenic apatites (e.g. bone; Ashhurst, 2004; Clement, 1992; Dean et al., 2015). The chemistry of these layers may therefore serve as a proxy for the chronology of environmental conditions experienced (or areas inhabited) throughout an animal's lifetime. Recent studies have shown vertebral chemistry is an effective tool in the determination of nursery origin of elasmobranchs (Lewis, Patterson, Carlson, & McLachlin, 2016; McMillan, Huvener, Semmens, & Gillanders, 2018; Smith, Miller, Márquez-Farías, & Heppell, 2016), particularly when nurseries are distributed along strong physicochemical (e.g. salinity) gradients. Similarly, genetic markers may also provide insight into the relative contributions of specific nurseries integrated across multiple generations. Coastal elasmobranchs often exhibit reproductive philopatry, with individuals returning to their natal nursery/region to breed (Chapman, Feldheim, Papastamatiou, & Hueter, 2014). As elasmobranch offspring typically do not experience the same level of passive dispersal as newly fertilized or hatched teleost larvae (Dudgeon et al., 2012) multi-generational fidelity of females to breeding sites could create genetic heterogeneity over relatively small spatial scales, despite the potential for highly migratory behaviour later in life. Therefore, natural tags, including vertebral chemistry and genetic markers, can be effectively applied to address questions of relative value of discrete estuarine habitats for coastal elasmobranchs.

Bull sharks *Carcharhinus leucas* are euryhaline elasmobranchs that spend their first several (2–4) years of life in estuaries (Heupel, Yeiser, Collins, Ortega, & Simpfendorfer, 2010; Matich & Heithaus, 2015).

Previous studies have reported significant heterogeneity in bull shark genetics (Karl, Castro, Lopez, Charvet, & Burgess, 2011; Tillett, Meekan, Field, Thorburn, & Ovenden, 2012) and vertebral chemistry among putative nurseries (Tillett et al., 2011; Werry, Lee, Otway, Hu, & Sumpton, 2011), making them a model species to evaluate combining genetic and chemical markers to identify nursery origin in coastal elasmobranchs. Here, young-of-the-year (YOY) bull sharks from estuaries along the Texas coast of the northwestern GoM were characterized in terms of the trace element and stable isotope composition of their vertebrae, and genotyped using single nucleotide polymorphisms (SNPs). Region-specific baselines developed from these data were then assessed to determine their ability to accurately identify the nursery origin of bull sharks.

## 2 | MATERIALS AND METHODS

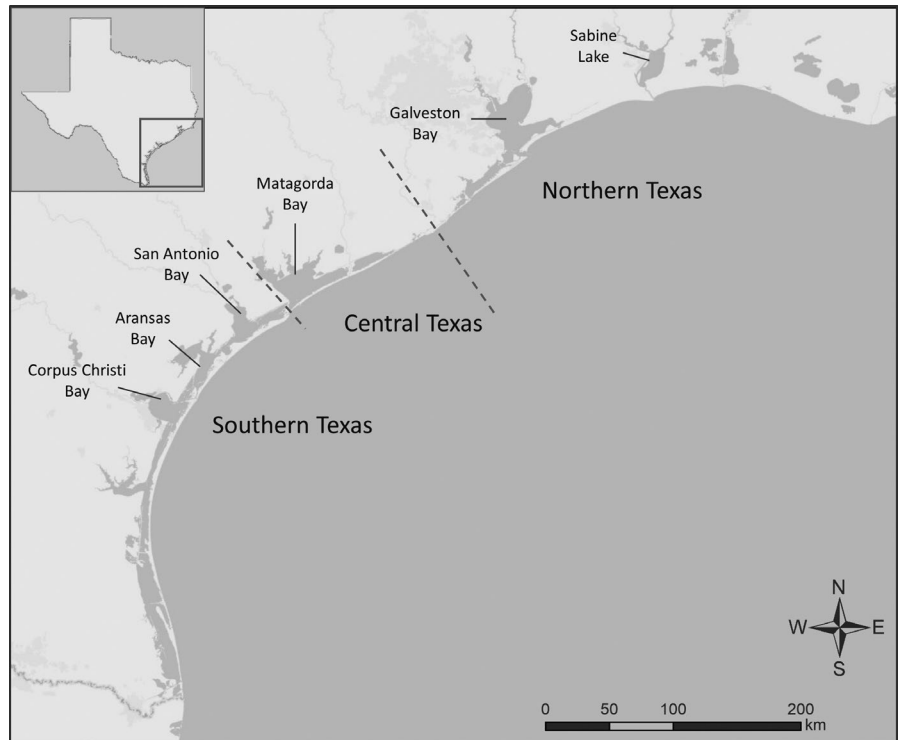
### 2.1 | Sample collection

A total of 207 YOY bull sharks (106 female, 101 male;  $76.9 \pm 11.7$  cm fork length [FL]) were collected, from August 2013 to November 2016, from four major estuarine complexes (hereafter estuaries) in Texas: Sabine Lake, Galveston Bay, Matagorda Bay and Aransas-Corpus Christi Bay (comprising San Antonio, Aransas and Corpus Christi Bays; Figure 1). Sharks included in this study were incidental mortalities, retained by Texas Parks and Wildlife Department during biannual (spring, fall) gillnet surveys in Texas estuaries (Martinez-Andrade, Fisher, Bowling, & Balboa, 2009). Samples from each estuary were pooled into regional groups based on similarities in hydrological characteristics (e.g. temperature, salinity, sources of freshwater input), resulting in a three-region grouping (Northern Texas<sub>3-reg</sub> [Sabine Lake, Galveston Bay], Central Texas<sub>3-reg</sub> [Matagorda Bay], and Southern Texas<sub>3-reg</sub> [Aransas-Corpus Christi Bay]) and a two-region grouping (Northern Texas<sub>2-reg</sub> [Sabine Lake, Galveston Bay, Matagorda Bay] and Southern Texas<sub>2-reg</sub> [Aransas-Corpus Christi Bay]).

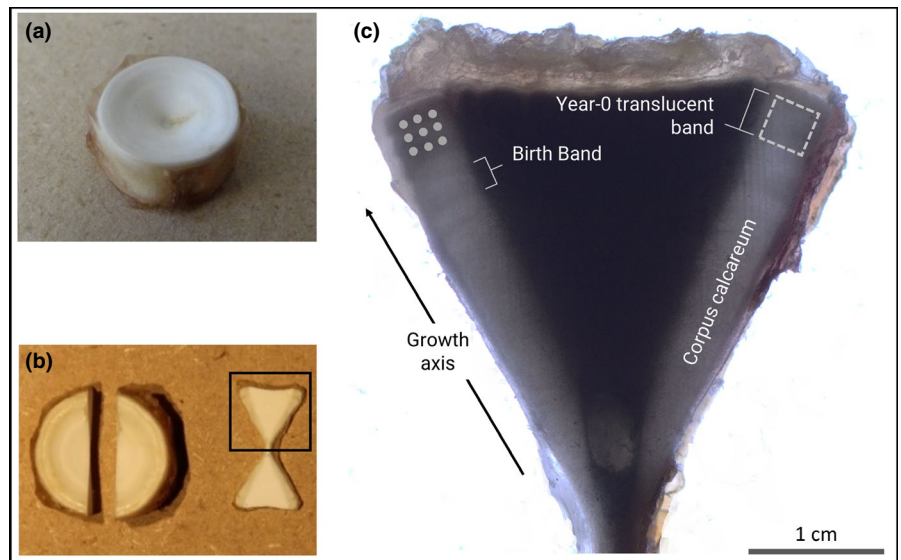
### 2.2 | Vertebral chemistry

Vertebrae from each shark were dried, cross-sectioned to a 0.75 mm thickness, and viewed under a dissecting microscope using transmitted light. Translucent/opaque band pairs occurring along the corpus calcareum and intermedialia of vertebrae were counted to estimate the age of each shark (Figure 2). Sharks lacking a complete band pair after the birth band were assumed to have been captured within their first year of life (age-0; Branstetter & Stiles, 1987; Cruz-Martinez, Chiappa-Carrara, & Arenas-Fuentes, 2005) in their natal estuary. Concentrations of seven elements (<sup>7</sup>Li, <sup>24</sup>Mg, <sup>43</sup>Ca, <sup>55</sup>Mn, <sup>88</sup>Sr, <sup>137</sup>Ba, <sup>65</sup>Cu) and two stable isotope ratios (<sup>13</sup>C:<sup>12</sup>C, <sup>18</sup>O:<sup>16</sup>O) were quantified from the age-0 translucent band in the corpus calcareum (hereafter 'age-0 band'; Figure 2b) of YOY bull sharks.

**FIGURE 1** Sample locations for bull sharks collected in Texas estuaries. Dashed lines indicate regional groupings used throughout data analysis. In two-region baselines, Southern Texas<sub>3-reg</sub> and Central Texas<sub>3-reg</sub> regions are combined into a single region (Southern Texas<sub>2-reg</sub>)



**FIGURE 2** (a) Vertebra centrum, (b) 'bow-tie' frontal cross-section, (c) anatomy of a vertebral cross-section, position and orientation of tissue extracted for carbonate stable isotope analysis (white rectangle) and LA-ICP-MS spot ablations (white filled circles) for elemental analysis



Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) was used to quantify element concentrations from a series of nine spot ablations evenly distributed across the region of interest. Variability in elemental concentrations among samples, due to variation in ablation yield, was corrected through use of  $^{43}\text{Ca}$  as an internal reference standard and expressed as element:Ca molar ratios (Supporting Information 1). Approximately 1.25 mg of powdered tissue was removed from the region of interest and sent to the University of Arizona Environmental Isotope Laboratory for stable isotope analysis of carbonates ( $^{13}\text{C}$ : $^{12}\text{C}$ ,  $^{18}\text{O}$ : $^{16}\text{O}$ ). Results were then reported in

delta notation ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ), the ratio of heavy to light isotopes in a sample, divided by the ratio of heavy to light isotopes of a reference material (Vienna PeeDee Belemnite for  $\delta^{13}\text{C}$ , and Vienna Standard Mean Ocean water for  $\delta^{18}\text{O}$ ).

### 2.3 | Genotyping

DNA was extracted from dermal tissue using Mag-Bind Blood and Tissue kits (Omega Bio-Tek). For assembly of a reduced representation reference, double digest restriction site-associated DNA

sequencing (ddRAD) following Portnoy et al. (2015) was performed to create a single library consisting of a subset of 24 sharks from all sampled estuaries, which was sequenced on a single lane of a MiSeq DNA sequencer (Illumina), producing 300 bp paired-end reads. Raw reads were demultiplexed using *process\_radtags* (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013) Reference contiguous sequence alignments (contigs) were reconstructed using the overlapping read assembly option in the *dDocent* pipeline (Puritz, Hollenbeck, & Gold, 2014) for a range of combinations of threshold values for minimum within ( $K_1$ ) and among individual ( $K_2$ ) occurrence and required similarity to cluster ( $c$ ). A subset of the HiSeq data set described below was used to identify the optimum reference that maximizes the number of reads mapped, while minimizing the number of reads for which read pairs are mapped to two different contigs. Final parameters selected were  $K_1 = 5$ ,  $K_2 = 6$  and  $c = 0.8$ .

For genotyping, three ddRAD libraries were constructed and sequenced on two separate lanes of an Illumina HiSeq 4000 (Illumina). Raw sequences were demultiplexed using *process\_radtags*, quality trimming, read mapping to the reference, and SNP calling were performed using the *dDocent* pipeline. Raw SNPs were rigorously filtered using *VCFtools* (Danecek et al., 2011) and custom scripts following O'Leary, Puritz, Willis, Hollenbeck, and Portnoy (2018). Final thresholds include minimum locus/genotype quality = 20, minimum genotype call rate per locus by estuary = 90%, minor allele count = 3, minimum genotype depth = 5, mean depth per locus = 15–180. Individuals with >10% missing data were removed (Supporting Information 2). SNPs on the same contig were collapsed into haplotypes using *rad\_haplotyper* (Willis, Hollenbeck, Puritz, Gold, & Portnoy, 2017) which was also used to remove loci exhibiting patterns indicative of paralogs or genotyping error from the final data set.

## 2.4 | Test for heterogeneity in natural tags

Tests for heterogeneity in vertebral chemistry among nurseries and years were performed using two-way multivariate analysis of variance (MANOVA, Type III), including estuary, year and their interactions as factors followed by two-way ANOVAs (estuary, year, estuary  $\times$  year), and one-way ANOVAs performed for each year to determine which element:Ca ratios and stable isotope values differed significantly among estuaries. Global  $F_{ST}$  (Weir & Cockerham, 1984) was estimated to test for genetic heterogeneity among estuaries. A 95% confidence interval (CI) was generated by bootstrapping across loci, with replacement 1,000 times and significance determined by permuting individuals among nurseries 1,000 times, as implemented in *hierfstat* (Goudet, 2005) and *assigner* (Gosselin, Anderson, & Bradbury, 2016). To test for differences among estuaries and regions, pairwise  $F_{ST}$  was estimated post hoc, with 95% CIs calculated and significance tested as above. Rare alleles may bias  $F_{ST}$ -estimates; therefore, loci with a major allele frequency >95% were removed prior to analyses.

## 2.5 | Predictions of nursery origin

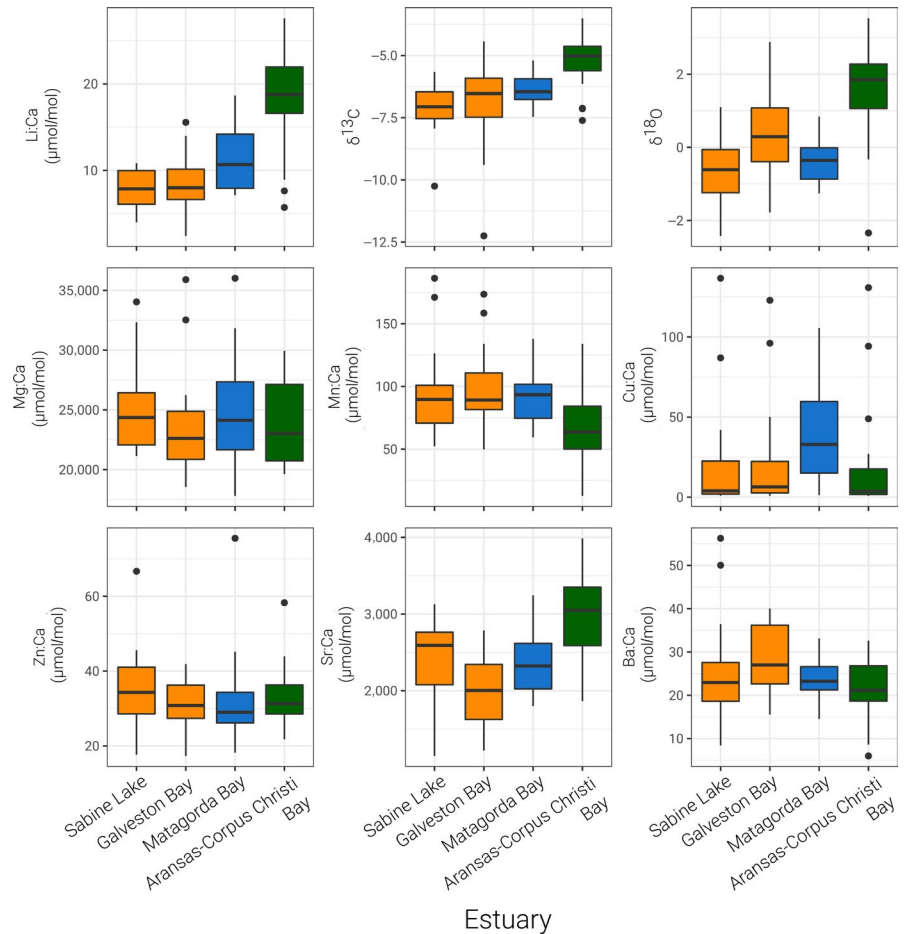
The ability to assign individuals of unknown origins to estuarine complexes and regions was evaluated by testing the accuracy of baseline data sets, using different combinations of natural tags: (a) elements and stable isotopes (vertebral chemistry), (b) allele frequencies of genetic loci (genetics) and (c) genetics and vertebral chemistry (combined). Mechanisms driving genetic heterogeneity are thought to operate over longer time scales. By contrast, drivers of heterogeneity in vertebral chemistry may vary among years. Thus, quadratic discriminant analysis (QDA) and leave-one-out-cross-validation (Supporting Information 2) were used to test the accuracy of year-specific vertebral chemistry baselines. Classification accuracy was reported as % of total samples correctly classified. Klecka's tau ( $\tau$ ), a chance-corrected measure of accuracy accounting for potential biases arising from unequal group sizes (Klecka, 1980) was also reported for year-specific vertebral chemistry baselines. While year-specific vertebral chemistry baselines were assessed using QDA, classification accuracy of baselines across years for all data sets (vertebral chemistry, genetics, and combined) was evaluated in *assignPOP* (Chen et al., 2018), which implements a machine-learning framework to create predictive models. Monte-Carlo cross-validation determines classification accuracy by sampling a subset of individuals as a training set, creating a baseline, and then determining the proportion of remaining individuals (test set) that are correctly classified, resolving bias due to self-assignment (Anderson, 2010; Waples, 2010). The same number of training individuals was used for each baseline to eliminate potential bias associated with unequal sample sizes (Puechmaille, 2016; Wang, 2017; Supporting Information 2). Classification accuracy may be affected by low-variance loci and small sample sizes leading to inaccurate estimates of allele frequencies. Therefore, loci with a major allele frequency >95% or with >5% missing data and estuaries with <20 individuals were removed (Chen et al., 2018). Sharks from San Antonio Bay with genotype ( $n = 7$ ) and vertebral chemistry ( $n = 6$ ) data available were therefore omitted from estuary baselines but included in both Southern Texas<sub>2-reg</sub> and Southern Texas<sub>3-reg</sub> baselines (Supporting Information 2). To test if subsets of informative loci had equal discriminatory power, loci were ranked by  $F_{ST}$ , and the top 1%, 5%, 10%, 25%, 50%, 75%, 90% and 100% were used as training loci. Mean classification accuracy and standard deviation (overall and per baseline) was calculated for 30 iterations as recommended by Chen et al. (2018) and the model with the highest overall classification accuracy selected. Unless otherwise specified, analyses were performed in R (R Core Team, 2013) using cited packages and custom scripts. Figures were generated using *ggplot2* (Wickham, 2009).

## 3 | RESULTS

### 3.1 | Heterogeneity in natural markers among nurseries

Vertebral chemistry of YOY bull sharks varied significantly among estuaries (MANOVA,  $p < 0.01$ ), among collection years, and among

**FIGURE 3** Boxplots of element: Ca molar ratios, and carbonate isotope ratios ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ) in vertebral tissue of juvenile bull sharks collected from three estuarine regions along the Texas coast (Northern Texas: Sabine Lake and Galveston Bay in orange; Central Texas: Matagorda Bay in blue; and Southern Texas: Aransas Bay and Corpus Christi Bay in green). Boxes represent 25th%, 50th% and 75th% percentile, while whisker lengths represent  $1.5 \times$  interquartile range. Outliers (filled circles) are observations falling beyond the range defined by the whiskers



estuary  $\times$  year ( $p < 0.01$ , Table S1). Li:Ca,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and Sr:Ca varied significantly among regions in all years (ANOVA,  $p < 0.05$ ; Figure 3, Table S2); for pairwise comparisons, values in the southernmost estuarine complex (Aransas-Corpus Christi Bay) were greater (ANOVA, Li:Ca:  $p < 0.0001$ ;  $\delta^{13}\text{C}$ :  $p < 0.01$ ;  $\delta^{18}\text{O}$ :  $p < 0.0001$ ; Sr:Ca:  $p < 0.01$ ; Figure 3). Similarly, vertebral chemistry differed significantly across regional groupings for both three-region (MANOVA,  $p < 0.0001$ ) and two-region ( $p < 0.0001$ ) groupings; again values in the southernmost regions (Southern Texas<sub>3-reg</sub>, Southern Texas<sub>2-reg</sub>) were larger compared to other regions (ANOVA,  $p < 0.0001$ , for both comparisons, Figure 1; Figures S1 and S2).

The final reduced-representation reference genome consisted of 26,395 RAD fragments (mean length = 318 bp; total = 8.4 Mbp). The filtered data set consisted of 207 individuals genotyped for 14,663 SNP-containing loci, hereafter 'loci' (39,129 alleles). After removing loci with major allele frequencies  $>95\%$  and subsequently loci with  $>5\%$  missing data, the data set consisted of 8,844 (26,106 alleles) and 8,783 (25,928 alleles) loci respectively. Genetic heterogeneity among estuaries was significant ( $F_{\text{ST}} = 0.00034$ , 95% CI: 0.00015–0.00055,  $p < 0.05$ ). Aransas-Corpus Christi Bay was significantly different from all estuaries, apart from San Antonio Bay ( $F_{\text{ST}} = 0.00040$ –0.00047,  $p < 0.05$ ), and Matagorda Bay was significantly different from San Antonio Bay ( $F_{\text{ST}} = 0.00144$ , 95% CI: 0.00033–0.0027,  $p < 0.05$ , Table S2). Pairwise comparisons among three regional groups revealed significant genetic heterogeneity

between the Northern Texas<sub>3-reg</sub> and Southern Texas<sub>3-reg</sub> regions ( $F_{\text{ST}} = 0.00033$ , 95% CI: 0.00017–0.00051,  $p < 0.01$ ), and the Central Texas<sub>3-reg</sub> and Southern Texas<sub>3-reg</sub> regions ( $F_{\text{ST}} = 0.00049$ , 95% CI: 0.00014–0.00085,  $p < 0.05$ ; Table S3). However, Northern and Central Texas did not differ significantly ( $F_{\text{ST}} = 0.00029$ , 95% CI: 0.0000–0.00065,  $p > 0.05$ ). When sharks from Central Texas<sub>3-reg</sub> were pooled with Northern Texas<sub>3-reg</sub> to create Northern Texas<sub>2-reg</sub>, this group differed significantly from Southern Texas<sub>2-reg</sub> ( $F_{\text{ST}} = 0.00030$ , 95% CI: 0.00016–0.00045,  $p < 0.01$ ).

### 3.2 | Classification among estuaries and regions

Overall classification accuracy using vertebral chemistry baselines ranged from 58.4% to 83.9%; group-specific classification accuracy was consistently higher in the southernmost groups (Aransas-Corpus Christi Bay, Southern Texas<sub>3-reg</sub>, Southern Texas<sub>2-reg</sub>, Table 1). Year-specific classification accuracy of vertebral chemistry baselines ranged from 64.7% to 76.5% at the estuarine scale, 75.0% to 88.2% among three regions, and 82.1%–100.0% between two regions. Chance-corrected success rates ranged from  $\tau = 0.52$  to 1.0; the greatest values ( $\tau = 1.0$ ) were observed in two-region baselines from 2013 and 2014 (Table S5). Overall classification accuracies of genetic baselines ranged from 29.5% to 58.2% and were, at all three spatial scales, lower than those of vertebral chemistry and combined baselines (Table 1). Accuracy of genetic

**TABLE 1** Classification accuracies of baselines developed for bull shark nurseries, incorporating genetic, vertebral chemistry and combined (genetic + vertebral chemistry) data at three spatial scales. Models used to develop baselines were linear discriminant analysis (LDA) and support vector machine (SVM)

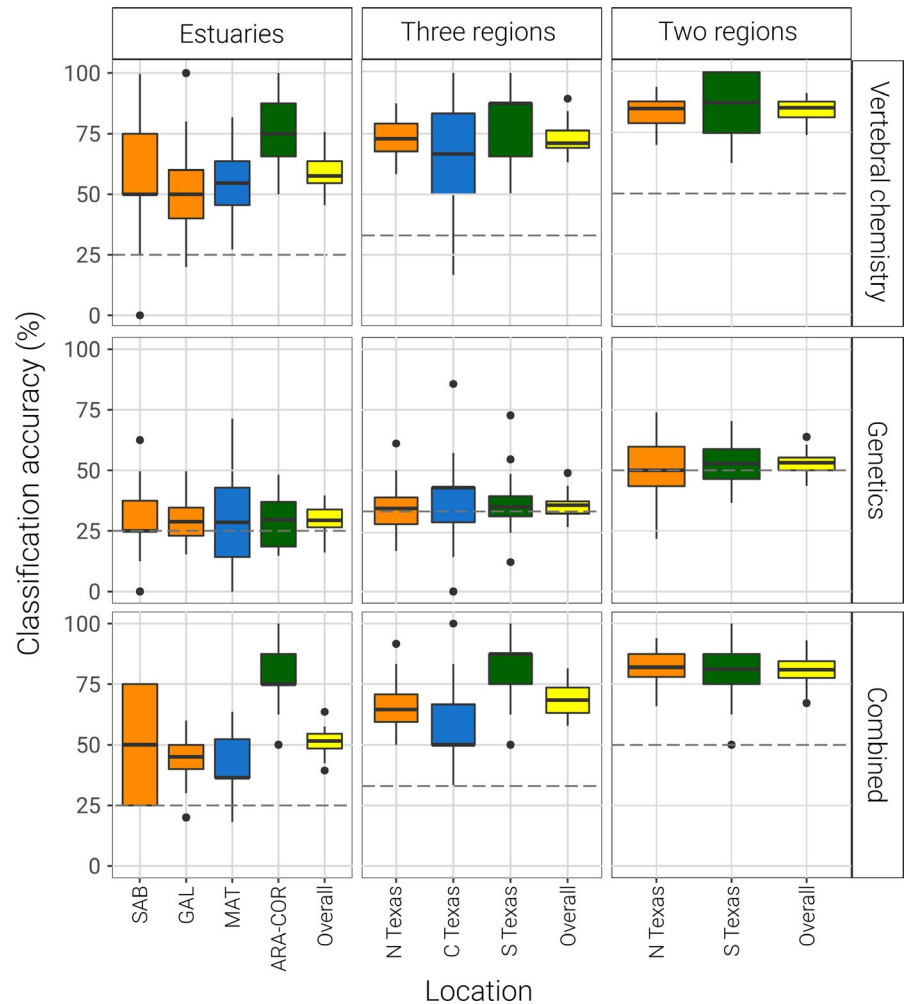
	Spatial scale	Sample size	Proportion of loci used (%)	Model	Overall classification accuracy ( $M \pm SD$ %)	Regions	Classification accuracy (%)	
							M	SD
Vertebral chemistry	Estuaries	93	—	LDA	58.4 $\pm$ 6.6	Sabine Lake	54.2	22.8
						Galveston Bay	49.7	16.9
						Matagorda Bay	54.2	12.1
						Aransas/Corpus Christi Bays	77.1	13.6
	Three regions	98	—	LDA	73.6 $\pm$ 5.9	North	74.4	7.7
						Central	62.2	21.4
						South	79.6	14.9
	Two regions	98	—	LDA	83.9 $\pm$ 5.2	North	83.7	6.2
						South	85.0	13.7
Genetics	Estuaries	148	75	SVM	29.5 $\pm$ 6.0	Sabine Lake	27.1	13.2
						Galveston Bay	29.4	9.2
						Matagorda Bay	32.4	20.5
						Aransas/Corpus Christi Bays	29.6	11.0
	Three regions	154	90	SVM	39.2 $\pm$ 7.9	North	42.5	12.4
						Central	27.1	23.2
						South	36.4	17.5
	Two regions	154	50	LDA	58.2 $\pm$ 7.0	North	58.6	9.4
						South	55.0	19.6
Combined	Estuaries	93	50	SVM	52.2 $\pm$ 5.6	Sabine Lake	42.5	19.9
						Galveston Bay	40.0	15.3
						Matagorda Bay	48.2	13.3
						Aransas/Corpus Christi Bays	77.9	13.4
	Three regions	98	100	SVM	70.8 $\pm$ 5.6	North	71.5	9.2
						Central	51.7	18.7
						South	82.9	5.6
	Two regions	98	100	LDA	89.3 $\pm$ 4.9	North	90.3	5.1
						South	82.5	11.7

baselines at all three spatial scales was only marginally greater than expected with random classification (Figure 4). Overall classification accuracy of combined baselines ranged from 52.2% to 89.3% and, at estuary and three-region spatial scales, were similar to those of vertebral chemistry baselines (52.2% vs. 58.4% and 70.8% vs. 73.6% respectively). At the two-region spatial scale, baselines using combined data yielded the highest overall classification accuracy of all years (89.3  $\pm$  4.9%;  $M \pm SD$ ). In general, classification accuracy increased with spatial scale (Table 1). Estimates of classification accuracy were similar in precision (standard deviation of estimates) across baselines (4.9%–7.9%). However, estimates were less precise for baselines using vertebral chemistry (5.2%–6.6%) or genetic data (6.0%–7.9%) compared to combined data (4.9%–5.6%).

## 4 | DISCUSSION

Significant heterogeneity in vertebral chemistry and genetics was observed for YOY bull sharks collected from estuaries in Texas with sufficient discriminatory power to assign individuals to nurseries at a spatial scale of 100s of kilometres. Observed classification accuracy to nursery sites was comparable to previous assessments of the origin of coastal sharks (~81%–85%—Lewis et al., 2016; >75%—McMillan et al., 2018; 29%–80%—Smith et al., 2016), teleosts (82%–92%—Rooker, Stunz, Holt, & Minello, 2010) and migratory birds (>80%—Cattray et al., 2016) utilizing natural tags, and conducted over larger spatial scales (100–1,000s of km). Baselines using vertebral chemistry and combined data performed similarly,

**FIGURE 4** Percentage of young-of-year (YOY) bull sharks correctly classified to baselines generated at three spatial scales (estuaries, three regions, two regions) using genetic data, vertebral chemistry data and combined (genetic + vertebral chemistry) data. SAB: Sabine Lake, GAL: Galveston Bay, MAT: Matagorda Bay, ARA-COR: Aransas and Corpus Christi Bays. Box colors represent regional groupings of estuaries. (Northern Texas: Sabine Lake and Galveston Bay in orange; Central Texas: Matagorda Bay in blue; and Southern Texas: Aransas Bay and Corpus Christi Bay in green). For baselines generated for two regions, Matagorda Bay is pooled with Sabine Lake and Galveston Bay to form the Northern Texas region. Horizontal dashed lines represent expected accuracy of randomly made classifications (100% divided by the number of groups). Boxes represent 25th%, 50th% and 75th% percentile, while whisker lengths represent 1.5\*interquartile range. Outliers (filled circles) are observations falling beyond the range defined by the whiskers



with vertebral chemistry having the highest overall classification rates for nurseries and three regions and combined having the highest classification accuracy at the two-region scale. However, at coarser spatial resolutions (e.g. two regions), baselines combining genetic and vertebral chemistry data improved classification accuracy, and the inclusion of genetic data may counteract bias due to year-specific variations in vertebral chemistry arising from interannual fluctuations in environmental conditions (Rundel et al., 2013; Taillebois et al., 2017).

Elemental incorporation of the calcified tissues of fishes occurs as a function of the ambient concentrations of these elements in surrounding waters, and are affected by several extrinsic and intrinsic processes (Barnes & Gillanders, 2013). These processes are undoubtedly better studied in teleosts than elasmobranchs and to date only one study has thoroughly described such processes in elasmobranchs (Smith, Miller, & Heppell, 2013). Latitudinal gradients in Li:Ca and Sr:Ca were likely driven by similar gradients in salinity, as both elements are thought to increase in calcified structures in relation to ambient salinity (Fleishman, Saulus, & Vasilieva, 1986). However, due to a lack of direct measurements of dissolved trace elements from estuaries in which animals were collected, it was not possible to account for elemental availability or attribute patterns of elements in shark vertebrae to specific

environmental factors. The carbonate ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ) isotope composition of calcified tissues of fishes has been linked to variance in salinity and temperature (Dorval, Jones, Hannigan, & Van Montfrans, 2007; Elsdon & Gillanders, 2002). Ratios of heavy to light isotopes present in ambient water are subject to alteration by physical, biological and interactive effects (e.g. evaporation,  $\text{C}_3$  vs.  $\text{C}_4$  primary producer abundance, allochthonous carbon input). Here we observed  $\delta^{18}\text{O}$  shifts of  $\sim 1.7\text{‰}$  in shark vertebrae consistent with regional differences in mean salinity (e.g. Northern Texas<sub>3-reg</sub>:  $\sim 12$ , Southern Texas<sub>3-reg</sub>:  $\sim 24$ ; Tolan, 2007), and the estimated increase of  $\sim 0.1\text{‰}$  with each salinity unit (Bastow, Jackson, & Edmonds, 2002). Rooker et al. (2010) identified similar latitudinal gradients in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in the otoliths of red drum *Sciaenops ocellatus* from along the Texas coast, and suggested that freshwater inflow (as a source of  $^{13}\text{C}$ -depleted terrestrial carbon) and the abundance of seagrass beds ( $^{13}\text{C}$ -enriched producers) in southern estuaries were the primary drivers of the north-south increase in  $\delta^{13}\text{C}$  values. Though the gradient in  $\delta^{18}\text{O}$  in the present study may have been influenced in part by a north-south increase in mean salinity, it was more likely driven by the comparatively large gradient in temperature among regions.

Significant genetic heterogeneity was detected among regional estuaries, with significant pairwise differences between estuaries in

Southern, Central and Northern Texas<sub>3-reg</sub>, demonstrating genetic population structure at a finer spatial scale than previously observed in bull sharks (Sandoval-Castillo, Robinson, Hart, Strain, & Beheregaray, 2018; Tillett et al., 2012). These patterns likely indicate philopatry at a local scale, with females returning to their natal or neighbouring estuary. Sex-biased dispersal, with females exhibiting natal or regional philopatry, is frequently observed in coastal sharks (Chapman et al., 2014; Feldheim et al., 2014), though regional philopatry is more commonly reported (potentially due to the difficulty of precisely identifying natal sites, and resultantly, natal philopatry), and it is difficult to quantify exact levels of female straying. Despite significant genetic differentiation at estuary and regional levels, classification accuracy rates of genetic baselines were only marginally higher than expected for random classification. For vertebral chemistry, genetics and combined baselines alike, three-region baselines consistently had the highest misclassification rates for sharks from Central Texas<sub>3-reg</sub>. This is unsurprising, given the lack of heterogeneity between Sabine Lake, Galveston Bay and Matagorda Bay in estuary-scale vertebral chemistry, and Northern Texas<sub>3-reg</sub> versus Central Texas<sub>3-reg</sub> (Figures S1 and S2). Low differentiation across northern and central estuaries of the Texas coast may be in part driven by the limited number of samples obtained from Matagorda Bay. Alternatively, heterogeneity in genetic and vertebral chemistry tracers may be limited among these regions, as a result of similar physicochemical conditions (reducing differences in vertebral chemistry), female straying (reducing genetic structure), or juvenile movement among estuaries (reducing genetic structure and differences in vertebral chemistry). Juvenile bull sharks are known to make temporary forays from natal estuaries (Heupel & Simpfendorfer, 2008), but given the increased predation risks and energetic costs associated with movement into open marine environments, movement among connected estuaries may be more common.

Though novel in application for elasmobranchs, the integration of multiple natural markers has been well-implemented for addressing questions of origin or population connectivity in other highly mobile taxa (e.g. teleost fishes, Taillebois et al., 2017; birds, Rundel et al., 2013). A key advantage of such integrated approaches is that different markers may arise via dissimilar mechanisms (genetic differentiation vs. accretion of calcified tissue), and over different time scales (evolutionary vs. ecological), potentially mitigating the biases of any single technique (Welch et al., 2015). One potential limitation of the models presented here is the possibility of unsampled nurseries, and the inherent difficulty the models presented here may have handling animals not originating from any of the sampled nurseries/regions. We therefore recommend that any future implementation of the approach presented here seeks to sample all potential contributing sources. Furthermore, to produce sufficiently discriminatory baselines from vertebral chemistry data, animals must spend the majority of the assayed time period (in this study, approx. 6–8 months) inhabiting their natal estuary, and greater heterogeneity in physicochemical characteristics must exist among estuaries than within them. Assuming chemical signatures within vertebral tissue are metabolically inert (Ashhurst, 2004; Clement, 1992; Dean et al., 2015; McMillan, Izzo, Wade, & Gillanders, 2016), even with

relatively high degrees of adult mixing or female straying, vertebral chemistry will reflect conditions of the natal nursery in their first year. By contrast, drift processes resulting in genetic differentiation operate over longer time scales, and many generations of restricted gene flow and/or natal philopatry of females are required for differences to accrue. Consequently, markers may disagree if individuals move across spatially heterogeneous environments, or if strong temporal environmental heterogeneity exists in the habitats used by independent genetic units. Strong gradients in environmental conditions along the Texas coast likely drove the high classification accuracy of vertebral chemistry baselines, while baselines combining natural tags only performed marginally better. Thus, where sample processing costs, time, and effort are a concern, vertebral chemistry analyses may be preferred. Nevertheless, the inclusion of genetic tags may serve to buffer the observed interannual variability (Table S5) in environmental gradients, and for other regions with weaker gradients, genetic differentiation across estuaries might still play an important role in the determination of nursery contribution rates. Effective implementation of the framework detailed here will, however, require the periodic collection of vertebrae and dermal tissue from adult sharks across the region of interest. Such efforts would also benefit from the routine archiving of vertebrae and dermal tissues from YOY individuals captured across discrete nurseries, which would permit age-matching of baselines to natural tracers from adult tissues. The framework presented here may be used to generate habitat/nursery-specific estimates of production, information of high priority in ecosystem-based management practices (e.g. Australia, New Zealand; UK; USA; ANZECC TFMPA, 1999; MMO, 2016; NOAA, 2002). Estimates of production also represent the highest level of analysis (Level 4; Able, 1999) sought in United States federal designations of essential fish habitat, after which such habitats are protected from specific fishing gears/practices, or other human activities with anticipated adverse effects (NOAA, 2002). However, for coastal elasmobranchs (or any other taxa with prolonged juvenile stages), demographic analyses demonstrating the relative importance of specific age-classes are required prior to implementation of species-specific management actions seeking to protect nurseries (Cortés, 1998; Kinney & Simpfendorfer, 2009).

## 5 | CONCLUSIONS

Here we evaluated the application of natural tags to identify nursery origin of a coastal elasmobranch over spatial scales of 100s of kilometres. A combination of complementary tags (elements and stable isotopes in vertebral tissue, genetics) provided the most accurate means of determining nursery origin, despite interannual variability in the data set. Furthermore, the presence of significant genetic differentiation among even adjacent estuaries indicates that combining vertebral chemistry and genetic data to develop nursery-specific baselines could be a powerful approach to estimate contribution rates of natal estuaries throughout the GoM, informing spatially explicit management efforts.



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## AUTHORS' CONTRIBUTIONS

All authors conceived the ideas and designed methodology. T.C.T. and S.J.O. collected and analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts, agreed to be accountable for the accuracy and integrity of their contributions, and gave final approval for publication.

## DATA AVAILABILITY STATEMENT

Raw, demultiplexed sequence reads are available from NCBI's Short Read Archive BioProject <https://www.ncbi.nlm.nih.gov/bioproject/612548>, SRA accession PRJNA612548 (TinHan et al., 2020a). The demultiplexed sequence reads are under embargo with NCBI and will be available from 1 April 2021. R notebooks and scripts containing fully reproducible code are available at <https://doi.org/10.5281/zenodo.3710520> (TinHan et al., 2020b).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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