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Sex ratio of black caiman (*Melanosuchus niger*) hatchlings in a sample of the wild managed population from the Mamirauá Reserve, Central Amazon

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ABSTRACT

Sex determination in crocodilians is dependent on environmental temperature, in which extreme temperatures favor the development of females, while intermediate temperatures favor males. Understanding such temperature dependence in light of current climate change is urgent, as males are the main targets in species' sustainable management. Here we analyze the sex ratio of newly-hatched Black caiman (*Melanosuchus niger*) hatchlings from a wild managed population in Central Amazonia. We captured 86 newly-hatched hatchlings and determined their sex through histological analysis of the gonads. We identified 58% males and 42% females among the 86 hatchlings analyzed (male-to-female ratio of 25:18) We identified 58% males and 42% females among the 86 hatchlings analyzed (male-to-female ratio of 25:18) suggesting that the sex ratio of Black caiman hatchlings in 2023 in the area was relatively balanced. This balance contrasts with expectations of a female bias under recent warming conditions and indicates the potential recruitment of males into the population, which is essential to ensure population equilibrium and the long-term viability of harvest quotas.

KEYWORDS: crocodilians; sustainable management; nesting

Razão sexual de filhotes de jacaré-açu (*Melanosuchus niger*) recém eclodidos em uma amostra da população silvestre manejada da Reserva Mamirauá, Amazônia Central

RESUMO

A determinação do sexo em crocodilianos é dependente da temperatura do ambiente, com temperaturas extremas produzindo fêmeas e temperaturas intermediárias produzindo machos. Entender essa dependência da temperatura diante dos alertas atuais de mudanças climáticas é urgente, uma vez que os machos são os principais alvos do manejo sustentável da espécie. Nesse estudo, nós analisamos a proporção sexual de filhotes de jacaré-açu (*Melanosuchus niger*) recém-eclodidos em uma população silvestre e manejada na Amazônia Central. Capturamos 86 filhotes recém-eclodidos e determinamos o sexo por análise histológica das gônadas. Nós identificamos 58% de machos e 42% de fêmeas entre os 86 filhotes analisados (razão macho-fêmea de 25:18), o que sugere que a proporção sexual dos filhotes de jacaré-açu eclodidos em 2023 na área foi relativamente equilibrada. Esse equilíbrio contrasta com nossas expectativas de um viés feminino devido ao aquecimento ambiental recente e indica o recrutamento potencial de machos para a população, o que é essencial para garantir o equilíbrio populacional e a viabilidade de cotas de manejo a longo-prazo.

PALAVRAS-CHAVE: crocodilianos; manejo sustentável; nidificação

Effectively-sustainable wildlife management requires knowledge of the population dynamics of managed species. This includes understanding and monitoring animal populations from birth, through the recruitment of young individuals that become reproductive, to adults that will determine quotas for future harvest. The sustainable management of Amazonian caimans for the commercialization of meat and by-products has been widely discussed in recent years (Marioni *et al.* 2021), with specific harvest planned in Brazilian laws (Amazonas 2011, Franco *et al.* 2019), especially for populations of Black caimans (*Melanosuchus niger* Spix 1825) from the middle Solimões river region, Central Amazonia.

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Although the Black caiman is quite a conspicuous species, there is a lack of basic population dynamics data for its management. For example, it is known that only about 10% of the eggs and juveniles produced annually by females are recruited into the population and reach reproductive age (Grigg 2015), but, to the best of our knowledge, the sex ratio of hatchlings has not been documented.

Sex determination in crocodilians is temperature-dependent (Bull 1980, Lang and Andrews 1994). Environmental factors, including climatic conditions, can directly influence the exposure of eggs to temperature variation (Ferguson & Joanen, 1983; López-Luna *et al*., 2020). While high or low temperatures tend to favor the development of females, males are predominantly produced at intermediate temperatures (FMF pattern), although precise estimates of this pattern are unknown for *Melanosuchus niger* (González *et al*. 2019).

This temperature dependence raises concerns in light of climate change scenarios, as rising temperatures could lead to an increase in the proportion of females within the population. This shift could significantly impact the generation of new individuals in the population, reflecting on the established harvesting quotas, which primarily target males (Amazonas 2011).

We monitored nine nests built in three lakes within the Mamirauá Reserve, situated at the confluence of the Solimões and Japurá Rivers, Central Amazonia, Brazil (Figure 1). Mamirauá Reserve is covered by floodplain habitats subject to a large monomodal flooding pulse (Ramalho *et al*. 2009) and composed of a mosaic of forests and water bodies (Irion *et al*. 1997).

All lakes are located in the sector (a socio-political division used in the Reserve) named Jarauá, and are utilized in sustainable management actions for *Melanosuchus niger* (Franco *et al*. 2019). The nests monitored in this study are a subset (5% to 42% of the nests) of the those present in each lake: (I) Lake Sarapião, 42% (total nests in the lake = 7); (II) Lake Tracajá, 17% (total nests in the lake = 23); and (III) Lake Tucunarezinho, 5% (total nests in the lake = 38).

Monitoring was conducted during the nesting period (October 2022–January 2023) to prevent predation or flooding (Villamarin *et al*. 2011, Torralvo *et al*. 2017). During the estimated hatching period (January/2023), we revisited the lakes and, using forceps, we captured 86 hatchlings from nine nests distributed across three lakes (Figure 1). All hatchlings were already in the water, grouped together, where they were captured. Some were close to an adult caiman, which we presumed to be the mother. The relationship between the captured hatchlings and the nest identity, assigned during the monitoring stage, was determined used a GPS device according to their proximity. Monitored nests were spaced about 100 meters apart, and hatchlings stay near their nest after hatching, making nest origin identification reliable.

Vegetation cover above the nest influences nest temperature, as shaded nests tend to have lower temperatures (López-Luna *et al*., 2020). Therefore, we visually estimated the percentage of vegetation cover above the nest, 0% indicating no vegetation cover above the nest, up to 100% when the nest was completely covered by vegetation.

The captured hatchlings were translocated to the Reptiles Laboratory of the Mamirauá Institute, weighed using an

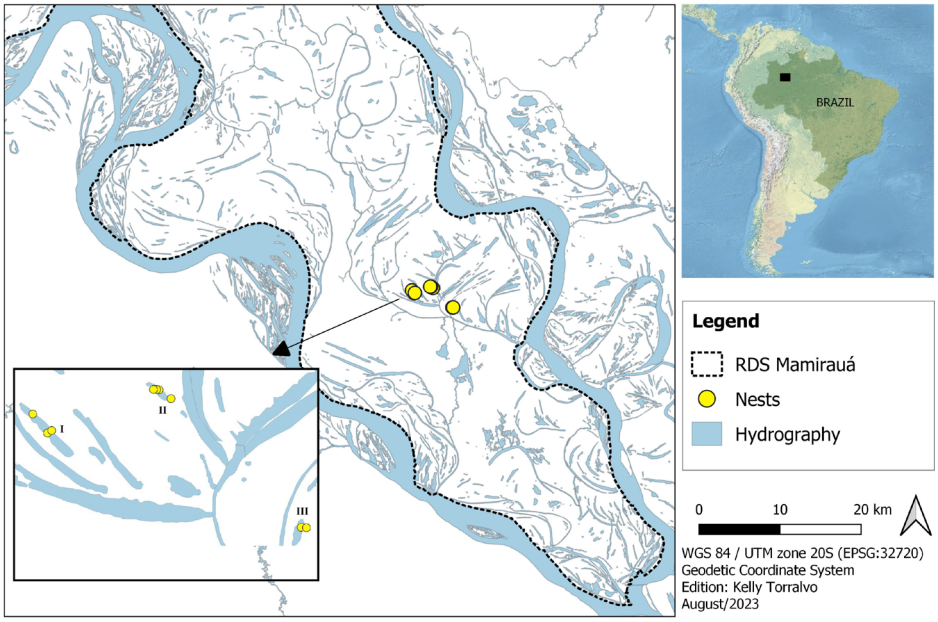

Figure 1. Mamirauá Sustainable Development Reserve in the Middle Solimões, Central Amazonia, showing monitored nests of the black caiman *Melanosuchus niger*. (I) Lake Sarapião; (II) Lake Tracajá; and (III) Lake Tucunarezinho.

analytical scale (500 g capacity, 5 g accuracy), had their total length (TL) measured with tape and head length with a digital caliper. They were subsequently euthanized for the removal of the Gonad-Adrenal-Mesonephros (GAM) complex, which was used for histological sex determination. Euthanasia was performed using the anesthetic Propofol and liquid Lidocaine, administered intravenously at a dose of 50 mg/kg. Death was confirmed by cardiac auscultation. The procedures were conducted under the authorizations registered with CEUA/IDSM (protocol no. 10/2022) and ICMBio/SISBIO (license no. 83966-1). The specimens are deposited in the Collections of the Mamirauá Institute.

Initially, the GAM was transversely cleaved and fixed for 24 hours in 10% formalin. After this fixation period, tissues were dehydrated in sequences of immersion in ethanol, clarified in xylene, and embedded in liquid paraffin. Finally, histological sections of 5-µm thickness were made, mounted on glass slides, and stained with Hematoxylin and Eosin (HE) (Pereira dos Santos *et al*. 2021). Sex determination was conducted through histological examination of the GAM complex, attached to the sexual duct. Differentiation was based on the presence or absence of ovarian and testicular structures, with females retaining the Müllerian duct and males exhibiting only its remnants (Stoker *et al*. 2003, Moore *et al*. 2010) (Figure 2). While adult individuals of the species *M. niger* exhibit sexual dimorphism in body size, little is known about this characteristic in their hatchlings (Verdade, 2003). Therefore, we also include morphometric data in the analyses of this study.

The sex ratio of the hatchlings was presented as the percentages of males and females found in nests, in lakes, as well as for the area considering all lakes together. For the general analysis of how the male-female sex ratio differ from 1:1 we used a chi-square test. We also compared total length (cm) and body weight (g) between males and females using the Kruskal-Wallis test, and head length (cm) using the Student's t-test. Additionally, we tested the relationship between the sex ratio of the hatchlings and the percentage of vegetation cover of each nest. For this, we used a binomial GLM with a quadratic term to capture non-linear effects, expecting extreme values of vegetation cover (colder or warmer due to solar exposure) to favor female hatching, and intermediate values to favor males, consistent with the FMF pattern.

We identified a total of 58% males and 42% females in the 86 analyzed hatchlings from nine nests, with a male-to-female ratio of 25:18, as derived from the 1:1 sex ratio analysis. However, this deviation from a 1:1 sex ratio was not statistically significant ($\chi^2 = 2.28$, df = 1, p = 0.13). The sex ratio of hatchlings varied between 40–75% males and 25–60% females among lakes, while individual nests showed wider variation, ranging from 0 to 100% for both sexes (Table 1).

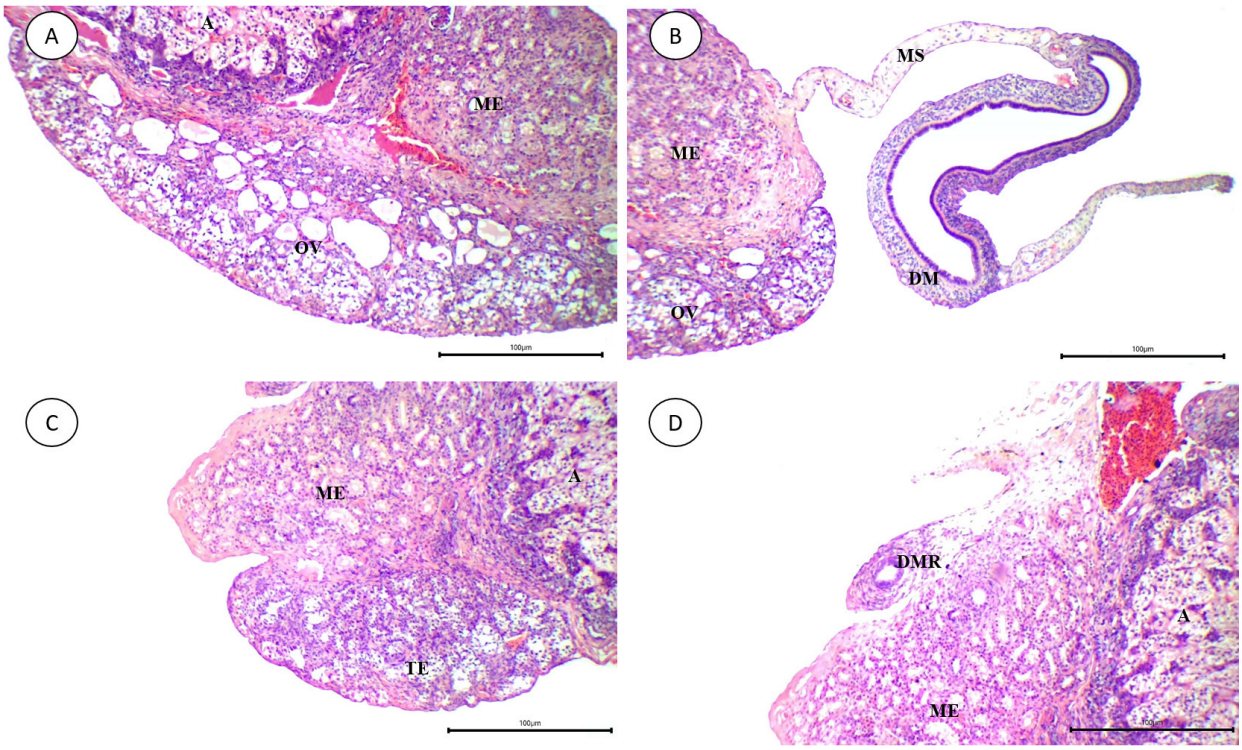

Figure 2. Photomicrographs (40x magnification) of the Gonad-Adrenal-Mesonephros (GAM) complex of juveniles *Melanosuchus niger*. A and B represent the GAM of a female, distinguishing the ovary (OV), mesonephros (ME), adrenal (A), mesosalpinx (MS), and Müllerian duct (DM). C and D represent the GAM of a male, distinguishing the testicle (TE), adrenal (A), mesonephros (ME), and the remnant Müllerian duct (DMR). Transverse sections stained with Hematoxylin and Eozin.

Table 1. Sex ratio of Black Caiman (*Melanosuchus niger*) hatchlings and vegetation cover above the nest from nine nests located across three lakes in the Mamirauá Sustainable Development Reserve, Central Amazon.

Sex ratio by nest			Sex ratio by lake			Vegetation cover %
ID_Nests	Male %	Female %	ID_Lake	Male %	Female %	
JM07O12	40	60	Sarapião	50	50	50
JM09O12	20	80				60
JM11O12	90	10				50
JM13O13	100	0	Tracajá	75	25	20
JM21O13	90	10				30
JM22O13	80	20				0
JM25O13	40	60				30
JM25O11	80	20	Tucunare-zinho	40	60	15
JM02N25	0	100				30

There was a difference in body weight between the sexes of the hatchlings (H = 4.67, p = 0.03), with males (84.1 g ± 6.5 SD) being heavier than females (81.9 g ± 5.6 SD). They also differ by mean total length (H = 5.78, p = 0.01); females had and average and SD of 32.9 ± 1.3 cm and males 33.6 ± 0.9 cm, but not by head length (t = -1.29, p > 0.05; females with 4.5 ± 0.19 cm and males 4.6 ± 0.1 cm) (Figure 3). However, there was no significant relationship between the sex ratio of each nest and vegetation cover above nests (linear term: β = 0.046, p = 0.77; quadratic term: β = –0.00017, p = 0.94).

Temperature-dependent sex determination (TSD) in crocodilians has been the focus of research for over three decades and is supported by strong evidence (González *et al*., 2019). Although this study did not include temperature data, the region has faced extreme droughts and rising temperatures in recent years (Fleischmann *et al*. 2025). We therefore expected a female-biased sex ratio, but our results indicate a relatively balanced proportion of males and females hatched in 2023 at the study site.

The presence of males observed in our sample represents recruitment of potential breeders for the population, maintaining the replacement of individuals harvested. Survival and reproductive success of hatchlings depend on multiple factors, making the annual sex ratio a key metric for management. To support this, ongoing studies are collecting nest temperature data absent from this study. Together, this information contributes to the understanding of the species' population dynamics and should underpin actions for effective sustainable management and conservation efforts.

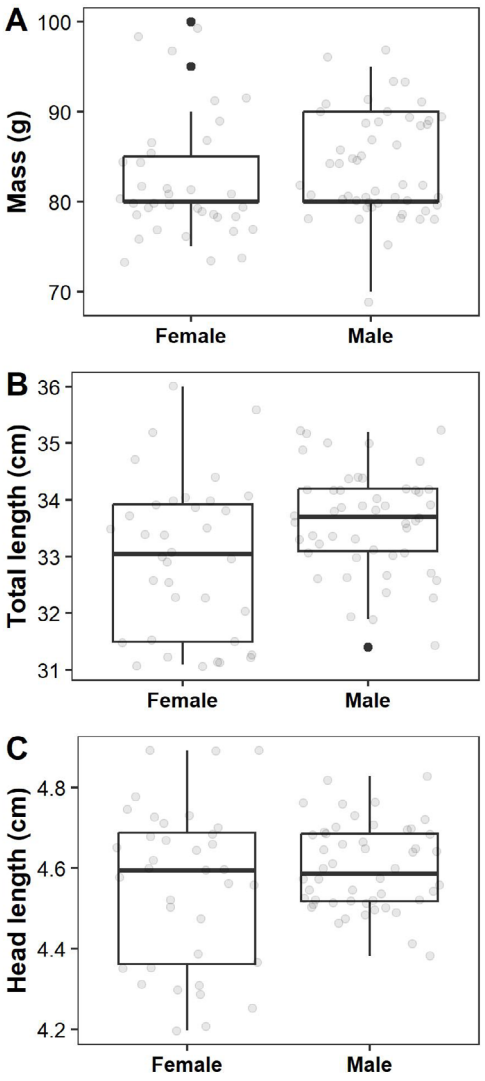

Figure 3. Morphological traits of Black caiman hatchlings in relation to sex: (**A**) Mass and (**B**) Size compared using Kruskal-Wallis test, and (**C**) Head length compared with Student's t-test.

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