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# Sexual dimorphism, deformations, and epibionts of *Phrynops tuberosus* (Testudines, Chelidae)

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**Abstract**

Studies focusing on the natural history of species are essential for developing effective conservation measures and evaluating ecological hypotheses. To this end, we describe natural history data of the Cotinga River toadhead turtle, *Phrynops tuberosus*, in the Banabuiú River in Ceará, Brazil, and evaluated sexual dimorphism, epibionts, and mutilation effects. We hand-captured 134 individuals by snorkeling, over a period of one year, resulting in the capture of 94 males, 24 females, and 16 juveniles. Females had larger head width and body mass than males, while males had longer tail length. One quarter of the turtles captured had some sort of injury or deformation, most common injuries being missing claws, mutilations, and shell deformations. We found no difference in body condition index between mutilated and non-mutilated animals. Mollusks, insects, and leeches were found as epibionts on *P. tuberosus* and most of the captured turtles had extensive algal cover. Future studies should focus on understanding the effect of mutilations on animal fitness and reproductive success.

**Keywords**

Body condition index; Cotinga River toadhead turtle; ecology; mutilation effect; natural history

## Introduction

Natural history studies are important to develop effective conservation actions for a species and to evaluate ecological hypotheses (Bury, 2006). However, basic aspects of the natural history of many species of turtles, such as sexual dimorphism, ef-

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fects of mutilations and epibionts, among others, on animal's fitness are still poorly studied.

Sexual dimorphism may be driven by ecological causes, fecundity selection, and sexual selection (Slatkin, 1984; Shine, 1989, 1991; Bulté et al., 2008; Liao et al., 2015). In most chelonians, females are larger than males which can be explained by differences in maturation time between the sexes (a proximate cause; Gibbons & Lovich, 1990) or a positive relationship between female size and reproductive output (an ultimate cause; Cox et al., 2007). Other hypotheses that can explain why males are smaller than females in some turtles are i) small males are agile and can quickly find partners and ii) males invest their energy in chasing females rather than in growing (Berry & Shine, 1980).

Mutilations and amputations may have negative effects on animals, reducing survival rates and affecting their performance (Coble, 1971; McCarthy & Parris, 2004; Waddle et al., 2008; Schmidt & Schwarzkopf, 2010), but such effects are not found in all species (Zymonas & McMahon, 2006; Waddle et al., 2008). Lacerations, mutilations, scars, amputations, and deformations are commonly found in turtle populations (Bell et al., 2006; Davy & Murphy, 2009; Bulté et al., 2010; Famelli et al., 2011; Rodrigues & Silva, 2013). Missing claws, shell deformities, and limb mutilations (amputations) are examples of these injuries. However, the influence of these injuries on aspects of individual performance or body condition is not generally known for turtles.

*Phrynops tuberosus* is a freshwater chelid turtle distributed in Venezuela, Guyana, and northern and northeastern Brazil (Rueda-Almonacid et al., 2007). This species is active throughout the whole year and its reproduction is related to precipitation seasonality along the year (Rueda-Almonacid et al., 2007; Rodrigues & Silva, 2014, 2015). Apart from individual reports (e.g., Rodrigues & Silva, 2013; Coelho et al., 2014), natural history information is scarce for freshwater turtles living in Brazilian semiarid regions including *P. tuberosus* (Souza, 2004); a species that has not been assessed by IUCN status risk. We present information on the natural history of *Phrynops tuberosus* in the Banabuiú River, focusing on sexual dimorphism, reproduction, injuries, and epibionts. We also evaluated the effect of amputations on the body condition of these animals. As far as we know, this is the first time that the effect of amputations has been evaluated in turtles.

## Materials and methods

We captured *Phrynops tuberosus* on the Banabuiú River at a perennial stretch near the village of Laranjeiras (5°17′45″S, 38°51′54″W, WGS84), Banabuiú, Ceará, Brazil, between December 2011 and November 2012. We actively searched for the turtles in a 41 m × 100 m stretch of the river in which the maximum depth was 2.5 m. Floating aquatic macrophytes were abundant in shallow portions of the river, and there were also dense macrophytes attached to the substrate. The climate in the region is tropical and semi-arid, with a mean temperature of 26-28°C and mean annual rainfall of about 815 mm occurring mainly from February to April (IPECE, 2012).

We hand-captured animals while snorkeling. This method provides good capture rates and detection probabilities in clear water (Sterrett et al., 2010). We performed a total capture effort of 250 person-hours, with three people searching for turtles one day per month. Sampling effort for each day was approximately eight hours.

We measured straight-line carapace length (CL), carapace width (CW), plastron length at the medium-ventral suture (PL), tail length base to vent (TL), and head width (HW). The measurements were made with 300 mm analog calipers with an accuracy of 0.05 mm. We also determined body mass (BM) using a digital balance with an accuracy of 1.0 g. We marked turtles as described by Cagle (1939), sexed them following Rueda-Almonacid et al. (2007), and then released them at the end of the survey day. We weighed an animal after recording all its linear measurements in order to wait longer to see if the turtle released some additional water retained in its bladder. We used palpation to assess whether female turtles had shelled eggs in their abdominal cavities (Rueda-Almonacid et al., 2007).

We recorded amputations (lack of any limb), shell deformation, other general lacerations, and epizoic invertebrates. Most invertebrates found on the carapace and soft parts were collected, identified to the lowest taxonomic level possible, preserved in 70% ethanol, and deposited in the collection of the Coleção de Herpetologia da Universidade Federal do Ceará – Coleção de Anexos (vouchers E0005-E0027). A specialist identified the mollusks and leeches were identified following Sawyer (1972) and Davies & Govedich (2001). We also visually estimated whether an individual had 0-25, 25-50, 50-75, or 75-100% of algal cover on the carapace in order to evaluate the frequency of this epibiont on turtles using photographs to classify individuals into each group (Garbary et al., 2007). Some animals were not grouped into these categories due to the poor picture quality.

We assessed sexual dimorphism in CW, TL, HW, and BM using Analysis of Covariance (ANCOVA). Plastron length was used as a covariate in these analyses. All measured variables were log-transformed ($\log_{10}$) in order to improve normality, homoscedasticity, and linearity. Sexual dimorphism in plastron length and carapace length were evaluated using $t$-tests. Some animals were not included in the analyses of sexual dimorphism because their plastron lengths could not be properly measured. Juveniles were not subjected to these analyses, because they did not have clear secondary sexual characteristics.

We tested the hypothesis that amputated animals had a body condition index (BCI, estimated as the ratio $BM/CL^3$, following Bjorndal et al. (2001)) similar to unmutilated animals. We used a bootstrap approach with 10 000 randomizations and calculated confidence intervals of 95% (0.0275-0.9725) in order to compare the mean BCI of amputated animals with the BCI of 10 animals randomly sampled from the population. We also performed additional bootstrap replicates considering the different probabilities of taking a juvenile, a male, or a female in the random sampling because we found sexual dimorphism (see Results) among these groups.

We observed that wounds were completely healed in all amputated animals, indicating that the animals had lived with their mutilations for a long time.

Statistical analyses were performed using the software R ver. 3.0.1 (R Core Team, 2013) and descriptive statistics are presented as mean ± standard deviation.

## Results

We captured 134 animals (16 juveniles, 94 males, and 24 females). We found no differences between the sexes in CL or PL (table 1). Males and females were significantly different in HW, TL, and BM (fig. 1). Females were larger than males in HW (fig. 1A), but TL was greater in males (fig. 1B). The interaction between sex and PL was significant in the ANCOVA model of BM, with small males heavier than small females, while large females were heavier than large males (table 1, fig. 1C).

Five female turtles had shelled eggs in their abdominal cavities. These animals were found in July (n = 1), August (n = 1), October (n = 2), and November (n = 1). Mean carapace length of gravid females was 27.85 cm (range 27.36-28.69 cm).

We found deformations or mutilations in 26% of the captured animals (35 of 134). Most of the injured animals (28) had only one type of injury, but seven had

**Table 1.**
Measurements (means and standard deviations) and sexual dimorphism of *Phrynops tuberosus* captured in the Banabuiú River, Ceará, Brazil.

| Measure | Males ($N = 86$) mean ± SD | Females ($N = 22$) mean ± SD | Statistics |
|---|---|---|---|
| CL (cm) | 20.83 ± 3.61 | 22.52 ± 5.37 | $t_{106} = 1.25$, $P = 0.21$ |
| PL (cm) | 16.65 ± 2.74 | 18.20 ± 4.13 | $t_{106} = 1.55$, $P = 0.12$ |
| CW (cm) | 15.15 ± 2.32 | 16.52 ± 3.64 | PL:Sex – $F_{1,104} < 0.01$, $P = 0.99$<br>Sex – $F_{1,105} = 0.30$, $P = 0.58$<br>PL – $F_{1,105} = 1434$, $P < 0.01$ |
| TL (cm) | 4.43 ± 0.81 | 2.71 ± 0.85 | PL:Sex – $F_{1,104} = 1.55$, $P = 0.22$<br>Sex – $F_{1,105} = 595$, $P < 0.01$<br>PL – $F_{1,105} = 321$, $P < 0.01$ |
| HW (cm) | 3.62 ± 0.53 | 4.22 ± 0.92 | PL:Sex – $F_{1,104} = 0.94$, $P = 0.33$<br>Sex – $F_{1,105} = 54.16$, $P < 0.01$<br>PL – $F_{1,105} = 1832$, $P < 0.01$ |
| BM (g) | 822.68 ± 330.21 | 1240.36 ± 680.57 | PL:Sex – $F_{1,104} = 4.86$, $P = 0.02$<br>Sex – $F_{1,104} = 18.72$, $P < 0.01$<br>PL – $F_{1,104} = 6454$, $P < 0.01$ |

Abbreviations: CL, carapace length; CW, carapace width; PL, plastron length; TL, distance between the base of the tail and the vent-tail length; HW, head width; BM, body mass. We used Analysis of Covariance (ANCOVA) with PL as the covariate to assess sexual dimorphism in CW, TL, HW, and BM. We compared CL and PL between males and females using $t$-test. For each ANCOVA analysis, we reported three $F$-values: 1) interaction between PL and sex (PL:Sex); 2) difference between males and females (Sex); and 3) influence of the covariate (PL). When the interaction was not significant, this variable was removed from the model and the $F$-values were recalculated.

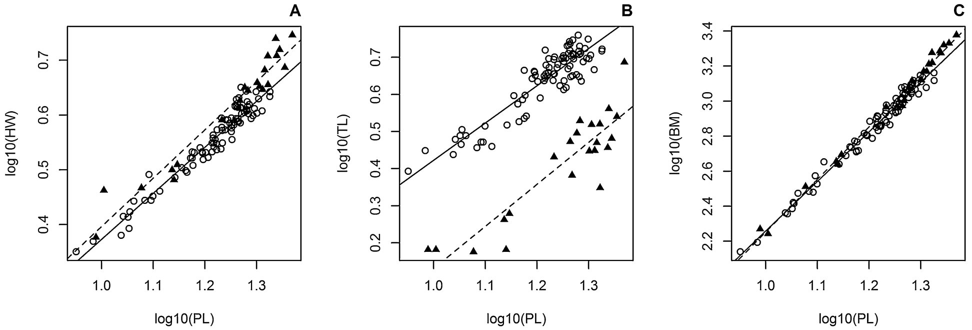

**Figure 1.** Sexual dimorphism in head width (HW, panel A), tail length (TL, panel B) and body mass (BM, panel C) of *Phrynops tuberosus* captured in the Banabuiú River, Ceará, Brazil. We used plastron length (PL) as a covariate in the Analysis of Covariance (ANCOVA) models. The measured variables were log-transformed ($\log_{10}$) in order to improve normality, homoscedasticity, and linearity. Open circles and full lines (males); black triangles and dotted lines (females).

two or three. Shell deformations (n = 11), missing claws (n = 10), limb mutilations or amputation (n = 10), eyes or nostril damage (n = 3), holes in marginal scutes (n = 3), lacking barbells (n = 3), and lacking toes (n = 1) were found. Some injuries seemed to be caused by humans, such as holes in the marginal scutes and an animal that lost its left forelimb due to a nylon wire wrapped around its limb.

We captured 10 animals (7% of captured animals) lacking a limb [two females (8% of captured females), three juveniles (19%), and five males (5%)]. The mean BCI of these animals (mean = 0.0922, range 0.0755-0.1085) was within the confidence interval calculated using 10 000 randomizations for a total random sampling (mean = 0.0912; CI = 0.0835-0.1007) and for samplings in which we kept the frequencies of males, females, and juveniles equal to those of the amputated group (mean = 0.0893, CI = 0.0820-0.0990). Hence, the BCI of turtles with missing limbs was not significantly different from the BCI of the studied population sample considering the 134 captured animals.

We found 28 different turtles with epifauna and some animals (six) had two different species of epibionts. The epizoic fauna was mainly composed of mollusks (Ancylidae or limpet-like shell snails: n = 12; the snail *Biomphalaria straminea*: n = 10; unidentified mollusks: n = 2), but insects (n = 3) were also found on some animals between the patches of algae attached to the carapace. We found two morphotypes of glossiphoniid leeches in 11 turtles, and the largest one was identified as *Placobdella* sp. These ectoparasites were found attached to the soft parts next to the limbs (inguinal region), neck, and tail, mainly on the underside of marginal scutes. Some leeches were also directly attached to the ventral part of the carapace, next to the soft parts, on the bony tissue. We analyzed the photographs of 93 turtles and all had algae attached to their carapace; most animals had more than 50% of their carapace covered. Six animals had 0-25% of their carapace covered, 17 had 25-50%, 31 had 50-75%, and 39 had 75-100%.

## Discussion

Fecundity selection favoring large females is common among ectothermic vertebrates (Cox et al., 2007; Stephens & Wiens, 2009; Liao et al., 2015). Female freshwater turtles are also commonly larger and heavier than males (e.g., Gibbons & Lovich, 1990; Rueda-Almonacid et al., 2007; Brito et al., 2009b; Marques et al., 2013), and reproductive advantages for large females and different energetic allocation may explain this dimorphism in these reptiles (Berry & Shine, 1980; Stephens & Wiens, 2009). The difference in the slope of the relationship between plastron length and body mass between males and females in *P. tuberosus* is an interesting finding not reported in other turtle studies, but is consistent with reproductive advantages of adult females storing energy to reproduce and adult males investing energy to catch females (Berry & Shine, 1980; Cox et al., 2007). Sexual dimorphism in tail length has already been reported for *Phrynops tuberosus*, and it is one of the few characteristics which are dimorphic in virtually all chelonians (Rueda-Almonacid et al., 2007).

Females had wider heads than males of similar size. This result has been found in species of the genus *Graptemys*, and differences in diet and reduction of intersexual competition explain sexual dimorphism in head width in these turtles (Lindeman, 2000; Lindeman & Sharkey, 2001; Bulté et al., 2008; Stephens & Wiens, 2009). Studies focusing on the diet of *Phrynops tuberosus* may improve the understanding of this difference in head width.

The reproduction of freshwater turtles is related to precipitation, and this relationship is stronger in semiarid regions (Souza, 2004; Rodrigues & Silva, 2014). In our study site (Banabuiú, Ceará, "Caatinga" biome), females of *Phrynops tuberosus* lay eggs and seem to be in reproductive condition from July to November, which is the end of the dry season in the region. This result is consistent to findings that hatchlings of *P. tuberosus* in "Caatinga" are more abundant in February and March, which is the peak of the rainy season in the region (Rodrigues & Silva, 2014), reinforcing the influence of precipitation on the reproduction of freshwater turtles from semiarid regions.

Our BCI results suggest that amputated animals are able to perform as well as their intact conspecifics in relation to energy acquisition. However, these injuries may have a negative effect on courtship behavior, when, for example, the male has to pursue and dominate the female before copulation. Pursuit and pre-copulation phases are common in chelid species (Molina, 1996; Brito et al., 2009a). In this situation, the lack of a limb could reduce swimming performance or hamper female domination, decreasing the reproductive success of males. Future studies should aim to test the occurrence of negative factors related to swimming performance and reproductive output.

Deformations and mutilations found in turtles may be a consequence of malformation (Davy & Murphy, 2009), predation attempts (Rueda-Almonacid et al., 2007; Famelli et al., 2011), or human actions (Bulté et al., 2010). Some *P. tuberosus* captured in the Banabuiú River had semicircular deformations on the posterior or anterior part of their carapace, consistent with a predator's bite. Agonistic interactions and courtship behavior of turtles of the family Chelidae involve bites and other dominance behaviors (Molina, 1992, 1996; Brito et al., 2009a). Hence, minor injuries may result from intra- or intersexual interactions. The holes in the marginal scutes of some animals and the limb amputated by a nylon wire represent some negative interactions between humans and these animals.

We found that the majority of turtles had a high algal cover (>50% of their carapace covered). A similar pattern was found in *Emydoidea blandingii* and *Chelydra serpentina* from Nova Scotia (Garbary et al., 2007). The mollusks and insects found on *P. tuberosus* were commonly attached to this algal cover. The association between these animals and *P. tuberosus* may be indirectly advantageous to these invertebrates because they can disperse long distances using turtles as vectors. Schärer & Epler (2007), for example, found that Hawksbill Turtles (*Eretmochelys imbricata*) might disperse chironomid species associated with their shell.

Leeches of the genus *Placobdella* are common parasites of turtles (Sawyer, 1972; Brites & Rantin, 2004; Siddal & Gaffney, 2004; Ryan & Lambert, 2005). We found leeches on many soft parts of *P. tuberosus*, a pattern already found in *Phrynops geoffroanus* and *Hydromedusa tectifera* (Brites & Rantin, 2004; Huckembeck & Quintela, 2013), and almost all the observed leeches were found on the underside of the marginal scutes. These findings reinforce a hypothesis proposed by McCoy et al. (2007), who stated that these hidden positions might reduce the desiccation risks of the leeches when turtles emerge from the water. Some leeches are able to feed on blood vessels found in the turtle's shell (Siddall & Gaffney, 2004), which may explain why these ectoparasites were also found attached to the carapace and plastron of *P. tuberosus*.

Finally, the sexual dimorphism found in *Phrynops tuberosus* followed the general pattern present in turtles regarding tail length, but the patterns in body mass and head width provide interesting scenarios for future studies of causal relationships on sexual dimorphism. We also described possible ecological associations (parasitism and commensalism) between *P. tuberosus* and invertebrates. In addition, we found that amputations did not reduce the body condition index of turtles.

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