Insights into the Ecology and Evolutionary Success of Crocodilians Revealed through Bite-Force and Tooth-Pressure Experimentation

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Abstract

Background: Crocodilians have dominated predatory niches at the water-land interface for over 85 million years. Like their ancestors, living species show substantial variation in their jaw proportions, dental form and body size. These differences are often assumed to reflect anatomical specialization related to feeding and niche occupation, but quantified data are scant. How these factors relate to biomechanical performance during feeding and their relevance to crocodilian evolutionary success are not known.

Methodology/Principal Findings: We measured adult bite forces and tooth pressures in all 23 extant crocodilian species and analyzed the results in ecological and phylogenetic contexts. We demonstrate that these reptiles generate the highest bite forces and tooth pressures known for any living animals. Bite forces strongly correlate with body size, and size changes are a major mechanism of feeding evolution in this group. Jaw shape demonstrates surprisingly little correlation to bite force and pressures. Bite forces can now be predicted in fossil crocodilians using the regression equations generated in this research.

Conclusions/Significance: Critical to crocodilian long-term success was the evolution of a high bite-force generating musculo-skeletal architecture. Once achieved, the relative force capacities of this system went essentially unmodified throughout subsequent diversification. Rampan changes in body size and concurrent changes in bite force served as a mechanism to allow access to differing prey types and sizes. Further access to the diversity of near-shore prey was gained primarily through changes in tooth pressure via the evolution of dental form and distributions of the teeth within the jaws. Rostral proportions changed substantially throughout crocodilian evolution, but not in correspondence with bite forces. The biomechanical and ecological ramifications of such changes need further examination.

Introduction

Despite their large size (1.2–6.7 m total length [1]), crocodilians (Crocodylia: Alligatoridae: [alligators and caimans]; Crocodyliidae: [crocodiles]; Gavialidae: [Indian and Malay (“false”) gharials]; [2], [3]) are remarkably stealthy predators – adept at stalking and ambushing prey in and around freshwater and estuarine environments. For the most part, their post-cranial anatomy is opportunistic feeders with diets that can include invertebrates, fish, snakes, turtles, birds and mammals [1], [7]. This is especially true of dietary generalists with teeth and snouts that occupy the middle ground among crocodilians with regard to sharpness and width, respectively. These include taxa such as the saltwater crocodile (Crocodylus porosus) and American alligator (Alligator mississippiensis) (Figures 1 and 2). On the other hand, those with extreme rostro-dental morphology tend to have more specialized diets. Several extremely slender-snouted forms with needle-like teeth, such as the Australian freshwater crocodile (Crocodylus johnsoni) and the Indian gharial (Gavialis gangeticus), consume a preponderance of small prey such as fish, insects, and crustaceans [1], [7], [8] (Figure 1). Their elongated jaws, although...
structurally weak in bending [9–11], afford a broad strike zone during side-to-side head motions, rapid distal jaw closure, and we suspect, less obstructed vision when targeting prey. The broad-snouted caiman (*Caiman latirostris*) and Chinese alligator (*Alligator sinensis*) have blunt rostra and dull bulbous teeth for consuming hard-shelled mollusks [1], [7], [12] (Figure 1). This rostro-dental morphology helps to ensure enhanced structural rigidity through lower bending moments [9]. Additionally, high bite forces occur at all tooth positions due to their proximity to the jaw joint [9–11]. Finally, the dwarf caimans (*Paleosuchus trigonatus* and *Paleosuchus palpebrosus*) have dog-like vaulted rostra, and teeth with intermediate sharpness (Figure 1). Both feed at the water’s edge; *Paleosuchus trigonatus* also forages on land for snakes, pacas and porcupines [1], [7], [13]. Their dorso-ventrally expanded snouts enhance rigidity in the plane of biting through increased area moments of inertia [9], [10].

The biomechanics of crocodilian feeding is poorly understood. Most notably it is not known how crocodilian bite forces and tooth pressures (bite force/tooth contact area) relate to rostro-dental and body size variance, dietary ecology, or evolutionary diversifications. Adult bite forces are only known for *Alligator mississippiensis* [14], [15], but are assumed to vary considerably among taxa. This is because of marked differences in the robustness of crocodilian teeth and jaws, dietary constituency (e.g. hard versus compliant prey [16]), and perhaps myology [17–19]. Recent computerized

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**Figure 1. Phylogenetic hypothesis for extant Crocodylia showing variation in rostral proportions.** The cladogram is based on reanalysis (see Materials and Methods) of molecular data from Gatesy and colleagues [2] using maximum likelihood and non-parametric rate-smoothing with branch lengths proportional to time. Lineages shown in blue represent caiman (a–e) and alligators (f,g) (= Alligatoridae), and those in green crocodiles (h–t) and gharials (u,v) (= Crocodyliidae+Gavialidae). The Yacare caiman, *Caiman yacare* is not shown for it was not utilized in the Gatesy et al. [2] analysis. Dorsal views of heads are modified from Wermuth and Fuchs [53] and standardized to the same length to show relative differences in rostral form. Bracketed numbers following taxon names are the mean rostral proportions or RP (= mid-rostral width/snout length) for each taxon from our study. Phylogenetic Independent Contrasts were performed on these 22 species; however, bite force, tooth pressure, and morphometric measurements and subsequent TIPs analyses were performed for all 23 extant taxa, including *Caiman yacare*.  

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Finite element modeling of crocodilian skulls supports this hypothesis [10] [11]. Bite forces are predicted to vary nine-fold among animals scaled to the same head size. Extremely low forces are posited in the delicate, slender-snouted forms and highest values in the robust, blunt-snouted taxa. The concurrent effects of interspecific differences in body size on bite force have not been explored, nor have the effects of phylogeny. Likewise, to our knowledge, tooth pressures (which reflect how such forces are actually transmitted to the prey) have not been studied in reptiles. (They are however known or estimated for humans, and a few animals such as sharks and other fish [20–22].)

Here we formally tested the longstanding hypothesis that crocodilian rostral proportions positively correlate with the capacity for bite-force generation. In addition, owing to the lack of speculation on how absolute bite forces and tooth pressures differ among extant crocodilians, we tested the hypothesis that these values scale isometrically with body mass.

We directly measured bite forces in sexually mature adults of all 25 extant crocodilian species [23] (Table 1) and inferred their peak tooth pressures at the prominent upper caniniform teeth (at the maxilla convexity near the front of the jaws) where prey are initially seized, and at the prominent upper molariform teeth (at the maxilla convexity near the back of the jaws) where food items are orally processed (Figure 2; also see Materials and Methods). We then tested the extent to which variation in forces and pressures could be explained by body size and rostral type. Spurious correlations were avoided by examining the effects of phylogenetic relationships using independent contrasts. Finally, the biomechanical-performance traits were mapped onto a highly robust, re-estimated DNA sequence phylogeny to visualize character evolution and make evolutionary inferences about the role feeding biomechanics played in crocodilian ecological diversifications (see Materials and Methods).

Results

The results of our study revealed taxon representative molariform bite forces ranging from 900 to 8,903 N (202 to 2,019 lbs) (Paleosuchus palpebrosus and Crocodylus porosus respectively; Table 1; Figure 3A). Body mass is the primary determinant of crocodilian force generation in both the raw data analysis (TIPs: R² = 0.92) and phylogenetically corrected analysis (PIC: R² = 0.87, p < 0.0001). The reduced major axis (RMA) scaling coefficient for log-transformed taxon representative bite force regressed against log-transformed body mass was 0.708 ± 0.111 (95% CI), which is not statistically different from isometry (scaling coefficient = 0.667). Only the forces for Gavialis gangeticus in the TIPS analysis are significantly atypical (lower) than those of extant Crocodylia as a whole (Figure 3A). Those for the Malay gharial (Tomistoma schlegeli) are moderately low. Interspecific differences in rostral proportions (Figure 1; Table 1) explain just 19% of the remaining variance in the size-standardized phylogenetically corrected data set (Figure 3B). This represents only 2.5% of the total variance from the aforementioned phylogenetically corrected analysis. Thus, the hypothesis that crocodilian rostral proportions positively correlate with bite-force capacity, while statistically significant (p = 0.03), is not supported as a major predictor of force even after correcting for size.

The taxon representative caniniform tooth-pressure values ranged from 195 to 1,344 MPa (28,282 to 194,931 psi) (Morelet’s crocodile – Crocodylus moreletii, and the Orinoco crocodile – Crocodylus intermedius, respectively; Table 2, Figure 4A). These values also trend positively with increasing body mass, but are highly variable (TIPs: R² = 0.20; PIC: R² = 0.19, p = 0.09). The RMA scaling coefficient for log-transformed taxon representative caniniform tooth pressure regressed against log-transformed body mass was 0.490 ± 0.203 (95% CI), which is greater (i.e. positively allometric) than isometry (scaling coefficient = 0.000), and so did not support our hypothesis. Exceptionally high values stand out in the slender-snouted, semi-piscivorous Crocodylus intermedius, and highly piscivorous Gavialis gangeticus (the latter generates the lowest relative bite force but also has exceptionally slender teeth with negligible contact area). The values for Crocodylus johnsoni are moderately high. All other ecomorph representatives show similar relative values. Size-standardized caniniform tooth pressures changed independently on multiple lineages (Figure 4B) and were uncorrelated with rostral proportions (PIC R² = 0.001; Figure 5A).
Taxon representative molariform tooth-pressure values ranged from 203 to 1,388 MPa (29,443 to 201,312 psi) (Dwarf crocodile – Osteolaemus tetraspis, and Crocodylus intermedius, respectively; Table 2, Figure 6A). These are more strongly correlated with body mass than the caniniform data (TIPs: $R^2 = 0.54$; PIC: $R^2 = 0.293$, $p = 0.008$). The RMA scaling coefficient for log-transformed taxon representative molariform tooth pressures regressed against log-transformed body mass was 0.553 ± 0.180 (95% CI), which is greater (i.e. positively allometric) than isometry (scaling coefficient = 0.000), and therefore did not support our hypothesis. None of the molariform pressure values are statistical outliers. Nevertheless, those for the slender-snouted Crocodylus johnsoni and Crocodylus intermedius are relatively high, and those for the broader-snouted generalists, the mugger (Crocodylus palustris), and Morelet’s crocodile (Crocodylus moreletii) are relatively low. Pressures for all other ecomorphs, including Gavialis gangeticus, are comparable. Size-standardized molariform tooth pressures changed repeatedly in the phylogeny (Figure 6B) and were not significantly correlated with rostral proportions (PIC $R^2 = 0.094$; Figure 5B).

### Discussion

The results of our investigation into the biomechanics and evolutionary ecology of crocodilian feeding revealed a number of unexpected findings. We found negligible support for the commonly held view that bite forces correlate strongly with rostral form – a proxy for strength. Rather, bite forces vary independently of rostral morphology, so much so that in some cases same-sized taxa from the extreme bounds of crocodilian rostal morphology and dietary ecology (e.g. the slender-snouted, Crocodylus johnsoni and robust-snouted, Caiman latirostris; Figure 1) show “pound for pound” comparable molariform bite forces (Figure 3A). During

### Table 1. Anatomical measurements, and bite-force performance for extant Crocodylia.

| Taxon                | N  | MRP | MBM  | RBM  | MTL  | RTL  | MBF  | RMBF | MTFR  | CBF   | RCBF |
|----------------------|----|-----|------|------|------|------|------|------|-------|-------|-------|
| Crocodylidae         |    |     |      |      |      |      |      |      |       |       |       |
| Crocodylus acutus    | 2  | 0.40| 132  | 100–164 | 294 | 270–318 | 3999 | 3643–4355 | 1.54  | 2599  | 2368–2830 |
| Crocodylus intermedius| 1  | 0.35| 182  | 182  | 340  | 340  | 6276 | 6276  | 1.59  | 4283  | 4283  |
| Crocodylus johnsoni  | 5  | 0.24| 20   | 7–43 | 167  | 134–215 | 1292 | 859–1863 | 2.05  | 629   | 418–856 |
| Crocodylus mindorensis| 1  | 0.65| 69   | 69   | 244  | 244  | 2736 | 2736  | 2.05  | 4283  | 4283  |
| Crocodylus moreletii | 1  | 0.75| 110  | 110  | 284  | 284  | 4399 | 4399  | 1.43  | 3069  | 3069  |
| Crocodylus niloticus  | 2  | 0.47| 86   | 86–87 | 250  | 240–261 | 3043 | 2914–3172 | 1.51  | 2007  | 1991–2023 |
| Crocodylus novaeguineae| 1  | 0.55| 154  | 123–186 | 303 | 291–315 | 5360 | 4782–5938 | 1.36  | 3928  | 3547–4310 |
| Crocodylus palustris  | 1  | 0.66| 207  | 207  | 332  | 332  | 7295 | 7295  | 1.74  | 4194  | 4194  |
| Crocodylus porosus    | 7  | 0.41| 272  | 36–531 | 344 | 202–459 | 8983 | 1646–16144 | 1.57  | 5792  | 930–11216 |
| Crocodylus rhabdiceri | 3  | 0.51| 52   | 30–65 | 214  | 187–246 | 2107 | 1392–3127 | 1.52  | 1379  | 917–2035 |
| Crocodylus siamensis  | 3  | 0.64| 69   | 40–87 | 238  | 212–263 | 3415 | 2073–4577 | 1.53  | 2227  | 1357–2891 |
| Mecistops cataphractus| 3  | 0.25| 67   | 50–95 | 247  | 231–262 | 2082 | 1704–2447 | —     | —     | —     |
| Osteolaemus tetraspis | 5  | 0.66| 17   | 9–34 | 147  | 124–183 | 1787 | 1375–2509 | 1.53  | 1164  | 902–1588 |
| Gavialidae            |    |     |      |      |      |      |      |      |       |       |       |
| Gavialis gangeticus   | 2  | 0.10| 112  | 103–121 | 326 | 318–334 | 1895 | 1784–2006 | 2.06  | 924   | 819–1030 |
| Tomistoma schlegelii  | 3  | 0.18| 142  | 79–255 | 347 | 290–405 | 3397 | 1704–6450 | 1.62  | 2099  | 1052–3985 |
| Alligatoridae         |    |     |      |      |      |      |      |      |       |       |       |
| Alligator mississippiensis| 15 | 0.69| 142  | 47–297 | 285 | 213–372 | 5117 | 2442–9452 | 1.54  | 3340  | 1414–6162 |
| Alligator sinensis    | 4  | 0.83| 14   | 12–15 | 150  | 140–155 | 1084 | 894–1357 | 1.48  | 735   | 555–963  |
| Caiman crocodilus     | 4  | 0.58| 20   | 18–25 | 166  | 166   | 1215 | 1148–1303 | 1.48  | 821   | 759–894 |
| Caiman latirostris    | 5  | 0.89| 30   | 16–45 | 167  | 157–177 | 1467 | 1050–2420 | 1.37  | 1063  | 777–1672 |
| Caiman yacare         | 5  | 0.69| 18   | 17–23 | 162  | 162   | 971  | 712–1192  | 1.50  | 646   | 485–900 |
| Melanosuchus niger    | 3  | 0.73| 59   | 31–103 | 246 | 204  | 2696 | 1779–4310 | 1.42  | 1911  | 1180–3112 |
| Paleosuchus palpebrosi| 3  | 0.69| 13   | 12–14 | 133  | 133   | 900  | 667–1125  | 1.56  | 576   | 436–711 |
| Paleosuchus trigonatus| 3  | 0.51| 22   | 19–28 | 150  | 143–156 | 1082 | 1058–1125 | 1.59  | 682   | 658–720 |
| Total                | 83  | 7–531| 124–459 | 667–16414 | 436–11216 |

N = Number of Specimens.  
MRP = Mean Rostral Proportion (mid-rostral width/snout length).  
MBM = Mean Body Mass (kg).  
RBM = Range of Body Masses (kg).  
MTL = Mean Total Length (cm).  
RTL = Range of Total lengths (cm).  
MBF = Taxon Representative Molariform Bite Force (N).  
RMBF = Range of Molariform Bite Forces (N).  
MTFR = Mean Tooth-Fulcrum Ratio (QA joint-Caniniform tooth/QA Joint-Molariform tooth).  
CBF = Estimated Taxon Representative Caniniform Bite Force (N).  
RCBF = Range of Estimated Caniniform Bite Forces (N).  

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cladogenic events, when rostral form was modified into different types (presumably to allow access to different prey), bite forces were just as likely to increase as decrease.

Our findings suggest that for crocodilians of similar body mass, the same absolute bite forces will be generated at equal distances from the quadrate-articular joint. A consequence of this is that more slender-snouted forms will at the same time experience higher stresses to their jaws since they have lower area moments of inertia with which to resist bending. Furthermore, since they have relatively longer snouts, equal loads applied at the tip of the jaws will lead to higher absolute bending moments than in shorter-snouted forms. This begs the question: How do slender-snouted species sustain bite forces typical of more robust-snouted crocodilians? We suspect the answer lies primarily in their prey selection. They target small prey relative to their size (e.g. fish and crustaceans, and/or birds and small mammals by the larger species) whose low inertia contributes little to resistance forces. It is also plausible that their jaws experience stresses closer to rupture strength (i.e. lower safety factor [24]) during feeding than the other ecomorphs. This is certainly the case during other behaviors such as fighting and defense, where they show a much greater propensity to sustain broken jaws [25].

Body size actually accounts for nearly all interspecific variance in adult crocodilian bite-force capacity, and these forces scale isometrically to body mass. Clearly a major factor in the evolutionary success of crocodilians stems from their long-term retention of a cranial musculoskeletal system that can generate sufficient force to procure and process near-shore prey across a broad range of body sizes. Only in the extremely slender-snouted Gavialis gangeticus, arguably the only truly piscivorous species, is there evidence of significant departure in performance, and this is reflected in their anatomy. These low-force biters independently evolved extremely hypertrophied, low-mechanical advantage adductor mandibulare muscles, and small, fusiform-fibered posterior pterygoid muscles that presumably accentuate rapid jaw closure [17], [19]. This enhanced jaw-closing performance was likely afforded at the cost of diminished bite-force capacity, which is consistent with our empirical findings for both molariform and estimated caniniform bite forces in Gavialis gangeticus.

The retention of relative bite-force capacity among crocodilians makes it apparent that the remarkably high bite forces first documented in adult Alligator mississippiensis [14], [15] are typical of most comparable-sized species, regardless of rostro-dental anatomy or diet. Even higher forces are to be found in larger species like the slender-snouted, semi-piscivorous Crocodylus intermedius and the medium-snouted generalist Crocodylus porosus – the largest extant crocodilian. (Our datum for one Crocodylus porosus individual, 16,414 N [3,689 lbs] represents the highest bite force measured in any animal. This value eclipses the highest recorded value in carnivoran mammals, 4,500 N [1,011 lbs] in the spotted hyena – Crocuta crocuta [26].)

Crocodilian bite-force retention can be used to predict forces in other specimens and species, including taxa known only from fossils (see Materials and Methods). For instance, scientifically documented 6.7-meter long Crocodylus porosus individuals [1] were likely capable of molariform bite forces of approximately 27,531 N to 34,424 N (6,187 to 7,736 lbs). In addition the historical range of adult bite-force values for Crocodylia as a whole may have spanned from 628 N to 102,803 N [141 to 23,102 lbs; in extinct Gavialis gangeticus [22]], and 11 m TL, 3,450 kg [3,689 lbs] represents the highest bite force measured in any animal. This value eclipses the highest recorded value in carnivoran mammals, 4,500 N [1,011 lbs] in the spotted hyena – Crocuta crocuta [26].)

No previous hypotheses exist regarding tooth pressures in crocodilians. Thus, the data we report provide new insights into how bite forces are conveyed through the most prominent teeth to allow these animals to seize prey, and initially puncture or drive cracks through their tissues. We found that both caniniform and molariform pressures scaled with positive allometry versus the expected isometric scaling value of 0.000. Notably the absolute pressures at both tooth positions were remarkably high. Values for all taxa exceeded the highest reported previously (147 MPa [21,321 psi]) for the giant extinct placoderm fish Dunkleosteus [22]), and pressures for some individuals were as much as 17-fold higher (Table 2). In addition we discovered that the caniniform and molariform teeth showed similar peak pressure values within individuals and species (Table 2). This occurred despite differing shapes and functions relative to one another (Figure 2) and unequal bite forces (Table 1). The caniniform forces are 36%
lower on average because they are further from the quadrato-articular joint fulcrum; Table 1.) We suspect the reason for the similarity is that both tooth types are composed of the same dental constituents (enamel and von Ebner’s dentine) and must be able to damage, and yet sustain impacts with the same tissue types when feeding. Notably, the pressure values in all taxa considerably exceed the ultimate shear strength of bone (65–71 MPa; Figures 4 and 6), the strongest of the hard constituents (incl. dentine, enamel, calcium carbonate) they encounter in their potential prey [29]. This holds true even during the seizing of prey underwater where initial tooth pressures could be less since jaw-closing velocity diminishes by up to two-fold from pressure and frictional drag (see Materials and Methods). Clearly this biomechanical capacity is integral to the dietary plasticity of all living crocodilians. It was also certainly vital to the occupation of near-shore habitats by crocodilians over millennia – although prey types changed, the materials of which they were composed did not (e.g. [30]).

Crocodilian tooth pressures show negligible correlation with phylogeny (low K values, significant deviation from a Brownian motion model). This result suggests that convergent adaptation is contributing more signal than phylogenetic relatedness. Presumably, evolutionary changes that allowed dietary niche occupations were responsible for much of the variation. Nevertheless, ecomorph-specific tooth-pressure values are ambiguous. Only highly piscivorous *Gavialis gangeticus* and semi-piscivorous *Crocodylus intermedius* and *Crocodylus johnsoni* [1], stand out with respect to caniniform pressure generation in showing relatively high values (Figure 4A). (This is remarkable in the cases of *Gavialis gangeticus* and *Crocodylus johnsoni*. Their most prominent caniniform teeth are located more rostrally than in all other crocodilians where bite forces are relatively low; Table 1. Furthermore, *Gavialis gangeticus* generates the lowest relative bite forces among living crocodilians; Figure 3.) All other crocodilian ecomorphs (molluscivores, terrestrial foragers, broad-snouted generalists, and the slender-

### Table 2. Dental measurements and pressure generation for extant Crocodylia.

| Taxon                  | N   | CCA   | RCCA   | CP     | RCP   | MCA   | RMCA   | MP     | RMP   |
|------------------------|-----|-------|--------|--------|-------|-------|--------|--------|-------|
| Crocodylidae           |     |       |        |        |       |       |        |        |       |
| *Crocodylus acutus*    | 2   | 5.78  | 5.78   | 410    | 410   | 5.50  | 4.93–6.08 | 728    | 716–740 |
| *Crocodylus intermedius* | 1   | 3.19  | 3.19   | 1344   | 1344  | 4.52  | 4.52   | 1388   | 1388  |
| *Crocodylus johnsoni*  | 5   | 1.01  | 0.52–1.80 | 624  | 381–1078 | 1.56  | 0.51–2.14 | 832    | 565–1871 |
| *Crocodylus mindorensis* | 1   | —     | —      | —      | —     | 8.02  | 8.02   | 341    | 341   |
| *Crocodylus moreletti*  | 15  | 15.72 | 15.72  | 195    | 195   | 14.24 | 14.24  | 309    | 309   |
| *Crocodylus niloticus*  | 2   | 6.18  | 2.89–9.48 | 451  | 213–689 | 5.41  | 5.06–5.77 | 566    | 505–627 |
| *Crocodylus novaeguineae* | 2  | 9.30  | 6.99–11.61 | 439  | 371–508 | 5.04  | 4.91–5.17 | 1061   | 973–1149 |
| *Crocodylus palustris*  | 1   | 10.97 | 10.97  | 382    | 382   | 14.42 | 14.42  | 506    | 506   |
| *Crocodylus porosus*    | 7   | 8.33  | 3.98–9.57 | 679  | 234–1343 | 7.44  | 2.22–16.87 | 1207   | 300–2473 |
| *Crocodylus rhombifer*  | 3   | 4.55  | 3.08–7.10 | 312  | 263–385 | 4.48  | 2.93–6.18 | 466    | 414–506 |
| *Crocodylus siamensis*  | 3   | 6.59  | 3.32–9.85 | 513  | 293–732 | 5.52  | 4.73–6.67 | 616    | 402–760 |
| *Mecistops cataphractus* | 3   | —     | —      | —      | —     | 4.19  | 4.19   | 406    | 406   |
| *Osteolaemus tetraspis* | 5   | 2.94  | 1.37–5.07 | 400  | 241–660 | 8.79  | 4.73–13.91 | 203    | 139–343 |
| Gavialidae              |     |       |        |        |       |       |        |        |       |
| *Gavialis gangeticus*   | 2   | 0.81  | 0.76–0.86 | 1154 | 958–1349 | 2.98  | 1.41–4.56 | 855    | 440–1270 |
| *Tomistoma schlegeli*   | 3   | 2.99  | 1.72–1.84 | 678  | 613–737 | 4.28  | 3.14–5.05 | 790    | 337–1384 |
| Alligatoridae           |     |       |        |        |       |       |        |        |       |
| *Alligator mississippiensis* | 15 | 8.42  | 4.16–14.67 | 383  | 209–722 | 6.43  | 4.18–8.16 | 806    | 299–1568 |
| *Alligator sinensis*    | 4   | 3.60  | 2.67–4.59 | 207  | 153–258 | 5.34  | 3.81–6.97 | 211    | 150–278 |
| *Caiman crocodilus*     | 4   | 3.04  | 2.52–3.53 | 275  | 228–329 | 3.58  | 2.60–4.40 | 351    | 296–455 |
| *Caiman latirostris*    | 5   | 3.99  | 2.21–6.47 | 298  | 173–487 | 4.18  | 2.43–6.79 | 372    | 226–489 |
| *Caiman yacare*         | 5   | 2.60  | 1.56–4.23 | 276  | 164–387 | 3.46  | 1.76–5.92 | 319    | 185–515 |
| *Melanosuchus niger*    | 3   | 4.15  | 3.04–5.71 | 446  | 320–545 | 6.55  | 3.92–9.22 | 417    | 274–509 |
| *Paleosuchus palpebrosus* | 3   | 1.58  | 1.25–2.01 | 365  | 350–393 | 2.18  | 1.15–3.63 | 493    | 309–793 |
| *Paleosuchus trigonatus* | 3   | 2.24  | 0.88–3.08 | 424  | 216–815 | 3.91  | 3.05–4.64 | 287    | 228–369 |
| Total                   | 83  | 0.52–15.72 | 153–1349 | 0.51–16.87 | 139–2473 |

**N** = Number of Specimens.

CCA = Taxon Representative Caniniform Contact Area @1 mm depth (mm²).

RCCA = Range of Caniniform Contact Areas @1 mm depth (mm²).

CP = Taxon Representative Caniniform Pressure (MPa).

RCP = Range of Caniniform Pressures (MPa).

MCA = Taxon Representative Molariform Contact Area @1 mm depth (mm²).

RMCA = Range of Molariform Contact Areas @1 mm depth (mm²).

MP = Taxon Representative Molariform Pressure (MPa).

RMP = Range of Molariform Pressures (MPa).

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snouted generalists – such as Tomistoma schlegelii and the American crocodile – Crocodylus acutus [1], [7] – show similar values to each other that are relatively lower. What unite these ecomorphs are caniniform teeth that abruptly broaden – moving from the crown apex to the tooth neck. Should substantial, hard constituents be impacted during biting, or off-axis forces experienced, this tooth morphology provides for structural rigidity through reduced bending moments and increased area moments of inertia. However, this is afforded at the cost of rapidly diminishing tooth pressure following initial contact [31]. Conversely, the slender caniniform teeth of the piscivorous and semi-piscivorous ecomorphs ensure that less force is required to drive the teeth through prey. However, higher bending moments and low area moments of inertia put their long, narrow tooth crowns at risk of breakage. Tooth failure is presumably circumvented to some degree through the selection of prey with negligible hard tissues and low inertia (see above).

Molariform tooth-pressure values vary widely among crocodilians. For example the data for the similar-sized durophagous Alligator sinensis and Caiman latirostris span much of the range for

Figure 4. Caniniform pressure values for extant Crocodylia, their phylogenetic distribution, and inferred ancestral character states. (A) Members of the Alligatoridae are shown in blue, and members of the Crocodylidae+Gavialidae in green. The OLS regression equation describes the weak relationship between body mass and caniniform pressure. Note that slender-snouted piscivorous to semi-piscivorous ecomorphs (Gavialis gangeticus and Crocodylus intermedius, respectively) show exceptionally high-pressure values (outside the 95% confidence interval), and Crocodylus johnsoni shows pressures expected of animals nearly a magnitude in size larger. Other ecomorphs show much lower and similar relative values. The arrow indicates the typical ultimate shear strength of bone. (B) Ancestral character-state reconstruction using squared-change parsimony of size-standardized caniniform pressures. Residual caniniform pressure values are color coded to MPa (squared-change parsimony; squared length = 19.491). Vertical scale is in relative time, with the outgroup/ingroup root arbitrarily set to 1.0. High relative pressures were achieved independently in Crocodylus intermedius, Gavialis gangeticus, and Crocodylus johnsoni. Uncolored branches represent taxa for which the caniniform teeth were shed or broken, and so pressure estimation was not possible. doi:10.1371/journal.pone.0031781.g004

Figure 5. Linear regressions of residual caniniform tooth pressures, and residual molariform tooth pressures versus rostral proportion phylogenetic independent contrasts. The (A) residual caniniform, and (B) residual molariform regressions show the low correlation between these parameters after accounting for body mass and phylogenetic relatedness. doi:10.1371/journal.pone.0031781.g005
other same-sized crocodilians (Figure 6A). No values are statistical outliers, and no definitive ecomorphological groupings exist. As mentioned above, crocodilian molariform pressures are comparable to those in the caniniform teeth. However, the bite forces at the molariform tooth positions are much higher since they are closer to the jaw’s fulcrum. Because the molariform teeth are stouter, they are well suited for enduring higher resistance forces while at the same time generating pressures that, like the caniniform teeth, are initially sufficient to damage the hard constituents in their prey. Catastrophic failure of the prey’s tissues is subsequently dictated contact areas, which act in concert with bite forces to explain more fully by its relevance to the positioning and numbers of teeth, jaw hydrodynamics, and resistance to torsion or bending during prey capture and processing [11]. Collectively, the data and methods from this study provide the quantitative biomechanical foundation for further exploration (particularly in fossil taxa) of the remarkable evolutionary success of these long-term predatory denizens of the water-land interface.

Materials and Methods

Data Collection

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The research protocol was approved by the Animal Care and Use Committee of The Florida State University (Permit Number: 0011). The animals were manually secured and strapped down to a testing platform prior to bite-force experimentation, and all efforts were made to minimize suffering. No animals were injured during the execution of this research.

We tested all available sexually mature adult crocodilian specimens from research, conservation, and display specimens housed at the St. Augustine Alligator Farm Zoological Park, St. Augustine Florida, USA and Crocodylus Park, Darwin, AU (Table 1). In total 83 adult (sexually mature) specimens representing all 23 extant crocodilian species currently recognized by the IUCN-SSC (Species Survival Commission of the International Union for Conservation) Crocodile Specialist Group ([23]; size range 1.24–4.59 m, 7–531 kg) were accessed. Multiple individuals were studied for 19 species (Table 1). Our analysis included both male and female specimens since prior studies on wild and captive *Alligator mississippiensis* bite forces revealed statistically indistinguishable performance in same-sized individuals (i.e. body mass, SVL, TL) [14], [15]. The results from the present study confirmed these findings (data not presented).
The bite forces were recorded using sandwich transducers and a portable charge amplification system specifically designed for use on crocodilians [14]. Two preliminary studies on growth series of captive and wild Alligator mississippiensis using this system showed that specimens consistently bit at values near the yield point of the dentition (safety factor = 1.0–1.4) and hence near maximal structural capacity (Note: ~10% of wild Alligator mississippiensis teeth are fractured during normal usage prior to shedding: [33]), and bite-force values for captive specimens can be used to accurately model those in wild individuals when standardized to body mass [15]. Three to five bites were recorded for each animal, the highest of which was used in post-hoc analyses.

Forces were measured on land with the transducer centered below either the left or right most prominent maxillary molariform tooth (located at the maxilla convexity nearer the back of the jaws; Figure 2). This is an ecologically relevant location since it is where these animals primarily crush prey. Crocodilians stereotypically seize prey contacted by the teeth and jaws as the head is swiped to the side. They also process food on one side of the jaw. Thus, unilateral rather than bilateral tooth engagement best mimics natural feeding behavior. Additionally, unilateral crushing of prey at the molariform teeth commonly occurs with the head out of water in all species. Similarly, the seizing of prey using the caniniform teeth often occurs with the head out of water. Fortuitously the prominent molariform tooth position is at a comparable relative distance from the fulcrum across taxa, as an RMA plot of log-transformed fulcrum to molariform distance regressed against log-transformed body mass showed a scaling coefficient of 0.342±0.029 (95% CI), which is not different than isometry at 0.333. Therefore it provided a useful biomechanical standard of comparison in our testing. We took into consideration the effects of drag on force (and pressure generation; see below) during underwater feeding. Maximal velocity differences during terrestrial versus aquatic biting are no more than two-fold intra-specifically regardless of rostral form [31]. [Note: The effective bite force applied during sub-aquatic or terrestrial clenching bites [i.e. where the bite-force transducer or prey has already been seized and a new bite initiated] would be unaffected by drag. Our data show that the forces generated during such bites are at least 90% of the maximum values recorded during initial, defensive bites [14], [15], [31].]

Standard measures of size and morphometrics pertinent to feeding biology were then recorded (Table 1). These included body mass (BM), total length (TL), and rostral proportion (RP, = mid-rostral width/snout length [measured midway between the anterior borders of the orbits to the tip of the rostrum]). In addition, dental putty molds (Knead-A-Mold; Townsend Atelier Inc., Chattanooga, TN) were made for the most prominent caniniform tooth, the primary tooth used to initially contact and seize prey, as well as for the most prominent molariform tooth used in the crushing of prey (Figure 2). These prominent teeth reside in alveoli at the apex of the maxilla convexities. They primarily act in initiation to initiate contact with the prey during seizing or crushing feeding behaviors. Their initial biomechanical performance can be directly linked to morphology and/or dietary ecology before the adjacent teeth become engaged as the tooth descends into prey or the padded transducer. (Note: the caniniform teeth in particular are also employed in defense and aggression where the same biomechanical performance measures studied here are also pertinent). The most pristine of any tooth pair was molded. Specimens for which both teeth were heavily damaged were not used in our analysis. Epoxy casts were made from the molds for use in post-hoc interspecific comparisons of absolute initial maximal tooth pressures.

The casts were indented normally to a depth of one mm in modeling clay. ([We found that measurements <5% crown height were imprecise for the teeth of small taxa. Because of this we opted to use the minimal depth for which repeatable measurements of area could be made for all specimens.) The indentations were digitally photographed and the realized contact area normal to the direction of loading determined using NIH Image software (Imagej64 v.1.42q, National Institute of Health, Bethesda, MD, USA). Initial molariform tooth pressures were determined by dividing the one mm contact area for each individual’s molariform tooth into its respective molariform bite-force value. Initial caniniform pressure estimates were determined by dividing the one mm contact area for each individual’s caniniform tooth into its respective estimated bite-force value. We analyzed the significance of the tooth-pressure values with respect to the shear strengths of the hardest constituents found in crocodilian prey. (Note: shearing is the primary means of failure for hard materials in biological systems [29].)
Phylogeny. Some species were not represented for all genes. Most because the published trees did not include branch lengths, and intervening glutamine tRNA, control region, 12S, and 16S [2].

28S rflp, and portions of the mitochondrial genes nd6, cyt b, the BDNF, ATP7A, LDHa, c-myc, c-mos, DMP1, ODC, and 18S/28S regions. No sequence consisting of published sequences for the nuclear genes RAG-1, from John Gatesy (University of California, Santa Barbara), Small modifications were made to maintain codon integrity in analyses. However these were pruned from the tree prior to

Paleognathae and Neognathae as outgroups in all phylogenetic

competing hypotheses is beyond the scope of the present study, but tionary and ecological ramifications of our data using other

contains time-correlated branch lengths (necessary for PIC. (Note: it was, however, included in analyses of the raw [TIPs] data.) We followed Gatesy et al. [2] in designing Paleognathae and Neognathae as outgroups in all phylogenetic analyses. However these were pruned from the tree prior to conducting the PIC analyses. Alignments were checked by eye. Small modifications were made to maintain codon integrity in reference to the translated amino acid sequence using MacClade [42], but sequences otherwise conformed to the published alignments. A maximum likelihood (ML) search was conducted using PAUP [43] under the GTR+I+G model as indicated by Modeltest [44] and the Akaike Information Criterion. Parameters were estimated from a randomly chosen tree among the most parsimonious trees found under equal weighting parsimony. Starting trees included the set of most parsimonious trees in addition to 10 random addition sequence replicates. All searches found the same single tree (Figure 1), congruent with the slightly less resolved tree in Gatesy et al. [2], and identical to that in the more recent Gatesy and Amato [45] analysis with the exception of our tree resolving one trichotomy near the tip in Crocodylus. The ML tree was made ultrametric using penalized likelihood in r8s [46, 47] and the ML branch lengths. Cross validation was conducted on the ML tree using a range of smoothing parameters from 1 to 1000. A smoothing parameter value of 3.2 was found to minimize deviations and was the value used in the final analysis.

Phylogenetic Independent Contrasts

All morphological and mechanical variables (see Tables 1 and 2) were log-transformed with the exception of rostral proportions (RP). Transformations were done to normalize these data, which spanned a large, 21-fold size range in mean body mass (Table 1). Because rostral proportions are a ratio, and therefore are already normalized to body size, log-transformation was unnecessary. These proportions were not significantly correlated with size, unlike all other variables. Phylogenetic signal was estimated by the K statistic using the picante package [48] in R [49]. K statistics ranged from 0.347 to 0.838. A value of 1.0 indicates these data are fit by a Brownian motion model, whereas values close to 0 indicate closely related taxa are less similar than expected under Brownian motion, as might be caused by adaptation or measurement error [50]. All variables exhibited significant phylogenetic signal (K>0.5) except log-molariform contact area and the log-pressure variables (K = 0.347-0.443; p = 0.209-0.344). Caniniform and molariform pressures were mapped onto the phylogeny using the Mk1 model for likelihood in Mesquite 2.5 [35]. PIC analyses were conducted using the ultrametric tree with the PDAP module [51] in Mesquite 2.5 [35]. The contrasts were standardized through division with their standard deviations (square-root of summed branch lengths). This effectively converted them to evolutionary rates. PDAP diagnostics (standardized contrasts regressed against their standard deviations) showed that only log-molariform contact area and log-caniniform pressure deviated significantly from a Brownian motion model (p<0.05). Because the tree contained one trichotomy, the degrees of freedom for the diagnostics were reduced by one. Since bite force was strongly correlated with body mass for both raw data (R² = 0.92) and phylogenetically corrected log-transformed data (R² = 0.87), we first created size-standardized variables. This was achieved by regressing contrasts of mean rostral proportions as well as log-transformed taxon representative bite forces, tooth contact areas, and tooth pressures against contrasts of log-mass and saving the residuals. To remove the effects of tooth size, the size-standardized residuals for pressure were also regressed against the size-standardized residuals of contact area. These size- and contact area-standardized residuals were saved. This effectively removed the evolutionary variance in pressure associated with changes in body mass and tooth cross-sectional area. These residuals for performance measures were then regressed against the rostral proportions. All regressions on PICs were constrained to pass through the origin. Throughout, we report TIPs results for ease of visualization, but due to
non-independence of the raw data, statistical significance is reported only for PICs.

Estimations of Bite Forces in Fossil Crocodilians and Large Extant Individuals

Our range of bite-force estimates for 6.7 m specimens of Crocodylus porosus was based upon the interspecific regression of mean body mass versus mean bite force \( (Y_{\text{bite force}}, N_1 = 29.632x) \) with an estimated mass of 1,308 kg from the intraspecific regressions of wild Crocodylus porosus from Webb and Messel [52]. A second bite-force estimate was acquired using an intraspecific regression for a captive growth series of this taxon \( (\text{range} = 0.96–531 \text{ kg}; [31]) \) where \( Y_{\text{bite force}}, N_1 = 115.39x \) body mass, kg, \( R^2 = 0.96 \). Note: Our previous research has shown that bite-force generation is statistically indistinguishable between same-sized (i.e. body mass, SVL, or TL) captive and wild Alligator mississippiensis [14], [15].

Our estimates of the upper and lower historical bounds of adult crocodile bite forces were based on the interspecific regression of mean mass versus mean bite force \( (Y_{\text{bite force}}, N_1 = 29.632x) \) with an estimated mean mass of 6.7 m individuals compared to captives of equal TL. We used the 0.8 m TL Precaimanoidea kaiy [27] to represent the lowermost known size for Crocodylia. The 11 m TL Deinonychus rigignadensis [28] was used to represent the upper bound. The mean largest adult body masses for these taxa were estimated from our interspecific regression of mean mass and TL for adults of extant taxa \( (Y_{\text{body mass}}, kg = 5.00x\text{total length}, m^2; R^2 = 0.93) \). (Note: the upper bound bite-force estimate for Deinonychus rigignadensis is more tenuous since the largest known fossil crocodilian specimens greatly exceed the neontological size range studied here.)

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Author Contributions

Conceived and designed the experiments: GME, PMG AKL KAV. Performed the experiments: GME PMG AKL KAV JDB DJWW. Analyzed the data: GME PMG JSJ AKL BDI. Contributed reagents/materials/analysis tools: GME JSJ JDB BMJWW. Wrote the paper: GME PMG JSJ AKL BDI.

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