The Utility of Captive Animals in Actualistic Research: A Geometric Morphometric Exploration of the Tooth Row of *Alligator mississippiensis* Suggesting Ecophenotypic Influences and Functional Constraints

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ABSTRACT

Captive broad snouted crocodylians are generally thought to have wider, shorter rostra than their wild counterparts. Interpreted to reflect morphological change in response to the conditions of captivity, this qualitative pattern could affect the utility of these animals in a variety of fields of research. However, due to relative ease of access and availability of life history data, captive animals are often utilized in actualistic research. Thus, this issue should be addressed in more detail. Here we explore snout shape variation between captive and wild members of *Alligator mississippiensis* using two-dimensional (2D) morphometric techniques. Several landmark schemes are used to assess the utility of different aspects of morphology in distinguishing the groups. While statistical analyses consistently differentiated between the groups, the area of morphospace occupied by wild members of *A. mississippiensis* generally overlapped with the larger area encompassing the captive specimens. This indicates that the captive condition is not as uniform as previously thought and instead encompasses a large spectrum of morphologies, ranging from the stereotypical broad, shortened snouts to outlines that are indistinguishable from the wild morphotype. These results align well with the interpretation that this change reflects an extreme example of ecophenotypy, since ranched, farmed, or zoo organisms are held in an array of enclosures, ranging from indoor, climate controlled pens to outdoor, more natural areas. This variation in environments should be reflected in different reactions to the animals’ surroundings, resulting in a broad spectrum of morphotypes. While wild specimens are still preferred, especially for fine scale analyses, these results indicate that not all captive members of *A. mississippiensis* exhibit the extreme morphological alterations often cited in the literature. Weighing the conditions in which the animals are held and exploring the possibility of morphological differences against the benefits of using captive specimens should be part of any actualistic study.

INTRODUCTION

Captive animals often differ morphologically from their wild relatives (e.g., Hard et al., 2000; McPhee, 2004; Zuccarelli, 2004; O’Regan and Kitchener, 2005; Guay and Iwaniuk, 2008). Differences in behavior (e.g., Flemming et al., 1996; Geiser and Ferguson, 2001; McPhee, 2003), functional performance (e.g., Erickson et al., 2004), and rate and type of pathologies (Fitch and Fagan, 1982; Munson et al., 2005) all have been noted between captive and wild individuals. Even so, animals kept in farms and zoos are regularly used to study a wide variety of topics, from physiology to functional morphology to taphonomy (e.g., Schachner et al., 2013). Within the bite mark literature, captive animals have been used extensively (e.g., Haynes, 1983; Willey and Snyder, 1989; Marenan and Spencer, 1991; Pickering and Wallis, 1997; Njau and Blumenschine, 2006; D’Amore and Blumenschine, 2009; Drumheller, 2012; Drumheller and Brochu, 2014) even though the potential for biases has been recognized in some taxa (Haynes, 1982).

KEY WORDS: Crocodylia; intraspecific variation; ecophenotypy; rostral morphology; captivity
However, captive animals have a variety of characteristics that make them attractive as research specimens. Living organisms held in zoological parks or farms are often more accessible for research. When dealing with threatened or endangered species, these animals may be the only viable source of data. Also, many of these facilities keep long-term veterinary and physiological records of their animals. Some of these variables, such as mass or sex, can be collected in the field, but others, such as exact age and measurements through ontogeny, may be difficult to impossible to collect from wild animals. Furthermore, captive specimens have habitats and routines that include regular human interaction, a potentially complicating factor if this fosters novel behaviors, but one which can make data collection safer for animals and researchers alike. Therefore, if captivity can be shown to have a negligible effect on ensuing data, these animals can prove to be productive sources of information.

Thanks to highly successful conservation efforts and extensive research collections, *Alligator mississippiensis* (Daudin, 1802) is one of the most rigorously studied species of extant crocodylians (Rowe et al., 1999). Therefore, this group is a strong candidate for studying the validity of using captive crocodylians as proxies for their wild—living or extinct—relatives. Among crocodylians, a variety of morphological differences between captive and wild individuals have been cited anecdotally in the literature. Captive *A. mississippiensis* are said to have wider, blunter snouts, teeth that splay outwards (Fig. 1), and larger fat deposits (Erickson et al., 2004). This pattern is generally thought to apply to other generalized and blunt-snouted taxa. Captive tube-snouted crocodylians, such as *Gavialis gangeticus* and *Tomistoma schlegeli*, often exhibit unusual bending along the rostrum, both laterally and dorsally. Previous morphometric analyses of *A. mississippiensis* have also revealed that captive animals exhibit a raised lip along the margins of the skull table, comparatively short basioccipitals and wide lacrimal bones, closely set eyes, and an upturned rim around the orbits (Sadleir, 2009). Prey acquisition behavior among captives does not substantially deviate from that seen in the wild (SKD personal observation, Cleuren and DeVree, 1992, 2000), but bite force performance does differ between captive and wild individuals, becoming more pronounced among older, larger animals, suggesting that this difference may stem from morphological changes driven by the conditions of their disparate environments, i.e., a possible ecophenotypic pattern (Erickson et al., 2004). Such observations raise concerns over the use of farm or zoo animals in research, and some institutions have responded by limiting or avoiding accession and curation of captive specimens (SKD personal observation).

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**Fig. 1.** *Alligator mississippiensis.* (A) FLMNH 61483, captive adult. (B) FLMNH 34886, wild adult. (C) Generalized ‘captive’ morphotype with arrows indicating the regions commonly cited as differing from the wild form. Scale bars = 10 cm.
This study aims to explore the effects of captivity on the morphology of *A. mississippiensis* and assess the potential implications for actualistic research. We utilize 2D geometric morphometric methods to explore differences in tooth placement and snout shape between captive and wild members of *A. mississippiensis*. Several different analytical techniques and landmarking schemes are used to quantify these differences allowing us to assess the potential influence of ecophenotypy and functional constraints.

**MATERIALS AND METHODS**

The specimens used in this study are held in three collections: the American Museum of Natural History (AMNH), Florida Museum of Natural History (FMNH), and a research collection from the Rockefeller Wildlife Station (RWS) held at the Field Museum of Natural History (FMNH). Associated metadata were often limited (i.e., details of animal sex, age, etc. were rarely recorded), so only specimens with known provenance and which lacked extensive pathologies, postmortem damage, and remaining soft tissue obscuring areas of interest were included in the analysis (supplementary online material, Table S1). We took images of the ventral view of each cranium, representing 19 captive and 66 wild individuals, with an Olympus® Stylus brand, model 760 still-image camera. Both camera and specimen were oriented on a copy stand using a two-way level centered on the suture between the specimens’ maxillae to ensure a uniform camera angle during image and data collection.

To capture the morphology of the snout and toothrow, we collected three sets of landmarks: snout outline, alveolar positions, and sutural intersections of the bones of the snout. Images for the analysis were compiled using tpsUTIL, version 1.60 (Rohlf, 2010). We performed landmark and semilandmark digitization in tpsDIG2, version 2.18 (Rohlf, 2010). Snout shape was assessed using a semilandmark approach to outline analysis (Bookstein, 1996, 1997). As employed in this study, semilandmarks are nondiscrete anatomical loci representing a biologically homologous curve within a single species, in this case *A. mississippiensis*. We chose not to slide our semilandmarks for this study (i.e., allow the semilandmarks to move to minimize Procrustes distance or bending energy between individual specimen configurations and the Procrustes reference configuration). The justification for sliding of semilandmarks has been questioned for a number of reasons (see MacLeod, 2013). Currently employed sliding protocols only allow the semilandmark to travel along a line tangent to the outline curve, rather than along the curve itself. Thus, any sliding moves the semilandmark off of the original outline; forcing it away from the actual biological curve we are interested in quantifying (MacLeod, 2013). Additionally, when curves have been well sampled, there is little space between semilandmarks within which they can slide, resulting in only minor differences from the original semilandmark placement (Tennant and MacLeod, 2014). Snout profile outlines were traced from the posterior-most point of the right maxilla, around the right to left premaxillae, to the posterior-most point of the left maxilla of 84 *A. mississippiensis* skulls. We then resampled this curve for 60 equidistant points (Fig. 2). We deemed this resolution sufficient to faithfully represent the outline of these structures based on visual inspection of the digitized specimens in tpsDIG2.

Landmarks selected to assess tooth position included all five premaxillary and the first seven maxillary teeth of the same 84 skulls (Fig. 2). More distal maxillary teeth were not sampled, following the landmark selection scheme outlined by Sadleir (2009), for multiple reasons related to ontogeny. Tooth placement and, to a lesser extent, tooth number vary somewhat through ontogeny. More importantly, in young individuals, alveoli are often not differentiated distal to the seventh maxillary tooth, making accurate landmark placement problematical. The center of the socket was selected for each landmark instead of a feature of the tooth itself for two reasons: crocodilians continuously shed teeth, making eruption and wear patterns irregular and the vast majority of the specimens available for this study had lost most, if not all, of their teeth during natural decay or the collection and cleaning processes.

The third set of landmarks assessed differences in sutural positions of the bones of the snout (Fig. 2). This data set includes far fewer specimens relative to the outline and alveolar data sets (n = 40; includes one specimen excluded from the outline and alveolar datasets due to pathological closure of some alveoli). This decrease in sample size is due to either insufficient closure of the secondary palate (particularly in the region of the intersection of the premaxillae and maxillae), or damage, that hinders accurate landmark placement. We
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concentrated on ventral cranial landmarks, as differences between dorsal cranial features in captive and wild individuals have one, already been explored using these techniques and two, yield results that are driven by features other than snout shape (Sadleir, 2009).

Dimension reduction is necessary in MorphoJ, rather than on PCA, as size differences between the groups are often not well represented by this method. Procrustes superimposition in the software package MorphoJ (Klingenberg, 2011). Procrustes superimposition translates landmark configurations by positioning their centroid to the origin of a Cartesian coordinate space and scaling the landmark configurations to unit centroid size. The average of all landmark configurations is used as a reference, and then all configurations are rotated to minimize summed squared distances between homologous landmarks (Gower, 1975; Rohlf and Slice, 1990; Zelditch et al., 2004), converting the 2D coordinates to Procrustes shape coordinates. Alligator skulls are bilaterally symmetrical, so the total shape variation of the sample will include both symmetrical and asymmetrical components. The symmetrical component of shape variation was isolated in MorphoJ and all further analyses were performed on the symmetrical component of shape. Specimens utilized in this study span a broad size range (6.6–55.8 cm as measured from quadrate condyle to otic notch). To remove the overall effects of size on shape (i.e., allometry), Procrustes shape coordinates were regressed on log-transformed centroid size. The amount of shape variation accounted for by the regression is quantified as a percentage of the total Procrustes sums of squares (Klingenberg and McIntyre, 1998). We employed a permutation method to test the significance of the correlation between size and shape against a null hypothesis of complete independence using MorphoJ (Drake and Klingenberg, 2008). To explore the distribution of the captive and wild group shapes, we performed a principal component analysis (PCA) on the residuals from this regression in MorphoJ. Symmetrization, regression, and PCA were applied to the snout outline and alveoli positions, snout outline only, and alveoli positions only, to test for differences in signal between datasets. We applied the same analytical procedures to the reduced sutural intersection data set.

To assess differences between captive and wild groups, we performed a linear discriminant analysis (LDA) for each of the four datasets. Discriminant analysis requires an inversion of the pooled within-group variance-covariance matrix (Sheets et al., 2006). Because of this requirement, the number of variables cannot exceed the number of specimens. The number of variables in the snout outline, alveoli positions, and combined snout outline and alveoli position datasets approached, or exceeded, the number of specimens. Therefore, a PCA on the shape data directly was not possible. PCA was used to reduce the statistical dimensionality of the data, by utilizing a limited number of principal component (PC) scores. We employed the method of Sheets et al. (2006) for objectively selecting an appropriate number of PCs to include by maximizing cross-validation success rate. This involves performing the discriminant analysis and cross-validation iteratively, varying the number of PCs up to the maximum number of PCs generated in each analysis (which, in all cases was less than the number of specimens) and selecting the number of axes that produce the highest success rate. In instances where multiple numbers of PCs achieved the same maximum success rate, we selected the lowest number. We performed discriminant analyses of the selected PCs in PAST v.3.04 (Hammer et al., 2001). Given the small number of variables relative to the degrees of freedom in the sutural intersection data set, the LDA was performed directly on the regression residuals (Procrustes coordinates vs. log-transformed centroid size). We used Procrustes coordinates of each group were regressed on log-transformed centroid size separately in MorphoJ to linearize the exponential relationships often observed in growth-series data. We utilized a permutation test to assess significance of the regression based on the null hypothesis of complete independence between the variables, also implemented in MorphoJ. To visualize the changes in morphology associated with the discriminant axis (characteristics separating groups), we computed canonical discriminant function scores on the selected PCs, which aggregate the variables relative to the degrees of freedom in the sutural intersection data set. We applied the same analytical procedure to the reduced sutural intersection data set.

To test the significance of the shape differences between captive and wild groups, we performed a permutational MANOVA, multivariate analysis of variance (Anderson, 2001) on the regression residuals for each data set based on the assumption that the measured groups were indistinguishable. A permutational MANOVA was required as the data were not normally distributed. We performed the MANOVA using the Euclidian distance measure in PAST with 10,000 replicates of group membership permutation.

Patterns of ontogenetic shape change between captive and wild Alligator were also tested. We compared ontogenetic trajectories by dividing the data into captive and wild groups. Because both captive and wild datasets poorly sample very small individuals, the smallest individuals, regardless of group membership, were pooled and included in both data sets (i.e., the smallest captive was added to the wild group and the smallest two wild individuals were added to the captive group). For the reduced sutural intersection landmark dataset, only the smallest captive was added to the wild data set (the two smallest wild specimens were unusable for this analysis as the secondary palate had yet to fully close). As shape differences between captive and wild Alligator are expected to result primarily from environmental effects, the differences are assumed to be minimal in the youngest/smallest individuals. Differences in shape between captive and wild individuals of the smallest size class should represent natural variation, rather than environmentally induced shape change. This methodology provides the same ontogenetic shape starting point for each group, and yields comparable group size ranges. (While no previous studies of the effects of captivity on crocodylians have noted differences in neonates, this assumption has not yet been fully tested. Therefore, we performed a second set of analyses, keeping the smallest captive and wild individuals separate. This yielded similar results, and the majority of the calculated difference stemmed from the variation in smallest sampled size between the groups, not their ensuing ontogenetic trajectories.) The Procrustes coordinates of each group were regressed on log-transformed centroid size separately in MorphoJ to linearize the exponential relationships often observed in growth-series data. We utilized a permutation test to assess significance of the regression based on the null hypothesis of complete independence between the variables, also implemented in MorphoJ. To visualize the relationship between size and shape (Drake and Klingenberg, 2008). These shape scores are variables associated with the shape change predicted by the regression (including the residual variation along that direction in shape space). To test the hypothesis of equivalent rates of ontogenetic change, we then regressed the shape scores for the captive and wild Alligator groups against the size scores.
RESULTS

Comparison of Captive vs. Wild Shape

In all analyses the first two PC axes accounted for a great majority of the variation (74.6–93.5%; see supplementary online material, Table S2 for detailed results). These two PC axes define a lower-dimensional shape subspace that will be used to describe the major aspects of variation among captive and wild Alligator specimens. The first two PC axes of the combined analysis of snout outline and alveoli landmark data encompass 82.96% of the variation in the sample (PC1: 63.96%; PC2: 19.0%). A bivariate plot of these two axes (Fig. 3A) shows broad overlap of the captive and wild specimens, with captives generally exhibiting more negative values along PC1. The 95% equal frequency ellipse for the captive sample shows a larger variance along both axes than the wild sample. The first PC axis is associated with the length and breadth of the snout, with more positive values representing a longer and narrower snout, while negative values indicate a shorter and broader snout (Fig. 3B). Associated with the elongation of the snout is an elongation of the diastema separating the fifth premaxillary alveolus from the first maxillary alveolus. The second PC axis is associated with, in part, the length of the toothrow, relative to the length of the snout. More negative values along PC2 show a more posteriorly elongated toothrow, placing the maximum curvature of the snout lateral to the fourth maxillary tooth in a more posterior position.

The first two PC axes of the outline-only analysis account for 93.5% of the sample variance (PC1: 85.4%; PC2: 8.1%). A bivariate plot of PC1 vs. PC2 shows extensive overlap between captive and wild samples (Fig. 3C). However, captive samples tend toward shorter, broader snouts (negative values on PC1), while wild samples possess longer, narrower snouts (positive values along PC1). The two groups show no separation along PC2. The 95% equal frequency ellipses show that the captive sample exhibits higher variance along PC1 despite smaller sample size, while captive and wild samples show similar variance along PC2. Variation along PC1 primarily reflects differences in snout length and breadth, with a lesser contribution of the extent of festooning along the maxillary margin (Fig. 3D). PC axis two describes differences in the maximum width of the snout around the level of the fifth maxillary tooth and differences in width at the posterior end of the maxilla.

For the analysis of alveolar positions, the first two PC axes account for 76.98% of the variation (PC1: 61.38%; PC2: 15.6%). Both captive and wild samples again show broad overlap along PC1 and PC2 (Fig. 3E). On average, captives show more negative values along PC1 and slightly more negative values along PC2. The captive sample also shows greater variance along PC1, but lower variance along PC2. The first PC axis primarily describes the length and breadth of the toothrow with a minor contribution from variation in the distance between the left and right first premaxillary teeth (Fig. 3F). The second PC axis involves variation in length of the diastema between the fifth premaxillary alveolus and first maxillary alveolus and the breadth of the posterior region of the toothrow.

The first two PC axes of the analysis of suture intersection data account for 74.64% of the variation (PC1: 59.94%; PC2: 14.7%). On a bivariate plot of PC1 vs. PC2, the captive and wild samples overlap extensively, with captives generally having more negative values along PC1 and more positive values along PC2 (Fig. 3G). The captive sample also exhibits much greater variance along PC1 and slightly greater variance along PC2 than does the wild sample. The first PC axis is primarily associated with variation in snout breadth, with minor contributions from variation in the length of the premaxilla and anterioposterior position of the anterior margin of the suborbital fenestrae (Fig. 3H). Axis two encompasses variation in the anteroposterior position of the midline maxilla/palatine suture and the anterior border of the suborbital fenestrae. It also includes a minor component of snout length.

Iterative LDA followed by cross-validation identified the optimal number of PCs to include for each data set (Table 1; full results of the iterative analyses available in supplementary online material, Table S3). The discriminant function was generally successful in distinguishing captive from wild specimens (RS classification success rates: 95.24–80%; Table 1). The high classification success rate of RS demonstrates some amount of overfitting as cross-validation success rates are uniformly lower (90.48–70%; Table 2), though the difference is small. In all analyses the correct classification rates exceed those expected by chance. Results of the permutational MANOVA indicate significant differences between group means of captive and wild specimens in all cases (Table 1).

The morphological features associated with the discriminant functions are shown in Figure 4. In all cases, the features distinguishing the groups are subtle. For the combined snout outline and alveoli position dataset, these features include increased undulations along the maxillary margins, slight shortening and broadening of the snout, and slight posterior expansion of the alveoli positions in captives relative to wild specimens (Fig. 4A). In the outline analysis, captives show broader snouts with a greater undulation of the maxillary margin and a slight decrease in snout...
Fig. 3. Results of PC analyses: (A) Morphospace related to the first two PC axes for the combined snout outline and alveoli position data; (B) Shape change associated with positive and negative extremes of the first two PC scores; (C) Morphospace related to the first two PC axes for the snout outline data; (D) Shape change associated with positive and negative extremes of the first two PC axes; (E) Morphospace related to the first two PC axes for the alveoli position data; (F) Shape change associated with positive and negative extremes of the first two PC axes; (G) Morphospace related to the first two PC axes for the sutural intersection data; (H) Shape change associated with positive and negative extremes of the first two PC axes. Percentages on PC axes indicate percentage of total variance explained by that PC. Ellipses represent 95% equal frequency ellipse for each group. For PC plots, black circles represent wild specimens, gray squares represent captive specimens. For the shape change wireframes, black represents positive change along the PC axis, while gray indicates negative change. Arrows on (B) and (F) indicate the location of the diastema between the fifth premaxillary and first maxillary teeth.
length (Fig. 4B). Analysis of the alveoli positions demonstrates a slight narrowing of the premaxillary portion of the tooth row, a decrease in length of the diastema between the last premaxillary and first maxillary alveolus, and a broadening of the posterior portion of the tooth row among captive specimens (Fig. 4C). The morphological features differentiating groups in the sutural intersection data include an overall broadening of the snout, a slightly more posteriorly positioned midline premaxilla/maxilla suture, and anterior margins of the suborbital fenestrae somewhat more anterolaterally placed in captives relative to wild specimens (Fig. 4D).

Patterns of Ontogenetic Shape Change

Regression of Procrustes shape coordinates on log-transformed centroid size indicated a variable, yet significant, amount of shape variance attributable to size in all datasets (range: 6.37–32.89%; Table 2). ANCOVA indicate that patterns of shape change differ significantly between captive and wild samples for all datasets except the sutural intersections (Table 1). For the significant datasets, the captives tend to change shape at a faster rate than wild specimens (Fig. 5). The variation within given size classes appears to be greater among captive specimens as well, though it has been suggested that at least part of the difference seen in captive morphologies is related to an accelerated growth rate, and therefore size should not be considered a trustworthy proxy for age in these animals (Sadleir, 2009). The large sample of farmed captives from Rockefeller Wildlife Station, all culled at approximately the same size, exhibit a very wide spread along the shape score axis; much larger than the similar number of wild specimens of comparable size.

DISCUSSION

Most, though not all, of the analyses comparing snout shapes across captive and wild groups yielded statistically significant results. However, there is a great deal of overlap between the groups in all cases. At face value, these results are unexpected. One of the principle differences often cited between captive and wild crocodylians is snout shape. General wisdom holds that among generalist crocodylians, like A. mississippiensis, captive individuals will exhibit shorter, wider snouts (Erickson et al., 2004). Previous analyses of A. mississippiensis crania demonstrated strongly divergent morphologies between the two groups, though much of this signal was preserved elsewhere in the skull (Sadleir, 2009). If the differences between these animals are so evident that

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**TABLE 1. Results of linear discriminant analyses, permutational MANOVA, and ANCOVA**

| Data set                  | Classification success rate (%) | Cross-validation |
|---------------------------|---------------------------------|------------------|
|                           | C<sub>pro</sub> | Resubstitution |                |
| Outline + Alveoli (11PCs) | 66.32             | 92.86           | 90.48           |
| Outline only (15PCs)      | 66.32             | 95.24           | 89.29           |
| Alveoli only (9PCs)       | 66.32             | 80.95           | 75.00           |
| Sutural landmarks         | 56.12             | 80.0            | 70.00           |

**TABLE 2. Results of regression of shape scores on log-transformed centroid size**

| Data set                      | % predicted | P value |
|-------------------------------|-------------|---------|
| Outline + Alveoli: total sample | 7.36        | 0.0020  |
| Outline + Alveoli: captive     | 19.75       | 0.0167  |
| Outline + Alveoli: wild        | 5.35        | 0.0158  |
| Outline only: total sample     | 7.91        | 0.0047  |
| Outline only: captive          | 20.54       | 0.0288  |
| Outline only: wild             | 6.37        | 0.0193  |
| Alveoli only: total sample     | 11.17       | <0.0001 |
| Alveoli only: captive          | 27.46       | 0.0055  |
| Alveoli only: wild             | 6.37        | 0.0072  |
| Sutural landmarks: total sample| 29.71       | <0.0001 |
| Sutural landmarks: captive     | 30.90       | 0.0086  |
| Sutural landmarks: wild        | 32.89       | <0.0001 |

C<sub>pro</sub>, Proportional chance criterion (classification success threshold expected from randomly assigning samples to groups in proportion to their size). P values in bold are nonsignificant. For graphical representation of morphology associated with LDA see Figure 4; For ANCOVA plots, see Figure 5.
they can be observed qualitatively, why would a quantitative method have more difficulty differentiating between the groups?

The problem seems to stem from the fact that captive vs. wild morphologies have been treated as a binary condition, when a spectrum would better describe the observed patterns. Across the board, these analyses demonstrate that captive animals occupy a larger area of morphospace than their wild counterparts (Fig. 3). However, there is also a great deal of overlap between the groups, and the area of morphospace occupied by the wild animals falls largely within the range of the captive sample. These results may reflect a combination of compounding factors in the development of the crocodylian snout: ontogeny, ecophenotypy, and functional constraints.

Differences between juvenile and adult crocodylians long have been recognized (McIlhenny, 1935). These qualitative observations of morphology and behavior since have been subjected to more rigorous analysis, both within and between captive and wild animals. Erickson and colleagues (2004) recognized that bite forces in captive and wild *A. mississippiensis* were highly similar among juveniles, but that differences arose in older, larger animals. Potential explanations given for differences in bite forces, cranial morphology (Sadleir, 2009), biomechanical performance of humeri (Meers, 2002) and geometric dimensions of femora (Farlow et al., 2005) in captives of different crocodylian species were all related to the long-term effects of unnatural habitats, behavior, and diet (Meers, 2002; Sadleir, 2009), pressures which act over time and ontogeny. During growth, the ecological pressures of captivity introduce increasingly aberrant morphologies the longer the conditions persist. Thus, the differences in captive morphologies should be viewed as a rather extreme kind of ecophenotypy. This interpretation is further supported by the results of most of the ANCOVA analyses (Fig. 5). The changes in shape when size is taken into account were statistically significant in all analyses except the sutural intersections (Table 1). Among the significant analyses, shape change among captives occurred at a higher rate than wild specimens throughout ontogeny.

However, even this explanation still oversimplifies the conditions that could lead to the observed patterns in crocodylian snout shape. Qualitative observations and in-depth morphometric analyses of populations within single species have revealed the breadth of variation possible under natural conditions (Sadleir, 2009), and yet all captives are

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**Fig. 4.** Wireframes demonstrating shape change between group means (left column) and between maximally divergent specimens (i.e., range; right column) along the discriminant axis. (A) Combined outline and alveoli analysis; (B) snout outline analysis; (C) alveoli position analysis; (D) sutural intersection landmark analysis. Black wireframes indicate wild samples, while gray indicates captive. See Table 1 for numerical results of the discriminant analysis.

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often lumped together as a single state. Living conditions for crocodylians at zoological parks and farms can range from bare concrete enclosures to fenced off areas of largely unchanged natural habitats. Animals that are held in indoor facilities often live under highly climate controlled conditions, with unchanging temperature and light exposure (Sadleir, 2009), while those kept in open air enclosures experience essentially the same light and temperature changes as their wild counterparts. Differences in captive diet and behavior level have been noted by researchers (Meers, 2002; Farlow et al., 2005), but have not yet been surveyed or studied in much detail. With so much possible variation in the conditions of captivity, a broad spectrum of captive animals’ morphological reactions to these pressures would be expected, and in fact, has been observed (Figs. 6 and 7).

Further interesting patterns emerge when we compare the relative strength of the signal generated across the different analyses of snout shape: alveoli position, snout outline, alveoli plus outline, and sutural intersections (Table 1). The MANOVA results for all four analyses were statistically significant. The LDA successfully differentiated the sample into the a priori defined captive and wild groups. This was further supported when cross-validation analyses correctly classified specimens at rates exceeding 70% in all cases. Yet the cross-validation rate differed between analyses with the lowest success rates coming from the analyses of alveoli position (75%) and of sutural intersections (70%). When placed in an ontogenetic context, all analyses yielded statistically significant results except the exploration of suture contacts.

Crocodylians’ snouts are their main tool for interacting with their environments, so much so that major differences in overall snout shape are often used as shorthand for the ecological role of different taxa (Busbey, 1995; Brochu, 2001; McHenry et al., 2006; Pierce et al., 2008; Sadleir and Makovicky, 2008). Ultimately, an individual crocodylian must

Fig. 5. Results of ANCOVA comparing patterns of ontogenetic shape change. (A) Combined outline and alveoli analysis; (B) snout outline analysis; (C) alveoli position analysis; (D) sutural intersection landmark analysis. Black circles indicate wild specimens; gray squares indicate captive specimens. See Table 1 for numerical results of the ANCOVA.
Fig. 6. *Alligator sinensis*, range of variation. From left to right: wild FMNH 31302, 'typical' captive FMNH 197946, 'extreme' captive FMNH 229974. Scale bars = 5 cm.

Fig. 7. *Alligator mississippiensis*, diversity of captive cranial morphologies. Left to right, top to bottom: FLMNH 34886 (wild exemplar), FLMNH 35129, FLMNH 61483, AMNH 9112, FLMNH 95386, AMNH 7141, AMNH 119220, FMNH RWSF 7, FMNH RWSF 4, AMNH 138124. Scale bars = 2 cm.
maintain a snout and mouth morphology that is functional enough to perform the daily requirements of, for example, feeding. The patterns observed between analyses yielding significant results and those that did not suggest that a functional constraint such as this might be in play.

When the covariation between size and shape are taken into account, the comparison of sutural contacts between captive and wild Alligator yielded a $P$ value of 0.9406. This analysis was performed on a reduced dataset, due to problems with full sutural closure in some of the specimens (supplementary online material Table S1). Nevertheless, the results provide a clear, robust signal: the points of contact between the major bones of the snout vary little between these groups. This leaves snout outline and tooth position to explain the majority of the differences between the two groups. Each of these variables in isolation and when combined can differentiate captive and wild specimens. In general, captive animals exhibit slightly shorter, blunter snouts with an increased undulation to the lateral margins of the maxilla. The tooth row flares posteriorly, and the spacing between the last premaxillary tooth and the first maxillary tooth also changes between the groups.

Visually, it seems that tooth position is shifting to accommodate the changes to the surrounding bone, but the pattern is more complicated than the alveoli simply following the outline of the snout. For example, the margin of the captive animals' snouts has a more distinct lateral undulation than their wild counterparts (Fig. 4). The teeth do not directly follow the curvature of the undulation in the captives though, and instead the space between the teeth increases as the tooth row extends further posteriorly to bridge the distance made by the increased curvature. The individual position of each tooth changes in the captives, but they are collectively doing so in a manner that cleaves closer to the line of their wild counterparts' tooth rows than the lateral margins of the jaws might suggest.

This dovetails interestingly with another oft cited feature of captive crocodylians, and one that this study was not able to directly address: tooth splay. Qualitatively, it has been noted that the teeth of captive crocodylians, at least those in the anterior half of the snout, splay outward instead of occupying their usual, more dorso-ventral orientation (Erickson et al., 2004). The current dataset makes measuring tooth splay difficult, because the vast majority of teeth have fallen out of their sockets during preparation for accession into skeletal collections. Left with only the alveoli to observe, quantitatively assessing tooth splay in this sample becomes challenging. Preliminary measurements of the diameter of the first five maxillary teeth indicate that the teeth are rotating in place rather than migrating towards the outer margin of the snout, resulting in a widening of the alveoli in the labio-lingual axis (Drumheller, 2012). These findings align with the results presented here, and may suggest an explanation for tooth splay. The teeth do not initially follow the lateral expansion and increased curvature of the margins of the snout, possibly being functionally constrained by the need to maintain some level of occlusion with the teeth of the lower jaw. To do otherwise could inhibit the primary feeding and grasping utility of this anatomical structure (Busbey, 1995; Brochu, 2001; McHenry et al., 2006; Pierce et al., 2008; Sadleir and Makovicky, 2008). However, the laterally expanding margins of the skull increasingly overhang the tooth row, a process which could start to force the teeth to accommodate the expansion by rotating in the labial direction—i.e., splaying. This pattern requires further exploration though, preferable utilizing living animals or specimens preserved with teeth in situ.

This study also does not address the rate and type of pathology in captive and wild crocodylians. While none of the wild specimens utilized in this study exhibited obvious significant pathologies in the tooth row, two of the 18 sampled captive specimens did. However, prior research indicates that wild A. mississippiensis are certainly not immune from injuries and infections that affect the tooth row (Erickson, 1996). Broken teeth and related alveolar damage and infections commonly result in partial to complete edentulism in wild members of many crocodylian species, including A. mississippiensis. In fact, one survey of crocodylian dental conditions directly compared captive and wild specimens, and found that the wild animals actually exhibited significantly more pathologies than their captive counterparts (Erickson, 1996). While differences in rate and type of pathologies between captive and wild animals have been observed in other taxa (Fitch and Fagan, 1982; Munson et al., 2005), without a more in depth study among crocodylians, all that can be confidently stated is that one, both captive and wild crocodylians experience these types of pathologies and two, any animals exhibiting major pathologies in the snout should probably be excluded from analyses related to jaw use and function.

With regards to the utility of captive animals in bite force, mechanics, and mark studies, the results of this analysis were mixed. However, taken in context, they can provide suggestions on how to approach working with these animals. Even among the analyses that produced statistically significant results, wild and captive Alligator ranges exhibited wide overlap, with the captive animals occupying an area of morphospace that encompassed, but also extended beyond, the wild range (Fig. 3), a pattern that is not entirely explained by ontogeny (Fig. 5). When viewed as an example of ecophenotypy, a likely explanation for
this pattern takes form. When the conditions of captivity more closely mimic wild conditions (e.g., outdoor, more natural enclosures), the ensuing morphology should cleave more closely to the wild morphotype. Similarly, less natural conditions (e.g., divergent climate conditions, artificial indoor enclosures) would be expected to result in more divergent morphologies (Fig. 7).

This indicates that captive crocodylians should not be wholesale avoided by researchers, especially for gross scale studies of function, if the individuals have been kept under healthier, more naturalistic conditions in their captivity and if existing datasets sampling both captive and wild animals yield congruent results, such as within the crocodylian bite mark literature (e.g., Njau and Blumenschine, 2006; Drumheller and Brochu, 2014) or when a method exists for standardizing data collected between the groups, such as within the bite force literature (Erickson et al., 2004). Wild animals are still preferable for studies in which the potential biasing effects of captivity have not yet been explored, such as systematic analyses, or in which biases previously have been identified, such as morphometric (Sadleir, 2009) and biomechanical analyses (Erickson et al., 2004). This paper should serve as a case study and a suggestion for how potentially biasing effects of captivity can be addressed and assessed in the future when determining whether captive animals will serve as a reasonable proxy for their wild counterparts.

Author Contributions
SKD collected the data and performed preliminary analyses. EWW performed the final analyses. SKD and EWW generated the figures and wrote the manuscript. RWS contributed to the analyses used, improved the manuscript, and provided access to specimens.

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