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Correlation between increased postpubertal phallic growth and the initiation of cranial sexual dimorphisms in male Morelet's crocodile

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Abstract

While puberty is an animal commonality, little is known of its timing or process in crocodylians. Males copulate with an intromittent phallus that has a distinct glans morphology which directly interacts with the female cloaca, putatively effecting effective semen transfer and ultimately increased fecundity. Here we present, during the Morelet's crocodile lifecycle, a well-defined body length (65 cm snout-vent length) inflection point that marks a subsequent increase of phallic glans growth rates. Putatively, this postpubescent growth produces a copulatory-effective phallus. While not as robust of a trend as snout-vent length, this growth inflection concomitantly begins with a body condition index ($CI = BM/SVL^3$) between 2.0 and 2.5 and is most distinct above a CI of 2.5. Also, in males, this 65 cm size threshold also aligns with the initiation of more robust growth in caniniform alveoli associated with prominent maxillary and mandibular teeth. This inflection was not observed in females, thus marking a sexual dimorphism that begins to present with the onset of puberty. This bodily manifestation of puberty other than those changes observed in the reproductive tracts is a novel observation for crocodylians and lays a foundation for further study among species of how changing endocrine signaling within sexually maturing males may also influence a broader range of secondary sex characteristics.

KEYWORDS
caniniform, crocodile, glans, phallus, puberty, sexual dimorphism

1 | INTRODUCTION

Puberty is a profound hormonal and physical transformation into a mature sexual phenotype that impacts almost every species (Chakradhar, 2018). Crocodylians are long lived and undergo a prolonged puberty after an immature stage lasting several years (Briggs-Gonzalez et al., 2017). For example, in South Carolina, USA wild male American alligators (*Alligator mississippiensis*) mature at approximately 12 years of age (Wilkinson, Carter, Leone, Woodward, & Rainwater, 2016), but among crocodylians the specific age for individual maturation is species-specific and greatly dependent on resource availability and

abiotic factors, such as climatic temperature (Da Silveira, Campos, Thorbjarnarson, & Magnusson, 2013; Wilkinson & Rhodes, 1997; Wilkinson et al., 2016).

Little is known about the timing, control, and results of the endocrine and phenotypic changes associated with crocodylian puberty (Ball & Wade, 2013; Grigg & Kirshner, 2015). As with other vertebrates, gonadotropin-releasing hormone and gonadotropins (follicle-stimulating hormone [FSH], luteinizing hormone) control the crocodylian hypothalamic–pituitary–gonadal axis (Lance, 1989). Among crocodylians, this neuroendocrine maturation process has been most characterized in alligators. Four-year-old alligators

respond to exogenous FSH treatments with increased circulating testosterone concentrations (Edwards, Gunderson, Milnes, & Guillette, 2004). Within this experimental group, the stimulatory response was greater in males larger than 58 cm snout-vent length (SVL), thus body condition has a correlation with puberty onset. Immature male alligators begin to show seasonal androgen cyclicity at ~45–50 cm SVL, but at lower concentrations than observed in mature adults (Guillette, Pickford, Crain, Rooney, & Percival, 1996; Hamlin, Lowers, & Guillette, 2011; Lance, Elsey, & Trosclair, 2015) and these periodic hormonal pulses increase each season in proportion with body size increases, thus suggesting a slow and gradual sexual maturation process.

Chronic injections of crude pituitary extracts or androgens into immature male and female alligators (Forbes, 1934, 1937, 1939) and testosterone propionate into male mugger crocodiles (*Crocodylus palustris*; Ramaswami & Jacob, 1965) result in profound penile/clitoral hypertrophy. Thus, immature external genitalia are competent to respond to endocrine signals and await puberty neuroendocrine maturation with the associated increases in androgenetic and other proliferative signals. Taken together, we can posit that gonadotrophin-stimulated increases in circulating androgens result in increased peripubertal male alligator penis size, and these seasonal incremental increases begin modestly, then intensify in older juveniles with greater seasonal androgen exposures. While some endocrine aspects of crocodylian puberty have been the subject of limited research, no attention has yet been paid to changes in male genital morphology during this time period.

Male crocodylians use a phallus (penis) as an intromittent organ to place sperm in proximity of the female reproductive tract (Kelly & Moore, 2016). During copulation, the phallus everts from the male cloaca (Kelly, 2013) and enters the female cloaca (Kuchel & Franklin, 2000). The distal phallus has an inflatable, elaborated, and cup-like glans that is the terminus of the gamete-conducting sulcus spermaticus (Johnston et al., 2014; Moore, Spears, Mascari, & Kelly, 2016). Roughly, the glans can be functionally subdivided into the bulbous body with a broad inflatable ridge (alternatively termed cuff) and the more gracile distal tip. Putatively, the globular portion of the glans interacts with the uroprocodeal fold of the female cloaca, anchoring its placement during intromission, thus achieving a copulatory lock and also acting to exclude external water from the site of insemination (Johnston et al., 2014). The distal tip extends the sulcus spermaticus and putatively focuses or directs semen release to maximize transfer efficiency and ultimately increase the chance of fertilization (Moore & Kelly, 2015). These prominent structures are critical to male fertility, develop via androgenic signaling, and therefore their morphologies are appropriate markers for the puberty initiation.

Among vertebrates, rising androgen exposure not only stimulates male genital morphology, but its circulation throughout the body also can influence the growth of a wide range of nonreproductive tissues. These changes in relative, postpubescent, somatic growth can manifest as changes in the size and shape of the skull and body in males compared with females, ultimately leading to sexual dimorphism.
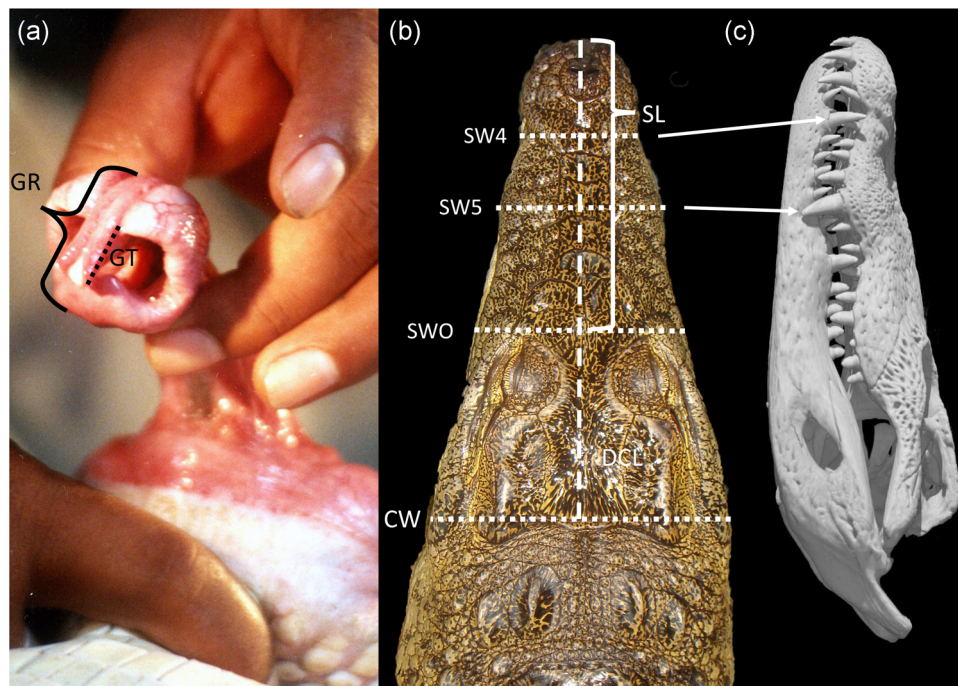

FIGURE 1 Morphometric measurement landmarks of Morelet's crocodile used in this study. (a) Male phallus: height of the glans ridge (GR), length of the glans tip (GT). (b) Dorsal view of head: cranial width (CW), dorsal cranial length (DCL: vertical dashed line), snout length (SL: defined by bracket), snout width anterior to the orbits (SWO), snout width at the 5th maxillary tooth (SW5), and snout width at the 4th mandibular tooth (SW4). (c) Computed tomography scan of *Crocodylus moreletii* skull (Dr. Jonathan R. Wagner, 2005, "*Crocodylus moreletii*," http://digimorph.org/specimens/Crocodylus_moreletii/) showing the corresponding tooth morphologies at the SW5, and SW4 landmarks [Color figure can be viewed at wileyonlinelibrary.com]

Sexual size dimorphism is known in several crocodilian species including American alligators (*Alligator mississippiensis*; Wilkinson & Rhodes, 1997) and Nile crocodile (*Crocodylus niloticus*; Detoeuf-Boulade, 2006; Platt et al., 2009). However, only a few studies have identified sexual dimorphism in crocodilian head size and shape (e.g., rostrum of gharials (*Gavialis gangeticus*; Hall & Portier, 1994); skull length and width in *Crocodylus moreletti*, (Barrios-Quiroz, Casas-Andreu, & Escobedo-Galván, 2012); interfrontal width of estuarine crocodiles (*C. porosus*; Webb & Messel, 1978); holistic skull shape of black caiman (*Melanosuchus niger*; Foth, Bona, & Desojo, 2015), whereas other morphometric studies of skull shape simply were not tested for dimorphism (e.g., Monteiro, Cavalcanti, & Sommer, 1997) or offered diet-influenced biomechanical adaptationist hypotheses to explain allometric changes in craniodental features (Blanco, Cassini, & Bona, 2018) rather than sexual selection. Although Barrios-Quiroz et al. (2012) reported allometric changes in snout shape particularly in the largest size class of captive *C. moreletii*, no study has sought to correlate pubescent endocrinology and genital development with the potential onset of cranial dimorphism in this or any other species of crocodilian.

In this study, we examined signs/indicators of puberty through measures of genital morphology in wild *C. moreletii*, a moderate-sized, freshwater crocodilian found in the Atlantic and Caribbean lowlands of Mexico, Guatemala, and Belize (Platt, Sigler, & Rainwater, 2010). In addition, to better explore signals of sexual dimorphism, we also investigated morphological correlations with a series of cranial measurements.

2 | MATERIALS AND METHODS

Crocodiles were captured and measured from two sites in northern Belize, Gold Button Lagoon (17°55′N, 88°45′W) and the New River Watershed (17°42′N, 88°38′W; ~23 km long), two of largest Morelet's crocodile populations in the country (Platt & Thorbjarnarson, 2000). From April through September, 1998–2000, crocodiles were hand- or noose-captured at night from a boat under permit from the Belize Ministry of Natural Resources. Measures of total length (TL; measured ventrally), SVL (measured ventrally from the tip of the snout to the anterior margin of the cloaca), and body mass (BM) were obtained from each animal (Platt et al., 2009). Sex was determined by cloacal examination of the genitalia (Allsteadt & Lang, 1995; Ziegler & Olbort, 2007). In *C. moreletii*, phalli of males are substantially larger than female clitori, as with other crocodilians. The vertical diameter of the male glans from the ventral surface to the dorsal ridge at its midpoint (hereafter glans ridge) and the length of the glans tip from its origin on the ventral glans to the distal edge of the tip on the lateral surface (hereafter glans tip; Figure 1a) were measured to the nearest 0.1 mm using a dial caliper with needle tips (see Guillette et al., 1996; Pickford, Guillette, Crain, Rooney, & Woodward, 2000). Each measurement was taken in triplicate, and a mean value was used in subsequent analyses. A single researcher (T. R. R.) took all measurements to minimize and standardize measurement error (Guillette et al., 1996). For a subset of the males (n = 110),

TABLE 1 Body morphometrics of Morelet's crocodiles of this study

Size metric	Male	Female
SVL (cm)	$\bar{x}$ = 57 (range: 17.9–148)	$\bar{x}$ = 50 (range: 16.9–100)
TL (cm)	$\bar{x}$ = 115 (range: 38–299)	$\bar{x}$ = 104 (range: 36–199)
Body mass (kg)	$\bar{x}$ = 86.6 (range: 0.9–70.7)	$\bar{x}$ = 53.6 (range: 10.3–31.7)

Abbreviations: SVL, snout-vent length; TL, total length.

measures of cranial width (CW), dorsal cranial length (DCL), and snout length (SL) were collected as described by (Platt et al., 2009; Figure 1b,c) as well as measures of snout width at three landmarks: anterior orbits (SWO), 5th maxillary tooth (SW5), and 4th mandibular tooth (SW4). Following data collection, crocodiles were marked and released at their respective capture sites.

Statistical analyses aimed to discriminate pre- from postpubescent males. A succession of two-phase linear regressions of glans tip
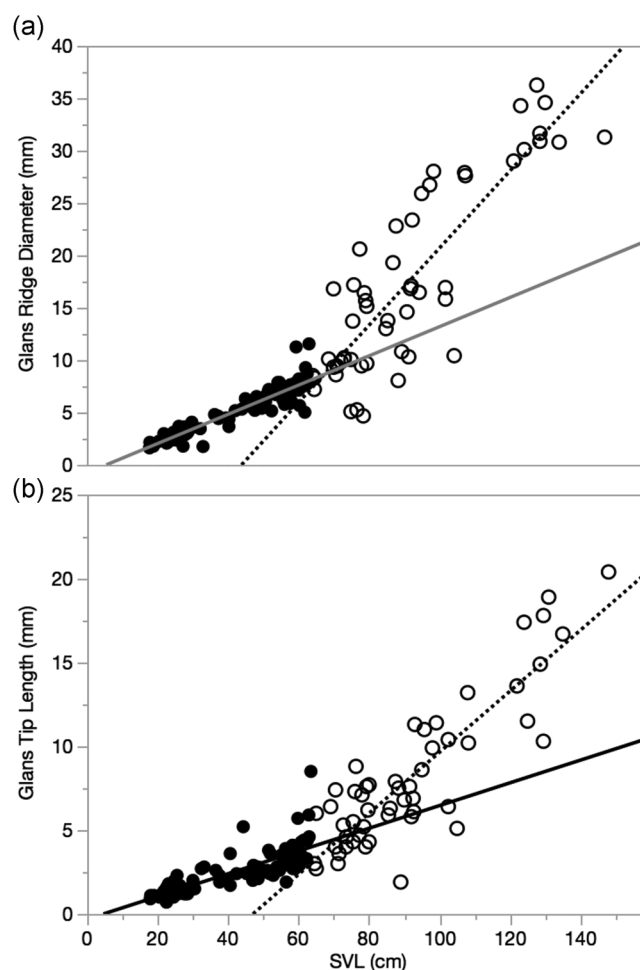

FIGURE 2 Scatterplot and biphasic linear regressions for phallic glans measurements of male Morelet's crocodiles with snout-vent lengths less than 65 cm (filled circles/solid line) and greater than 65 cm (open circles/dotted line). (a) Glans ridge diameter. (b) Glans tip length. SVL, snout-vent length

and ridge measurements to SVL were fit to the data wherein the intersection point was shifted along the SVL continuum with consecutive readings. The best two-phase fit was achieved for each phallic measurement at the regression line intersection that produced the minimum residual sum of squares via analysis of covariance (ANCOVA), so the biphasic breakpoint of the two separate linear regressions showed heterogeneous slopes (Eme et al. 2019; Snelling, Taggart, Maloney, Farrell, & Seymour, 2015).

The identified SVL breakpoint agreed between the two phallic measure analyses and was subsequently utilized in further two-phase linear regression analyses of Fulton's K body condition index (BM/SVL³), as previously applied to Morelet's crocodiles (Mazzotti et al., 2012) and cranial data. Alternately, captured females did not present an observable genital sign of puberty initiation. Lacking an overt external sign to use for cranial statistical tests, we used the male breakpoint as a proxy for female statistical analyses with a clear caveat of the ad hoc nature of this analysis. Unequal variance t tests were used to compare body condition indices between size classes.

3 | RESULTS

A total of 153 male and 65 female Morelet's crocodiles was examined in this study. The crocodiles sampled ranged from small juveniles to reproductive adults (Table 1). The breakpoint analyses for both male glans ridge diameter and tip length yielded equivalent minimum residual sum of squares between 65 and 70 cm SVL, of which a breakpoint of 65 cm SVL was chosen for subsequent two-phase linear regression analyses of smaller (<65 cm) and larger (>65 cm) crocodile body condition indices and head dimensions. This size class assignment is a proxy for smaller, prepubescent male crocodiles before initiation of external genital maturation and larger, postpubescent animals after the initiation of external genital maturation.

Both phallic glans ridge and tip measures showed biphasic scaling with SVL (Figure 2a,b, respectively and Table 2). For each measure, animals larger than the breakpoint displayed positive linear regression slopes that were roughly double than those of smaller animals.

TABLE 2 From linear regressions ($y = b$ (SVL) + intercept) represented in Figures 2–4: ANCOVA pairwise slope comparisons for each size group (small: >65 cm SVL, large: <65 cm)

Independent	Dependent	Group	Pairwise slope comparison	r^2	Int.	b	F	p value
Males								
SVL	Glans ridge	Small	<0.001**	0.87	−0.79	0.14	$_{1,99}$ 640.9	<.001**
		Large		0.71	−16.5	0.37	$_{1,49}$ 124.7	<.001**
SVL	Glans tip	Small	<0.001**	0.65	−0.37	0.07	$_{1,99}$ 185.4	<.001**
		Large		0.76	−9.21	0.19	$_{1,49}$ 141.4	<.001**
SVL CI	Glans ridge	Small	0.020*	0.18	−2.17	3.34	$_{1,99}$ 21.6	<.001**
		Large		0.33	27.8	17.50	$_{1,49}$ 23.1	<.001**
SVL CI	Glans tip	Small	0.002**	0.10	−0.74	1.51	$_{1,99}$ 12.6	<.001**
		Large		0.22	−9.16	6.64	$_{1,49}$ 14.9	.003**
SVL	Dorsal cranial length/cranial width	Small	0.371	0.42	2.21	0.004	$_{1,70}$ 52.1	<.001**
		Large		0.43	2.20	0.004	$_{1,36}$ 27.3	<.001**
SVL	Snout length/dorsal cranial length	Small	0.001**	0.37	0.45	0.003	$_{1,70}$ 41.2	<.001**
		Large		0.12	0.61	<0.001	$_{1,36}$ 4.8	.034*
SVL	Snout length/snout width at anterior orbital	Small	0.030*	0.05	1.91	0.002	$_{1,70}$ 3.5	.065
		Large		0.49	1.56	−0.004	$_{1,36}$ 34.5	<.001**
SVL	Snout length/snout width at 5th maxillary tooth	Small	0.027*	0.43	1.67	0.006	$_{1,70}$ 53.3	<.001**
		Large		0.46	2.41	−0.005	$_{1,36}$ 30.1	<.001**
SVL	Snout length/snout width at 4th mandibular tooth	Small	0.001*	0.33	2.60	0.010	$_{1,70}$ 35.1	<.001**
		Large		0.27	3.69	−0.006	$_{1,36}$ 13.4	<.001**
Females								
SVL	Dorsal cranial length/cranial width	Small	0.905	0.17	−0.004	2.18	$_{1,41}$ 25.1	<.001**
		Large		0.37	−0.004	2.17	$_{1,19}$ 3.8	.064
SVL	Snout length/dorsal cranial length	Small	0.001**	0.87	0.48	0.002	$_{1,41}$ 280	<.001**
		Large		0.031	0.56	0.001	$_{1,19}$ 8.3	.009*
SVL	Snout length/snout width at anterior orbital	Small	0.119	0.03	1.51	0.002	$_{1,41}$ 2.47	.124
		Large		0.02	1.89	0.002	$_{1,19}$ 1.34	.260
SVL	Snout length/snout width at 5th maxillary tooth	Small	0.021*	0.25	1.66	0.005	$_{1,41}$ 14.7	<.001**
		Large		0.02	2.17	0.001	$_{1,19}$ 0.47	.499
SVL	Snout length/snout width at 4th mandibular tooth	Small	0.074	0.39	2.60	0.009	$_{1,41}$ 27.4	<.001**
		Large		0.01	3.04	0.001	$_{1,19}$ 0.13	.718

Note: For each of the two biphasic linear regressions: coefficient of determination (r^2), line intercept (Int.), line slope (b), and ANOVA results for each linear regression.

Abbreviations: ANCOVA, analysis of covariance; CI, condition index; SVL, snout-vent length.
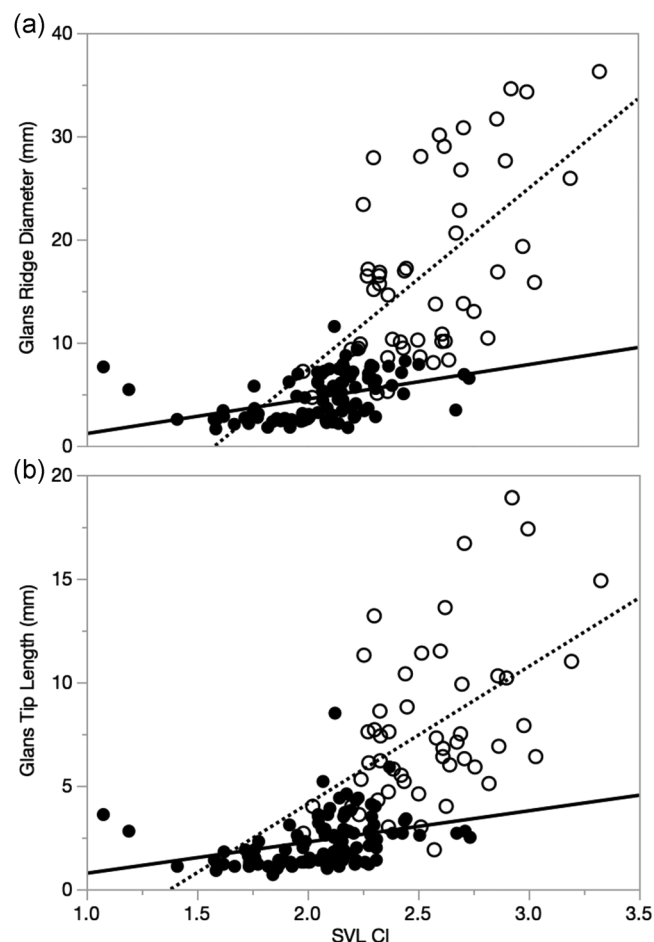
*$P \leq 0.05$
**$P \leq 0.01$

FIGURE 3 Scatterplot and biphasic linear regressions for phallic glans measurements for male Morelet's crocodiles with snout-vent length condition indices (BM/SVL^3) calculated from animals with snout-vent lengths less than 65 cm (filled circles/solid line) and greater than 65 cm (open circles/dotted line). (a) Glans ridge diameter. (b) Glans tip length

The coefficient of determination in all linear regressions explained between 65% and 87% of the variation (Table 2).

Regression of phallic measures by SVL body condition indices yielded similar significance, though with greater variance (Figure 3a,b, respectively and Table 1). The crocodiles with SVL larger than the 65 cm breakpoint had greater condition indices than those with SVL smaller than the breakpoint ($\bar{x}$ = 2.6, standard deviation [SD] = 0.29; $\bar{x}$ = 2.1, SD. The linear re- = 0.28, respectively; t (92.7) = −10.1, p < .001) gression slopes of the larger animals were ~4–5 times steeper than those of the smaller animals; however, they explain a lesser amount of observed variance with coefficients of determination of 22% and 33% (Table 2). Linear regression lines of smaller animals only explained 18% and 10% of the observed variance.

Analysis of male and female cranial measurements yielded differing patterns than those observed for male phallic measures. Assessing the DCL:CW ratio across SVL sizes and sexes yielded significant negative slope regression lines for both crocodile size classes, but no heterogeneity between the regression lines

(Figure 4a,b and Table 2). In contrast, assessing the SL:DCL ratio by SVL yielded differing regression lines by size classes for both male and female crocodiles (Figure 4c,d and Table 2). Males of the small size class displayed a steep positive slope explaining 37% of the variance, as compared with the larger size class which exhibited a regression line approximately threefold less steep and describing only 12% of variance. Additionally, smaller size class females displayed a steep positive slope explaining 87% of the variance, as compared with the larger size class females which exhibited a weaker regression line approximately one-half as steep and describing only 3% of variance.

Analysis of the interaction of SVL and SL ratios at the three width landmarks (SL/SWO, SL/SW5, SL/SW4) yielded sexually dimorphic trends. For all width landmarks, large male crocodiles showed negative linear regression slopes that explained between 27% and 49% of the variation (Figure 5a,c,e and Table 2). In contrast, small crocodiles displayed a positive linear regression slope for this ratio when utilizing SW5 and SW4 landmarks, but not the SWO landmark. These comparisons explained 33% and 43% of variance in small crocodiles, respectively. Comparing heterogeneity of slopes for each regression pair, the SW4 and SW5 regression lines differed between small and large crocodiles, but not when incorporating the SWO landmark.

For width landmarks, large female crocodiles did not show significant negative linear regression relationships (Figure 5b,d,f and Table 2). In contrast, small female crocodiles displayed a positive linear regression slope for this ratio when utilizing SW4 and SW5 landmarks, but not the SWO landmark. These comparisons explained 39% and 25% of variance, respectively.

4 | DISCUSSION

Our study examined male genital size measures as an indicator of crocodylian puberty initiation. We utilized wild Morelet's crocodile populations in northern Belize over a 3-year period with all the inherent genetic, biotic–biotic interaction, and dynamic environmental stress variabilities (Bundy, Davey, & Viant, 2009; Milnes & Woodward, 2001; Orlando, 2001; Serna-Lagunes et al., 2010). Despite these heterogeneous conditions, we observed evidence of an ~65 cm SVL (~130 cm TL) threshold for the initiation of male genital maturation. Above this size, the growth rate for two key glans features tied to reproductive competence markedly increases, though with individual variability. These changes in male genital morphology corresponded with the onset of hypertrophied craniodental features not found in females indicating sexual dimorphism in skull shape. While females lacked clear morphological puberty cues during field measurements, previous observation had identified the smallest nesting female of approximately 75 cm SVL (Platt, Rainwater, Thorbjarnarson, & McMurry, 2008). Therefore, the projected female initiation of puberty around 65 cm SVL is likely.

Assessing these two phallic measures against a condition index yielded similar results, albeit with increased variation around the
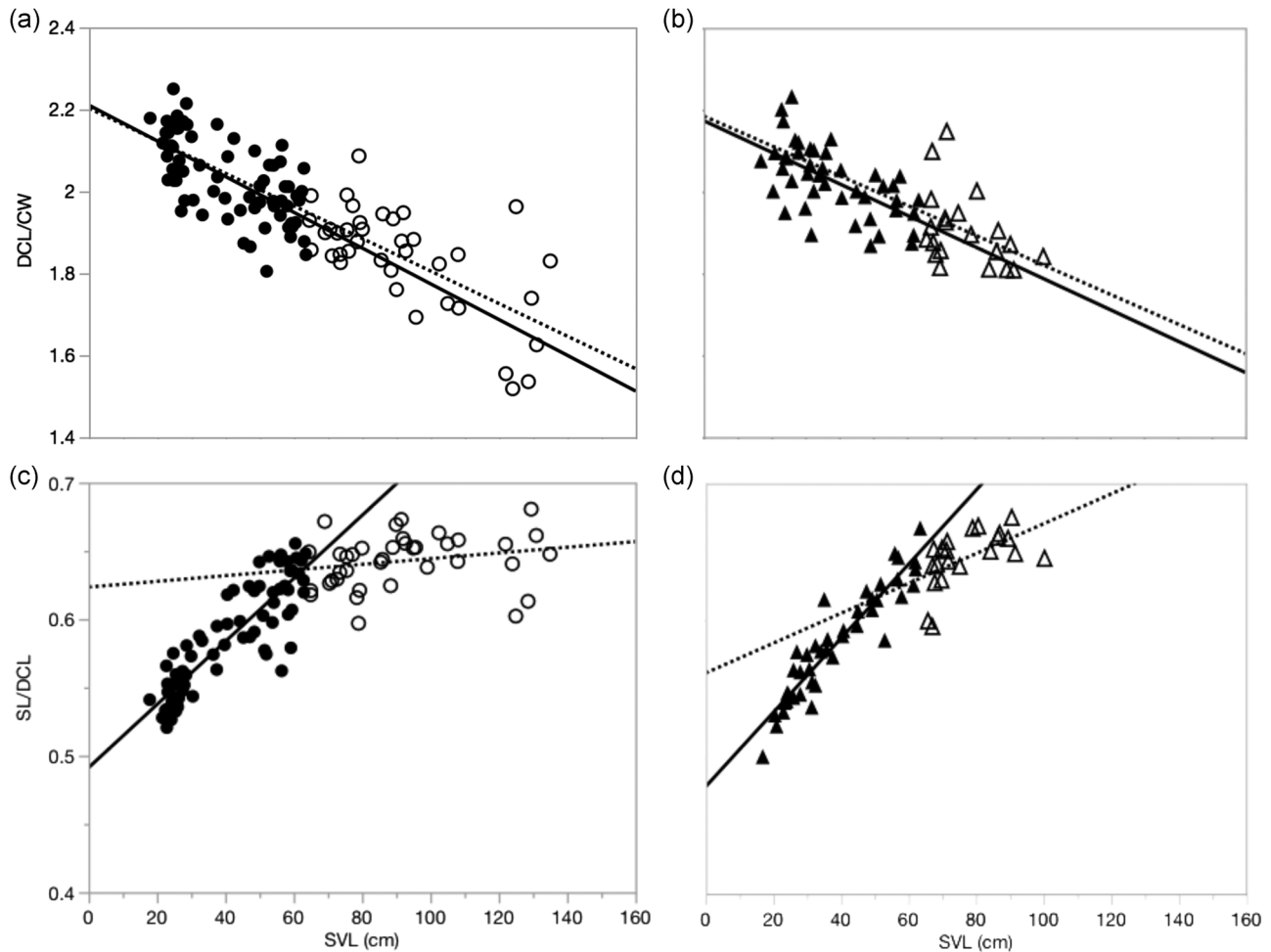
FIGURE 4 Scatterplot and biphasic linear regressions of head measurement ratios for male (circles) and female (triangles) Morelet's crocodiles with snout-vent lengths less than 65 cm (filled shapes/solid lines) and greater than 65 cm (open shapes/dotted lines). (a,b) Dorsal cranial length (DCL) divided by cranial width (CW). (c,d) Snout length (SL) divided by dorsal cranial length

central trends as compared to the analysis with the SVL measure alone. We observed that SVL condition indices below 2.0 characterize small immature genitalia, 2.0–2.5 appear to represent a transition zone, and indices above 2.5 almost exclusively indicate animals with more rapidly growing glans. This agrees with a previous study of condition index in wild Morelet's crocodiles (Mazzotti et al., 2012) showing adults have higher condition indices, but a range of external factors impact individuals.

Body condition indices may predict an individual's fitness and denote the ability to allocate excess resources (above maintenance) toward reproduction. However, sometimes energy reserves have been found to be generally independent or weakly dependent on body size (Schulte-Hostedde, Zinner, Millar, & Hickling, 2005) and not entirely indicative of male reproductive capacity (Aubret, Bonnet, Shine, & Lourdais, 2002). Therefore, while at face value, a body condition index could be predicted expected to better predict male puberty than body length, many factors confound these implications and interpretation of the index.

Reconciling our observed differences between the SVL and SVL condition index results is further challenged because the ages of

individual animals are unknown. Aging crocodylians in the wild is difficult and impractical because size variation between similaraged animals can be substantial (Wilkinson et al., 2016). Further, BM, particularly in smaller animals, can fluctuate markedly with periodic feeding.

In addition to sexual dimorphism in *C. moreletii* body size (Platt et al., 2009), our measurements of skull shape change across puberty revealed more evidence of sexual dimorphism. Similar to changes in the robusticity of caniniform teeth and other cranial features in mature male *C. novaeguineae* (Hall & Portier, 1994), we found that male *C. moreletii* also develop larger, more robust maxillary and mandibular caniniform teeth relative to those of female crocodiles, as shown in our measurements across caniniform alveoli (SW4, SW5) relative to measurements of DCL and CW.

Like in other crocodylians (e.g., Hall & Portier, 1994), male–male *C. moreletii* interactions, including social ordering, territoriality, and access to breeding females may favor larger male individuals with more robust teeth and heads, making them targets for sexual selection. Larger head size in crocodylians has been shown to correlate with higher bite forces and thus access to a greater
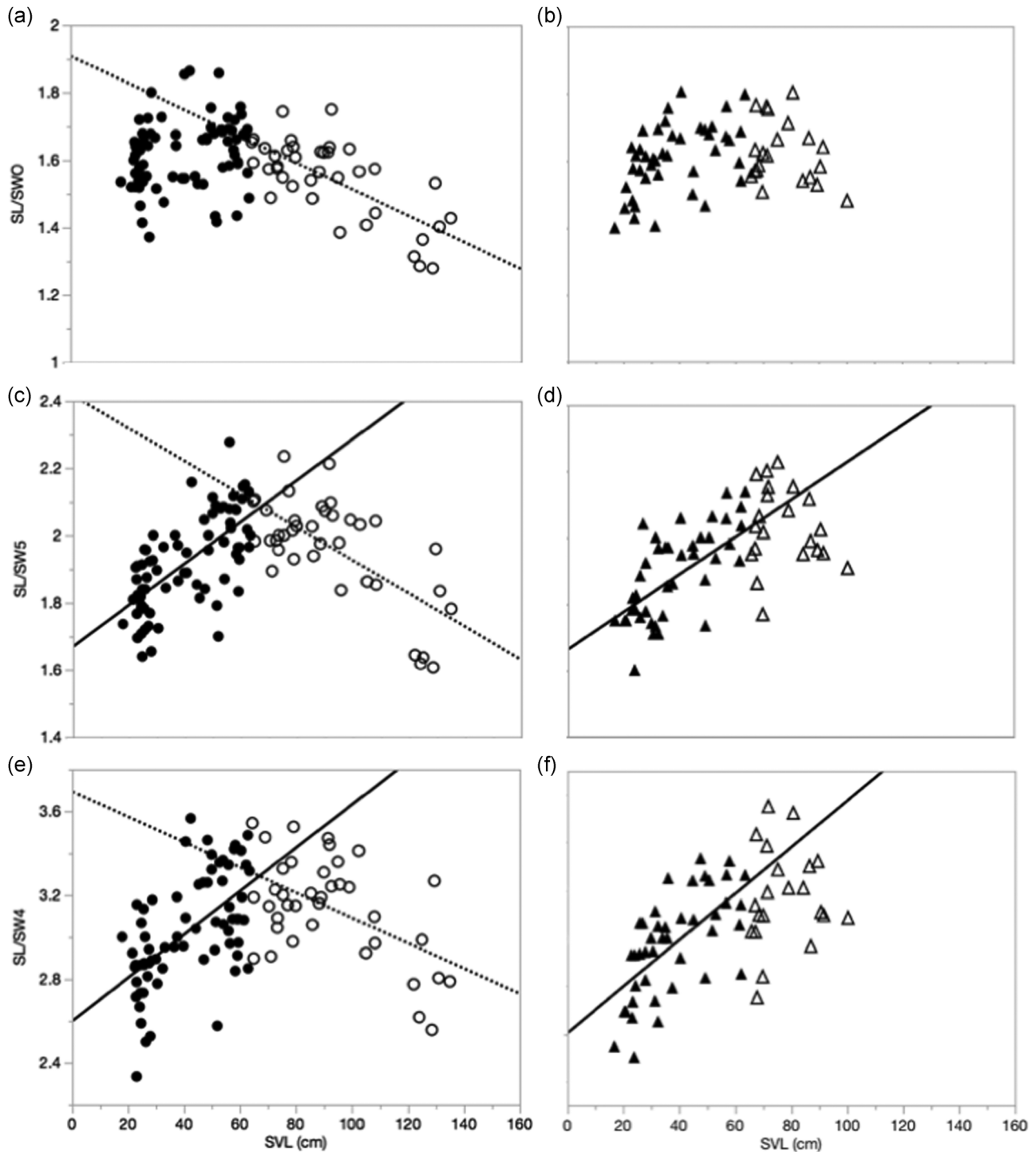
FIGURE 5 Scatterplot and biphasic linear regressions of head measurement ratios for male (circles) and female (triangles) Morelet's crocodiles with snout-vent lengths less than 65 cm (filled shapes/solid lines) and greater than 65 cm (open shapes/dotted lines). Snout lengths (SLs) divided by snout widths at (a) anterior orbits (AO), (b) 5th maxillary tooth (SW5), and (c) 4th mandibular tooth (SW4). SVL,

diversity of prey items (Erickson et al., 2003); however, these performance feats may simply be a byproduct of other selective pressures, for example, male-to-male combat (Rainwater et al., 2011). Although sexual dimorphism in cranial shape in crocodylians has only been previously reported in *C. novaeguinea*, it is possible that other species are similarly dimorphic. Foth et al. (2013) found

putative differences in cranial shape between sexes of *M. niger*, but shied away from assertion given the nature of their landmark data. Indeed, cranial shape landmarks, measurements, and their analytical framework (e.g., general Procrustes analysis, Euclidean distance matrix analysis) vary considerably among studies of extant crocodilian intraspecific head shape (Foth et al., 2015; Pearcy &

Wijtten, 2011; Sadleir & Makovicky, 2008) muddying signals of cranial shape dimorphism.

Other phylogenetic analyses of crocodyliform head shape also eschew testing for sexual dimorphism in head shape, instead offering tests and narratives of heterochronic shifts, dietary niche occupation or integration without testing for the effects of sex in their samples (e.g., Erickson et al., 2012; Gignac & O'Brien, 2016; Piras et al., 2014). Thus, we may be potentially missing signals of sexual dimorphism in other species of crocodylians and their extinct ancestors. New approaches to analysis of cranial shape in living and fossil crocodylians including the realities of pre-and postpubertal somatic conditions will help elucidate the distribution of cranial shape sexual dimorphism in the clade.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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