Relationship Between Egg Biometrics and Hatchling Size in a Wild Population of *Crocodylus acutus* in Guatemala

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Resumen

A nivel mundial, el cocodrilo americano (*Crocodylus acutus*) está catalogado como Vulnerable (VU) en la Lista Roja de la UICN debido a amenazas como la pérdida de hábitat, la caza furtiva, la captura incidental en pesquerías y la hibridación. En consecuencia, es esencial generar datos sobre las poblaciones silvestres, particularmente en etapas críticas del ciclo de vida como la reproducción y la anidación. El objetivo de este estudio fue evaluar la relación entre la biometría del huevo y el tamaño de las neonatos en una población silvestre de *C. acutus* en la Reserva Natural Privada Setal, Guatemala. El trabajo de campo se llevó a cabo de febrero a junio de 2025. Para cada nido, se registraron la longitud, el ancho y la masa del huevo; los huevos se marcaron individualmente y se devolvieron a sus respectivos nidos. En el caso de los neonatos, se registraron la longitud total, la longitud hocico-cloaca, la longitud de la cabeza, el ancho maxilar y la masa corporal. Todos los individuos fueron marcados mediante el corte de escamas en las quillas caudales y liberados en el canal adyacente. Se localizaron un total de 27 nidos, obteniendo 897 huevos y 515 neonatos, de las cuales 454 pudieron vincularse directamente en pares huevo-neonato. Los análisis revelaron una correlación significativa entre la masa del neonato y tanto la masa del huevo ($n = 454$; $r = 0.688$; $R^2 = 0.473$) como el volumen del huevo ($n = 454$; $r = 0.653$; $R^2 = 0.425$). Otros parámetros, como la longitud, no mostraron correlaciones significativas. Estos resultados sugieren que la masa y el volumen del huevo pueden ser indicadores confiables de la condición física inicial de la neonatos, proporcionando una herramienta práctica para los programas de conservación, aprovechamiento y monitoreo en áreas con recursos limitados.

Abstract

Globally, the American crocodile (*Crocodylus acutus*) is listed as Vulnerable (VU) on the IUCN Red List due to threats such as habitat loss, poaching, fisheries bycatch, and hybridization. Consequently, generating data on wild populations is essential, particularly during critical life cycle stages such as reproduction and nesting. The objective of this study was to evaluate the relationship between egg biometrics and hatchling size in a wild population of *C. acutus* at the Setal Private Nature Reserve, Guatemala. Fieldwork was conducted from February to June 2025. For each nest, egg length, width, and mass were recorded. Eggs were individually marked and returned to their respective nests. For the hatchlings, total length, snout-vent length, head length, maxillary width, and body mass were recorded. All individuals were marked by scute-clipping on the caudal whorls and released into the adjacent canal. A total of 27 nests were located, yielding 897 eggs and 515 hatchlings, 454 of which could be directly linked as egg-hatchling pairs. Analyses revealed a significant correlation between hatchling mass and both egg mass ($n = 454$; $r = 0.688$; $R^2 = 0.473$) and egg volume ($n = 454$; $r = 0.653$; $R^2 = 0.425$). Other parameters, such as length, showed no significant correlations. These results suggest that egg mass and volume can be reliable indicators of initial hatchling physical condition, providing a practical tool for conservation, management, and monitoring programs in resource-limited areas.

Introduction

In Guatemala, according to the List of Threatened Species (LEA) issued by the National Council of Protected Areas (CONAP)**,** the American crocodile (*Crocodylus acutus*) is classified under Category I, "Critically Endangered" (CR), primarily due to habitat loss and fragmentation, poaching, and illegal trade (CONAP, 2022). Furthermore, based on the criteria of the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, it is classified as Vulnerable (VU) (Rainwater et al., 2022). Within the country, populations of *C. acutus* have been only partially monitored. In the department of Izabal, in particular, there are only a few previous studies, which suggest that the

population is small and that the species does not fully utilize the entire lacustrine system, likely because of human activities (Corado-García, 2021; Javier A. Benítez-Moreno, pers. comm.). In this context, it is a priority to generate updated information on wild populations, especially during critical stages of their life cycle, such as reproduction and nesting, which are key determinants of long-term population viability. The objective of this study was to evaluate the relationship between the biometric characteristics of *C. acutus* eggs and hatchling size at hatching, to generate information that contributes to the understanding of the species' reproductive biology in Guatemala.

Maternal investment significantly impacts crocodilian hatchling characteristics, with egg size acting as a key determinant of reproductive success across species like *Alligator mississippiensis* (Deitz and Hines, 1980), *C. porosus* (Webb and Cooper-Preston, 1989), and *Caiman* spp. (Verdade, 2001; Larriera *et al.,* 2004; Campos *et al.,* 2008). While *C. acutus* exhibits high reproductive plasticity (Thorbjarnarson, 1989; Charruau *et al.,* 2010), data on egg-hatchling correlations in Central America are scarce, with existing research indicating that environmental factors may influence development alongside yolk resources (Gómez-González *et al.,* 2017). Given the vulnerability of the American crocodile and a significant knowledge gap in Guatemala's lacustrine systems, this study evaluated the relationship between egg biometrics and hatchling size at the Setal Private Natural Reserve during the 2024–2025 seasons.

Material and Methods

Study area

The Setal Private Natural Reserve (RNPS) covers approximately 617ha and is located at the municipality of El Estor, Izabal, Guatemala, within the Río Dulce–Lake Izabal basin, along the shoreline of the country's largest lake. Approximately 80% of its area lies within the Bocas del Polochic Wildlife Refuge, a wetland of international importance that fulfills key ecological functions, including sediment filtration and the maintenance of habitats for wildlife. The area is characterized by a warm, humid climate with marked seasonality, consisting of a dry season from December to April and a rainy season from May to November. The mean annual temperature is around 27 °C, with high relative humidity and annual precipitation that supports the development of highly biodiverse ecosystems typical of tropical very humid forest. The RNPS harbors species of conservation importance, such as the black howler monkey (*Alouatta pigra*) and *C. acutus*.

Additionally, the reserve includes an artificial canal approximately 1.2 km in length and between 1.5 and 2.5 m in depth, which is used by *C. acutus* as a nesting site and permanent habitat. This canal also serves as a refuge for aquatic birds and fish, further enhancing the ecological importance of the area (Figure 1).



Figure 1. Location of the artificial canal of the CGN processing plant. Map source: Compañía Guatemalteca de Níquel (CGN).

Field work

Data were collected during the nesting period of *C. acutus* in Guatemala, from February to June 2025. The sampling design was structured into three consecutive stages: (1) nest location and egg measurements, (2) inter-stage monitoring, and (3) hatching and hatchling measurements. During the nest-location phase, daily morning surveys were conducted along the canal to identify recently constructed nests. Once located, each nest was marked with tape indicating the laying date or date of discovery, along with a unique identification number based on the order of detection. Each nest was then carefully opened, and all eggs were individually marked with a pencil to indicate their original orientation, ensuring that they were returned to the same orientation. All eggs were counted to determine clutch size, and each egg was labeled individually. The major diameter (egg length, EL) and minor diameter (egg width, EW) were measured using an analog caliper (Truper), and egg mass (EM) was recorded using a digital jewelry scale (Diyife). Once measurements were completed, the eggs were carefully returned to the incubation chamber (Figure 2).

During the inter-stage monitoring phase, morning and afternoon surveys were conducted to monitor nest status. As the hatching period approached, survey frequency was increased to identify nests close to hatching.

In the third phase, once nests undergoing hatching were identified, hatchlings were removed and placed in labeled plastic containers corresponding to their egg number to maintain the egg–hatchling association (Figure 3). Subsequently, each hatchling was weighed using a spring scale, and hatching body mass (BM) was recorded. The following morphometric variables were recorded: total length (TL), snout–vent length (SVL), total cranial length (TCL), and maxillary width (MW), using a caliper. Each hatchling was marked using the caudal scute clipping method following the protocol described by Sánchez *et al*. (2011) and was subsequently released into the inlet channel as close as possible to its original nest (Figure 4). Hatching process and general appearance of hatchlings (Figure 5).



Figure 2. Nest location and egg measurements of *C. acutus***.** (a) Manual excavation of the nest. (b) Measurement of EL and EW using a caliper. (c) Recording of EM using a digital scale.

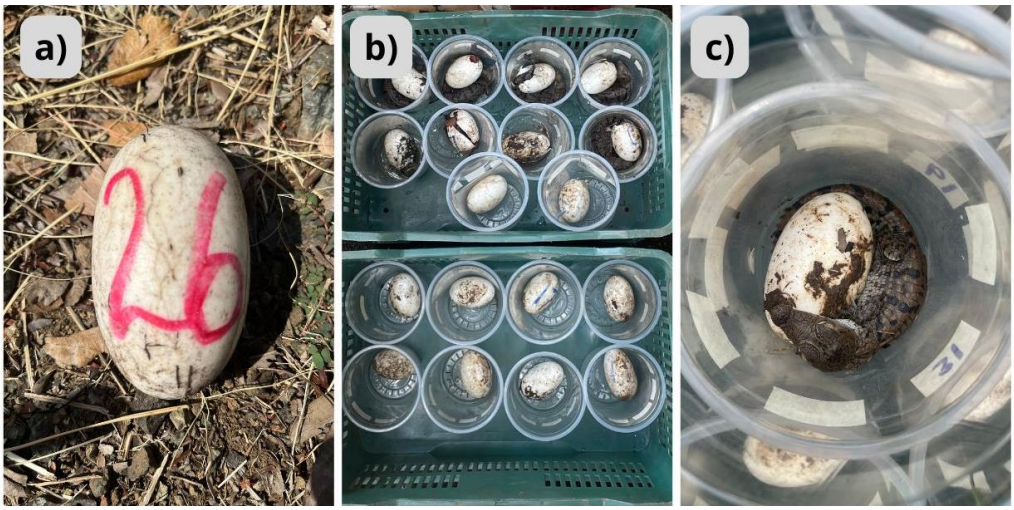


Figure 3. Handling and individual recording of *C. acutus* eggs and hatchlings. (a) Egg marked with an identification number; (b) arrangement of eggs in individual plastic containers; (c) newly hatched hatchlings alongside its corresponding egg.



Figure 4. Recording of morphometric variables in *C. acutus* hatchlings. (a) Measurement of TL; (b) Recording of BM; (c) Hatchling release at the original nest location.

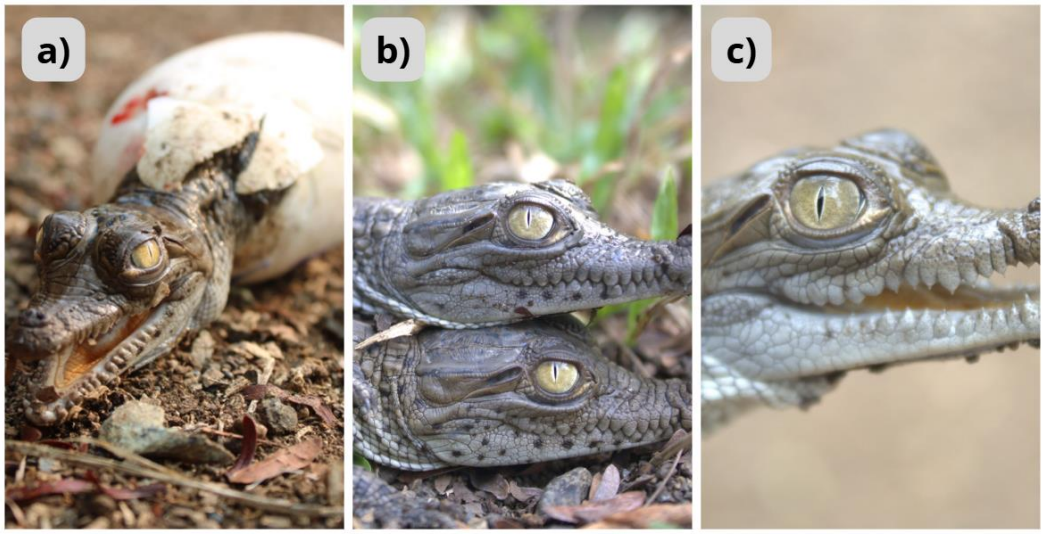


Figure 5. Hatching process and characteristics of *C. acutus* hatchlings. (a) Hatchling emerging from the egg; (b) Grouping of hatchlings; (c) Early-stage hatchling.

Results

Relationships between egg and hatchling morphometric variables were evaluated using Pearson correlation analysis to identify associations between egg measurements and hatchling traits.

The analysis revealed positive and statistically significant associations between BM and EM ($r = 0.688$, $p < 0.001$), egg volume (EV) ($r = 0.653$, $p < 0.001$), and EW ($r = 0.567$, $p < 0.001$). Among these, EM showed the strongest correlation with BM, followed by EV and EW. In contrast, some variables did not show significant correlations (Table 1). Based on these results, a simple linear regression analysis was performed to evaluate the relationship between EM and BM. The model was statistically significant ($p < 0.001$) and showed a coefficient of determination of $R^2 = 0.473$. Similarly, the relationship between EV and BM was analyzed, yielding a statistically significant model ($p < 0.001$) with a coefficient of determination of $R^2 = 0.425$. Finally, the relationship between EW and BM was evaluated, also showing a statistically significant model ($p < 0.001$) with a coefficient of determination of $R^2 = 0.321$ (Table 2, Figure 6).

Table 1. Pearson correlation analysis between egg morphometric variables (EW: egg width, EL: egg length, EV: egg volume, EM: egg mass) and hatchling morphometric variables (TL: total length, SVL: snout–vent length, TCL: total cranial length, MW: maxillary width, BM: body mass). Values represent correlation coefficients (r).

Pearson's correlation analysis					
	TL	SVL	TCL	MW	BM
EW	0.109	0.242	0.056	0.048	**0.567**
EL	0.132	0.257	0.073	0.183	0.493
EV	0.135	0.299	0.074	0.132	**0.653**
EM	0.157	0.333	0.094	0.174	**0.688**

Table 2. Simple linear regression models describing the relationship between egg morphometric variables (EM: egg mass, EW: egg width, EV: egg volume) and hatchling body mass (BM). Coefficients of determination (R^2), p-values, and fitted model equations-are presented.

INDEPENDET VARIABLE	DEPENDENT VARIABLE	R^2	P-VALUE	EQUATION
EW	BM	0.321	<0.001	Body mass (g) = –50.91 + 2.472 × Egg Width (mm)
EV	BM	0.425	<0.001	Body mass (g) = 17.73 + 0.0005 × Egg Volume (mm^3)
EM	BM	0.473	<0.001	Body mass (g) = 15.06 + 0.506 × Egg Mass (g)

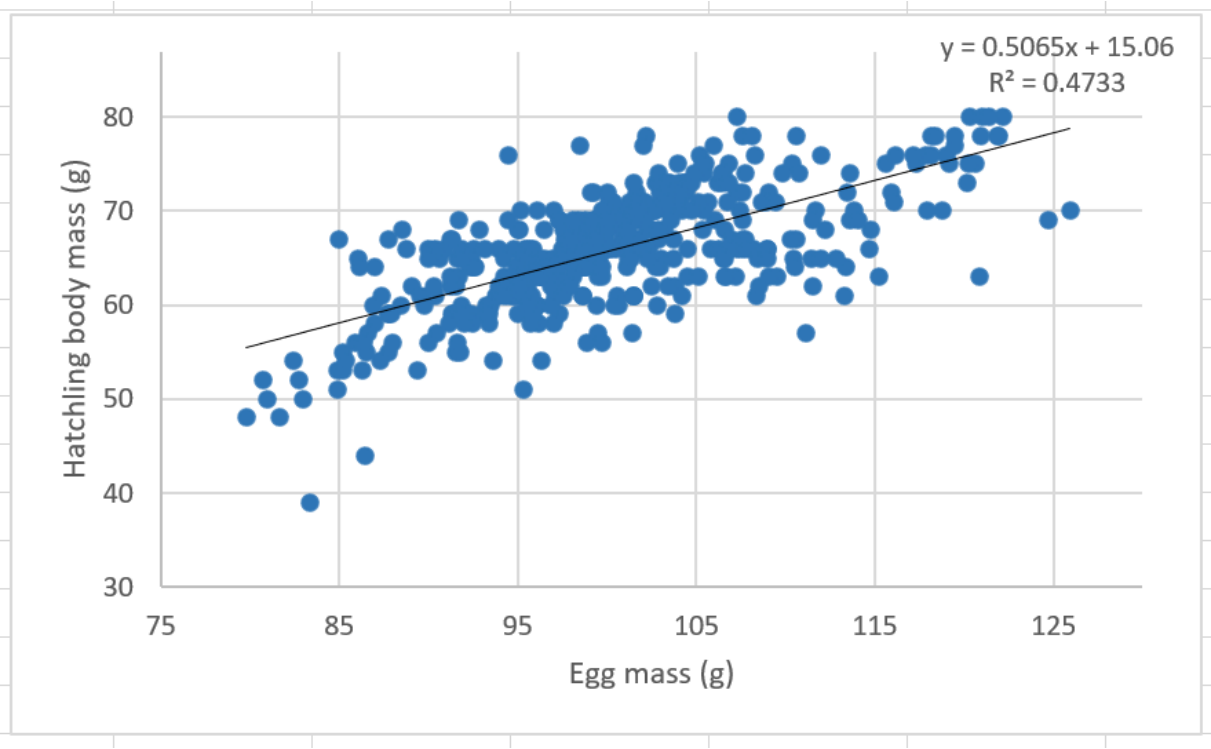


Figure 6. Relationship between egg mass and hatchling body mass of *C. acutus*. The line represents the fitted linear regression model, showing a positive and statistically significant relationship between both variables (R^2 = 0.473, $p < 0.001$).

Discussion

The results obtained in this study indicate positive relationships between egg morphometric variables and hatchling characteristics. Statistically significant associations were found between BM and EM (r = 0.688), egg volume (EV) (r = 0.653), and EW (r = 0.567). These results suggest that variation in egg size is associated with variation in hatchling size. Among the variables analyzed, EM showed the highest correlation with BM and the greatest explanatory power in the regression model (R^2 = 0.473), followed by EV (R^2 = 0.425) and EW (R^2 = 0.321).

These results suggest that egg mass better represents variation in hatchling body mass, as it showed the strongest association and highest explanatory power among the variables analyzed. Despite these associations, the strength of the relationships observed was moderate. The correlation coefficients indicate associations of intermediate strength, while the regression models show that a considerable proportion of the variation in hatchling body mass is not explained solely by egg variables ($R^2 \leq 0.47$). This suggests that, although egg size influences hatchling development and size, it is not the only factor determining size at hatching.

The relationship between egg size and hatchling size can be understood in terms of resource availability during embryonic development. Larger eggs, particularly those with greater egg mass (EM), may contain higher energy reserves, which can support embryo growth and result in larger hatchlings (BM). However, the variation observed in the data suggests that this effect is not consistent across all cases. In this context, it is likely that additional factors also influence hatchling development, such as environmental conditions during incubation (e.g., temperature and humidity), as well as maternal characteristics or differences in embryonic development. These factors may interact and contribute to the observed variation in hatchling size.

The relationship between egg size and hatchling size has been widely documented in other crocodilian species. In *C. porosus*, Webb and Cooper-Preston (1989) reported a high explanatory power of egg mass

for predicting hatchling mass ($R^2 > 0.80$), whereas in *A. mississippiensis*, Deitz and Hines (1980) found a very strong correlation between the same variables ($r = 0.95$). These findings indicate that, in some cases, egg size can serve as a highly accurate predictor of hatchling size. In the present study, although EM showed the strongest relationship with BM, the strength of this association was lower compared to those reported in other species, suggesting a more moderate relationship.

However, this relationship is not consistent across all species or under all conditions. Villegas and Reynoso (2019) did not find a significant relationship between egg mass and hatchling mass, length, or survival in *C. moreletii*, which may be associated with a limited sample size or the influence of unaccounted environmental factors. In contrast, Mejía-Reyes *et al*. (2023) reported a high explanatory power of egg volume for predicting hatchling mass ($R^2 > 0.75$), highlighting the importance of considering variables that integrate multiple dimensions of the egg to better understand their relationship with hatchling development.

Overall, the results of this study are consistent with the general trend reported in the literature, in which egg size is positively associated with hatchling size, although the strength of this relationship varies. In this case, the associations observed between egg variables (EM, EV, EW) and BM were moderate compared to other studies reporting stronger relationships, suggesting that, although egg size is an important factor, it is not the sole determinant of hatchling size.

Finally, although egg variables (EM, EV, EW) showed significant relationships with hatchling characteristics (BM), the results indicate that a considerable portion of the variation is not explained by the egg variables evaluated. This highlights the importance of considering additional biological and environmental factors in future studies to achieve a more comprehensive understanding of the processes influencing hatchling development.

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