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Do sharks exhibit heterodonty by tooth position and over ontogeny? A comparison using elliptic Fourier analysis

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ABSTRACT

Tooth morphology is often used to inform the feeding ecology of an organism as these structures are important to procure and process dietary resources. In sharks, differences in morphology may facilitate the capture and handling of prey with different physical properties. However, few studies have investigated differences in tooth morphology over ontogeny, throughout the jaws of a single species, or among species at multiple tooth positions. Bull (*Carcharhinus leucas*), blacktip (*Carcharhinus limbatus*), and bonnethead sharks (*Sphyrna tiburo*) are coastal predators that exhibit ontogenetic dietary shifts, but differ in their feeding ecologies. This study measured tooth morphology at six positions along the upper and lower jaws of each species using elliptic Fourier analysis to make comparisons within and among species over their ontogeny. Significant ontogenetic differences were detected at four of the six tooth positions in bull sharks, but only the posterior position on the lower jaw appeared to exhibit a functionally relevant shift in morphology. No ontogenetic changes in morphology were detected in blacktip or bonnethead sharks. Intraspecific comparisons found that most tooth positions significantly differed from one another across all species, but heterodonty was greatest in bull sharks. Additionally, interspecific comparisons found differences among all species at each tooth position except between bull and blacktip sharks at two positions. These morphological patterns within and among species may have implications for prey handling efficiency, as well as in providing insight for paleoichthyology studies and reevaluating heterodonty in sharks.

KEYWORDS

dentition, ecology, elasmobranchs, feeding, morphology

1 | INTRODUCTION

Morphology of the feeding apparatus can constrain the ecological niche of an organism through its performance and behavioral application during the acquisition of food items (Arnold, 1983; Lauder, 1982; Losos, 1990; Ricklefs & Miles, 1994; Wainwright, 1988). By integrating ecological signals over time, tooth morphology can serve as a useful indicator of diet (Erickson et al., 2012; Freeman, 2000; Ricklefs & Miles, 1994; Sage & Selander, 1975; Van Valkenburgh, 1988). The primary function of teeth is to transmit force from the jaw adductor muscles to dietary items, although other functions are also important (e.g., agonistic and mating behaviors; Herrel, Moore, Bredeweg, & Nelson, 2010; Le Boeuf & Mesnick, 1991; Pratt & Carrier, 2001). Additionally, teeth are used during stages of prey capture, retention, and processing in predatory organisms.

To facilitate these different purposes, some organisms have distinct functional units of teeth whose morphology and location along the jaw margin or elsewhere within the cranium (i.e., pharyngeal jaws, vomerine/palatine teeth) are adept for certain functions (Galloway, Anderson, Wilga, & Summers, 2016; Janis & Fortelius, 1988; Mehta & Wainwright, 2007; Norton, 1988). The attribution of form to function has been particularly useful in the extrapolation of diet to fossil species, especially in those with heterodont dentition (Underwood, Mitchell, & Veltkamp, 1999; van Valkenburgh, 1988).

A set of teeth are typically characterized as having either a similar or different morphology, which are termed homodont and heterodont, respectively (Liem, Bemis, Walker, & Grande, 2001). Examples of homodont dentitions are ubiquitous in most major vertebrate groups, but heterodonty is much less prevalent (with the exception of mammals;

Bertrand, 2014; D'Amore, 2015; Davit-Béal, Chisaka, Delgado, & Sire, 2007; Reif, 1982). It is likely that homodonty represents a plesiomorphic character in vertebrates (Bertrand, 2014; Huysseune & Sire, 1998; Tucker & Fraser, 2014; Ungar, 2010). Although elasmobranchs represent one of the most basal vertebrate lineages, heterodonty is prevalent within many of these fishes. Traditionally, tooth function in elasmobranchs has been inferred from morphology (Cappetta, 1986, 1987; Frazzetta, 1988), but recent studies that have incorporated measures of performance show that this relationship is complex (Corn, Farina, Brash, & Summers, 2016; Huber, Claes, Mallefet, & Herrel, 2009; Whitenack & Motta, 2010). The attribution of ecology to morphology has been straightforward in some species, such as white sharks (*Carcharodon carcharias*; Ferrara et al., 2011; French et al., 2017), sandtiger sharks (*Carcharias taurus*; Ferrara et al., 2011), horn sharks (*Heterodontus francisci*; Huber, Eason, Hueter, & Motta, 2005; Summers, Ketcham, & Rowe, 2004), bonnethead sharks (*Sphyrna tiburo*; Mara, Motta, & Huber, 2010; Wilga & Motta, 2000), and cownose rays (*Rhinoptera bonasus*; Kolmann, Huber, Motta, & Grubbs, 2015). However, the traditional method of attributing form to function has not been helpful for other elasmobranchs. This issue is best exemplified in batoids that possess a plate-like dentition and feed on soft-bodied stingrays (Dean, Bizzarro, Clark, Underwood, & Johanson, 2017). The cuspidate teeth of white-spotted bamboo sharks (*Chiloscyllium plagiosum*) have been difficult to characterize as well as these teeth can be reoriented to form crushing plates for hard prey (Ramsay & Wilga, 2007). In some cases, tooth morphology can even be modified on a seasonal basis. The dentition of mature male batoids can change from molariform to cuspidate teeth to facilitate a better grasp of females during copulation (Bigelow & Schroeder, 1953a; Gutteridge & Bennett, 2014; Kajiura & Tricas, 1996). Moreover, Whitenack and Motta (2010) found many different tooth morphologies to be functionally equivalent with respect to puncture and draw performance in extant and extinct elasmobranchs. Although, the relationship between tooth morphology and feeding ecology is complex, the dignathic heterodonty exhibited in many carcharhiniform sharks (Bigelow & Schroeder, 1948; Compagno, 1988; Frazzetta, 1994) may have functional importance depending on the stage of feeding.

In many large-bodied sharks, the differentiation in tooth morphology between the upper and lower jaws as well as along the tooth row (the mesio-distal direction parallel to the jaw margin; Bigelow & Schroeder, 1953b; Cappetta, 1987) has been hypothesized to differ in function during prey capture, retention, and processing (Applegate, 1965; Frazzetta, 1988; Liem et al., 2001; Lucifora, Menni, & Escalante, 2001). In carcharhiniform sharks, anterior teeth on the lower jaw are typically gracile, smooth-edged, and often make first contact with prey items during jaw closure. They have also been postulated to puncture and hold prey in place during feeding events (Frazzetta, 1994; Moss, 1972; Motta & Wilga, 2001; Springer, 1961). Once the teeth on the upper jaw have punctured the prey item, small prey are often swallowed whole, while large prey is processed into smaller-sized pieces (Frazzetta, 1994). Many carcharhiniform sharks use a head-shaking behavior to remove pieces of flesh from large prey, which is effective since the labio-lingually flattened teeth have sharp, blade-like edges in the majority of these species (Frazzetta, 1988; Frazzetta & Prange, 1987; Moss, 1972; Motta, Tricas, Hueter, & Summers, 1997). If differences in tooth morphology serve a functional purpose, as has often been hypothesized, it should have consequences for the time and energy required to process or handle prey. Prey handling efficiency may increase if a tooth's shape is suited to a particular function compared to one that is not (Anderson & LaBarbera, 2008; Emerson, Greene, & Charnov, 1994; Huber et al., 2009). This may be of particular importance for young conspecifics, whose prey selection can be constrained by gape, bite force, and the ability of their teeth to puncture and process prey items (Bergman, Lajeunesse, & Motta, 2017; Habegger, Motta, Huber, & Dean, 2012; Mara et al., 2010; Whitenack & Motta, 2010).

As ontogenetic dietary shifts in the diversity, size, and material properties of shark prey are common (Barry, Condrey, Driggers, & Jones, 2008; Bethea et al., 2007; Bethea, Buckel, & Carlson, 2004; Estrada, Rice, Natanson, & Skomal, 2006; Habegger et al., 2012; Lowe, Wetherbee, Crow, & Tester, 1996; Newman, Handy, & Gruber, 2012), it is likely that a concomitant change in tooth morphology (i.e., ontogenetic heterodonty) may occur to meet the functional demands of these dietary shifts. Ontogenetic changes in diet and dentition have been characterized in heterodontiform (Powter, Gladstone, & Platell, 2010; Reif, 1976; Summers et al., 2004) and lamniform (French et al., 2017; Powlik, 1995; Tricas & McCosker, 1984) sharks, but have not been fully investigated in the dignathic heterodont carcharhiniforms to date (but see Raschi, Musick, & Compagno, 1982). These studies have primarily evaluated ontogenetic heterodonty using qualitative methods (McCosker, 1985; Powlik, 1995; Raschi et al., 1982; Reif, 1976; Summers et al., 2004; Tricas & McCosker, 1984), but recent studies have begun using quantitative analyses as a more robust approach (French et al., 2017; Powter et al., 2010).

In general, studies of shark tooth morphology have often been conducted using linear or geometric morphometrics (French et al., 2017; Marramà & Kriwet, 2017; Nyberg, Ciampaglio, & Wray, 2010; Whitenack & Gottfried, 2010; Whitenack & Motta, 2010), but these methods do not fully capture the complexity of tooth morphology in most instances (Crampton, 1995). Unlike linear and geometric morphometrics, elliptic Fourier analysis (EFA) is able to create a more accurate representation of complex organismal morphologies by characterizing the whole outline of the structure of interest (Ferson, Rohlf, & Koehn, 1985; Kuhl & Giardina, 1982). This method would be preferable to investigate ontogenetic changes in shark tooth morphologies compared to landmark-based geometric morphometrics. The accuracy of outlines produced by EFA can be selected a priori, allowing the detail to be controlled for features of different resolutions. Previous studies have used EFA to characterize and classify the shape of fish otoliths (Tracey, Lyle, & Duhamel, 2006), bivalves (Ferson et al., 1985), plants (Neto, Meyer, Jones, & Samal, 2006), pinniped whiskers (Ginter, DeWitt, Fish, & Marshall, 2012), and shark body shape (Fu et al., 2016). As EFA enables a more accurate characterization of tooth morphology than previously used methods, interspecific and intraspecific comparisons are expected to be more accurate as well.

Bull sharks (*Carcharhinus leucas*), blacktip sharks (*Carcharhinus limbatus*), and bonnethead sharks (*Sphyrna tiburo*) are carcharhiniforms that exhibit dignathic heterodonty, differ in feeding ecology, and exhibit ontogenetic dietary shifts (Barry et al., 2008; Bethea et al., 2007; Cliff & Dudley, 1991). If differences in the performance of shark teeth during prey capture and handling are reflective of differences in morphology, then it is expected that tooth morphology will vary within and among species by relative crown height, base crown width, and notch angle to efficiently puncture, cut, or crush prey.

TABLE 1 Sample sizes (N), sex ratios, and mean (±SD) body length measurements (min—max) for each species

Species	N	Sex ratio F/M	TL (cm)	FL (cm)	PCL (cm)
Bull (*C. leucas*)	21	3/18	118.4 ± 43.6 (74.4–215.0)	94.5 ± 36.0 (59.5–174.5)	85.7 ± 33.0 (53.9–159.0)
Blacktip (*C. limbatus*)	28	15/13	124.4 ± 29.2 (67.4–171.1)	99.5 ± 23.2 (52.7–135.6)	90.1 ± 22.1 (47.8–122.5)
Bonnethead (*S. tiburo*)	24	17/7	85.5 ± 18.3 (51.7–125.4)	67.9 ± 15.3 (40.8–99.8)	62.1 ± 14.4 (36.6–92.4)

Note. TL = total length; FL = fork length; PCL = precaudal length; F = female; M = male.

Different combinations of these variables may potentially be tied to feeding behaviors such as biting and swallowing small, soft prey, cutting through large or functionally difficult prey, or crushing hard prey. It was hypothesized that ontogenetic heterodonty is exhibited in each species concomitant with an ontogenetic shift in diet. Additionally, the extent of heterodonty was hypothesized to be greatest in the generalist bull shark compared to the piscivorous blacktip and durophagous bonnethead sharks. This is because the extent of heterodonty is expected to serve as a potential measure of the number of different functional roles that the teeth perform. As dietary breadth and material properties of prey items differ for each species, it was also hypothesized that tooth morphology differs among species (for all size classes) at each of the six selected tooth positions.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Bull shark (*N* = 21), *Carcharhinus leucas* (Müller & Henle, 1839), *N* = 21, blacktip shark, *Carcharhinus limbatus* (Müller & Henle, 1839), *N* = 28, and bonnethead sharks, *Sphyrna tiburo* (Linnaeus, 1758), *N* = 24, were opportunistically sampled from fishing charters or from routine long-line surveys conducted by the Texas Parks and Wildlife Department in Galveston, Texas in March through October from 2014 to 2016. Sex was identified for each shark and measurements of total (TL, cm), fork (FL, cm), and precaudal length (PCL, cm) were recorded (Table 1). Four size classes were delineated for bull and blacktip sharks (young-of-the-year

[YoY], juvenile, subadult, adult), but only three were used for bonnethead sharks (YoY, juvenile, adult). Size classes for each species were based on previous studies from Texas or from a nearby location at a similar latitude, which has been shown to affect growth rates in bonnethead sharks (Branstetter, 1987; Branstetter & Stiles, 1987; Lombardi-Carlson, Cortés, Parsons, & Manire, 2003). In all species, teeth were extracted from the functional row at six positions on the left side of the head. To account for possible changes in morphology at different positions along the upper and lower jaws, three positions were sampled along each jaw margin. These positions included an anterior position on the upper (AntUp) and lower jaws (AntLow), a lateral position on the upper (LatUp) and lower jaws (LatLow), and a posterior position on the upper (PostUp) and lower jaws (PostLow), illustrated in Figure 1 and described in detail in Table 2. These positions were selected to be representative of the whole tooth row in the upper and lower jaws. Teeth were only extracted if there were no visible signs of damage. If teeth were not considered to be in good condition, the contralateral side of the head was used as a suitable alternative; images of these teeth were reflected to match the orientation of the teeth from the left side of the head. Missing or damaged teeth in each species resulted in a variation of sample sizes by tooth position (Table 3).

2.2 | Sample clean-up and processing

After extraction, all teeth were soaked in 9% hydrogen peroxide for 30 min to loosen soft tissue attached to the root for removal via scalpel. Digital images of teeth were collected using a SPOT Pursuit camera mounted on a Nikon SMZ 1500 stereomicroscope if they were
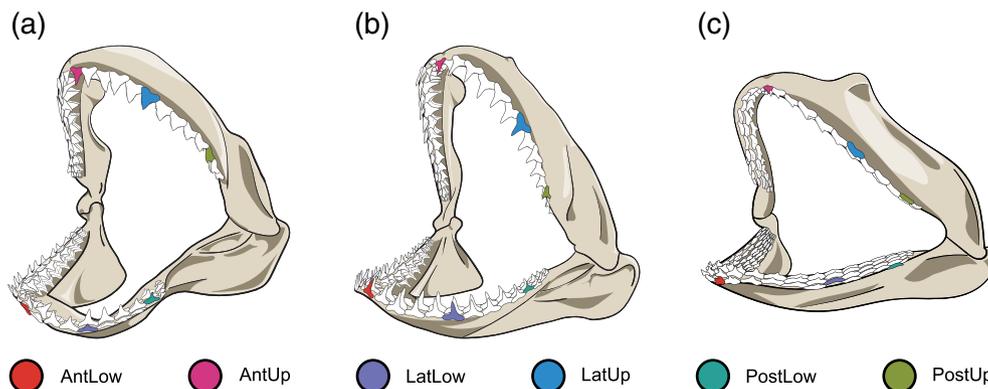
FIGURE 1 Positions of teeth sampled from the functional row of the upper and lower jaws are illustrated for bull (a), blacktip (b), and bonnethead sharks (c). These teeth include the anterior position on the lower (AntLow) and upper jaws (AntUp), the lateral position on the lower (LatLow) and upper jaws (LatUp), and the posterior position on the lower (PostLow) and upper jaws (PostUp). Further details regarding the exact positions can be found in Table 2
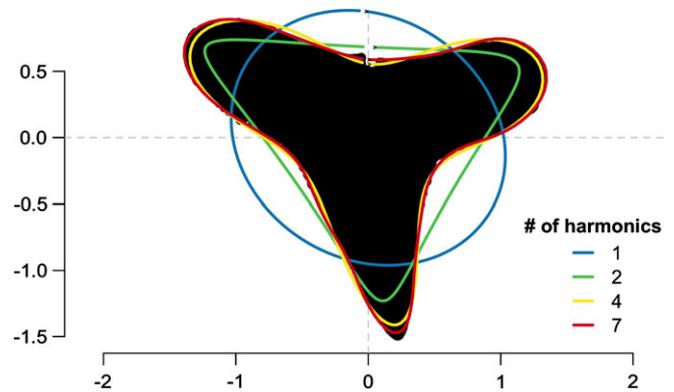
TABLE 2 Descriptions of tooth positions (from anterior to posterior) used for evaluating differences in morphology within and among species

| Position	Description
Anterior-upper jaw (AntUp)	The tooth position immediately lateral to the symphyseal tooth on the upper jaw (palatoquadrate).
Anterior-lower jaw (AntLow)	The tooth position immediately lateral to the symphyseal tooth on the lower jaw (Meckel's cartilage).
Lateral-upper jaw (LatUp)	The tooth position 50% of the jaw length (distance between the symphyseal tooth and the jaw joint) on the upper jaw.
Lateral-lower jaw (LatLow)	The tooth position 50% of the jaw length on the lower jaw.
Posterior-upper jaw (PostUp)	The tooth position 25% of the jaw length from the joint on the upper jaw.
Posterior-lower jaw (PostLow)	The tooth position 25% of the jaw length from the joint on the lower jaw.

FIGURE 2 A visual representation of elliptic Fourier analysis fitting the silhouette of a centered and scaled shark tooth. This demonstration uses one, two, four, and seven harmonics, which describe 90, 98, 99, and 99.9% of the total shape of the outline, respectively

small enough to fit within the field of view. These images were collected using SPOT Advanced (ver 5.2) software. Teeth that did not fit within the field of view of the stereomicroscope were imaged with a Canon EOS-1D Mark II camera fitted with a 50 mm Sigma EX macro lens that used a remote shutter release to ensure sharp images. All images were collected from the labial side of the tooth, which was oriented normal to the lens to reduce distortion caused by parallax. Images were prepared for EFA by creating silhouettes of all teeth in grayscale using Adobe Photoshop CC 2017 (Adobe Systems, San José, CA).

2.3 | Elliptic Fourier analysis and tooth morphometrics

EFA is a preferred method for capturing the outline of an object by fitting a function to an ordered set of coordinates within a Cartesian plane (Ferson et al., 1985; Kuhl & Giardina, 1982). This function consists of a sum of harmonics (trigonometric curves) produced by orthogonal Fourier decomposition that fits greater complexity of the outline with each successive harmonic. Each harmonic is also described by four coefficients (two Fourier coefficients each for the x and y components). These coefficients describe the size, shape, and orientation of each harmonic ellipse along the closed outline. Due to

the method by which these harmonics are generated, the lower-ordered harmonics roughly capture most of the variance in shape while the higher-ordered harmonics capture the finer details (Kuhl & Giardina, 1982; Crampton, 1995; Figure 2). The accuracy of the function used to fit an outline can be selected for a priori using an average Fourier power spectrum, which allows the average cumulative power of a set of harmonics to be chosen for a given analysis (Bonhomme, Picq, Gaucherel, & Claude, 2014; Crampton, 1995). To capture the greatest accuracy in tooth morphology, the number of harmonics chosen for each tooth comparison was selected to describe 99.9% of the total variation in shape. All EFA was conducted using the "Momocs" package (ver 1.2.9; Bonhomme et al., 2014) in the R statistical environment (R Core Team, 2018). All tooth outlines were centered and scaled to centroid size prior to EFA to align all teeth and remove the effect of tooth size for a given comparison, respectively (Figure 3). Smoothing was conducted on the curves produced by EFA using a simple moving average (nine iterations) to reduce any noise generated during this process (Haines & Crampton, 2000). As shape analysis using EFA is conducted on outlines generated from an automated algorithm using nearest neighbor values of pixels around the entire contour of the shape of interest (Claude, 2008; Rohlf, 1990), user-based error is decreased during the digitization process. This method

TABLE 3 Sample sizes (n) for each size class by tooth position within each species

Species	Size class	AntUp	AntLow	LatUp	LatLow	PostUp	PostLow
Bull (C. leucas)	YoY	6	6	6	6	6	6
	Juvenile	11	10	10	9	11	10
	Subadult	2	2	2	2	2	2
	Adult	2	2	2	2	2	1
Blacktip (C. limbatus)	YoY	3	3	3	3	3	3
	Juvenile	5	5	4	4	5	6
	Subadult	11	11	9	9	11	11
	Adult	8	8	7	7	8	8
Bonnethead (S. tiburo)	YoY	7	7	7	7	7	7
	Juvenile	8	8	8	8	8	8
	Adult	8	8	9	9	8	9

Note. YoY = young-of-the-year; refer to Table 2 for abbreviations of each tooth position.
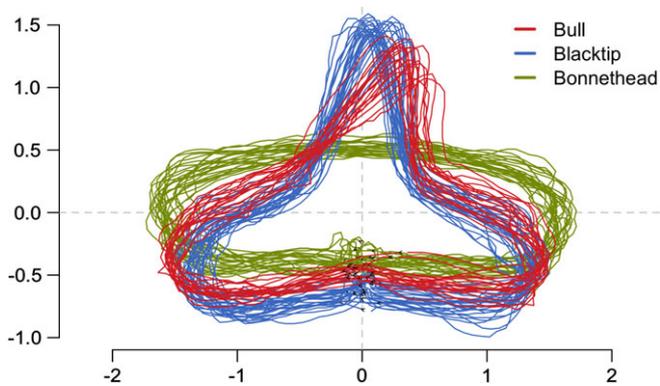
FIGURE 3 An example of raw centered and scaled tooth outlines from the posterior position along the lower jaw of each species (PostLow). These outline traces display the variation in morphology at this tooth position both within and among species

is generally quicker to conduct shape analysis than linear and geometric morphometrics due to the automated process, especially given a large number of selected landmarks.

As EFA uses harmonic coefficients to describe tooth shape rather than linear measurements common in traditional morphometrics, relative characteristics of tooth morphology are used to qualitatively describe these teeth. These characteristics include base crown width, crown height (perpendicular to base crown width), and notch angle (angle between the tip of the crown and base crown width on the distal edge of the tooth), which are expected to be functionally relevant characteristics (Figure 4; Anderson & LaBarbera, 2008; Crofts & Summers, 2014; Whitenack & Motta, 2010). Although these traditional tooth morphometrics were not explicitly measured, they were used to make qualitative comparisons among tooth outlines in support of the quantitative statistical analyses.

2.4 | Statistical analyses

All intraspecific and interspecific comparisons of tooth morphology were initially evaluated by principal component analysis (PCA) on the harmonic coefficients. The ordination of multivariate data is useful for
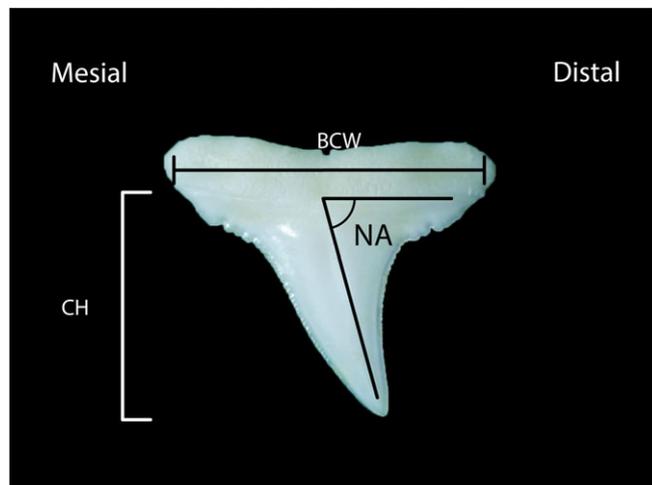
FIGURE 4 Morphometrics used to describe and make qualitative comparisons among teeth after statistical analyses. BCW = base crown width; CH = crown height; NA = notch angle

the exploratory visualization of individual teeth within morphological space (morphospace). Although unconstrained ordination methods (e.g., PCA) are useful for dimensional reduction, they are not able to directly test for differences among groups. Quantitative comparisons among groups (size class, tooth position, species) were conducted by permutational multivariate analysis of variance (PERMANOVA) with the "vegan" package (ver 2.5–2; Oksanen et al., 2018) using 1,000 permutations on selected PC scores (Anderson, 2001a; Anderson, 2001b). This method is a nonparametric analogue of MANOVA that is robust to violations of multivariate normality using a permutation procedure (Anderson, 2001a). The number of informative PC-axes were determined by comparing against randomly generated eigenvalues using 1,000 permutations, where eigenvalues from the original dataset were greater than the permuted dataset. Following significant results from the PERMANOVAs, pairwise comparisons (using 1,000 permutations) were calculated using Bonferroni-adjusted p-values. All size classes were grouped together during intraspecific comparisons among tooth positions as well as during interspecific comparisons by tooth position since it was expected that the variation over ontogeny would be much smaller than among tooth positions or species. Significance was set at $\alpha = 0.05$ for all tests.

3 | RESULTS

3.1 | Ontogenetic comparisons

Within bull sharks, significant ontogenetic differences in tooth morphology were detected at the LatLow (pseudo-$F_{3,15} = 2.55$, $p = 0.046$), LatUp (pseudo-$F_{3,16} = 3.62$, $p = 0.018$), PostLow (pseudo-$F_{3,15} = 6.51$, $p = 0.012$), and PostUp positions (pseudo-$F_{3,17} = 4.68$, $p = 0.003$), whereas AntLow and AntUp positions did not exhibit significant ontogenetic differences ($p > 0.05$; Table 4). Of the four tooth positions with significant ontogenetic differences, only pairwise comparisons between YoY and juvenile size classes were significant ($p < 0.05$; Supporting Information Table S1). However, these results may have been affected by the low sample sizes for subadult and adult bull sharks (Table 3). Teeth from YoY and juvenile size classes at the LatLow position were separated along the PC1 axis (53.8% explained variation), while subadult and adult conspecifics overlapped more with juveniles. Relative crown height slightly increased from YoY teeth on the negative side of the PC1 axis to the positive side where juvenile, subadult, and adult teeth were positioned in morphospace (Figure 5a). The PCA of the LatUp position showed greatest differences between YoY and juveniles along the PC1 axis as well, which explained 58.5% of the variation (Figure 5b). Differences in morphology appeared to be driven by a slight change in the notch angle, which increased (i.e., greater notch angle) from the negative side (YoY) to the positive side (juvenile, subadult, adult) of the PC1 axis. At the PostLow position, morphological differences were more pronounced compared to the other tooth positions, for which YoY and juvenile bull sharks were separated along the PC1 axis (82.5% of the variation; Figure 5c). This pattern of changes was primarily a result of increases in relative crown height from YoY (negative PC1 axis) to juvenile conspecifics (positive PC1 axis). At the PostUp position, YoY and juvenile size

TABLE 4 Results of PERMANOVA (1,000 permutations) for ontogenetic comparisons by tooth position within each species

Species	Tooth position	PCs Retained[a]	df	Pseudo-F	p
Bull (*C. leucas*)	AntLow	3	3,16	1.60	0.176
	AntUp	3	3,17	0.81	0.550
	LatLow	2	3,15	2.55	**0.046**
	LatUp	2	3,16	3.62	**0.018**
	PostLow	2	3,15	6.51	**0.012**
	PostUp	2	3,17	4.68	**0.003**
Blacktip (*C. limbatus*)	AntLow	3	3,23	1.50	0.195
	AntUp	3	3,23	0.57	0.746
	LatLow	3	3,19	1.36	0.260
	LatUp	3	3,19	1.47	0.204
	PostLow	2	3,24	0.07	0.996
	PostUp	2	3,23	0.02	1.000
Bonnethead (*S. tiburo*)	AntLow	2	2,20	1.29	0.291
	AntUp	2	2,20	0.93	0.419
	LatLow	2	2,21	2.38	0.087
	LatUp	3	2,21	0.89	0.432
	PostLow	2	2,21	0.18	0.918
	PostUp	2	2,20	1.26	0.282

Note. Refer to Table 2 for abbreviations of each tooth position; significant results are in bold.
[a]The number of informative principal components (PCs) that were used for PERMANOVA.

classes were separated along the PC1 axis as well, which explained 50.5% of the total variation (Figure 5d). YoY individuals on the negative side of the PC1 axis appeared to exhibit more pointed cusps compared to juveniles on the positive side based upon a qualitative assessment. Although subadult and adult size classes did not exhibit significant pairwise differences in any of these analyses, tooth
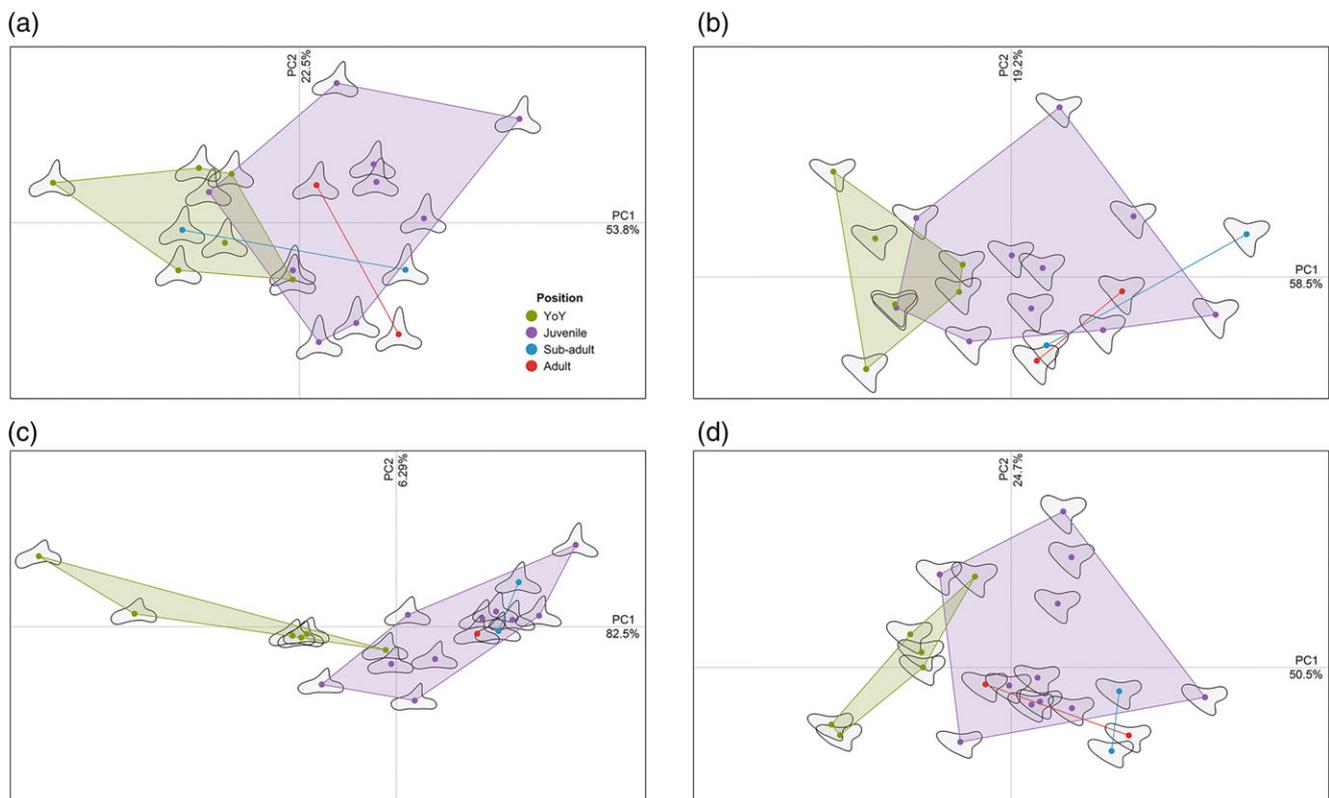
FIGURE 5 PCA-ordinations of significant ontogenetic differences in tooth morphology from bull sharks plotted in morphospace. Numbers next to axis labels indicate the percentage of explained variation in morphology for that axis in a given ordination. These plots display the ontogenetic comparisons in tooth morphology at the lateral position along the lower (LatLow; a) and upper jaws (LatUp; b), as well as at the posterior position along the lower (PostLow; c) and upper jaws (PostUp; d). Gray silhouettes of teeth depict the outline generated using the harmonic coefficients produced by elliptic Fourier analysis to achieve 99.9% of total harmonic power. Eigenvalues and loadings of the harmonic coefficients for each of the PCAs (a - d) can be found in the Supporting Information in Tables S4 - S7, respectively
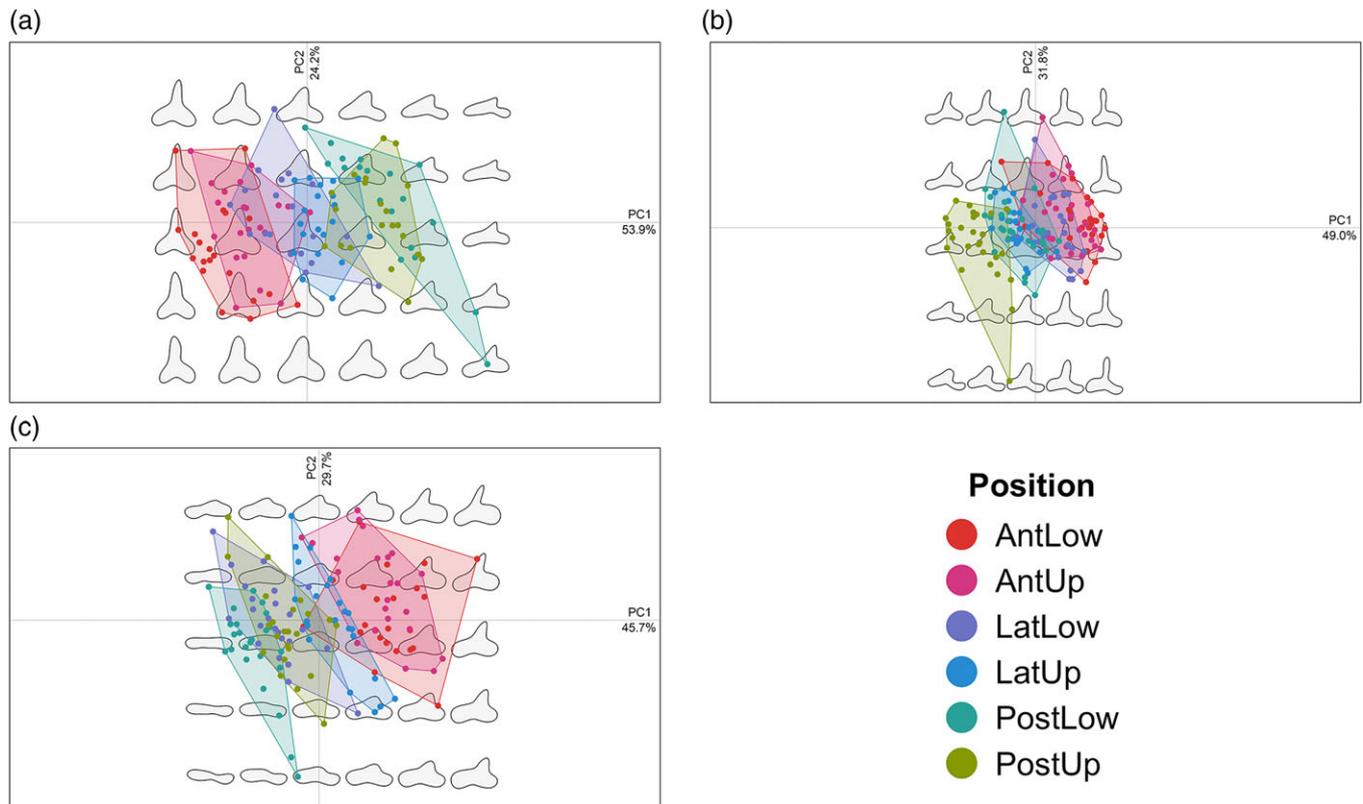
FIGURE 6 PCA-ordinations of tooth morphology among tooth positions in bull (a), blacktip (b), and bonnethead sharks (c). Numbers next to axis labels indicate the percentage of explained variation in morphology for that axis in a given ordination. Points that fall within the minimum convex polygons represent the realized morphology of each tooth position. Gray tooth silhouettes depict the full continuum of morphospace among all tooth positions for each species as calculated using the harmonic coefficients from elliptic Fourier analysis. Eigenvalues and loadings of the harmonic coefficients for each of the PCAs (a - c) can be found in the Supporting Information in Tables S8 - S10, respectively

morphology in both of these groups frequently clustered with juvenile conspecifics. All other ontogenetic comparisons by tooth position in blacktip and bonnethead sharks were not significant ($p > 0.05$; Table 4).

3.2 | Intraspecific comparisons among tooth positions

Significant differences in tooth morphology by position were detected in bull sharks following the PERMANOVA on four retained PCs (pseudo-$F_{5,114}$ = 28.50, $p < 0.001$). All 15 pairwise comparisons found significant differences with the exception of the PostLow-PostUp comparison ($p = 0.150$; Supporting Information Table S2). The bull shark PCA showed that PC1 accounted for morphological differences among tooth positions (53.9% of the total variation), whereas PC2 explained variation within each tooth position (24.2% of the variation; Figure 6a). Teeth in bull sharks change morphology from a greater relative crown height with an approximately 90° notch angle (on the negative side of the PC1 axis; AntLow, AntUp) toward a lower relative crown height and a more acute notch angle (on the positive side of PC1 axis; PostLow, PostUp). Variation in tooth morphology was reasonably consistent within each tooth position along the PC2 axis, which represented other small differences in morphology. This is indicative of a similar level of intrinsic morphological variability at each tooth position regardless of whether ontogenetic differences had been detected or not.

Tooth morphology also significantly differed among positions in blacktip sharks (using four retained PCs; pseudo-$F_{5,149}$ = 26.08, $p < 0.001$) with the exception of the AntLow-AntUp, AntLow-LatLow, AntUp-LatLow, and MidLow-PostUp pairwise comparisons ($p > 0.05$; Supporting Information Table S2). The PC1 axis explained 49.0% of the total variation and represented differences in relative crown height and notch angle (Figure 6b). A large overlap in morphospace was observed among AntLow, AntUp, and LatLow teeth on the positive side of the PC1 axis, which were all characterized by a large relative crown height and a notch angle that approximated 90°. Relative crown height decreased and the notch angle became more acute on the negative side of the PC1 axis where the PostUp teeth were clustered. Teeth from LatUp and PostLow were located near zero along the PC1 axis, which represented an intermediate morphotype between the AntLow, AntUp, and LatLow positions and the PostUp position. Similar to the comparison in bull sharks, the PC2 axis represented smaller differences in morphology within each tooth position and explained 31.8% of the total variation. This variability along the PC2 axis was relatively consistent with the exception of a single outlier for the PostUp position.

Significant differences were detected among all tooth positions in bonnethead sharks as well (using four retained PCs; pseudo-$F_{5,135}$ = 26.10, $p < 0.001$) with the exception of the LatLow-PostLow, LatLow-PostUp, and PostLow-PostUp pairwise comparisons ($p > 0.05$; Supporting Information Table S2). Tooth positions were separated by an increase in relative crown height from the negative to the positive side of the PC1 axis,

TABLE 5 Results of PERMANOVA (1,000 permutations) for interspecific comparisons by tooth position

Tooth position	PCs Retained[a]	df	Pseudo-*F*	*p*
AntLow	3	2,67	71.09	**<0.001**
AntUp	3	2,68	60.23	**<0.001**
LatLow	3	2,63	152.16	**<0.001**
LatUp	3	2,64	76.72	**<0.001**
PostLow	4	2,68	93.50	**<0.001**
PostUp	2	2,68	51.68	**<0.001**

Note. Refer to Table 2 for abbreviations of each tooth position; significant results are in bold.
[a]The number of informative principal components (PCs) that were used for PERMANOVA.

which explained 45.7% of the total variation (Figure 6c). AntLow and AntUp teeth clustered together on the positive PC1 axis (high crowns) while PostLow, PostUp, and LatLow were clustered on the negative PC1 axis (low crowns). Teeth from the LatUp position did not group together with any of the other tooth positions and was found near zero along the PC1 axis. Teeth from the AntLow position also appeared to display greater variation in shape compared to other tooth positions with respect to the PC1 axis, demonstrating greater variability in relative crown height. The PC2 axis explained 29.7% of the total variation and also explained smaller differences in tooth morphology, which was consistent across all tooth positions.
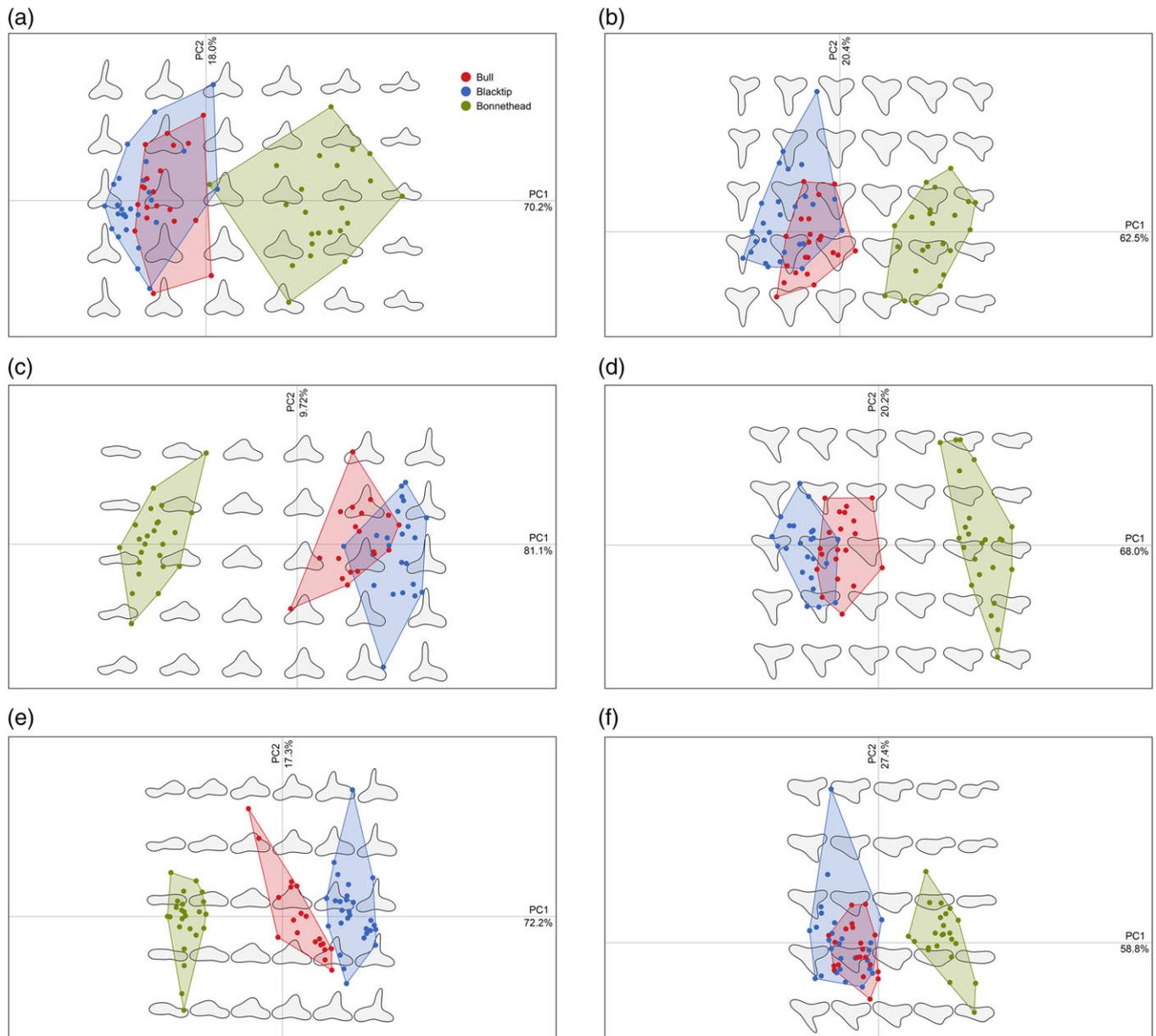
3.3 | Interspecific comparisons

Comparisons among species found significant differences at all six tooth positions (*p* < 0.001; Table 5), for which pairwise relationships varied (Supporting Information Table S3). At the AntLow position, bull, and blacktip sharks were both significantly different from bonnetheads (*p* = 0.003), but not from each other (*p* = 0.147). The PC1 axis (70.2% of variation) represented relative crown height, which was greater in bull and blacktip sharks compared to bonnetheads (Figure 7a). At the AntUp position, however, morphology among all three species significantly differed (*p* = 0.003) and PC1 explained 62.5% of the total variation. AntUp teeth in bonnethead sharks exhibited a lower relative crown height than the other species in addition to a more acute notch angle (Figure 7b). The primary difference between bull and blacktip shark teeth at the AntUp position appeared to be the greater relative width at the base of the crown in bulls compared to blacktips. Similarly, significant differences among all species were found at the LatLow (*p* = 0.003), LatUp (*p* = 0.003), and Post-Low positions (*p* = 0.003). Differences in tooth morphology at the LatLow position were characterized by relative crown height and separated along the PC1 axis (81.1% of variation; Figure 7c). Although tooth morphology at this position was most noticeably different in bonnetheads, bull sharks primarily differed from blacktips by possessing a greater relative base crown width. Teeth at the LatUp position decreased in relative crown height and notch angle from the negative to the positive side of the PC1 axis, which explained 68.0% of the variation (Figure 7d). At this position, blacktip sharks had slightly greater relative crown heights and notch angles than bull sharks, both of which were greater than in bonnethead teeth. PostLow tooth morphology varied greatly among all species and also separated along the

PC1 axis, which explained 72.2% of the variation (Figure 7e). Bonnethead sharks possessed molariform teeth (low relative crown height) at this position, whereas the teeth of bull and blacktip teeth were cusped. However, blacktip teeth were more gracile and exhibited a greater relative crown height than bull sharks. At the PostUp position, significant differences were only detected in the bull—bonnethead and blacktip—bonnethead pairwise comparisons (*p* = 0.003), but not between bull and blacktip sharks (*p* = 0.348). The PC1 axis (58.8% of variation) separated bonnethead teeth that were molariform from bull and blacktip sharks that both exhibited greater relative crown heights and acute notch angles (Figure 7f).

4 | DISCUSSION

Ontogenetic dietary shifts have been reported for each of the three species in this study, but these did not appear to be associated with a change in tooth morphology. Although bull sharks were the only species to exhibit statistically significant differences in morphology over ontogeny, most of these changes do not appear to be functionally significant. Of the four tooth positions in bull sharks with significant ontogenetic differences, only the PostLow position appeared to exhibit a functional shift in tooth shape. Although there may be implications with regard to cutting performance at this single position (e.g., an increase in relative crown height may more securely hold prey in place to be cut by teeth on the upper jaw), it is unclear why only one of the six evaluated tooth positions would exhibit these differences. This could possibly be the result of greater selection pressure at this tooth position since functionally difficult prey may need to be secured by teeth with higher crowns in the posterior region of the lower jaw during forceful head-shaking behavior. However, further functional testing would be required to support this hypothesis. Over ontogeny, the diet of bull sharks shifts from primarily small-bodied teleost prey as YoY and juveniles to including greater proportions of birds, marine mammals, and other elasmobranchs as subadults and adults (Cliff & Dudley, 1991; Snelson, Mulligan, & Williams, 1984; Snelson & Williams, 1981). It is possible that a functional change in tooth morphology could be useful for this dietary shift. The prey consumed by these larger size-classes are more difficult to process than that of younger conspecifics (Habegger et al., 2012) and a change in tooth morphology may assist in cutting through tough tissue (e.g., skin, scales, tendons/ligaments, bones, connective tissue) as opposed to the puncture of soft tissues by younger bull sharks. More specifically, an increase in relative crown height at the PostLow position over ontogeny may assist larger conspecifics to securely hold the prey in place during lateral head-shaking behavior. This may be difficult for some prey due to an integument that is compliant, thick, and/or covered in puncture-resistant scales. These interpretations of ontogenetic changes in tooth morphology (or lack thereof) are potentially limited as a result of the small sample size of sub-adult and adult conspecifics. In blacktip and bonnethead sharks, no significant differences in tooth morphology were found over ontogeny. However, these patterns likely reflect the consumption of prey items with comparable material properties. This is exemplified by sustained piscivory in blacktip sharks and an increase in the proportion of hard-shelled

FIGURE 7 PCA-ordinations of interspecific comparisons by tooth position, including the anterior position on the lower (AntLow; a) and upper jaws (AntUp; b), the lateral position on the lower (LatLow; c) and upper jaws (LatUp; d), and the posterior position on the lower (PostLow; e) and upper jaws (PostUp; f). Numbers next to axis labels indicate the percentage of explained variation in morphology for that axis in a given ordination. Points that fall within the minimum convex polygons represent the realized morphology of each species. Gray tooth silhouettes depict the full range of morphospace among all species for a given tooth position as calculated using the harmonic coefficients from elliptic Fourier analysis. Eigenvalues and loadings of the harmonic coefficients for each of the PCAs (a - f) can be found in the Supporting Information in Tables S11 - S16, respectively

crustaceans consumed by bonnetheads (Barry et al., 2008; Bethea et al., 2007).

When making intraspecific comparisons in morphology among tooth positions, few of the teeth displayed similarities within each species. In bull sharks, only teeth at the posterior positions along the tooth row (PostLow and PostUp) were morphologically equivalent while all other pairwise comparisons significantly differed. Teeth from the upper jaw typically have crowns with a broader labial face and serrated edges whereas teeth from the lower jaw are often gracile with smooth edges. These morphological differences may result in the partitioning of functions between the upper and lower jaws. With less surface area to make contact with the prey item and therefore less

friction during puncture, gracile teeth from the lower jaw can penetrate tissue more easily than teeth from the upper jaw of this species (Frazzetta, 1988). In blacktip sharks, however, gracile teeth at the AntLow, AntUp, and LatLow positions were morphologically equivalent. Patterns of morphological equivalency in bonnetheads were similar to blacktip sharks, in which molariform teeth at PostLow, PostUp, and LatLow positions did not significantly differ from one another. The blacktip pattern results in more teeth used to capture and retain elusive fish prey, but expands the dental battery of molariform teeth used to process hard-shelled prey in bonnethead sharks. Therefore, these results suggest that there are functional units of teeth along the jaws, which also exhibit species-specific patterns.

Given the intraspecific patterns of dissimilarity among tooth positions, bull sharks exhibited a slightly greater level of heterodonty than the other two species. These patterns of heterodonty among species may have implications for the duration and efficiency of prey handling, such that the dentition of a given species may confer an advantage to only certain types of prey. The dentition of blacktip and bonnethead sharks appear specialized for piscivory and durophagy, respectively, which is supported by the unit of morphologically equivalent gracile teeth in blacktips and molariform teeth in bonnetheads. By possessing a greater number of teeth with these respective morphologies, blacktip sharks may be able to capture and consume fishes more efficiently, whereas bonnethead sharks may be able to efficiently crush and consume crustaceans. The high level of heterodonty in bull sharks appears to fit with their status as a generalist consumer, which would require a diversity of tooth shapes that are appropriate for puncturing and cutting tissue of teleosts, elasmobranchs, and marine mammals. If these species attempted to capture and process atypical prey items (e.g., the consumption of hard-shelled prey by blacktips), however, it is expected that prey-processing would require longer durations and be more energetically expensive due to an unsuitable tooth morphology. To support these hypotheses, further functional testing must be conducted.

Prey handling efficiency is influenced by morphology of the feeding apparatus, which can dictate the type or size of prey that are selected (Emerson et al., 1994; Hampton, 2018; Hoyle & Keast, 1988; Werner, 1977). In sharks that use lateral head-shaking to process prey, the shape of teeth at the lateral and posterior regions along the jaws may substantially impact the cutting efficiency of functionally difficult tissue. Notched blades can greatly increase the cutting efficiency (up to 50%) through compliant material by concentrating the stress on the tissue at the base of that notch, which causes it to fracture (Abler, 1992; Anderson & LaBarbera, 2008). This results in less wasted energy and therefore decreases the level of stress needed for material fracture. Additionally, the cutting efficiency of a notched blade increases as the angle becomes more acute (Anderson & LaBarbera, 2008). A common pattern in many carcharhiniform sharks is a decrease in the notch angle from anterior to posterior along the tooth row (i.e., the angle becomes more acute), which would confer increased efficiency during draw at the lateral and posterior positions compared to the anterior positions. Therefore, anterior teeth are more suitable for initial prey capture whereas lateral and posterior teeth are advantageous for processing large prey.

Constraints related to prey handling efficiency may be strongest at smaller size classes. This is because smaller sharks are restricted by both gape and bite force, which limits their ability to puncture or fracture the prey item (Hernandez & Motta, 1997; Mara et al., 2010; Verwaijen, Van Damme, & Herrel, 2002; Wainwright, 1988). Although teeth from the upper jaw of young bull sharks would require more force to puncture the integument of a teleost fish compared to young blacktips (Whitenack & Motta, 2010), bite force in bull sharks is greater on average for all overlapping body lengths (Habegger et al., 2012; Huber, Weggelaar, & Motta, 2006). Therefore, the increased force required by bull sharks to puncture the same prey item as black tips is not expected to constrain their ability to capture and process prey. Additionally, young bull and black tip sharks may be limited in

their ability to puncture the integument of some teleost fishes (e.g., ladyfish *Elops saurus*) due to deformation of these compliant prey (Whitenack & Motta, 2010). This occurs when the deformation of prey tissue exceeds crown height of these small shark size classes, thereby preventing puncture of the integument (Whitenack & Motta, 2010). As bonnethead sharks have a smaller gape and lower bite force compared to the other two species, they are likely constrained by the size of their hard-shelled prey. This may particularly limit young conspecifics to smaller prey since the force required to fracture the shell of its primary prey item (blue crab *Callinectes sapidus*) increases with crab carapace length (Mara et al., 2010).

Interspecific comparisons by tooth position found species-specific patterns in morphology, which often differed by relative crown height and notch angle. This was most apparent in four of the six positions (AntUp, LatLow, LatUp, PostLow), in which all species significantly differed from one another. However, teeth from the AntLow and PostUp positions in bull and blacktip sharks were morphologically equivalent. If morphology does confer a particular function (or a difference in prey handling efficiency), the teeth in each of these species may reflect the functional properties of their prey and the mode of prey processing necessary for consumption. However, testing this hypothesis was beyond the scope of this study.

Based on observations of feeding behavior and diet in these species, inferences can be made regarding tooth function. The diet of adult bonnet head sharks consists almost entirely of portunid crabs (Bethea et al., 2007; Cortés, Manire, & Hueter, 1996; Plumlee & Wells, 2016), whose exoskeleton requires greater force to fracture than the integument of teleost fishes (Mara et al., 2010; Whitenack & Motta, 2010). Bonnet head teeth at the anterior region of the jaws have short relative crown heights compared to bull and black tip sharks, in addition to molariform teeth along the posterior margin of the jaws. Teeth from the AntLow position appear to match the ideal morphology to fracture hard-shelled prey, which was suggested by functional testing on snail shells (Crofts & Summers, 2014). This implies that the cusped AntLow teeth of bonnetheads are also suitable to crush crabs in addition to the posterior molariform teeth, although lower bite force at the anterior teeth may impose a constraint (Mara et al., 2010). However, large crabs are not always crushed before consumption. Bonnetheads often use lateral head-shaking to remove the legs of their prey before swallowing them whole (Myrberg & Gruber, 1974; Wilga & Motta, 2000). Large black tip size classes prey on small to medium-sized teleost fishes and a lower proportion of small elasmobranchs (Castro, 1996). Following the initial capture of small or medium-sized prey, black tip sharks typically readjust their grasp on the prey or may swallow it immediately (Frazzetta & Prange, 1987). The size and material properties of these soft-bodied prey items appears to only necessitate a secure grasp before consumption. Therefore, the gracile teeth located toward the anterior region of the jaws (AntLow, AntUp, LatLow) are suitable to capture elusive fishes. For the occasional large prey item, such as elasmobranchs, a slight decrease in notch angle from anterior to posterior along the tooth row may facilitate greater cutting efficiency prior to consumption. Compared to blacktips, common prey items of large bull sharks are difficult to process and may exceed maximum gape (Habegger et al., 2012; Werry, Lee, Otway, Hu, & Sumpton, 2011). Large base crown widths of teeth from the

upper jaw likely resist high lateral forces (Williams, 2001), which typically occur during head-shaking behavior. The presence of serrations and acute notch angles at the lateral and posterior positions are thought to increase cutting efficiency of compliant material (Abler, 1992; Anderson & LaBarbera, 2008). This is important for large bull sharks since they consume greater proportions of elasmobranchs, marine mammals, birds, and large teleost fishes compared to smaller conspecifics (Cliff & Dudley, 1991; Heithaus, 2001; Werry et al., 2011). Although the material properties of large bull shark prey have not yet been tested, it is expected that they are more difficult to process than small-bodied teleosts due to the presence of larger skeletal elements and an integument that requires greater force to puncture (Currey, 1987; Habegger et al., 2012; Horton & Summers, 2009; Whitenack & Motta, 2010). As characterized in each of these species, it appears that the interaction of prey processing behavior and material properties of the prey item is reflected by the collective morphology at all tooth positions.

While this study focuses on the tooth morphology of extant sharks, these methods and findings can be used to guide paleoichthyological studies and to test functional hypotheses in extant and extinct fishes. We recommend the use of EFA to evaluate the morphology of elasmobranch teeth, which may benefit from the fusion of traditional morphometrics (linear measurements) to quantitatively describe any significant differences among tooth outlines (sensu Ginter et al., 2012). Additionally, we suggest a cautious approach to the identification of isolated fossil elasmobranch teeth due to the intrinsic variables (sex, age, position in jaws) that may contribute to morphological differences within a single species. We suggest that future functional testing of shark teeth include the measurement of performance of a given morphology *in situ* using the upper and lower jaws to perform dynamic movements as used by the species of interest. This approach is likely to address any possible discrepancies in proposed function while maintaining the natural arrangement of teeth, which could be obscured by the analysis of an individual tooth (tooth vs. teeth). Prey handling efficiency could also be evaluated for a variety of prey types by measuring the duration required to process a prey item, as well as to measure the concomitant energy expenditure. Furthermore, this study proposes that there may be different levels of heterodonty within elasmobranchs. Future studies should evaluate other species with varying levels of heterodonty to discern whether there is a relationship between the extent of heterodonty and the properties of the primary prey that are consumed.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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