Ontogenetic changes in the tooth morphology of bull sharks

(*Carcharhinus leucas*)

Kyle Goodman

Department of Biological Sciences, Faculty of Science and Engineering, Macquarie

University, North Ryde, New South Wales 2113, Australia

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Declaration

I wish to thank the following people who assisted in the research detailed in this thesis:

All other research described in this report is my own original work

Kyle Goodman

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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, which raises the question: does tooth morphology change with ontogeny? Teeth were measured using traditional morphometrics and an Elliptic Fourier Analysis to determine if morphology varied with position in the jaw and if there was an ontogenetic morphological change concordant with this niche shift. Tooth shape, surface area and thickness were measured. 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 with an inflexion 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, ontogenetic shift, tooth morphology

1. Introduction

From embryogenic formation through to maturity, many species undergo distinct changes in anatomy, behaviour and physiology as part of their development (French *et al*., 2017; Habegger *et al.*, 2012; Olson, 1996). These changes from one basal state to a mature form are called ontogenetic shifts and are not uniform across populations or species, at the same rate, but derive from individual rates of development (French *et al*., 2017; Matich and Heithaus, 2015; Turner Tomaszewicz *et al*., 2017). Ontogenetic shifts maximise an individual's resource intake while minimising mortality through increased overall body growth and foraging ability, e.g. bite force, being responsible for divergences and overlaps of organisms at different sizes and niches (French *et al*., 2017; Grubbs, 2010; Matich and Heithaus, 2015). These shifts can occur either as gradual changes, e.g. a deposit-feeding polychaete *Pseudopolydora kempi japonica* Imajima and Hartman 1964 slowly transitions from a diet of both diatoms and macroalgae, to one dominated by macroalgae (Hentschel, 1998); or as a discrete shift, e.g. the blackbelly salamander *Desmognathus quadramaculatus* Holbrook 1840 transitions from an aquatic diet in larval stage to a terrestrial one post-metamorphosis (Davic, 1991; Hertz *et al*., 2016).

For many marine predators, prey capture and subsequent consumption is explicitly related to the mouth and its structure. Gape, bite strength and the structure of teeth are critical to the biomechanics of seizing prey, retaining and potentially dismembering it (Cullen and Marshall, 2019). 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, 1995; Ward-Campbell and Beamish, 2005; Wilga *et al*., 2007). Throughout the life of predatory fish, modifications in gape and dentition may occur which are linked to diet shifts for selection of different prey (Ward-Campbell and Beamish, 2005). Tooth morphology can provide insights into a species' diet composition based on structure, e.g. highly molariform teeth are better suited to consume 'hard' prey like crustaceans, while cuspid teeth are better suited to consume 'soft' items such as cephalopods (Corn *et al*., 2016; Ferrara *et al*., 2011; Powter *et al*., 2010; Whitenack and Motta, 2010). However, this classification of 'hard' or 'soft' prey may underestimate the overlap of prey types, with not all hard or soft prey presenting alike, e.g. a teleost, the ladyfish *Elops saurus* Linnaeus 1766 and a cephalopod, the common octopus *Octopus vulgaris* Cuvier 1797 are both soft-bodied animals, but very different from each other (Whitenack and Motta, 2010). Therefore,

inferring diet or change in diet solely from tooth-structure while referencing simple dietary terms is likely inaccurate. Ideally, dental morphometry should be paired with a dietary analysis, e.g. stable isotopes, fatty acids and/or stomach content analysis to provide a more comprehensive ecological understanding.

Many species of sharks are considered top order predators and capable of exploiting a diverse range of prey. Since feeding is the mechanism by which resource intake occurs, the teeth have an important ecological role for these organisms as tooth shape facilitates prey acquisition (Cullen and Marshall, 2019). There is 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. These sharks transition 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 which allow them to pierce and hold flesh, while the broader and more serrated teeth of adults enable gouging chunks of flesh (Ferrara *et al.*, 2011; French *et al.*, 2017; Wilga and Ferry, 2015). Teeth are not the only ontogenetic change occurring in *C. carcharias* feeding apparatus. There is also structural reinforcement of *C. carcharias* jaws resulting from additional mineralisation (Ferrara *et al.*, 2011; French *et al.*, 2017; Wroe *et al.*, 2008).

The bull shark *Carcharhinus leucas* Valenciennes 1839 is a versatile predator capable of inhabiting both freshwater and marine environments in tropical and temperate regions and exploiting diverse resources (French *et al.*, 2017; Heupel *et al.*, 2015; Lee *et al.*, 2019; Smoothey *et al.*, 2019; Thorburn and Rowland, 2008; Werry *et al.*, 2011). Juvenile *C. leucas* use estuaries as nursery areas (Heupel and Simpfendorfer, 2008; Matich and Heithaus, 2015), and while adults are considered apex predators, juveniles are not. The juveniles lack of size, speed, experience and feeding apparatus makes them vulnerable to predation (Fu *et al.*, 2016; Matich and Heithaus, 2015; Thorburn and Rowland, 2008). Habitat use is linked to body size and is a function of the mortality-resource trade-off, i.e. maximising 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 and Heithaus, 2015; Ramirez *et al.*, 2017). In seeking low risk environments, juvenile *C. leucas* spend significant amounts of time (i.e.

up to four years) in rivers and estuaries before transitioning to the marine environment (Thorburn and Rowland, 2008). This size-based segregation in space use consists of larger animals using open marine environments to follow resources and habitats with less competition, while the smallest individuals make use of environments with lower associated risk (Heupel and Simpfendorfer, 2008; Heupel *et al.*, 2015).

Ontogenetic shifts in habitat and associated available prey can result in structural changes in the feeding apparatus which increase the rate of capture or ability to process previously inaccessible prey. In turn, these shifts may incorporate hunting adaptations to acquire faster or more functionally difficult prey (Grubbs, 2010; Habegger *et al.*, 2012; Lowe *et al.*, 1996). Structural limitations on successful hunting bouts would include bite force and dentition. To create an effective bite, a predator must inflict pressure to grip the prey item and pierce its body deep enough to prevent escape. The total bite force required to effectively hunt prey is dictated by the structural components that constitute prey, e.g. skin, muscle, scales or skeletal elements, and each new prey can vary in thickness or resistance of bodily elements thereby potentially increasing processing difficulty and requisite bite force (Kolmann and Huber, 2009; Whitenack and Motta, 2010).

An ontogenetic shift in diet has been shown for *C. leucas* (Lowe *et al.*, 1996; Niella *et al.*, 2021; Werry *et al.*, 2011) so there may be an expectation that bite force will increase to compensate for increasingly complex or functionally difficult prey (Habegger *et al.*, 2012). 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, thereby increasing bite force by virtue of organism size (Habegger *et al.*, 2012; Kolmann *et al.*, 2015). Young *C. leucas* bite force changes at a positively allometric rate and tapers to isometry with age (Habegger *et al.*, 2012). This disproportionately high increase in bite force for the younger members of the species provides an early competitive advantage (Habegger *et al.*, 2012; Kolmann and Huber, 2009). *Carcharhinus leucas* are considered to have overpowered jaws for their dietary range, tested prey items such as *E. saurus* and the Chesapeake blue crab *Callinectes sapidus* Rathbun 1896 require 5 and 50 Newtons (N) to respectively puncture each item, with theoretical bite forces in juveniles ranging 36 – 170 N and mature individuals 2128 – 5914 N at anterior and posterior bite points, respectively (Habegger *et al.*, 2012; Whitenack and Motta, 2010). This implies that the isometry experienced in adults may be due to a lack of selective pressure to continuously increase their power (Habegger *et al.*, 2012).

Power and jaw strength are not the only components to potentially require ontogenetic change to enable prey shifts. Teeth are equally important to enable successful predation. *Carcharhinus leucas* exhibit two clearly distinct tooth shapes between the upper and lower jaws

(Habegger *et al.*, 2012; Wilga and Ferry, 2015). Upper jaw teeth are broad triangular, lingo-labially flattened with serrations and have overlapping bases, while lower jaw teeth are tall and narrow cusped (Frazzetta, 1988; Habegger *et al.*, 2012; Whitenack and Motta, 2010; Whitenack *et al.*, 2011). These characteristics typify the upper jaw as cutting teeth and lower jaw as tearing teeth for removal and penetrating of flesh, respectively (Frazzetta, 1988; Huber *et al.*, 2009; Motta and Wilga, 2001; Ramsay and Wilga, 2007; Whitenack and Motta, 2010; Wilga and 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; Huber *et al.*, 2006**,** 2009; Motta and Wilga, 1995; Whitenack and 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 chance of escape and the number of bites needed to separate flesh (Bergman *et al.*, 2017; Hocking *et al.*, 2017; Huber and Motta, 2004; Motta and Wilga, 2001). The two different tooth types on the upper and lower jaws would indicate a wide dietary breadth as a result of a non-specialised feeding structure, consistent with the known generalist predator status of *C. leucas* (Cullen and Marshall, 2019; Estupinan-montano *et al.*, 2017).

Mineralisation and strength characteristics of *C. leucas* jaws and teeth are not as well studied in comparison to those species which can be kept in captive environments where feeding apparatus morphology and manipulative capabilities can be thoroughly observed. To better understand the role of teeth in foraging ecology, tooth mineralisation and the manipulative capacity of shark jaws during feeding events need further investigation. This study investigates whether the niche change that *C. leucas* are known to undergo in life would be facilitated by an ontogenetic change in dentition. Ontogenetic shifts in *C. leucas* dentition have been identified by Cullen and Marshall (2019) who selected their own representative teeth to sample from *C. leucas* jaws to identify the presence of ontogenetic changes in morphology. No identification of where in the lifecycle this shift occurs was identified nor any relationship to diet. Here a fine-scale approach was used examining all teeth across all jaw positions and life development stages (i.e. neonate, juvenile and adult), to assess where morphological alterations occur and their subsequent potential implications for *C. leucas* foraging ecology. I therefore hypothesise that changes in the dental morphology of *C. leucas* would precede niche change, thus allowing these animals to explore new resources.

2. **Method**

2.1. *Sample collection*

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 (Figure 1). Adult *C. leucas* (n = 9) caught between March 1999 and February 2020 were provided by the NSW Shark Meshing (Bather Protection) Program (SMP) and NSW Shark Management Strategy north coast net trials (Figure 1) (NSW Government Department of Primary Industries, 2020). All captured individuals were measured and separated in age classes according to total length (L_T) (Table 1).
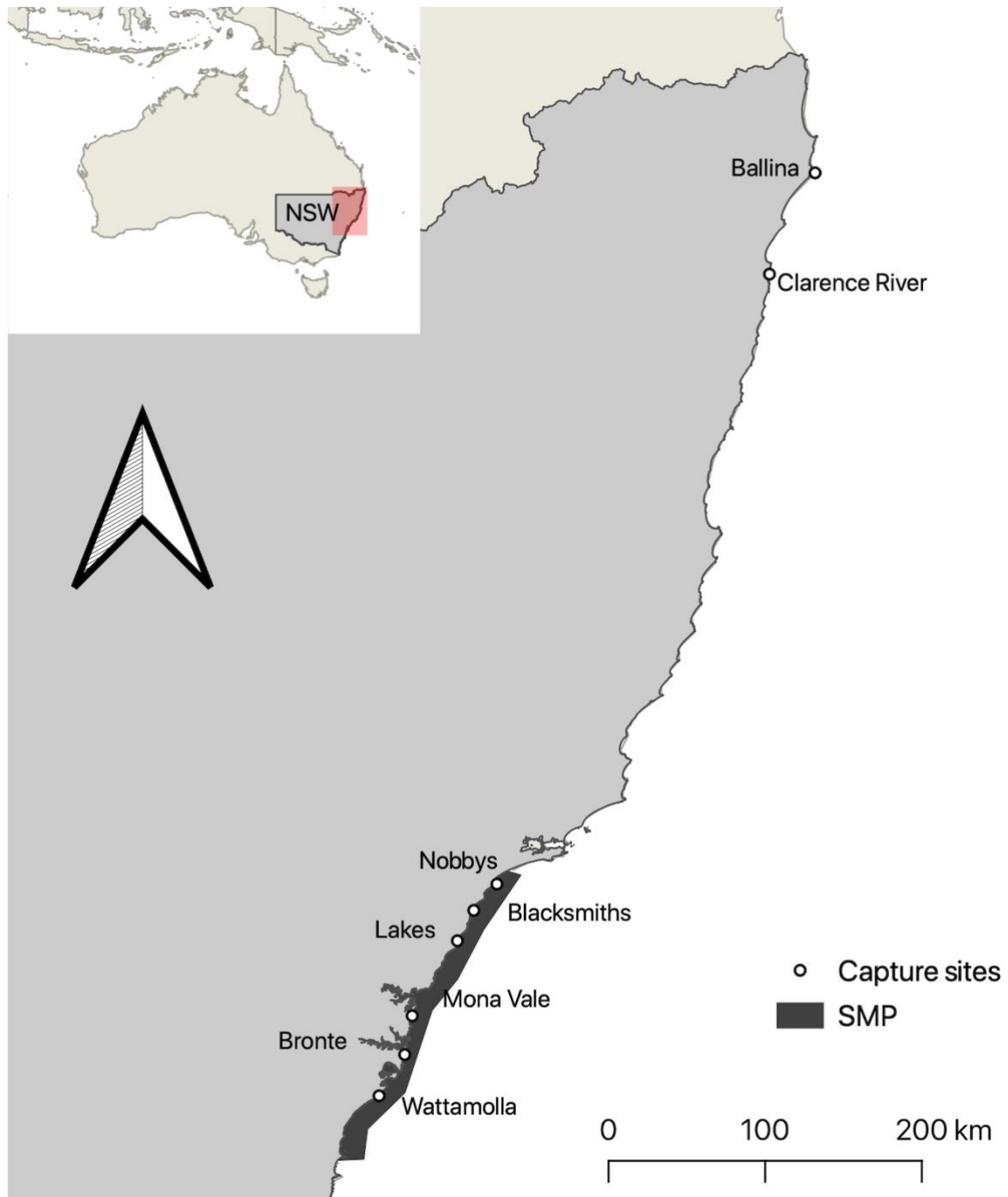
Figure 1. Capture of *Carcharhinus leucas* along the New South Wales (NSW) coastline. Neonate and juveniles were obtained from commercial catches in the Clarence River, while adults were acquired from the bather protection nets of the NSW Shark Meshing (Bather Protection) Program (SMP) and NSW Shark Management Strategy north coast net trials off Ballina 2016 – 2018.

Table 1. Sizes of *Carcharhinus leucas* sampled per age class including sample size (n), mean, standard deviation and range (min – max) 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)
Sub-adult	161 – 200	0	N/A
Adult	> 201	9	247.4 ± 33.6 (211 – 310)

The jaws were excised from all sharks and cleaned to expose the teeth. The focus was on the teeth which comprise the first (i.e. functional) row of teeth from the upper and lower jaws (Figure 2), however, if teeth in the functional row were damaged or missing, the corresponding tooth in the second series of the tooth file was used (Figures 2 and 3a).

(a)

Anterior — Symphysial tooth

Lateral

mesial

Upper Left

1 2 3 4 5 6 7 8 9 11 10 12 13

IDD

distal

Posterior

Functional row

Upper left tooth numbering

Tooth file

Lower left tooth numbering

12 11 10 9 8 7

Inter Dental Distance (IDD)

1 2 3 4 5 6

Lower Left

Symphysis

(b)

Fully formed functional tooth on forward edge of the jaw

Tooth file

2

①

3

Functional row

4

Row number

5

6

7

Buccal side
(providing a lingual view of the tooth if viewed from this side)

Labial side

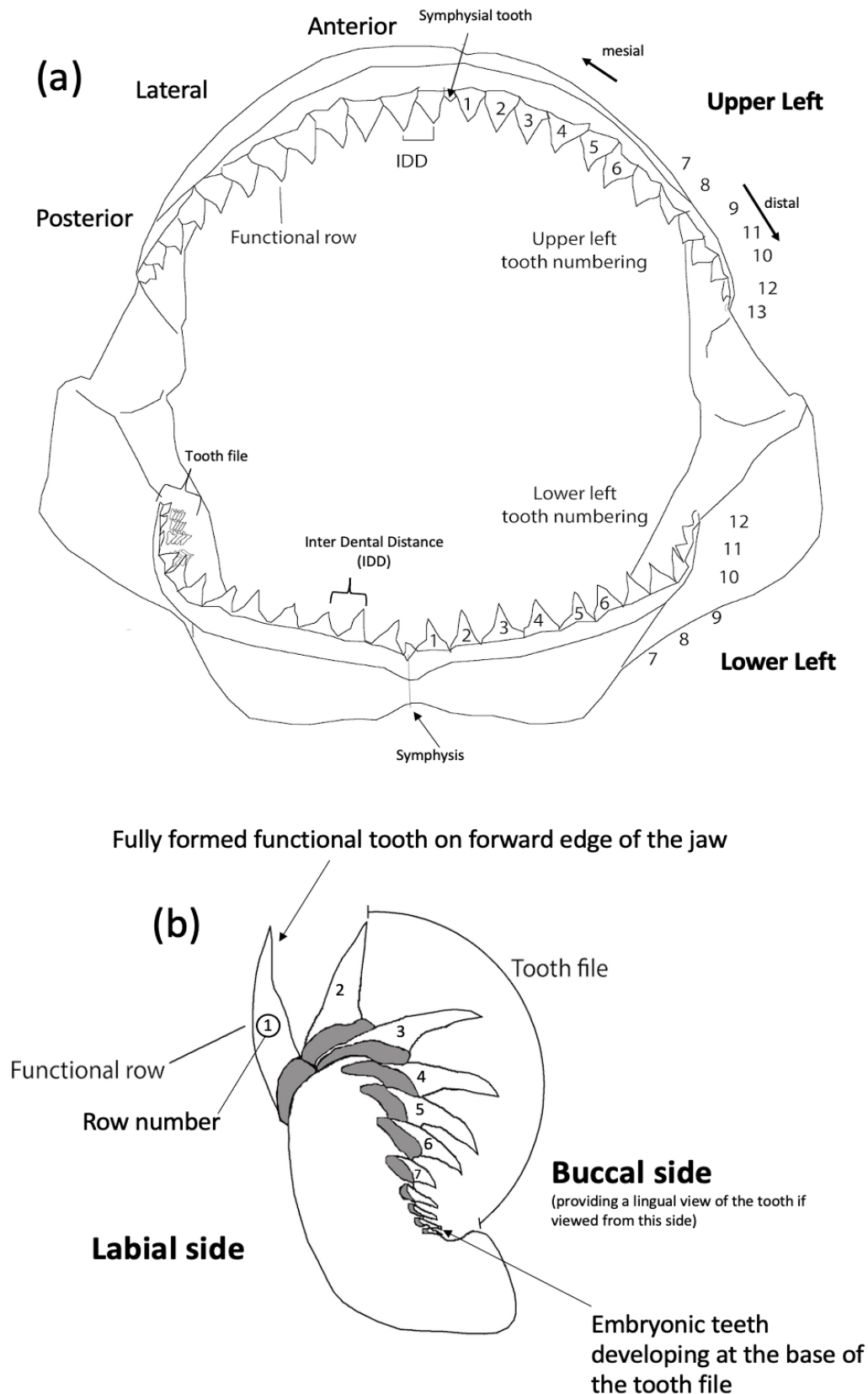Embryonic teeth developing at the base of the tooth file

Figure 2. (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 (functional row) and the conveyor belt of replacement teeth (tooth file) visible in the jaw.
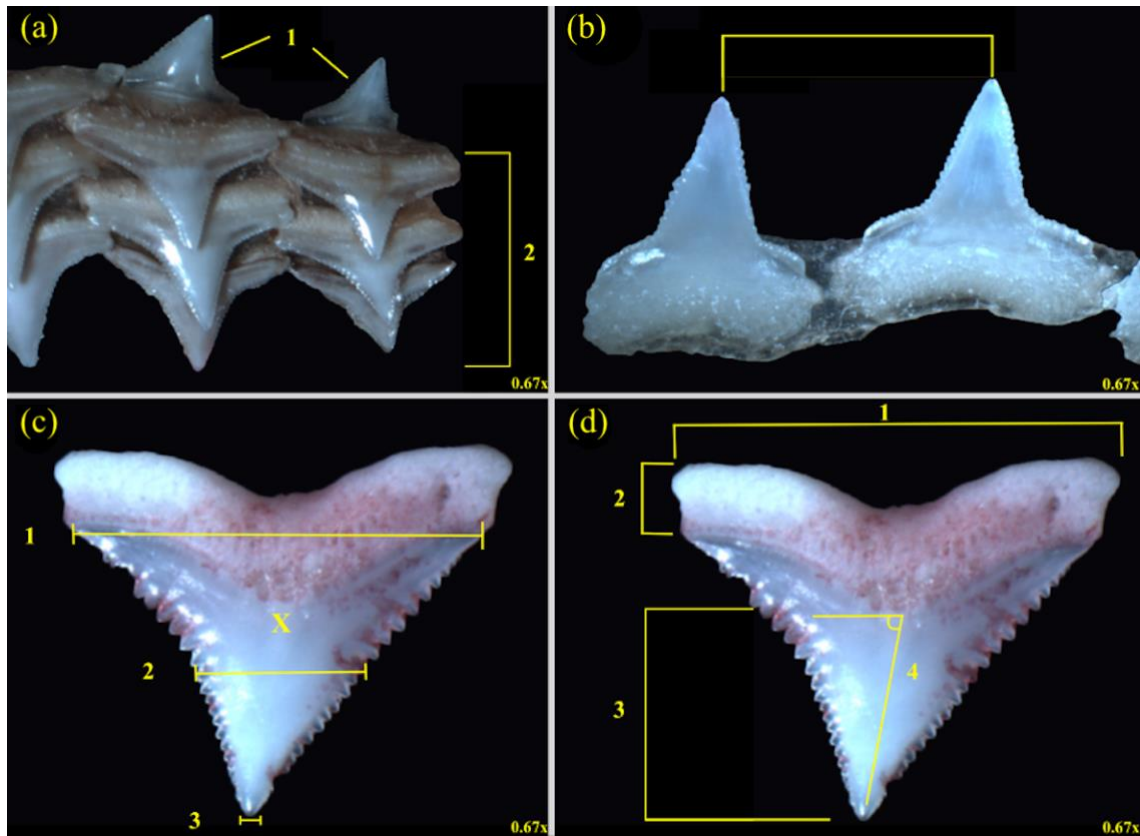
Figure 3. (a) Buccal view of *Carcharhinus leucas* teeth where 1 = the functional row and 2 = tooth file to be sampled in the case of a missing or damaged functional row. (b), (c) and (d) show the labial view of teeth describing landmark locations for morphometric measurements of teeth. (b) Interdental distance, (c) the three tooth width measurements where 1 = tooth base, 2 = mid-tooth and 3 = tooth tip width and X demarcates the position at which tooth thickness at tooth base was measured whilst mid-tooth and tooth tip thicknesses are measured along the tooth ridge at the same crown height position as width 2 and 3, (d) 1 = root width, 2 = root height, 3 = crown height and 4 = notch angle. Images captured on Olympus SZ61 stereomicroscope.

2.2. *Tooth morphology*

2.2.1. Traditional morphometrics

A series of 11 distinct measurements traditionally used to describe tooth shape (Figure 3b – d) were taken using a digital vernier caliper (0.01 mm accuracy). Notch angle (Figure 3d) and surface area were calculated with the open-source ImageJ (version 2.0.0-rc-43/1.52n) image processing software.

2.2.2. Elliptic Fourier Analysis

An alternative approach to obtain tooth shape data is the Elliptic Fourier Analysis (EFA) which uses a nearest pixel approach to automatically obtain scaled coordinates of an object, thus reducing the bias originated from intra-observer variation frequently observed with traditional methods (Cullen and 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 variation in tooth shape (Cullen and Marshall, 2019). The EFA was performed using the Momocs package (Bonhomme *et al.*, 2014) in R statistical environment (R Core Team, 2020; version 3.5.3) 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 belonging to neonates and juveniles < 130 cm L_T the entire suite of upper and lower jaw teeth were removed from the jaws for photographs. Juveniles > 130 cm L_T and adults allowed tooth photographs to be made *in situ*. 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 0.67x 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 error of parallax. All photos were edited using Adobe Photoshop^{TM} 2020 (version 21.2.0) to create silhouettes of each tooth for the EFA. As the gums of sharks > 130 cm L_T obscured the full extent of the tooth root it was not included in silhouette creation for the EFA.

2.3. *Statistical analysis*

2.3.1. Method quality control

To ensure accuracy of the physical measurements taken, 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). A paired t-test was done to determine if there was a significant difference among rounds of measurements.

To confirm protocols in running the EFA, a preliminary EFA was done using all 50 teeth in each jaw of one juvenile and one adult shark. Each of these teeth were photographed four times and the EFA repeated, the output was then prepared for a discrepancy analysis. An Analysis of Variance (ANOVA) was done on the EFA harmonic coefficients to determine if there was any significant difference in the outputs from the different rounds of photographs. The number of replicates for each preliminary analysis was determined based on the size of the error bars from initial measurements.

2.3.2. <u>Comparative analysis and morphology</u>

Two Principal Component Analysis (PCA) were performed, one including the traditional morphometric measurements and the other using all the EFA harmonic coefficients. This step was done to remove noise from the multiple variables comprised in each approach and standardise their values among all sharks sampled in relation to the biological factors possibly influencing tooth morphology. A Generalized Linear Model (GLM) approach was then used including 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), and gaussian families of error distribution were used. The data for surface area and thickness at tooth base (hereinafter referred to as thickness) were log transformed to fit a normal distribution. Similarly, for tooth shape (i.e. EFA), GLMs were used to investigate for the presence of allometric trends in tooth size. The PCA and GLM were run with the base R stats package (R Core Team, 2019). Generalised Additive Mixed Model (GAMM) was run with the mcgv package (Wood, 2011) and were 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 comprising shark size (L_T), and the interactive effect between jaw position (e.g. upper right) and tooth number (i.e. 1 – 13). The shark identification number was included in the GAMMs as a random effect to account for possible inter-individual 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 and Farrell, 2004) and visually inspected for a normal 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 morphological aspect. Due to 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 shark size (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 = tooth 1 and 4; posterior category = tooth 8 and 11. A post-hoc Tukey test was then used to investigate for possible significant differences between left and right sides of upper and lower jaws. Significance levels were set at p-value < 0.05 for all analyses.

3. **Results**

3.1. *Preliminary analysis and method selection*

A total of 34 *C. leucas* (min = 72.5 cm, max = 310 cm, mean ± SD = 141.26 ± 69.21cm L_T) were analysed (Table 1). Non-significant differences in values associated with measurements of tooth thickness were calculated from the preliminary analyses (p = 0.253). No significant differences were observed among the Elliptic Fourier Analysis (EFA) harmonic coefficients produced during the comparison of multiple photographs on the same individuals (Table S1), confirming this method to be suitable for future analysis. Since the EFA approach exhibited a lower AIC (22794.86) than the morphometric analysis (25599.35), the EFA was selected to further investigate changes in tooth morphology through ontogeny and by jaw position (Tables S2; S3 and S4).

3.2. *Tooth shape*

The Principal Component Analysis (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 4). The separation in shape between lower and upper jaw 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 4) 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 4), which constitutes part of the y-axis shape configuration (Claude, 2008).
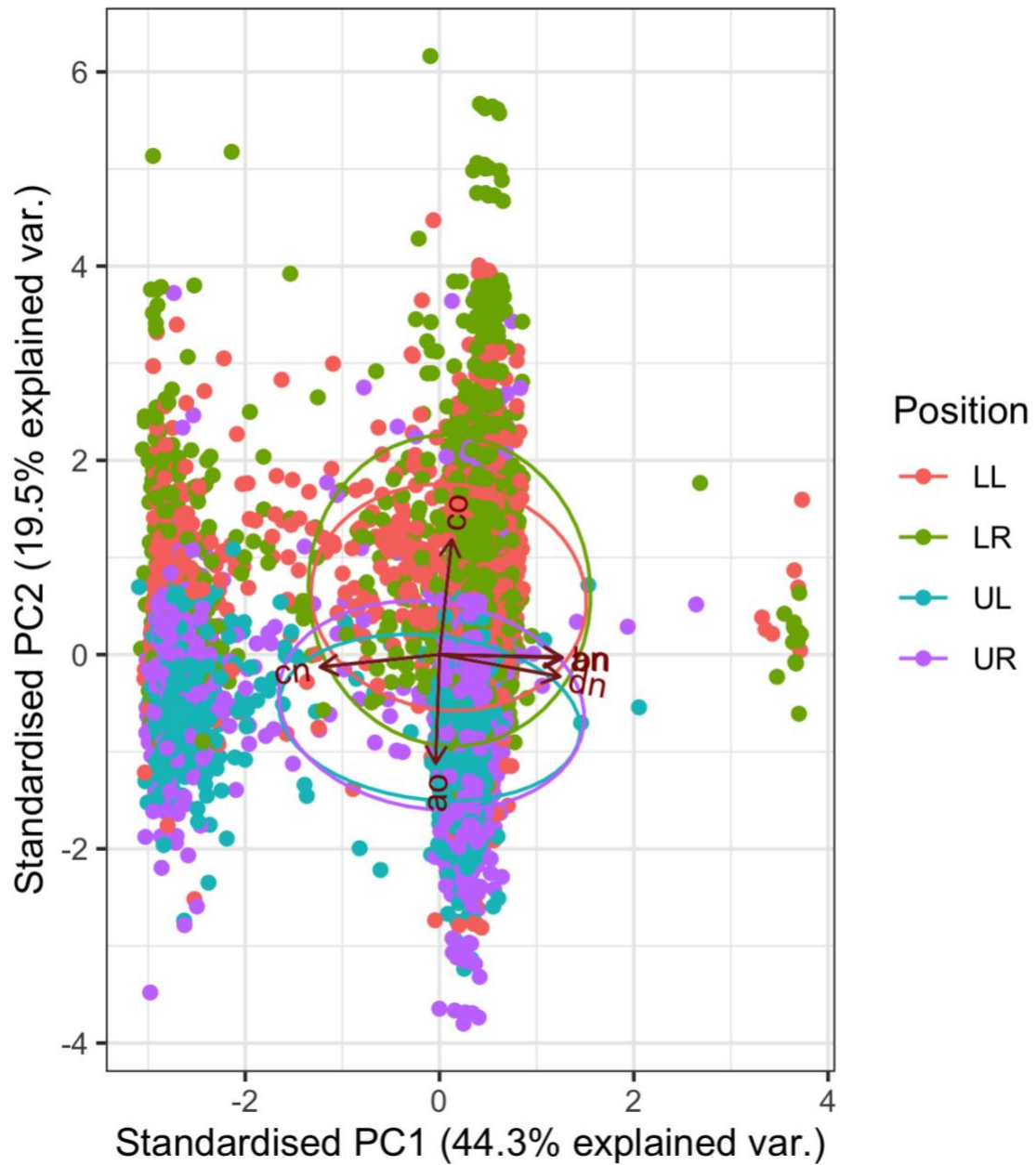
Figure 4. Principal Component Analysis of the Elliptic Fourier Analysis coefficients describing *Carcharhinus leucas* tooth shape by jaw position, i.e. LL (lower left), LR (lower right), UL (upper left) and UR (upper right). The loading labels represent the horizontal (a_n, b_n and a_0) and vertical (c_n, d_n and c_0) EFA coefficients while the coloured ellipses represent the distribution of each respective jaw position.

Significant differences in tooth shape were identified as a function of shark total length (L_T) and jaw position (Table 2). A significant difference in *C. leucas* tooth shape was observed for individuals smaller and larger than 135 cm L_T (Figures 5; 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 6 and 7). In contrast with the consistent changes in tooth shape as a function of body growth throughout all jaw positions (Figure 5), shape alterations were much more pronounced in the lower than in the upper *C. leucas* jaws (Figure 6).

Table 2. Generalised Linear Models of *Carcharhinus leucas* tooth shape, including the effects of total length (L_T) and interactions between jaw position (LL = lower left, LR = lower right, UL = upper left, and UR = upper right) and tooth number (Tooth.n). Coefficient estimates (Est.), Standard error (SE), t-values and p-values are included for each variable.

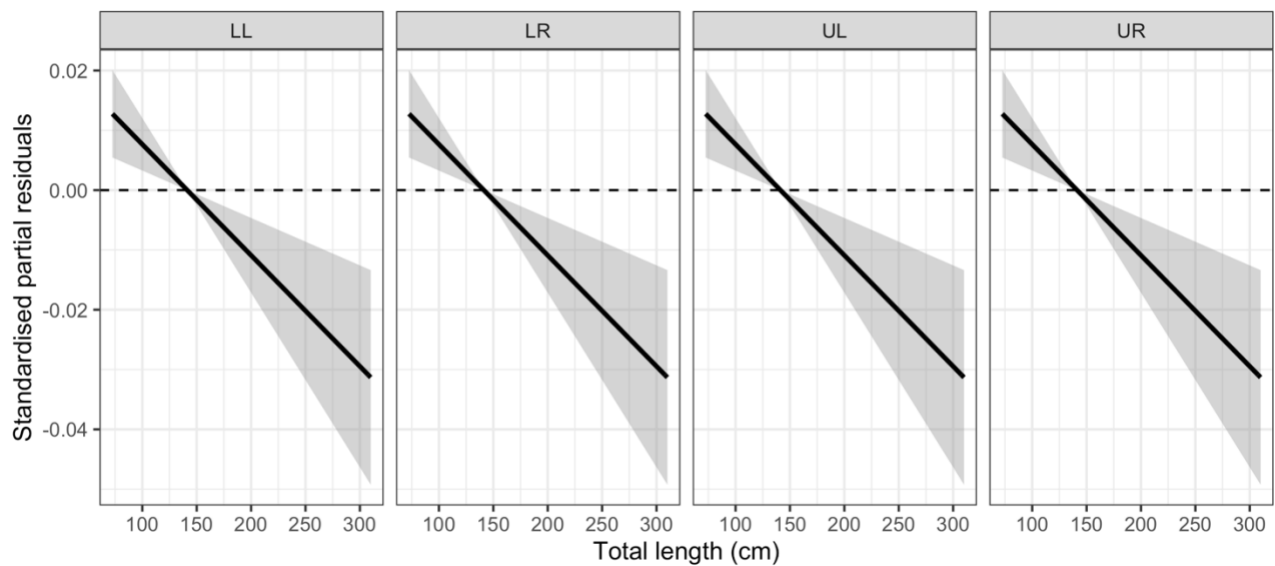| Variable	Est.	SE	t-value	p-value
Intercept	-7.76e-02	1.09e-02	-7.07	< 0.001
Total length	-1.86e-04	5.42e-05	-3.42	< 0.001
LL x Tooth.n	3.77e-02	1.38e-03	27.32	< 0.001
LR x Tooth.n	4.40e-02	1.39e-03	31.61	< 0.001
UL x Tooth.n	-8.37e-03	1.28e-03	-6.55	< 0.001
UR x Tooth.n	-5.72e-03	1.29e-03	-4.44	< 0.001

Figure 5. Generalised Linear Models of the coefficients describing *Carcharhinus leucas* tooth shape with the effect of total body length (cm). Shaded bands and dashed lines indicate the 95% confidence intervals and null effects, respectively.
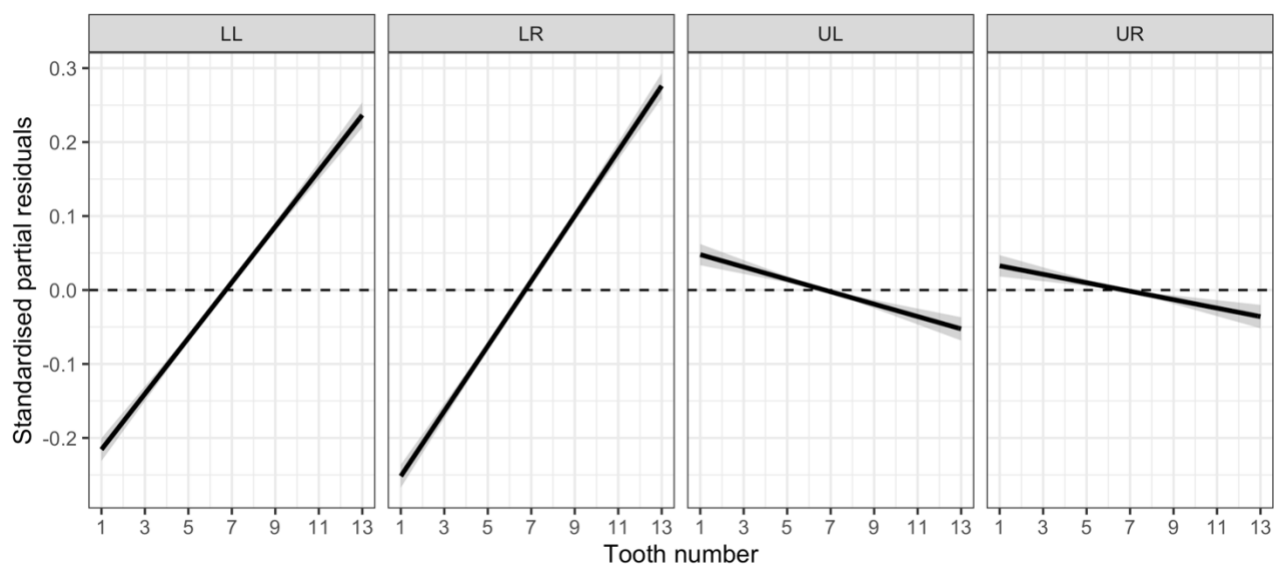

Figure 6. Generalised Linear Models of the coefficients describing *Carcharhinus leucas* tooth shape with the effect of tooth number by jaw position, i.e. LL (lower left), LR (lower right), UL (upper left) and UR (upper right). Shaded bands and dashed lines indicate the 95% confidence intervals and null effects, respectively.
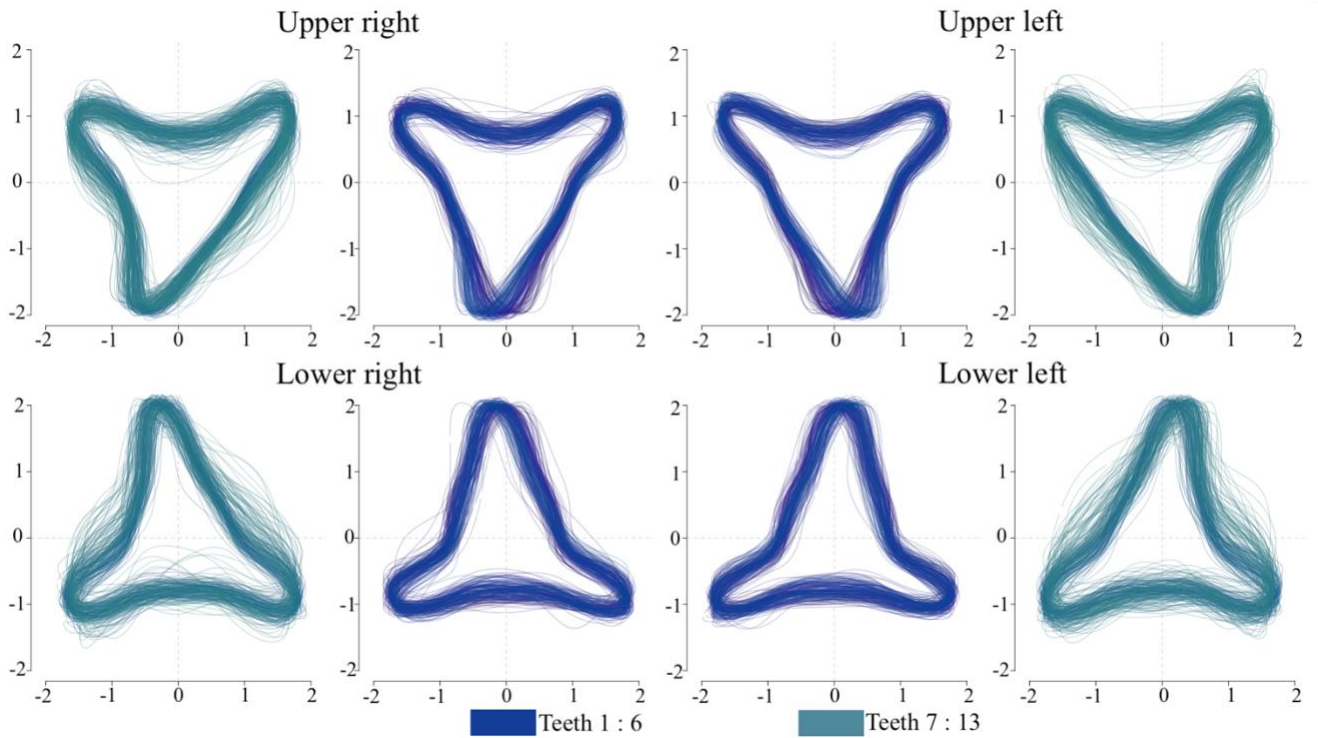
Figure 7. Individual raw centred and scaled outlines from all teeth (N = 1689 total tooth outlines) from *Carcharhinus leucas* jaws obtained with Elliptic Fourier Analysis. Each outline corresponds to one shark tooth, the display here shows the variation in morphology observed within the jaws. Anterior shape = teeth 1 to 6 and posterior shape = teeth 7 to 13 in all jaw hemispheres.

3.3. *Tooth size*

Size of tooth was found to significantly differ as a function of total length and position in the jaw (Tables 3 and 4). Tooth surface area and base thickness was significantly different for individuals shorter or longer than 135 cm L_T (Figures 8 and 9). 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 (Figures 8 and 9). A significant effect of shark identification number was observed in the Generalised Additive Mixed Models (Table 4). Within all jaw positions, *C. leucas* teeth could be split into three categories based on surface area, with the respective left and right sides of upper and lower jaws mirroring each other in tooth groupings, however, variation exists between the surface area classes representative of the upper and lower jaws (Figure 10). 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 are mesial = tooth 1, intermediate = teeth 2 – 6 and distal = teeth 7 – 13 (Figure 10). Within all jaw positions, the teeth could be split into two groups based on thickness with teeth

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1 – 6 identified significantly different to 7 – 13 (Figure 11). The changes in tooth size (surface area and thickness) as a function of tooth number (i.e. placement within the jaw position) in the jaw were found to be consistent throughout the entire jaw (Figures 10 and 11).

Table 3. Generalised Linear Models of *Carcharhinus leucas* tooth size by morphometric aspect, including the effects of total length (L_T). Coefficient estimates (Est.), Standard error (SE), t-values and p-values are included for each variable.

Morphometric aspect	Variable	Est.	SE	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

Table 4. Generalised Additive Mixed Model of *Carcharhinus leucas* tooth size by morphometric aspect, including the effects of total length (L_T), shark identification number (ID) and interaction between jaw position (LL = lower left, LR = lower right, UL = upper left, and UR = upper right) and tooth number (Tooth.n). Effective degrees of freedom (Edf.), reference degrees of freedom (Ref. df.), F-statistic and p-values are included for each variable.

Morphometric aspect	Variable	Edf.	Ref.df.	F	p-value
Surface area	Tooth.n x 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 x 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

Figure 8. Generalised Linear Models of log transformed *Carcharhinus leucas* tooth surface area (mm^2) with the effect of total body length (cm). Shaded bands and dashed lines indicate the 95% confidence intervals and null effects, respectively.
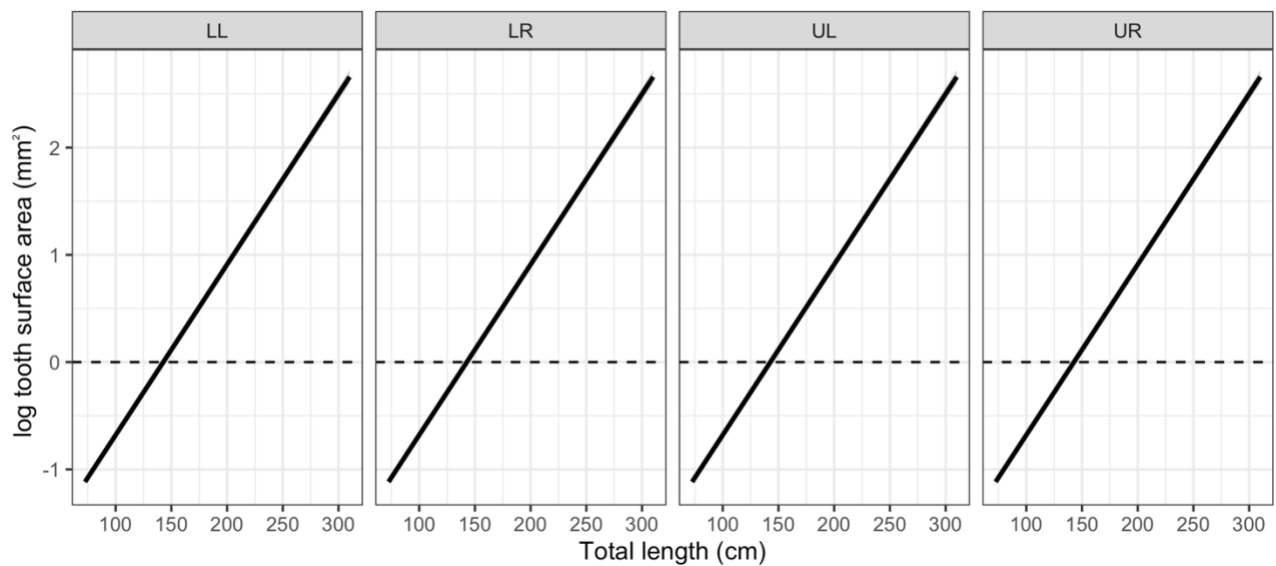

Figure 9. Generalised Linear Models of log transformed *Carcharhinus leucas* tooth thickness at the base (mm) with the effect of total body length (cm). Shaded bands and dashed lines indicate the 95% confidence intervals and null effects, respectively.

Figure 10. Generalised Additive Mixed Model of log transformed *Carcharhinus leucas* tooth surface area (mm^2) with the effect 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.
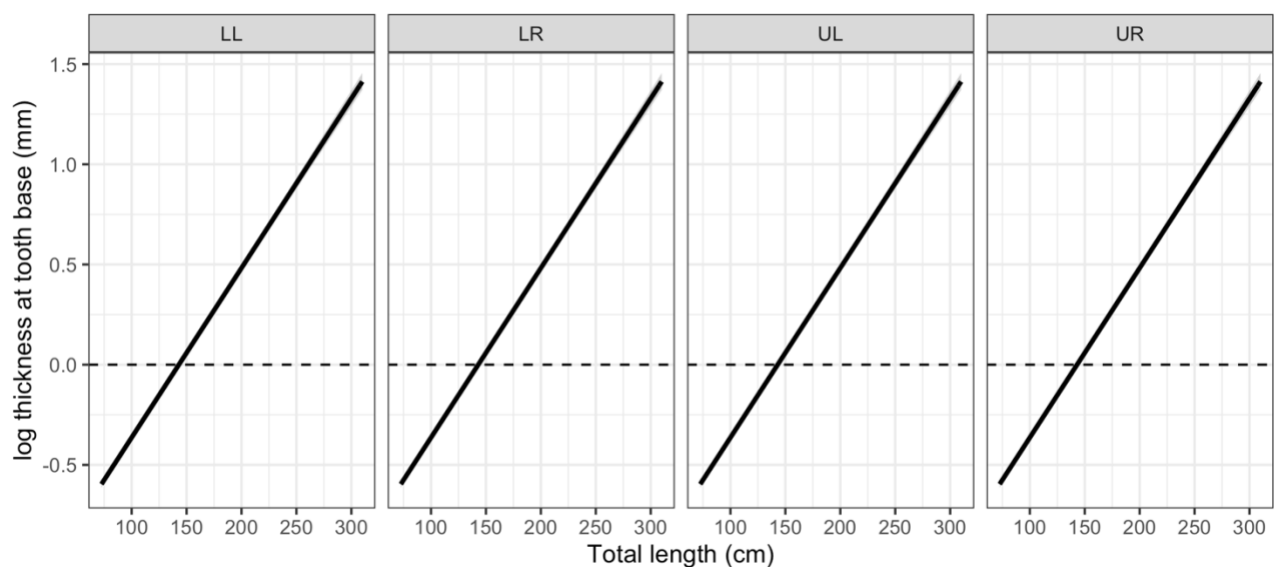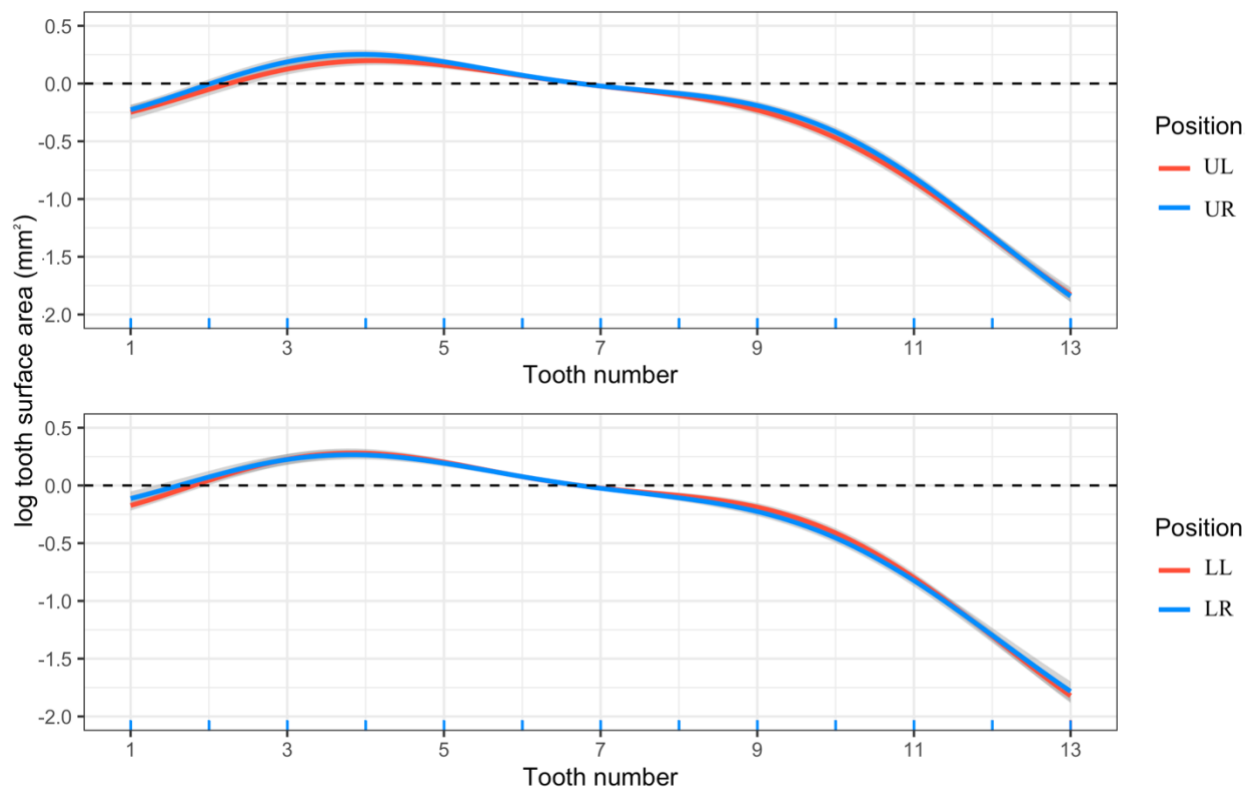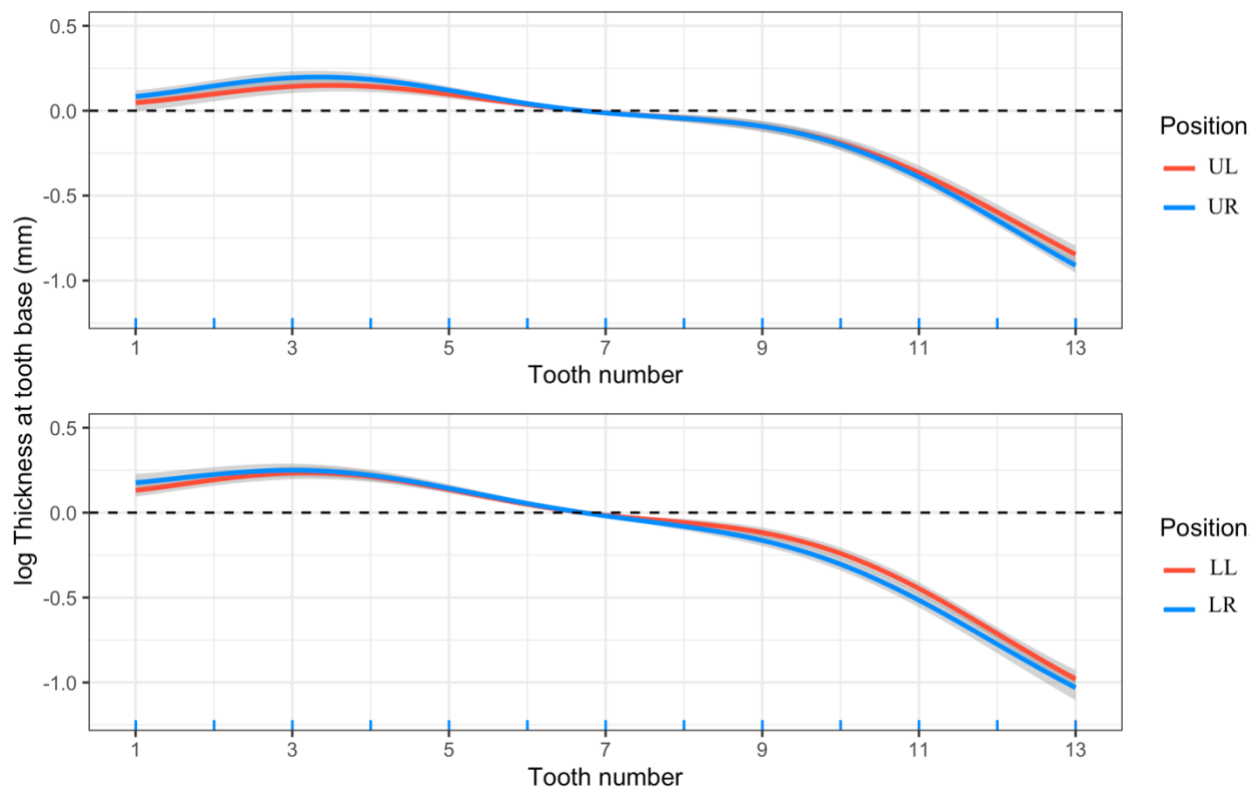
Figure 11. Generalised Additive Mixed Model of log transformed *Carcharhinus leucas* tooth thickness at the base (mm) with the effect of tooth number by jaw position, i.e. LL (lower left), LR (lower right), UL (upper left) and UR (upper right). Shaded bands and dashed lines indicate the 95% confidence intervals and null effects, respectively.

3.4. *Bilateral symmetry*

Significant modelled differences in tooth morphology as a function of jaw position in *C. leucas* were found (Table 5), however, Tukey's post hoc analyses revealed that significant differences associated with jaw position (i.e. left and right sides) are not present within upper and lower jaws (Table 6), thus indicating the presence of bilateral symmetry in tooth morphology.

Table 5. Analysis of Variance assessing the effect of jaw position on tooth morphology of *Carcharhinus leucas*. Representative teeth 1, 4, 8 and 11 were selected from each jaw position. Degrees of freedom (df), F-value and p-value are included for each morphological aspect

	Variable	df	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

Table 6. Tukey Honest Significant Difference comparison of mean results for *Carcharhinus leucas* tooth morphology including the effect of jaw position. Only the p-adjusted values corresponding to left and right comparisons for determination of bilateral symmetry are included, i.e. lower left (LL) v lower right (LR) and upper left (UL) v upper right (UR). 95% family-wise confidence interval

	Variable	Jaw position comparison	p-value
Shape	Jaw position	LL - LR	0.837
Shape	Jaw position	UL - UR	0.967
Surface area	Jaw position	LL - LR	0.986
Surface area	Jaw position	UL - UR	0.987
Thickness at tooth base	Jaw position	LL - LR	0.998
Thickness at tooth base	Jaw position	UL - UR	0.999

The total tooth count has been shown to vary in both Carcharhiniformes and Lamniformes, however, the difference in tooth count is negligible (Bass *et al.*, 1973; Shimada, 2002). The usual *C. leucas* tooth count is 13 tooth files in the upper jaw and 12 tooth files in the lower jaw on both left and right sides (Bass *et al.*, 1973). Eleven of the 34 *C. leucas* in this study had non-usual tooth counts: 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 lower left or right sides of the jaw.

4. **Discussion**

This study is the first to examine *C. leucas* tooth morphology using two complementary methods, i.e. traditional morphometrics and Elliptic Fourier Analysis (EFA), to identify patterns of dentition and extrapolate ecological consequences to morphology. This study discovered clear distinctions in tooth morphology, based on shark length, between immature and mature size classes. The bilateral symmetry of teeth within *C. leucas* jaws has been associated with genetically healthy individuals across the animal kingdom, from scorpionflies to people (Gomes *et al.*, 2011; Thornhill and Sauer 1992). Additionally, 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 and Sauer 1992). It is proposed, therefore, that this symmetrical distribution of multiple tooth morphs provides *C. leucas* a mechanical advantage which is further 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.*, 2001).

Research on Carcharhiniform dentition has lagged behind investigations of Lamniform dentition. Lamniformes have been shown to possess multiple tooth size groupings in the upper and lower jaws and it is hypothesised that the presence of a tapering dental bulla is the cause for these differential sizes observed (Shimada 2002). A dental bulla is described as an inflated and hollow section of jaw cartilage, appearing as a prominence along the inside of the jaw and allows/ supports the development of large teeth (Shimada 2002). One Carcharhiniform, the snaggletooth shark *Hemipristis elongata* Klunzinger 1871 is known to possess a dental bulla which produces noticeably narrower teeth than the subsequent distal ones (Chappell and Séret, 2020; Shimada, 2002). This bulla is variable in Carcharhiniformes, e.g. it is also present in the hooktooth shark *Chaenogaleus macrostoma* Bleeker 1852 but absent in the blacktip reef shark *C. melanopterus* Quoy & Gaimard 1824 and *Hemigaleus microsotoma* Bleeker 1852 (Chappell and Séret, 2020). It is not currently known if *C. leucas* possess a dental bulla that may dictate tooth size and shape. Further investigations into the presence of dental bulla across elasmobranchs exhibiting variations in tooth morphology are encouraged.

Traditional morphometrics are generally labour intensive and time-consuming approaches to obtain morphological data, considering the small size of neonate teeth (ranging 0.43 – 3.98 mm in crown height) and the need to maintain accurate measurements an alternative approach was adopted, the EFA. The size of jaw and teeth influenced the speed of data collection for both methods, specifically smaller individuals required greater time for both techniques. From start of photography through to image edits and running the EFA in R to producing results, this approached

proved to be ~ 2.5x faster than traditional morphometrics. If using archived or already edited images the time investment would be even shorter. However, lateral-view images of teeth could not be obtained and due to the scaling process involved in the EFA the influence of size was lost. Consequently, traditional morphometric measurements also proved useful to measure size metrics, i.e. surface area and thickness, thereby providing a complete understanding of both measures of shape (EFA) and size (traditional morphometrics).

4.1. *Bilateral symmetry*

Bilateral symmetry was found in *C. leucas* tooth morphology for both the upper and lower jaws. The EFA outlines of tooth shape had clear and discernible anterior and posterior tooth shapes in all jaw hemispheres. Two sharks possessed unusual teeth with torsions, but pathologic teeth are expected to exist in at least 1% of Carcharhiniformes (Becker *et al.*, 2000). Pathologic teeth are most likely a consequence of oral trauma, for example, from oral manipulation of spined or barbed prey and subsequent puncturing of tooth forming tissues causing irregularities in tooth development (Balbino and Antunes, 2007; Becker et al., 2000). Pathologic teeth were only observed in two adult *C. leucas* (5.9% of sharks), and it is unlikely that juveniles would target prey that could inflict enough damage to result in severe oral trauma.

4.2. *Tooth morphology*

4.2.1. Ontogenetic heterodonty

The findings here agree with Cullen and Marshall (2019) in that *C. leucas* undergoes an ontogenetic shift in tooth shape. Beyond determining the presence of a shift, I have identified a recognisable change in the shape, surface area and thickness of teeth at ~135cm total length (L_T) (Figures 5; 8 and 9). Although the general classification of cutting teeth in the upper jaw and tearing teeth in the lower jaw remain constant through all life stages of *C. leucas*, there are differences in tooth shapes between young and mature sharks (Figures S1and S2). Cullen and Marshall (2019) determined significant differences in shape between their young-of-the-year and juvenile age classes only. Those authors recognised that their results might be impacted by low sampling numbers of sub-adult and adult sharks. This study also lacked members of the sub-adult life stage but had a large number of replicates for the other age classes. There is an increase in overall broadness and bluntness of *C. leucas* teeth, appearing less cuspid and more robust with increasing shark length (Figures S1 and S2). The anterior teeth develop an expansion of the mid-tooth creating a clear hump on the lateral edges of the teeth with shark length, and the posterior teeth are not as

slender and appear to have an increase in notch angle, particularly at the most apical point of the teeth. Broader teeth facilitate increased purchase during feeding events while curved teeth increase the length of tooth cutting edge increasing predatory efficiency (French *et al.*, 2017; Martin *et al.*, 2005). *Carcharodon carcharias* teeth become broader and less cuspidate through ontogeny and this is the suggested catalyst for inclusion of marine mammals into their diet (French *et al.*, 2017). *Carcharhinus leucas* tooth broadness and dietary breadth also increases through ontogeny, beginning to include items of greater complexity with size, e.g. elasmobranchs, marine mammals, large teleost and sea turtles (Estupinan-montano *et al.*, 2017; Habegger *et al.*, 2012; Niella *et al.*, 2021). Given the evidence for paired ontogenetic change in *C. carcharias* diet and dentition, I surmise there is a similar ontogenetic trend in *C. leucas*.

The changes in both surface area and thickness occur at a positively allometric rate and given that young *C. leucas* experience a similar pattern of increase in bite force it would be appropriate for teeth to increase at the same disproportionate rate (Bergman *et al.*, 2017; Habegger *et al.*, 2012). Through ontogeny the jaws of *C. leucas* increase in mineralisation and this may increase their durability and aid in absorbing and accommodating large bite forces to prevent self-inflicted damage from structurally weak components (Dingerkus *et al.*, 1991; Ferrara *et al.*, 2011). Bite force (Bergman *et al.*, 2017; Habegger *et al.*, 2012), jaw architecture and muscles all increase in structural integrity with growth to prevent any one mechanism of the feeding apparatus overpowering another component (Dingerkus *et al.*, 1991; Ferrara *et al.*, 2011; Luczkovich *et al.*, 1995). As tooth surface area and thickness show a similar increase, it can be assumed that this aids tooth strength needed to maintain a hold on prey and ensure minimal damage to teeth during feeding (Lucifora *et al.* 2009). Investigating tooth structure through ontogeny could bring light to durability and potential strength changes in dentition with age.

4.2.2. Tooth position within the jaw

The results of this study support other reports that the tooth morphology of *C. leucas* is dignathic heterodontic, which is, they have distinct upper and lower jaw tooth shapes, (Cullen and Marshall, 2019). Here I further define the pattern of dentition as monognathic heterodontic, that is, they possess different teeth from the symphysis to the corner within one jaw and I categorised the teeth in the jaw. *Carcharhinus leucas* have cutting type teeth in the upper jaw and tearing type teeth in the lower jaw (Frazzetta, 1988; Huber *et al.*, 2009; Motta and Wilga, 2001; Ramsay and Wilga, 2007). This study further refines the classification of *C. leucas* teeth as I found clear shape changes along the jaw margin, with decreasing notch angle from the symphysis towards the back of the mouth, resulting in a change from a relatively equilateral triangle to more acute or hooked-shape

teeth (Figure 7). 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 capture and manipulation of prey (Lucifora *et al.*, 2001). 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 increases the draw effect from a reduced and focused slicing edge (Berthaume *et al.*, 2014; Lucifora *et al.*, 2001; Martin *et al.*, 2005; Wilga and 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 tooths' tearing effect (Habegger *et al.* 2011; Huber *et al.*, 2009; Wilga and Ferry, 2015). Neither the size nor the number of serrations present on *C. leucas* teeth were assessed but this could be a point of interest surrounding tooth morphology and function, either by jaw position or shark size. While optimal tooth shape is dependent on diet (Berthaume *et al.*, 2014), the acrodont dentition and overall shape of *C. leucas* teeth combined with a high bite force and headshaking appears to overcome physical limits imposed by prey morphology, e.g. scales, skin, muscle or bone (Habegger *et al.*, 2012; Huber *et al.*, 2009; Motta and Wilga, 2001; Whitenack and Motta, 2010).

Carcharhinus leucas possess multiple tooth size classes in their jaws with morphological groupings of teeth retained across the size range of sampled specimens. The front teeth (teeth 1 – 6) were all significantly larger and thicker than the back teeth (teeth 7 – 13), but of these front teeth the intermediate teeth were the largest with the mesial teeth having significantly smaller surface area. The back teeth were significantly smaller than the preceding teeth in both surface area and thickness. The two metrics, surface area and thickness, show the same trend (Figures 10 and 11), however, the most anterior teeth in both the upper and lower jaws exhibit a slightly higher surface area to thickness ratio, implying that these teeth are slightly more robust than laterally adjacent teeth. These most anterior teeth are likely the teeth first impacting prey during the bite, implying that the enhanced robustness of these teeth will provide advantage to the shark through reduced potential for breakage.

The upper jaw teeth had increased surface area compared to the lower jaw even though they are thinner. This may arise from the fact that the broad upper jaw teeth are better suited to handling and gouging prey (French *et al.*, 2017), while the lower jaw teeth are more effective at penetrating and grasping prey items and so increased thickness could offer greater purchase in prey retention (Whitenack and Motta, 2010), assuming thicker teeth equal stronger teeth. Determining what influences tooth strength would be worthwhile in addition to evaluating dental insertion angles as various tooth types and positions along the jaw can have different insertion angles affecting tooth role, e.g., grasping or puncturing (Lucifora *et al.*, 2001). An accompanying review of feeding

sequences to highlight jaw gape and manipulative capability should allow determination of which teeth are used in what part of prey capture and consumption, as well as determining how dental insertion angles or gape impacts *C. leucas* foraging ability.

4.3. *Ontogenetic niche shift*

Stable isotope analyses can identify occupied niches by analysing biological material and assessing the proportion of natural tracers in assimilated energy (Shiffman *et al.*, 2012; Tamburin *et al.*, 2019). Small *C. leucas* are known to reside primarily in the upper reaches of estuarine habitats and gradually move towards the marine realm as they grow, 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). Using fast turnover tissues (e.g. liver) can accurately determine an organisms trophic position, reflecting its most recent diet and, therefore, space and resource use at time of capture (Niella *et al.*, 2021). Niella *et al.* (2021) sampled the same neonate and juvenile specimens analysed in this study and found significant ontogenetic shifts in niche use along the Clarence River nursery area. A gradual shift in δ^{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 δ^{15}N and δ^{34}S (Niella et al. 2021), 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, associated with increases in use of saline environments (δ^{13}C), higher trophic level (δ^{15}N) and the contribution of more pelagic food items (δ^{34}S) (Hussey *et al.*, 2012; Shiffman *et al.*, 2012).

4.4. *Conclusions and future research*

Due to sample limitations, the sub-adult life stage of *C. leucas* could not be assessed and therefore I can only infer the tooth morphology for this age group. Investigations which examine ontogenetic shifts ideally should aim to assess all stages of development. Given the results of this study one can ascertain sub-adult *C. leucas* have a different tooth shape to the neonate/ juvenile sharks. It would be worthwhile to investigate other morphological features not available here which may contribute to *C. leucas* niche change and so identify other causal factors. Ontogenetic changes to head and caudal fin morphology with ecological consequences are evident in the tiger shark *Galeocerdo cuvier* Péron & Lesueur 1822 (Fu *et al*., 2016), and so other aspects of *C. leucas* body form may change with ontogeny and offer an increased competitive or predatory advantage.

However, my study did comprise the age classes when the most noticeable niche shifts occur in the species thus providing important results.

This study made use of already dead specimens but could be applied to living animals following French *et al*., (2017) who took photographs of live *C. carcharias* during cage diving and later obtained morphometric measurements of teeth using ImageJ. A combination of EFA and adapting the data acquisition of French *et al*., (2017) may make it possible to describe shark dentition without capturing sharks and so eliminate capture mortality in highly susceptible species, such as the great hammerhead shark *Sphyrna mokarran* Rüppell 1837 (Ellis *et al.*, 2017; Gallagher *et al.*, 2014; Gulak *et al.*, 2015). However, this approach is likely to be significantly less robust, but it may still have limited application in areas with global shark ecotourism and for endangered species.

EFA is recommended for investigations of tooth morphology as doing so would not only increase the rate at which research could be performed, but also increase the accuracy with EFA capable of describing 99.9% of an object outline while reducing bias from intra-observer variation (Cullen and Marshall, 2019). Supplementary morphometrics can be used in conjunction with EFA if tooth size cannot be determined when scaling of images occurs or the inability to obtain lateral view images, as seen here.

This study conclusively 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 developmental timing of ontogenetic shifts in niche (Niella *et al.*, 2021). Dentition and diet are inextricably linked. The size of sharks where this step change occurred may be region specific and care is needed if extrapolating to other coastlines, countries or continents where the rates of development may differ (Harry *et al*., 2011; Lombardi-Carlson *et al*., 2003; Tanaka *et al*., 2011). Future research is necessary to expand the knowledge on Carcharhiniform feeding apparatus in order to address the gaps identified by this research. For example, future research could include the effects that gape might have upon *C. leucas* ability to consume larger prey. Observations of teeth appearing to become less translucent and less broken with age suggest potential ontogenetic structural change. Future research into dental structure of shark teeth, e.g. thicker enamel in older animals, will provide further insight into ontogenetic changes in shark foraging strategies.

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Supporting Information

Table S1. Analysis of Variance of the Elliptic Fourier Analysis harmonic coefficients produced from the preliminary photographs including the effects of the variables jaw position (lower left, lower right, upper left and upper right) and an interaction between tooth number (Tooth.n) and round of photography (photo.n).

	Degrees of freedom	Sum of squares	Mean squares	F-value	p-value
Jaw position	3	0	0	0	1
Tooth.n:photo.n	51	0	0	0	1
Residuals	606370	606025	0.9994		

Table S2. Stepwise variable selection procedure for the Generalised Linear Model of biological traits influencing the traditional morphometric approach of *Carcharhinus leucas* tooth morphology. L_T = total length, Tooth.n = tooth number and Position = Jaw position. Included are all candidate models and their respective AIC, delta AIC (ΔAIC = model AIC – lower AIC) and AIC weights (wAIC). Significance levels of the Analysis of Variance between previous nested model and the new variable to be included are also included for each step (p-value).

Model	AIC	ΔAIC	wAIC	p-value
Null	27246.73	1357.35	<0.0001	
L_T	26756.46	867.08	<0.0001	
Tooth.n	26211.29	321.91	<0.0001	<0.0001
Position	27181.32	1291.94	<0.0001	
Tooth.n x L_T	25724.03	81.42	<0.0001	<0.0001
Tooth.n x L_T + Position*	25599.35	0	0.9999	<0.0001

*Final model

Table S3. Stepwise variable selection procedure for the Generalised Linear Model of biological traits influencing the Elliptic Fourier Analysis approach of *Carcharhinus leucas* tooth morphology. L_T = total length, Tooth.n = tooth number and Position = Jaw position. Included are all candidate models and their respective AIC, delta AIC (ΔAIC = model AIC – lower AIC) and AIC weights (wAIC). Significance levels of the Analysis of Variance between previous nested model and the new variable to be included are also included for each step (p-value).

Model	AIC	ΔAIC	wAIC	p-value
Null	23585.63	790.77	<0.0001	
L_T	22847.86	53	<0.0001	<0.0001
Tooth.n	23543.4	748.54	<0.0001	
Position	23577.82	782.96	<0.0001	
L_T x Position	22852.29	57.43	<0.0001	<0.0001
L_T x Position + Tooth.n*	22794.86	0	0.9999	<0.0001

*Final model

Table S4. Stepwise variable selection procedure for the Generalised Additive Mixed Model (GAMM) of biological traits influencing the Elliptic Fourier Analysis approach of *Carcharhinus leucas* tooth morphology. L_T = total length, Tooth.n = tooth number and Position = Jaw position. Included are all candidate models and their respective AIC, delta AIC (ΔAIC = model AIC – lower AIC) and AIC weights (wAIC). Significance levels of the Analysis of Variance between previous nested model and the new variable to be included are also included for each step (p-value). All iterations of the GAMM utilised the shark identification number as a random effect.

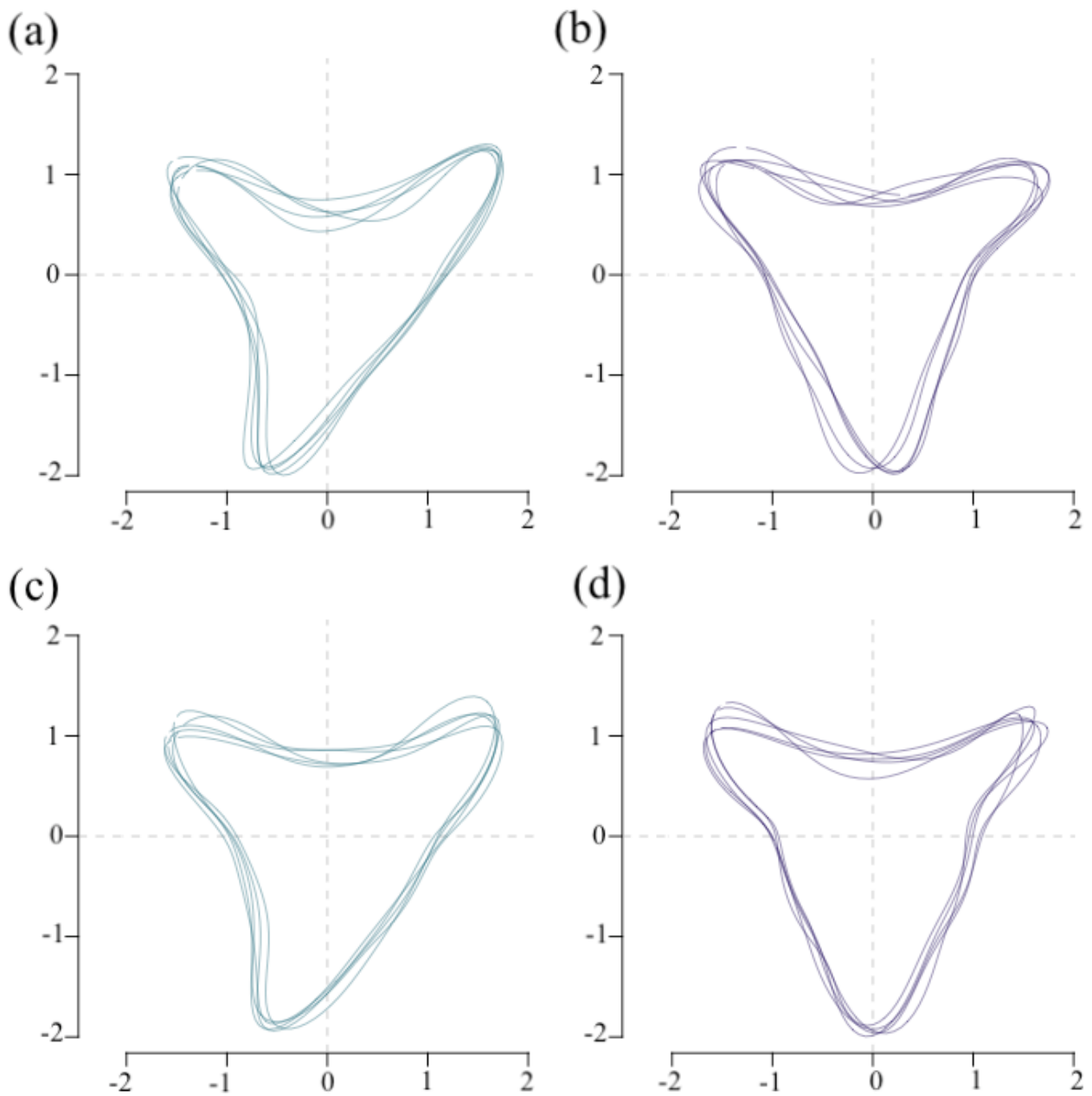| Model	AIC	ΔAIC	wAIC	p-value
Null	200187.9	2269.6	<0.0001	
L_T	200183.2	2264.9	<0.0001	
Tooth.n	199920.8	2002.5	<0.0001	
Position	198827.9	909.6	<0.0001	<0.0001
Position + Tooth.n	197922.8	4.5	0.0953	<0.0001
Position + Tooth.n x L_T *	197918.3	0	0.9046	<0.0001

*Final model

Figure S1. Individual raw centred and scaled tooth outlines from *Carcharhinus leucas* jaws obtained with Elliptic Fourier Analysis. Upper jaw tooth 9 (green) and 1 (purple) for each of five sharks for the neonate category (a – b) and adult category (c – d) highlighting the slight increase in robustness with age.
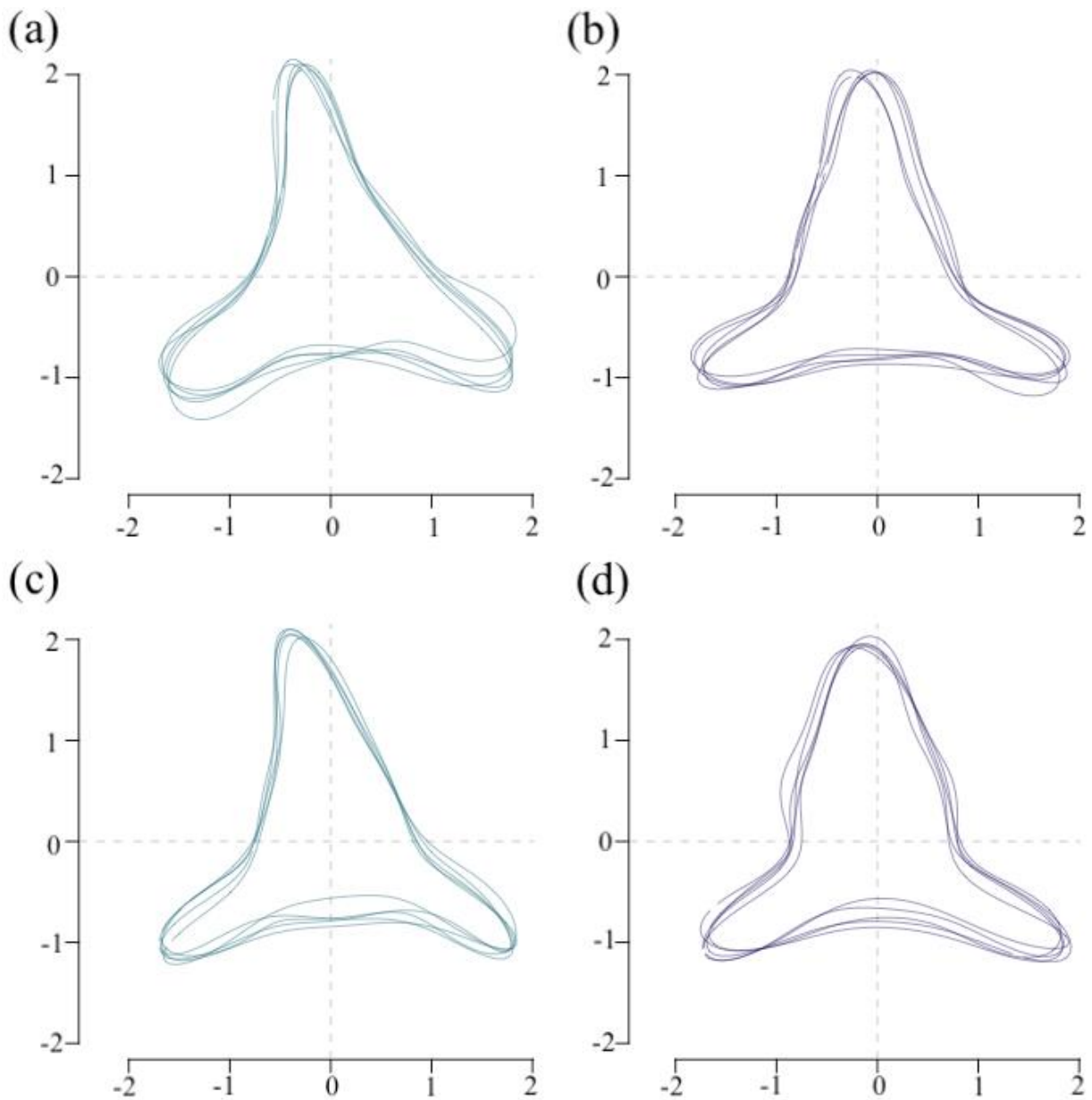
Figure S2. Individual raw centred and scaled tooth outlines from *Carcharhinus leucas* jaws obtained with Elliptic Fourier Analysis. Lower jaw tooth 9 (green) and 1 (purple) for each of five sharks for the neonate category (a – b) and adult category (c – d) highlighting the slight increase in robustness with age.