

Ontogenetic changes in the tooth morphology of bull sharks (*Carcharhinus leucas*)

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Abstract

Teeth are an integral component of feeding ecology, with a clear link between tooth morphology and diet, as without suitable dentition prey cannot be captured nor broken down for consumption. Bull sharks, *Carcharhinus leucas*, undergo an ontogenetic niche shift from freshwater to marine habitats, which raises the question: does tooth morphology change with ontogeny? Tooth shape, surface area and thickness were measured using both morphometrics and elliptic Fourier analysis to determine if morphology varied with position in the jaw and if there was an ontogenetic change concordant with this niche shift. Significant ontogenetic differences in tooth morphology as a function of position in the jaw and shark total length were found, with upper and lower jaws of bull sharks presenting two different tooth morphologies. Tooth shape and thickness fell into two groupings, anterior and posterior, in both the upper and lower jaws. Tooth surface area, however, indicated three groupings, mesial, intermediate and distal, in both the upper and lower jaws. While tooth morphology changed significantly with size, showing an inflection at sharks of 135 cm total length, each morphological aspect retained the same tooth groupings throughout. These ontogenetic differences in tooth morphologies reflect tooth strength, prey handling and heterodonty.

KEYWORDS

dentition, elasmobranchs, foraging ecology, jaws, ontogenetic shifts

1 | INTRODUCTION

From embryonic formation through to maturity, many species undergo distinct developmental changes in anatomy, behaviour and physiology (French *et al.*, 2017; Habegger *et al.*, 2011; Olson, 1996). Distinct developmental events that occur through the course of an organism's life are called ontogenetic shifts and are not uniform across

populations or species, as they are derived from individual rates of development (French *et al.*, 2017; Matich & Heithaus, 2015; Turner Tomaszewicz *et al.*, 2017). One example of an ontogenetic shift related to the overall growth and foraging ability of individual organisms is the bite force of sharks, which may be responsible for correlations between animal size and niche divergence (French *et al.*, 2017; Grubbs, 2010; Matich & Heithaus, 2015).

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For many marine predators, including sharks, prey capture and subsequent consumption are explicitly related to the mouth and its associated structures. Gape, bite strength and the structure of teeth are critical to the biomechanics of seizing prey, retaining it and potentially dismembering it (Cullen & Marshall, 2019; Ferrara *et al.*, 2011). Gape imposes limitations on suction and ram feeding predators as they engulf an entire food item, while species that use biting as a feeding mode can target organisms larger than their gape as they tend to remove smaller digestible units one at a time (Ferry *et al.*, 2015; Luczkovich *et al.*, 1995; Ward-Campbell & Beamish, 2005; Wilga *et al.*, 2007). Throughout the life of predatory fishes, ontogenetic modifications in gape and dentition may occur that enable dietary shifts and selection of different prey (Ward-Campbell & Beamish, 2005). Tooth morphology can provide insights into a species' diet composition based on structure, *e.g.*, highly molariform teeth are better suited to consuming 'hard' prey like crustaceans, while cuspid teeth are better suited to consuming 'soft' items such as cephalopods [Corn *et al.*, 2016; Ferrara *et al.*, 2011; Powter *et al.*, 2010, but see also Whitenack and Motta (2010), who found little evidence to support tooth morphology as a suitable predictor for biological roles in the 10 shark species they examined].

Many species of sharks are considered top order predators and capable of exploiting a diverse range of prey. Ontogenetic shifts in habitat use often correlate to ontogenetic dietary or trophic shifts, and these correlations are only just being understood thanks to recent advances in tracking technology (Hussey *et al.*, 2015). For example, the effect of habitat change on ontogenetic dietary shifts has been shown to be the proximate explanatory variable in blue stingrays, *Dasyatis chrysonota* Smith 1828 (Ebert & Cowley, 2003), albeit animal size may influence their ability to inhabit different habitats and so be a primary driver of elasmobranch ontogenetic dietary shifts. Habitat use is linked to body size and is a function of the mortality-resource trade-off, *i.e.*, maximizing predator evasion enables development and growth to a maximum allowable size that an environment can support before requiring transition to a new habitat with higher quality resources (Grubbs, 2010; Matich & Heithaus, 2015; Ramirez *et al.*, 2017).

Biting is an important feeding mode for many predator species and tooth shape plays an important ecological role for these animals once it facilitates prey acquisition (Cullen & Marshall, 2019). There is usually a positive relationship between shark size and trophic position, with morphological changes such as increases in body size enabling capture and handling of a greater diversity and size of prey species (Fu *et al.*, 2016; Grubbs, 2010; Lowe *et al.*, 1996). For example, the diet of juvenile sandbar sharks *Carcharhinus plumbeus* Nardo 1827 is mainly crustaceans and small teleosts, whereas the adult's diet is dominated by cephalopods, teleosts and other elasmobranchs (Grubbs, 2010; McElroy *et al.*, 2006). Similarly, the white shark *Carcharodon carcharias* Linnaeus 1758 exhibits a paired ontogenetic shift between diet and dentition, transitioning from a primarily piscivorous diet as juveniles to a diet that includes marine mammals as adults (Grainger *et al.*, 2020), with the latter only possible after a change in

dentition (French *et al.*, 2017; Grubbs, 2010; Wroe *et al.*, 2008). Juvenile *C. carcharias* have cuspid teeth (Bemis *et al.*, 2015) which allow them to pierce and hold flesh, while the broader and more serrated teeth of adults enable the gouging of chunks of flesh (Ferrara *et al.*, 2011; French *et al.*, 2017; Wilga & Ferry, 2015). Teeth are not the only ontogenetic change in the feeding apparatus of *C. carcharias*, as there is also structural reinforcement of the jaw that results from additional mineralization (Ferrara *et al.*, 2011; French *et al.*, 2017; Wroe *et al.*, 2008).

The bull shark *Carcharhinus leucas* Valenciennes, Müller & Henle 1839 is a versatile bite-feeding predator capable of inhabiting both freshwater and marine environments in tropical and temperate regions worldwide and exploiting diverse resources (Espinoza *et al.*, 2021; French *et al.*, 2017; Heupel *et al.*, 2015; Lee *et al.*, 2019; Livernois *et al.*, 2021; Niella *et al.*, 2017, 2021; Smoothey *et al.*, 2016, 2019; Thorburn & Rowland, 2008; Werry *et al.*, 2011). Juvenile *C. leucas* use estuaries as nursery areas (Heupel & Simpfendorfer, 2008; Matich & Heithaus, 2015) and while adults are considered apex predators, juveniles are not. The juveniles' lack of size, speed and experience makes them vulnerable to predation (Fu *et al.*, 2016; Matich & Heithaus, 2015; Thorburn & Rowland, 2008). In seeking low-risk environments, juvenile *C. leucas* spend significant amounts of time (*i.e.*, up to 4 years) in rivers and estuaries before transitioning to the marine environment (Pillans *et al.*, 2005; Thorburn & Rowland, 2008). This size-based segregation in space use consists of larger animals using open marine environments to follow resources (Espinoza *et al.*, 2021) and habitats with less competition, while the smallest individuals make use of environments with lower associated risk (Heupel *et al.*, 2015; Heupel & Simpfendorfer, 2008).

Bite force is a function of body size, particularly with respect to a wider head enabling an increase in the mass and cross-sectional area of jaw muscles (Habegger *et al.*, 2011; Irschick & Hammerschlag, 2015; Kolmann *et al.*, 2015). Young *C. leucas* bite force changes at a positively allometric rate and tapers to isometry with age (Habegger *et al.*, 2011; Kolmann & Huber, 2009). There is also an ontogenetic shift in *C. leucas* diet (Lowe *et al.*, 1996; Niella *et al.*, 2021; Werry *et al.*, 2011), so there may be an expectation that bite force would increase to compensate for increasingly complex or functionally difficult prey (Habegger *et al.*, 2011).

Power and jaw strength are not the only components to potentially require an ontogenetic change to enable prey shifts in macrophagous bite-feeding species. Teeth are also important to enable successful predation. *C. leucas* are considered to exhibit distinct tooth shapes between the upper and lower jaws (Habegger *et al.*, 2011; Wilga & Ferry, 2015). Upper jaw teeth are broad triangular, linguolabially flattened with serrations and have overlapping bases, while lower jaw teeth are tall and narrow cusped (Frazzetta, 1988; Whitenack & Motta, 2010). These characteristics impute the upper jaw as cutting teeth and lower jaw as tearing teeth for removal and penetrating of flesh, respectively (Frazzetta, 1988; Whitenack & Motta, 2010; Wilga & Ferry, 2015). The upper and lower jaws work in concert to clasp down on prey. The upper jaw is extended from the head, reducing time to contact and engage prey with teeth, whilst the

lower jaw closes and its teeth penetrate the body (Ferrara *et al.*, 2011; Whitenack & Motta, 2010). For many sharks, including *C. leucas*, inward pointing sharp teeth and a high bite force combined with lateral head shaking increase prey handling efficiency by reducing the chance of escape and the number of bites needed to separate flesh (Bergman *et al.*, 2017; Hocking *et al.*, 2017). The different tooth morphologies on the upper and lower jaws likely indicate a wide dietary breadth, and this nonspecialized feeding structure is consistent with the known generalist predator status of *C. leucas* (Estupiñán-Montaño *et al.*, 2017; Matich *et al.*, 2011).

The mineralization and strength characteristics of shark teeth have been shown to differ between taxonomic groups (Jambura *et al.*, 2018; Moyer & Bemis, 2017). Serrations provide an important contributor to enabling effective distribution of the stressors affiliated with biting through prey of different hardness, including absorption of forces generated by the lateral head shaking common in carcharhinids during prey processing (Moyer & Bemis, 2017), including in *C. leucas*. Carcharhiniform teeth have previously been described as orthodont teeth (Jambura *et al.*, 2018), including a triple layer enameloid crown which provides tooth strength (Moyer & Bemis, 2017). However, ontogenetic changes in tooth shape which may facilitate prey consumption have not previously been described for all teeth in the jaw. This study subsequently investigates whether the niche change that *C. leucas* are known to undergo is facilitated by an ontogenetic change in dentition. Ontogenetic shifts in *C. leucas* dentition have been proposed by Cullen and Marshall (2019), who selected representative teeth to sample from *C. leucas* jaws to identify ontogenetic changes in morphology, however, they did not identify where in the lifecycle this shift occurs nor any relationship to diet. Here, we examined all teeth across all jaw positions and life development stages (*i.e.*, neonate, juvenile and adult) to assess where morphological alterations occur and their potential implications for *C. leucas* foraging ecology. We hypothesise that changes in the dental morphology of *C. leucas* will precede niche change, thereby allowing these animals to exploit new resources.

2 | MATERIALS AND METHODS

2.1 | Sample collection

All animals used in this study were caught on commercial fishing gear with carcasses sold for human consumption in the case of juvenile sharks, whilst adults comprised individuals retrieved dead from the New South Wales bather protection program using large mesh gillnets (Reid *et al.*, 2011). Neonate ($n = 8$) and juvenile *C. leucas* ($n = 17$) were collected between June 2010 and July 2011 from the estuary general commercial fishery landing *C. leucas*, captured using setlines or gillnets in the Clarence River (29.42°S, 153.34°W), New South Wales (NSW), Australia. Adult *C. leucas* ($n = 9$) were caught in NSW bather protection nets between March 1999 and February 2020. No animals were killed directly for this research and their collection was covered through the NSW Animal Care and Ethics Committee permit number

TABLE 1 Sizes of *Carcharhinus leucas* sampled per age class, including sample size (n), mean, standard deviation and range (minimum–maximum) for total length (L_T)

	L_T range (cm)	n	L_T (cm)
Neonate	45–85	8	81.2 ± 3.7 (72.5–85)
Juvenile	86–160	17	114 ± 18 (87–150)
Subadult	161–200	0	N/A
Adult	>201	9	247.4 ± 33.6 (211–310)

Note. N/A, not available.

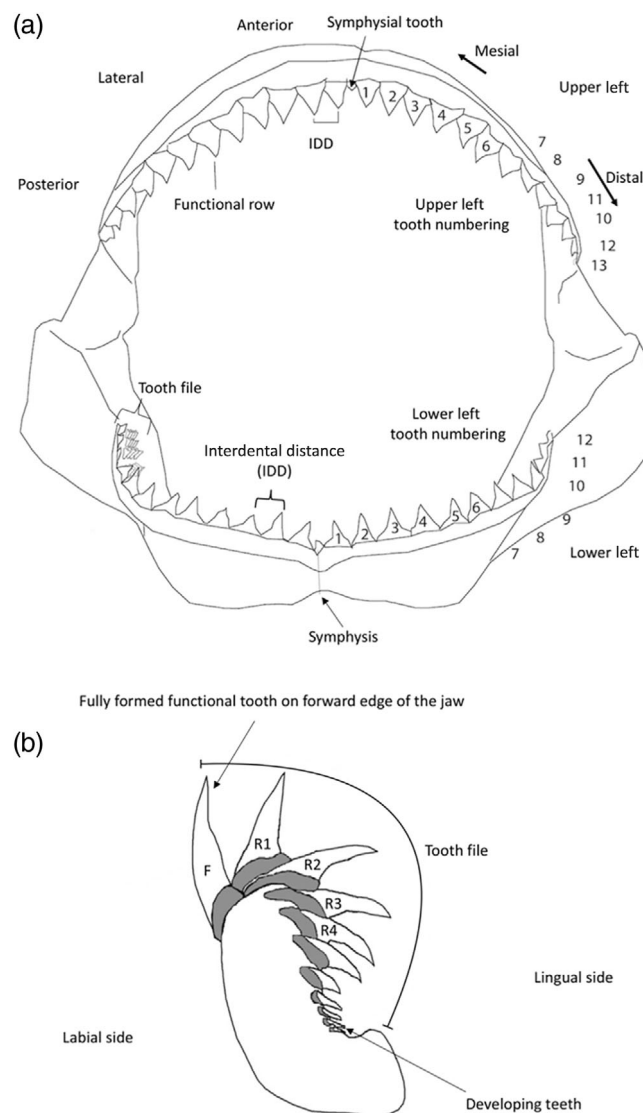


FIGURE 1 (a) Jaw structure including jaw layout of *Carcharhinus leucas*, tooth numbering, functional row and tooth file. (b) Cross-section of the lower jaw showing the teeth measured at the leading edge (F = functional tooth/row) and the conveyor belt of replacement teeth (R1, R2, R3 etc.) that comprise the tooth file visible in the jaw

08/06. All individuals were measured and separated into age classes according to total length (L_T) (Table 1) and kept frozen at -18°C until processing.

Samples were thawed and the jaws were excised with soft material sufficiently cleaned to expose the teeth. Jaws were left to air-dry for data collection. The focus was on the teeth which comprise the first (*i.e.*, functional) row of teeth from the upper and lower jaws (Figure 1), but if teeth in the functional row were damaged or missing, the corresponding replacement tooth next in the series of the tooth file was used (Figures 1 and 2a). An identification system was adopted to ensure on removal of teeth that they were accurately labelled and stored, and the jaw was divided into upper and lower hemispheres, and subdivided into left and right, *e.g.*, the upper right jaw = UR. The teeth were provided with a numbering scheme adapted from Moyer *et al.* (2015), beginning with the first tooth immediately adjacent to the symphysis (tooth series one) progressing to the last tooth series in said quarter, *e.g.*, UR1 through to UR13. In this study when a functional tooth proved inadequate for use only the first replacement tooth (R1 in Figure 1b) was ever necessary to be used from a given tooth file. A further subdivision could be made into defining functional (F) or replacement (R) teeth use from a specific tooth file, *e.g.*, UR1F or UR1R1, but this was not defined in this study. Numerous rows of replacement teeth reside in *C. leucas* jaws, where continual functional tooth shedding and eruption occurs at a frequent rate, with turnover rates between days or months (Bergman *et al.*, 2017; Whitenack & Motta, 2010). *C. plumbeus* displays fast tooth turnover rates, young individuals show an 18 day cycle while mature individuals show a 36 day cycle (Luer *et al.*, 1990). Given this, we assume that a given functional tooth is equivalent to the next immediate tooth in the file, *i.e.*, replacement tooth one, due to fast expected turnover rates in *C. leucas*.

2.2 | Tooth morphology

2.2.1 | Traditional morphometrics

A series of 11 distinct measurements were used to describe tooth shape, including (i) notch angle, (ii) root height, (iii) crown height, (iv) root width, (v) base, (vi) medial, (vii) apex (Figure 2b), (viii) width at the base, (ix) width at the medial, (x) width at the apex (Figure 2c) and (xi) tooth surface area. Notch angle and tooth surface area were calculated with open-source ImageJ (version 2.0.0-rc-43/1.52n) image processing software, while the remaining measurements were taken using a digital vernier calliper (0.01 mm accuracy).

2.2.2 | Elliptic Fourier analysis

An alternative approach to obtain tooth shape data is elliptic Fourier analysis (EFA), which uses a nearest pixel approach to automatically obtain scaled coordinates of an object, thereby reducing bias originating from intra-observer variation in traditional methods (Cullen & Marshall, 2019). This approach generates a series of harmonics (trigonometric curves) for the object being analysed, each consisting of four coefficients, *i.e.*, respectively two *x* and *y* components, which detail its

shape, size and orientation. A greater number of harmonics results in higher proportions of the object shape to be described, with seven harmonics being required to describe 99.9% of the variation in tooth shape (Cullen & Marshall, 2019). The EFA was performed using the Momocs package (Bonhomme *et al.*, 2014) in the R statistical environment (version 3.5.3; R Core Team, 2020) where all tooth outlines were centred and scaled, removing the effect of object size to make inter-class comparisons. See Cullen and Marshall (2019) for a full description.

Photographs of all teeth were taken from the labial side of the tooth to be used in the EFA. Due to the small size of teeth from neonates and juveniles <130 cm L_T , the entire suite of upper and lower jaw teeth were removed from the jaws for photographing. For juveniles >130 cm L_T and adults, all tooth photographs were made *in situ* as these jaws are used in shark attack forensic analyses (Clua & Reid, 2017) and cannot be destroyed. Teeth from sharks <130 cm L_T were photographed with a MicroPublisher 5.0 RTV digital camera mounted to an Olympus SZ61 stereomicroscope set to 0.67 \times zoom. Teeth from sharks >130 cm L_T were photographed with an Apple iPhone 7 (version 13.5.1) which was placed in a jig to reduce parallax error. As a standardization procedure to account for the differences in camera resolution between the two devices used, all photos were edited using Adobe Photoshop 2020 (version 21.2.0) to create black and white silhouettes of each tooth with 10 \times 10 cm and 300 dots per inch (DPI) prior to the EFA. As the gums of sharks >130 cm L_T obscured the full extent of the tooth root this structure was not included in silhouette creation for the EFA.

2.3 | Statistical analysis

2.3.1 | Method quality control

To ensure the accuracy of the physical measurements, traditional morphometric measurements were repeated twice for six teeth on each of the upper and lower jaws, evenly distributed on each of the right and left sides of nine sets of shark jaws (83–118 cm L_T). The teeth included in this preliminary analysis from the upper jaw hemisphere were at positions 1, 7 and 10 from both the left and right sides, while the lower jaw hemisphere teeth were at positions 1, 6 and 9 from the left and right sides. A paired *t*-test was used to determine if there was a significant difference among rounds of measurements.

The dental formula $\frac{13-1-13}{12-1-12}$ was observed in these east Australian *C. leucas*, as reported by Bass *et al.* (1973) for southern African animals. To confirm protocols in running the EFA, a preliminary EFA was done using all 50 functional teeth in each jaw of one juvenile and one adult shark. Symphyseal teeth were not included as functional teeth. Each of these teeth were photographed four times and the EFA repeated, and the output was then prepared for a discrepancy analysis. Since a Shapiro–Wilk test confirmed the data to be normally distributed (*P* value <0.05), an analysis of variance (ANOVA) was done on the EFA harmonic coefficients to determine if there was any

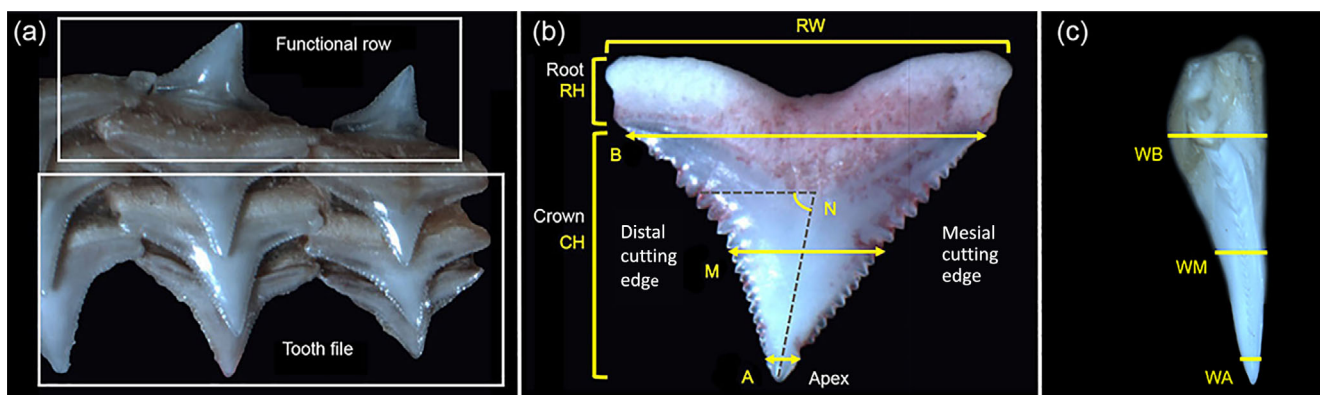


FIGURE 2 (a) Lingual view of *Carcharhinus leucas* teeth showing the functional row and tooth file to be sampled in the case of a missing or damaged functional row tooth. (b) Frontal view showing traditional tooth landmarks (white) (Moyer & Bemis, 2017), together with the measurements taken in the present study (yellow), adapted from Becker *et al.* (2000), Whitenack and Motta (2010), Ebert and Stehmann (2013) and French *et al.* (2017), including notch angle (N), root height (RH), crown height (CH), root width (RW), base (B), medial (M), half-way from the CH and apex (A), at the apex tip. (c) Sagittal view showing the width at the base (WB), width at the medial (WM) and width at the apex (WA). Images captured on an Olympus SZ61 stereomicroscope

significant difference in the outputs from the different rounds of photographs.

2.3.2 | Comparative analysis and morphology

Two principal component analyses (PCA) were performed, one including the morphometric measurements and the other using all the EFA harmonic coefficients. This step was done to remove noise from the multiple variables in each approach and standardize the values among all sharks sampled in relation to the biological factors possibly influencing tooth morphology, *i.e.*, shark total length and tooth number (as a proxy of jaw position). A generalized linear model (GLM) approach was then used with the PCA scores as the response variables to test for significant differences in tooth shape along the jaw position and across the size range of sharks. The potential explanatory variables tested included shark size (L_T), and the interaction between jaw position (*e.g.*, upper right) and tooth number (*i.e.*, 1–13), with Gaussian families of error distribution. The data for surface area and thickness at tooth base (hereafter referred to as thickness) were log transformed to fit a normal distribution. Similarly, for tooth shape (*i.e.*, EFA), GLMs were used to investigate the presence of allometric trends in tooth size. The PCA and GLM were run with the base R stats package (R Core Team, 2020). The generalized additive mixed model (GAMM) was run with the mcgv package (Wood, 2011) and used to assess the variation of tooth structure surface area and thickness within the jaw of *C. leucas*. The physical measurements were log transformed to meet normality and included as the response variables, with the explanatory variables also comprising L_T and an interaction between jaw position and tooth number. The shark identification number was included in the GAMM as a random effect to account for possible interindividual differences in tooth morphology. The inclusion of additional explanatory variables followed a stepwise variable selection procedure performed with the qpcR package in R (Spiess, 2018).

Starting from the null model, significant variables were progressively added to a previous nested model according to lower Akaike information criterion (AIC) and confirmed after a significant ANOVA. Final models were chosen based on higher AIC weights (Wagenmakers & Farrell, 2004) and visually inspected for a normal residual distribution.

To test for bilateral symmetry of tooth morphology in *C. leucas* jaws, an ANOVA with a *post hoc* Tukey's test was performed on each log-transformed morphological aspect. Because of the effect of extraneous comparison and loss of statistical power, representative teeth from each jaw position (upper left, upper right, lower left and lower right) were compared using an ANOVA to test for symmetry in tooth morphology in relation to the variables L_T and position in the jaw. These representative teeth of *C. leucas* dentition were selected based on the significant shape differences found in the EFA and comprised two teeth per identified tooth category selected from each jaw position, *e.g.*, anterior category = teeth 1 and 4, posterior category = teeth 8 and 11. A *post hoc* Tukey test was then used to investigate for possible significant differences between the left and right sides of the upper and lower jaws. Significance levels were set at $P < 0.05$ for all analyses.

3 | RESULTS

3.1 | Preliminary analysis and method selection

A total of 34 *C. leucas* (minimum = 72.5 cm, maximum = 310 cm, mean \pm s.d. = 141.26 ± 69.21 cm L_T) were analysed (Table 1). There were no significant differences in morphometric measurements of tooth thickness ($P = 0.253$) or among the EFA harmonic coefficients produced during the comparison of multiple photographs on the same individuals (Supporting Information Table S1). The EFA approach exhibited a lower AIC (22,794.86) than the morphometric analysis (25,599.35) and therefore was selected to further investigate changes

in tooth morphology through shark ontogeny and by position in the jaw (Supporting Information Tables S2–S4).

3.2 | Bilateral symmetry

Eleven of the 34 *C. leucas* in this study had tooth counts within the range in dental formula $\frac{13-1-13}{12-1-12}$ reported by Bass *et al.* (1973). Four were missing the 13th tooth file on both upper left and right sides, four were missing the 13th tooth file on either upper left or right sides, one was missing the 12th tooth file in the lower left jaw, and two had an additional (13th) tooth file in either the lower left or lower right sides of the jaw.

Significant modelled differences in tooth morphology as a function of jaw position in *C. leucas* were found (Table 2), but Tukey's *post hoc* analyses revealed that significant differences associated with jaw position (*i.e.*, left and right jaws) are not present within upper and lower jaws (Supporting Information Table S5), thus indicating the presence of bilateral symmetry in tooth morphology.

3.3 | Tooth shape

The PCA demonstrated that 63.8% of the total variation was explained by the first two principal component scores, PC1 = 44.29% and PC2 = 19.51% (Figure 3). The separation in shape between the lower and upper jaws occurred mostly in the vertical plane with left and right sides showing considerable similarity for both jaw positions, influenced mostly by the a_0 and c_0 EFA coefficients (Figure 3), which represent the x and y coordinates, respectively, in relation to the centroid position, *i.e.*, a_n and b_n provide a_0 , while c_n and d_n provide c_0 (Caple *et al.*, 2017; Claude, 2008). The remaining EFA coefficients were mostly responsible for the horizontal variation in the PCA scores with the greatest differences driven by c_n (Figure 3), which constitutes part of the y axis shape configuration (Claude, 2008).

Significant differences in tooth shape were identified as a function of L_T and jaw position (Table 3). A significant difference in *C. leucas* tooth shape was observed for individuals smaller and larger than 135 cm L_T (Figure 4, and Supporting Information Figures S1 and S2). The upper and lower jaws showed two main different tooth shapes, with teeth 1–6 significantly different to 7–13 across all jaw positions (Figures 5 and 6). In contrast with the consistent changes in tooth shape as a function of body growth throughout all jaw positions (Figure 4a), shape alterations were much more pronounced in the lower than in the upper *C. leucas* jaw (Figure 5).

3.4 | Tooth size

Size of tooth was found to significantly differ as a function of total length and position in the jaw (Tables 4 and 5). Tooth surface area and base thickness were significantly different for individuals shorter or

TABLE 2 Analysis of variance assessing the effect of jaw position on the tooth morphology of *Carcharhinus leucas*

	Variable	d.f.	F value	P value
Shape	Jaw position	3	178.12	<0.001
Surface area	Jaw position	3	66.17	<0.001
Thickness at tooth base	Jaw position	3	4.92	<0.003

Note. Representative teeth 1, 4, 8 and 11 were selected from each jaw position. d.f., DEGREES of freedom; F value and P value are included for each morphological aspect.

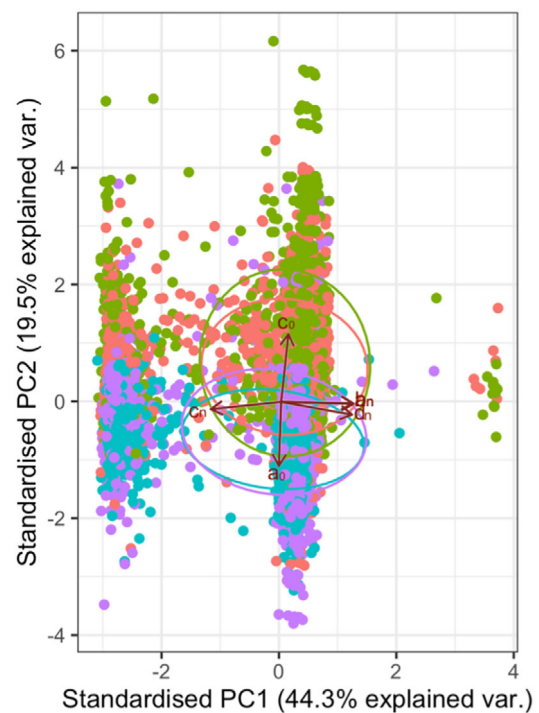


FIGURE 3 Principal component analysis of the elliptic Fourier analysis (EFA) coefficients describing *Carcharhinus leucas* tooth shape by jaw position, *i.e.*, LL (lower left), LR (lower right), UL (upper left) and UR (upper right). The loading labels represent the horizontal (a_n , b_n and a_0) and vertical (c_n , d_n and c_0) EFA coefficients while the coloured ellipses represent the distribution of each respective jaw position. Jaw position: (—●—) LL, (—●—) LR, (—●—) UL, (—●—) UR

TABLE 3 Generalized linear models of *Carcharhinus leucas* tooth shape, including the effects of total length and interactions between jaw position and tooth number

Variable	Est.	s.e.	t value	P value
Intercept	−7.76e-02	1.09e-02	−7.07	<0.001
Total length	−1.86e-04	5.42e-05	−3.42	<0.001
LL × Tooth.n	3.77e-02	1.38e-03	27.32	<0.001
LR × Tooth.n	4.40e-02	1.39e-03	31.61	<0.001
UL × Tooth.n	−8.37e-03	1.28e-03	−6.55	<0.001
UR × Tooth.n	−5.72e-03	1.29e-03	−4.44	<0.001

Note. Est., coefficient estimates; LL, lower left; LR, lower right; s.e., standard error; Tooth.n, tooth number; UL, upper left; UR, upper right. t values and P values are included for each variable.

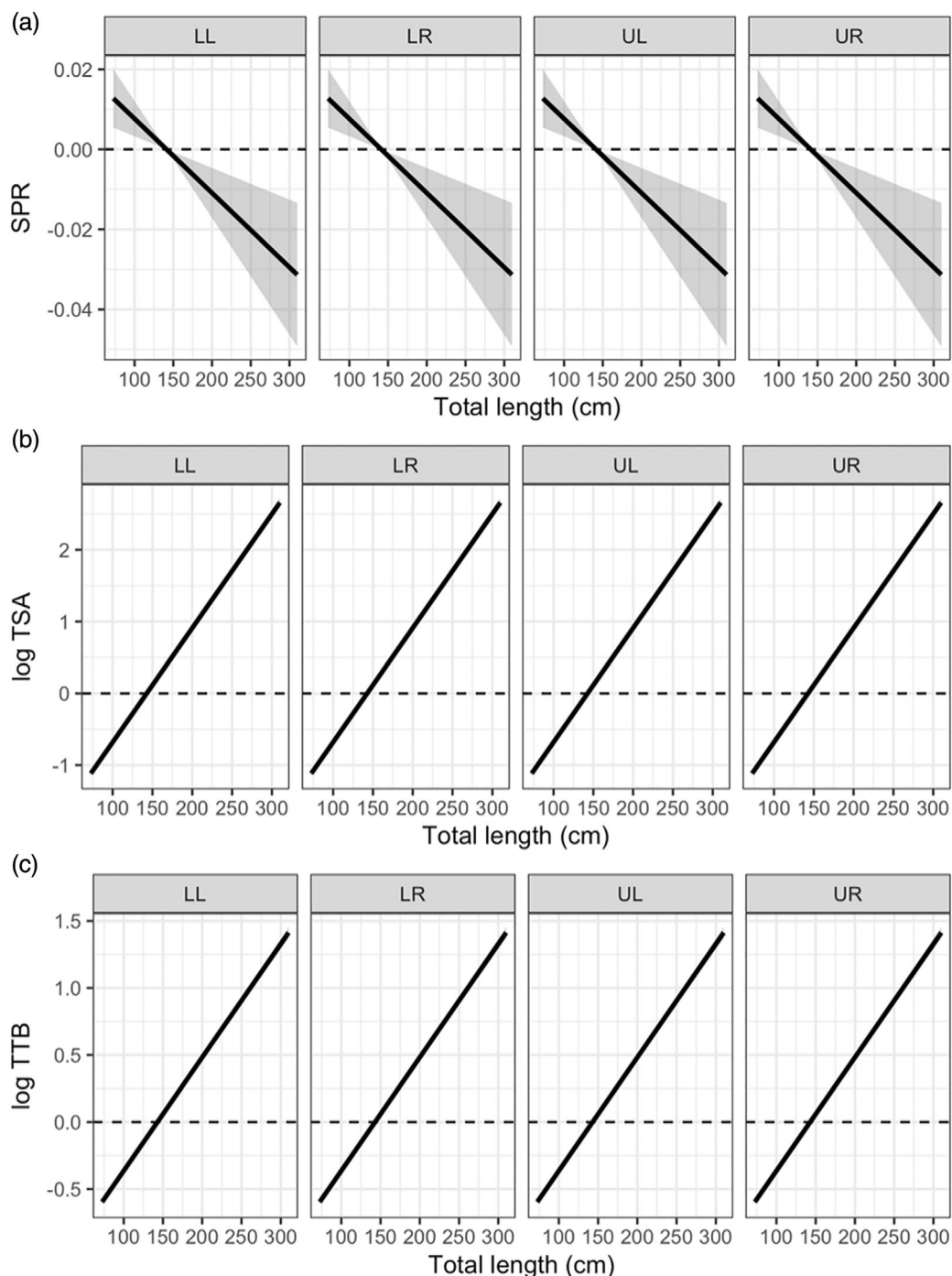


FIGURE 4 Generalized linear models of (a) the coefficients (SPR, standardized partial residuals) describing tooth shape, (b) tooth surface area (TSA) and (c) tooth thickness at the base (TTB), including the significant effects of *Carcharhinus leucas* total length (cm). Shaded bands and dashed lines indicate the 95% confidence intervals and null effects, respectively. LL, lower left; LR, lower right; UL, upper left; UR, upper right

longer than 135 cm L_T (Figure 4b,c). The changes observed in tooth size as a function of total length were consistent between tooth surface area and base thickness across all jaw positions (Figure 4b,c). Significant intra-individual variation was observed in the GAMMs (Table 5). Based on surface area, *C. leucas* teeth could be split into three categories along both upper and lower jaws, with the respective

left and right sides of upper and lower jaws mirroring each other in tooth groupings, but variation exists between the surface area classes representative of the upper and lower jaws (Figure 7a). The upper jaw was divided into mesial = teeth 1 and 2, intermediate = teeth 3–6 and distal = teeth 7–13, while the lower jaw divisions were mesial = tooth 1, intermediate = teeth 2–6 and distal = teeth 7–13

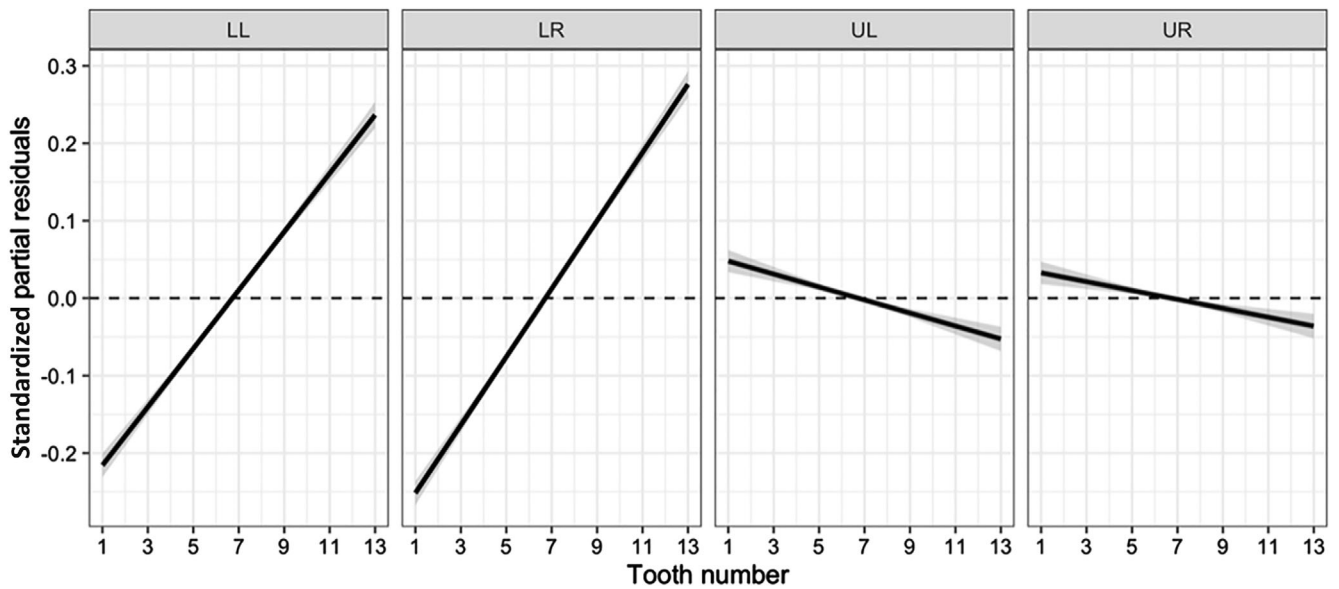


FIGURE 5 Generalized linear model of the coefficients describing *Carcharhinus leucas* tooth shape, including the significant effects of tooth number by jaw position, i.e., LL (lower left), LR (lower right), UL (upper left) and UR (upper right). Shaded bands and dashed lines indicate the 95% confidence intervals and null effects, respectively

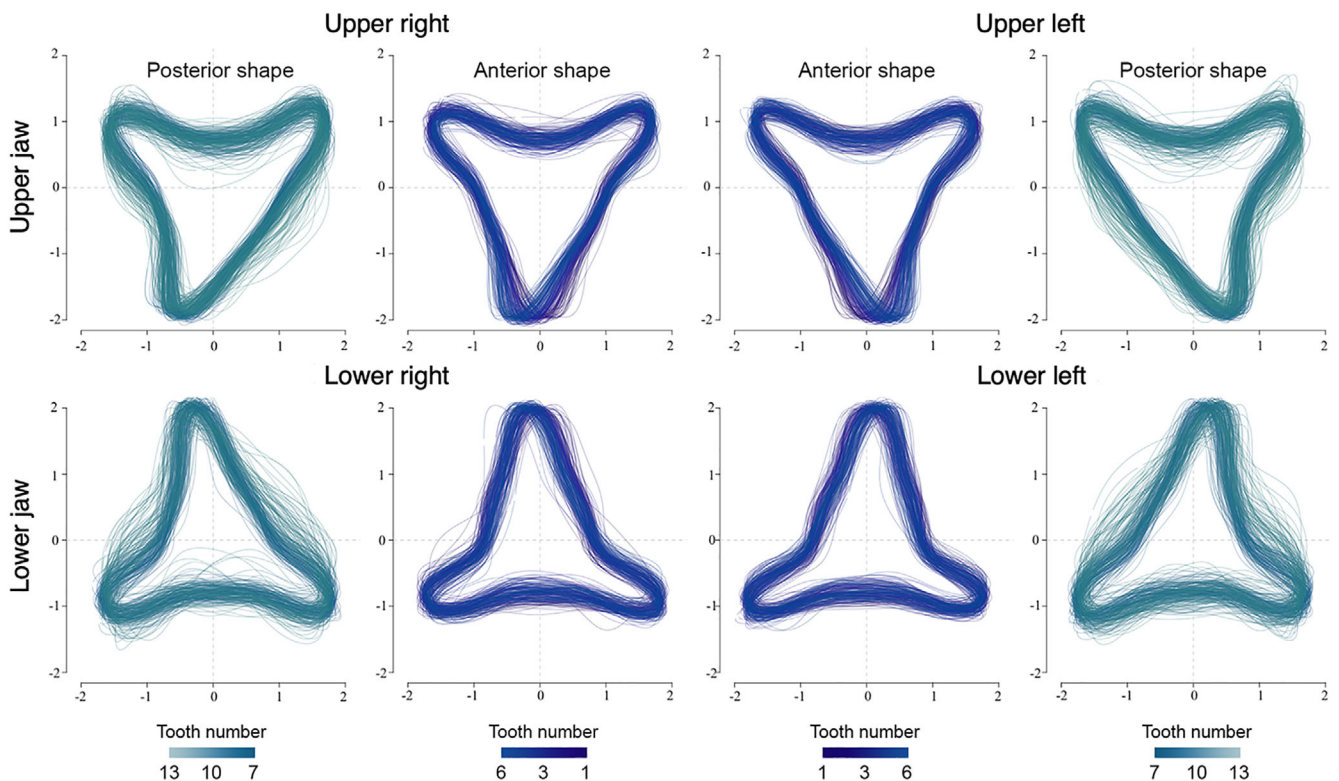


FIGURE 6 Individual raw centred and scaled outlines from all teeth ($N = 1689$ total tooth outlines) from *Carcharhinus leucas* jaws (all ontogenetic stages) obtained with elliptic Fourier analysis. Each outline corresponds to one shark tooth, and the display here shows the variation in morphology observed within the jaws (upper panels = upper jaws; lower panels = lower jaws) as identified in our models. Anterior shape (blue colour scale) = teeth 1 (darker) to 6 (lighter) and posterior shape (green colour scale) = teeth 7 (darker) to 13 (lighter)

(Figure 7a). Along each jaw, the teeth could be split into two groups based on thickness with teeth 1–6 identified significantly different to 7–13 (Figure 7b). The changes in tooth size (surface area and

thickness) as a function of tooth number (i.e., placement within the jaw were found to be consistent throughout the entire jaw (Figure 7).

TABLE 4 Generalized linear models of *Carcharhinus leucas* tooth size by morphometric aspect, including the effects of total length (L_T)

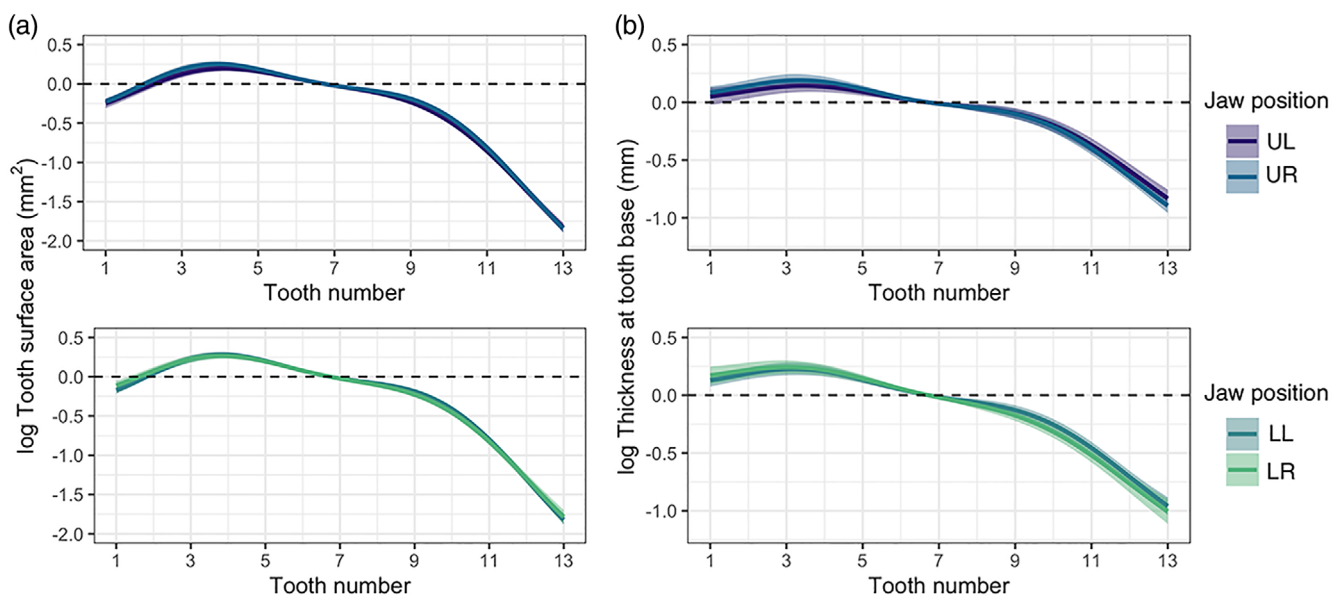
Morphometric aspect	Variable	Est.	s.e.	t value	P value
Surface area	Intercept	1.01	0.04	25.84	<0.001
	L_T	0.02	1.92e-4	82.84	<0.001
Thickness at tooth base	Intercept	-0.43	0.03	-16.46	<0.001
	L_T	0.01	1.29e-4	65.74	<0.001

Note. Est., coefficient estimates; s.e., standard error. t values and P values are included for each variable.

TABLE 5 Generalized additive mixed model of *Carcharhinus leucas* tooth size by morphometric aspect, including the effects of total length (L_T), individual (ID) and interaction between jaw position and tooth number

Morphometric aspect	Variable	E d.f.	Ref. d.f.	F	P value
Surface area	Tooth.n × position	8.89	8.99	1132.4	<0.001
	L_T	2.12	2.18	155.8	<0.001
	ID	29.57	31	55.2	<0.001
Thickness at tooth base	Tooth.n × position	8.78	8.99	458.88	<0.001
	L_T	2.12	2.12	67.56	<0.001
	ID	29.62	31	66.73	<0.001

Note. E d.f., effective degrees of freedom; LL, lower left; LR, lower right; Ref. d.f., reference degrees of freedom; Tooth.n, tooth number; UL, upper left; UR, upper right. F statistic and P values are included for each variable.

**FIGURE 7** Generalized additive mixed models of *Carcharhinus leucas* (a) tooth surface area and (b) tooth thickness at the base, including the significant interacting effects of tooth number by jaw position, i.e., LL (lower left), LR (lower right), UL (upper left) and UR (upper right). Shaded bands and dashed lines indicate the 95% confidence intervals and null effects, respectively. Jaw position: (—) UL, (—) UR, (—) LL, (—) LR

4 | DISCUSSION

This study is the first to examine the ontogenetic changes in the tooth morphology of *C. leucas* through the use and comparison of morphometrics and EFA to identify patterns of dentition and extrapolate ecological consequences to morphology. We discovered clear distinctions in tooth morphology between immature and mature size classes based on shark total length. Obtaining morphometrics data is generally labour intensive and time-consuming,

particularly when performed by human observers rather than electronic equipment such as photogrammetry or laser scanning. Considering the small size of neonate teeth (ranging from 0.43 to 3.98 mm in crown height) and the need to maintain accurate measurements, we opted for using an alternative approach, the EFA. EFA is a much cheaper alternative to obtain accurate measurements by removing interobserver bias (since it is automated) as it does not require the use of any hi-tech seldom-accessible equipment and can be performed using a mobile phone camera.

4.1 | Bilateral symmetry

Teeth within *C. leucas* jaws were bilaterally symmetrical for both upper and lower jaws, with EFA outlines identifying discernible anterior and posterior tooth shapes in all jaw hemispheres. The benefit of symmetrical feeding structures has been linked to sequestering and increased performance in oral manipulation of prey items (Gomes *et al.*, 2011; Thornhill & Sauer, 1992). This symmetrical distribution of multiple tooth morphs gives *C. leucas* a mechanical advantage enhanced by wide jaws, high bite force and headshaking behaviours during feeding, all of which increase bite efficiency, reduce handling time and prevent prey escape (Dean *et al.*, 2005; Lucifora *et al.*, 2009). Two adult sharks in our sample size had deformed teeth with torsions, which may be due to injuries to the dental lamina sustained while feeding on larger prey items and could have compromised feeding performance.

4.2 | Tooth morphology

4.2.1 | Ontogenetic heterodonty

East Australian *C. leucas* experienced an ontogenetic shift in tooth shape, corroborating previous reports from other regions (Cliff & Dudley, 1991; Cullen & Marshall, 2019). The teeth change in shape, surface area and thickness at ~ 135 cm L_T . Although the general classification of cutting teeth in the upper jaw and tearing teeth in the lower jaw remains constant through all life stages of *C. leucas*, there were differences in tooth shapes between young and mature sharks (Supporting Information Figures S1 and S2), extending the reported ontogenetic changes reported by Cullen and Marshall (2019), who determined significant differences in shape between young-of-the-year and juvenile age classes only. *C. leucas* teeth become less cuspidate and more robust with increasing shark length. The anterior teeth developed a lateral expansion of the mesial cutting edge with increased shark length, and the posterior teeth were not as slender and appeared to show an increase in notch angle, particularly towards the tooth apex (Supporting Information Figures S1 and S2). Broader teeth facilitate increased purchase during feeding events while curved teeth increase the length of tooth cutting edge, thereby increasing predatory efficiency (French *et al.*, 2017; Martin *et al.*, 2005). The inclusion of marine mammals into the diets of *C. carcharias* only occurs after their teeth become broader and less cuspidate through ontogeny (French *et al.*, 2017). Similarly, the *C. leucas* diet also changes through ontogeny, with larger individuals targeting elasmobranchs, marine mammals, large teleosts and sea turtles (Estupiñán-Montaño *et al.*, 2017; Habegger *et al.*, 2011; Niella *et al.*, 2021), which might be facilitated by the increases in tooth broadness described in our study.

Triangular and serrated teeth with a small edge radius are the characteristics responsible for disperse bite force and improve performance during prey processing (Moyer & Bemis, 2017). From an early age, the teeth of *C. leucas* have all these aspects and change little in overall shape throughout their life development (Figure 6). Jaw cartilage has been shown to ontogenetically increase in mineralization,

which may improve its durability and aid in absorbing impacts to prevent damage (Dingerkus *et al.*, 1991; Ferrara *et al.*, 2011; Seidel *et al.*, 2017). In addition, juvenile *C. leucas* already possess ~ 3.5 times the necessary force required to puncture hard prey such as crabs (Habegger *et al.*, 2011; Whitenack & Motta, 2010). Such a prominent muscular development coupled with a favourable tooth shape may confer a physical advantage to young *C. leucas* feeding performance. Investigating tooth shape through ontogeny could enhance our understanding of possible changes in feeding performance with age.

4.2.2 | Tooth position within the jaw

Our results support other studies that describe the tooth morphology of *C. leucas* as dignathic heterodontic, *i.e.*, they have distinct upper and lower jaw tooth shapes (Cullen & Marshall, 2019). *C. leucas* have cutting-type teeth in the upper jaw and tearing-type teeth in the lower jaw (Frazzetta, 1988; Huber *et al.*, 2009; Motta & Wilga, 2001; Ramsay & Wilga, 2007). Here we further defined the pattern of dentition as dignathic heterodontic in that there are different teeth shapes from the symphysis to the angle of the jaw within each side. Naylor and Marcus (1994) found that among the upper jaws of Carcharhinid sharks, tooth shape changed between species, similarly to the indicated by our GAMMs of surface area for intra-specific variation in *C. leucas*, *i.e.*, positions 1 and 2 are similar, 3 and 8 are different, and 10–13 are similar. However, Naylor and Marcus (1994) only found slight intra-specific changes in tooth shape with ontogeny, probably due to the limitations of their methods, including the lack of a technological approach compared to more recent studies.

Variable sizes and shapes of teeth along the jaw margin increase the effectiveness of bites, with different tooth shapes and jaw positions playing different roles during the capture and manipulation of prey (Lucifora *et al.*, 2009). Variation along the jaw margin for a more efficient bite can be inferred from sharper cusps and curved cutting edges, which will reduce energy expenditure and increase the draw effect from a reduced and focused slicing edge (Berthaume *et al.*, 2014; Lucifora *et al.*, 2009; Martin *et al.*, 2005; Wilga & Ferry, 2015). Serrated and pointed anterior teeth increase ease of penetrating prey and are likely used in capture efforts with the decreasing notch angle in the posterior directing and increasing the teeth' tearing effect (Habegger *et al.*, 2011; Huber *et al.*, 2009; Wilga & Ferry, 2015). Neither the size nor the number of serrations present on *C. leucas* teeth were assessed, but these could be important for tooth function. While optimal tooth shape is dependent on diet (Berthaume *et al.*, 2014), the lyodont dentition and overall shape of *C. leucas* teeth combined with a high bite force and headshaking appear to overcome physical limits imposed by prey morphology, *e.g.*, scales, skin, muscle or bone (Whitenack & Motta, 2010).

4.3 | Ontogenetic niche shift

To determine whether our identification of an ontogenetic change in tooth shape, surface area and thickness at ~ 135 cm L_T is related to

changes in prey and a potential niche shift, we compared our results with those of Niella *et al.* (2021, 2022), who conducted a stable isotope analysis on the same sample of juvenile *C. leucas*. Stable isotope analyses can identify occupied niches by analysing biological material to assess the proportions of natural tracers present (Shiffman *et al.*, 2012; Tamburin *et al.*, 2019). Small *C. leucas* are known to reside primarily in the upper reaches of river and estuarine habitats, and gradually move towards the marine realm as they grow (Werry *et al.*, 2018). At ~ 135 cm L_T *C. leucas* are considered large juveniles and will primarily inhabit lower river and estuarine habitats (Werry *et al.*, 2011, 2012; Yeiser *et al.*, 2008). Niella *et al.* (2021, 2022) found significant ontogenetic shifts in niche use within the Clarence River nursery area. A gradual shift in $\delta^{13}C$ indicated sharks <130 cm L_T prefer less saline environments upstream, while larger sharks inhabit increasingly more saline environments. There were significant inflection points in $\delta^{15}N$ and $\delta^{34}S$ (Niella *et al.*, 2021), indicating juvenile *C. leucas* moved towards the river mouth and relied on different habitats for food (Niella *et al.*, 2022). These findings are consistent with our findings of ontogenetic changes in tooth morphology at similar size/age, indicating a niche shift of *C. leucas* at ~ 130 cm L_T is associated with an increase in their use of saline environments ($\delta^{13}C$), higher trophic level ($\delta^{15}N$) and the concomitant contribution of more pelagic food items ($\delta^{34}S$) in their diet (Hussey *et al.*, 2012; Shiffman *et al.*, 2012).

5 | CONCLUSION

This study shows that *C. leucas* undergo ontogenetic heterodonty and that the changes in this species' diet are paired to growth, specifically with changes in feeding structure matching the developmental timing of ontogenetic niche shifts (Niella *et al.*, 2021). Dentition and diet are inextricably linked in *C. leucas*. The size of sharks where this change in diet occurred may be region specific and care is needed if extrapolating to other regions where the rates of development may differ (Harry *et al.*, 2011; Lombardi-Carlson *et al.*, 2003; Tanaka *et al.*, 2011). It is evident that investigations which examine ontogenetic shifts ideally should aim to assess all stages of development. Despite limited subadult *C. leucas* samples, our study comprised the age classes when the most noticeable niche shifts occur in the species.

AUTHOR CONTRIBUTIONS

Conceptualization: K.G., A.F.S., R.H., V.M.P. and Y.N. Data generation: K.G. and T.B.-H. Analysis: K.G., T.B.-H. and Y.N. Led the writing of the manuscript: K.G. Writing, review and editing: K.G., A.F.S., R.H., T.B.-H., V.M.P. and Y.N.

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DATA AVAILABILITY

The teeth measurement data that support the findings of this study are openly available in zenodo at <https://doi.org/10.5281/zenodo.5506920> (Niella & Goodman, 2021).

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SUPPORTING INFORMATION

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