



COMPARATIVE EVALUATION OF CLUTCH FREQUENCIES ON EGG

PROPERTIES OF FOUR SPECIES OF TORTOISES IN ECOLOGICAL ZONES, NIGERIA

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Abstract

Egg characteristics of tortoise species in ecological zones **were observed** by traversing line transects to locate nesting females between January to November in **2024–2025**. The objective of this study was to evaluate **clutch frequencies on egg features of tortoises' species**. Sixty (60) adult tortoises were allocated into 4 groups (*Centrochlys sulcata*; *Pelusios sinuatus*; *Testudo marginata*; and *Pelusios castaneus*).

Clutch size (CLUTS) averaged 6 (range: 4–8) with elliptical, spherical or oval albescent eggs. The mean egg shape index (ESI) $\pm$ SD and egg volume (EV) $\pm$ SD were 1.16 ± 0.02 and $18.22 \pm 1.02 \text{ cm}^3$

Keywords. Relative clutch mass, clutch frequency, egg length, egg volume, egg mass, egg shape index

respectively. Mean Straight Carapace Length (SCL) and egg mass (EM) were 186.00 mm and 2245.50 g respectively. The mean egg length (ELT) and egg width (EWD) ranged from 33.89mm – 38.50mm and 25.29mm – 31.21mm respectively with higher mean values and standard error of $38.50 \pm 1.20 \text{ mm}$ and $31.21 \pm 0.53 \text{ mm}$ for *P. castaneus* and *T. marginata* respectively. *P. sinuatus* species had the highest CLUTS of 5 and total egg count of 25 was recorded throughout the seasonal periods of the experiment. The mean relative egg mass (REM),

relative clutch mass (RCM), relative egg length (REL), and relative egg width (REW) were calculated as 1.31%, 1.29%, 19.43% and 16.78%, respectively. Differences in clutch frequency of the four tortoise species studied was a significant source of variation in clutch number, CLUTS	and total egg production. It is only the <i>P. sinuatus</i> that exhibited significantly different values in all parameters studied.
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Introduction

Various tortoise species are distributed across the three ecological zones in Delta State of Nigeria (Kperegbeyi *et al.*, 2024). It can tolerate extreme weather conditions such as dry and wet season of an environment. The study animals live on lake, rivers, swamp, burrow pits and occupy habitats such as dry open steppes, coasts, badlands, or dry forests (Ayaz and Çicek, 2011; Baran *et al.*, 2012). The populations are threatened by habitat loss due to agricultural activity, fragmentation, industry, pressures of urbanization, and pollution (Anadón *et al.*, 2006; Lapid *et al.*, 2004).

The frequency of genes related to egg characteristics in tortoises can influence factors like egg size, shape, and shell thickness, and this can affect hatching success and hatchling survival. While research have shown that various environmental and maternal factors also play a role, the genetic component of these traits is also important.

All tortoises have a bimodal annual activity cycle in which during aestivation and hibernation metabolic activity ceases. These features pose a temporal difference in respect to its activity pattern of dimorphism between genders; males emerge from hibernation earlier than females, and females enter aestivation later than males (Bertolero *et al.*, 2011). Females tortoise generally undergo reproduction annually, by laying up to four (4) clutches from April to June (Thompson and Speake, 2002; Lam *et al.*, 2006; Belitz *et al.*, 2009; Miller and Dinkelacker, 2008 and Bellairs and Osmond, 2014). CLUTS vary between 1 and 7 eggs (Fritz *et al.*, 2006). They preferred seclusion areas to nesting in sharp sand, nest box under shaded area from sun (Kohler 2006). The unfulfilled yolk sac absorption and predators are some of the factors that reduces hatching and survival ratio in tortoise's nest (Speake *et al.*, 2001; Zeidler, 2002; Wallace *et al.*, 2006 and Tunsaringkarn *et al.*, 2011).

In Nigeria, various species of tortoises had been discovered in the ecological zones of Nigeria, among are Marginated tortoise (*Testudo marginata*), African spurred tortoise (*Centrochlys sulcata*), Sarrated hinged terrapin tortoise (*Pelusios sinuatus*), Greek tortoise (*Testudo graeca*), West African mud tortoise (*Pelusios castaneus*), and Pancake tortoise (*Malacochersus tornieri*) are unique species inhabiting in Niger Delta region (Kperegbeyi *et al.*, 2025).

Tortoise species are characterized by long-lived with high and constant survival ratio. Egg properties and survival of hatchlings are vital factors in tortoise's population (Diaz-Paniagua *et al.*, 2001; Zeidler, 2002 and Anadon *et al.*, 2006). Therefore, the egg quality traits, and survival of hatchlings in nests may be good factors for population variation (Hewavisenthi and Parmente, 2002; Prajanban *et al.*, 2012; Hosen *et al.*, 2013; and Bellairs and Osmond, 2014).

Thus, this study aims at evaluating clutch frequencies on egg characteristics of tortoises' species, and recommending the most productive and reproductive species to farmers for successful tortoise production.

Materials and Methods

Experimental Site

The research was conducted at the Department of Animal Production Research Farm (DAPRF), Southern Delta University (SDU), Ozoro. Latitude 5° 32' N and Longitude 6° 15' E of Greenwich meridian place the center in mid-western Nigeria's rainforest. Humidity averages 2500-3000 mm per year and 27.4°C and 85 % are the mean temperature and RH (SDU, 2024).

Experimental Animal and Management Practices

Sixty (60) adult tortoises were used for the experiment, and are allocated into 4 groups (African spurred tortoise (*Centrochlys sulcata*); Serrated hinged terrapin (*Pelusios sinuatus*); Marginated tortoise (*Testudo marginata*); and West African mud tortoise (*Pelusios castaneus*). Tortoises of different species were obtained from different ecological zone in Delta State of Nigeria. Intensive housing system was used for this experiment. The dimension of the area is 12ft x 12ft. The house was ant proof to prevent ants not to invade the unit. A pen (ditched) was constructed measuring 8ft x 2ft. The tortoise was fed with concentrate diet (Top super feed), cooked meat, fermented cassava (Akpu), and palm fruits. Feeding was done twice and thrice (10-hourly and 6-hourly) per day. The animal was grouped into four (4) treatments e,g TRT 1, TRT 2, TRT 3, and TRT 4 respectively. The tortoises were given the same treatment throughout the experimental period under the same managerial conditions. The ditch was filled with enough water to reach the bridge where top shell (carapace) meets the bottom shell (plastron). Some routine preventive hygiene was done frequently such as providing fresh water, feeding, changing of water regularly at all times, observing the behaviour of the tortoises, signs or symptoms of any disease condition should be observed by physical examination. The tortoises were properly identified with an indelible mark on their back labeling T1, T2, T3 and so on.

Data Collection

All tortoises were weighed individually with an electronic scale measured in grams (g) to obtain the BW. The morphometric was also gauged with a tape calibrated in centimeters (cm). The experiment lasted for a period of 52 weeks.

BW: The BW was taken with a digital scale on a monthly basis.

SCL: Measured the SCL from the head region to the dorsal view.

CW: The carapace width was taken between the bridges.

PL: Measured the straight plastron length between the tips of the head end to the V-shape of the anus region.

PW: It was taken between bridges of the ventral view.

Egg collection

The experiment lasted for 52 weeks between January and November in 2024–2025. The female's tortoise lay their eggs on a heap sharp sand between May and July. Eggs were carefully examined to records the number of eggs produce per clutch; measurement of ELT and EWD with a digital calliper (mm); and EWT was measured by an electronic scale (g). Thereafter, the eggs were returned to the nest. Egg parameters like EV, REL, RCM, REM, REW, and ESI were computed. Werner *et al.*, 2016 postulated the formula for egg volume V (in ml) = $RLRW^2\pi 4:3:1000$,

Where,

RL = the long radius

RW = short radius (mm).

REM and RCM = calculated as a proportion of the female BWT

REW and REL = calculated as a proportion of female SCL

ESI= calculated as an ELT to EWD ratio (Hichami *et al.*, 2016).

SCL= egg-laying female was measured by a vernier caliper (Rouag *et al.*, 2007).

Statistical Analysis

Data collected was statistically analysed by general linear model ANOVA and further post-hoc tests were done with a significant ANOVA using the Turkey's Honestly Significant Difference (HSD).

Results

Egg Properties

The mean $\pm$ Standard Error (S. E) of descriptive statistics of egg properties of tortoise species are presented in Table 1. The result revealed that the egg quality traits of tortoise

species on average SCL and EM for females ranged from 177mm – 208mm and 1979.35g – 2245.50g respectively with higher mean values and standard error of 208.03 ± 10.04 mm and 2245.50 ± 168.12 g for *P. castaneus* and *P. sinuatus*. The CLUTS was 5 for five clutches with total of 25 eggs. Tortoise eggs are typically hard-shelled, elliptical or spherical, and white or slightly creamy in colour when laid. The average ESI and EV obtained were 1.31 ± 0.04 and 21.26 ± 0.87 cm³ for *P. castaneus* and *C. sulcata* respectively. The mean ELT and EWD ranged from 33.89mm – 38.50mm and 25.29mm – 31.21mm respectively with higher mean values and standard error of 38.50 ± 1.20 mm and 31.21 ± 0.53 mm for *P. castaneus* and *T. marginata* respectively. *P. sinuatus* species had the highest CLUTS of 5 and total egg count of 25 was recorded throughout the seasonal periods of the experiment. While *P. castaneus* had the lowest value of 4 CLUTS and total count of 8 eggs (Figure 1).

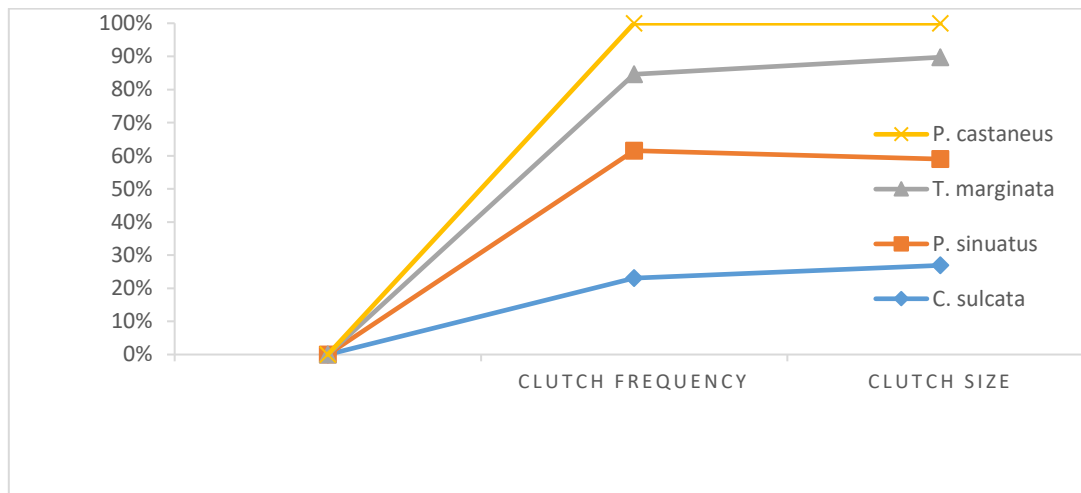


Figure 1: Clutch frequencies and CLUTS

Table 1. Mean $\pm$ Standard Error of egg quality traits of tortoise from different species

Species	N	CLUTS	Total No. of eggs	EWD (mm)	ELT (mm)	EV (cm ³)	ESI	EM (g)	Female SCL (mm)	Female Mass (g)
African spurred tortoise (<i>Centrochlys sulcata</i>)	3	7	21	30.70 $\pm 0.42^a$	34.76 $\pm 1.08^c$	21.26 $\pm 0.87^a$	1.13 $\pm 0.03^a$	28.60 $\pm 0.35^a$	204.00 $\pm 6.25^b$	2198.63 $\pm 256.08^b$
Serrated hinged terrapin (<i>Pelusios sinuata</i>)	5	5	25	31.21 $\pm 0.53^a$	36.15 $\pm 1.12^b$	18.22 $\pm 1.02^d$	1.16 $\pm 0.04^a$	29.47 $\pm 0.28^a$	186.00 $\pm 8.24^c$	2245.50 $\pm 168.12^a$
Marginated tortoise (<i>Testudo marginata</i>)	3	8	24	29.50 $\pm 0.63^b$	38.50 $\pm 1.20^a$	20.18 $\pm 0.55^b$	1.30 $\pm 0.04^a$	24.56 $\pm 0.53^b$	177.01 $\pm 4.06^d$	1979.35 $\pm 192.09^d$
West African mud tortoise (<i>Pelusios castaneus</i>)	2	4	8	25.79 $\pm 1.41^c$	33.89 $\pm 1.14^d$	19.32 $\pm 1.23^c$	1.31 $\pm 0.04^a$	25.08 $\pm 0.19^b$	208.03 $\pm 10.04^a$	2076.27 $\pm 308.18^c$

N= Clutch frequency, CLUTS = Clutch Size, EWD= Egg Width, ELT= Egg Length, EV = Egg Volume, ESI= Egg Shape Index,
EM= Egg Mass, SCL= Straight carapace Length,

Table 2. Measure proportion of egg parameters of tortoise from different species in ecological zone

Species	Ecologic al zones	N	Total No. of egg	Clutch size	Egg									
					Femal e SCL (mm)	EM (g)	ELT (mm)	EWD (mm)	EV (m ³)	ESI	REL (%)	REW (%)	REM (%)	RCM (%)
African spurred tortoise (<i>Centrochyls sulcata</i>)	LLR	3	21	7	204.0 0	28.6 0	34.7 6	30.7 0	21.2 6	1.13	17.04 ±1.02 c	15.05 ±1.01 a	1.30 ±0.01 a	1.28 ±0.02 a
Serrated hinged terrapin (<i>Pelusios sinuata</i>)	LLR	5	25	5	186.0 0	29.4 7	36.1 5	31.21	18.2 2	1.1 6	19.43 ±1.04 b	16.78 ±1.02 a	1.31 ±0.02 a	1.29 ±0.03 a
Marginate d tortoise (<i>Testudo marginata</i>)	LLR	3	24	8	177.01	24.5 6	38.5 0	29.5 0	20.1 8	1.3 0	21.75 ±1.08 a	16.67 ±1.04 a	1.24 ±0.02 b	1.23 ±0.01 b
West African mud tortoise (<i>Pelusios castaneus</i>)	FWS	2	8	4	208.0 3	25.0 8	33.8 9	25.7 9	19.3 2	1.31	16.29 ±1.06 c	12.40 ±1.02 c	1.21 ±0.01 c	1.19 ±0.01 c

REL= Relative egg length; REW = Relative egg width; REM = Relative egg mass; RCM = Relative clutch mass.

Table 2 showed the measure proportion of egg quality traits of tortoise from different species. The mean REM and RCM ranged from $1.21 \pm 0.01\%$ - $1.31 \pm 0.01\%$ and $1.19 \pm 0.01\%$ - $1.29 \pm 0.03\%$ respectively. Also, REL and REW ranged from $16.29 \pm 1.06\%$ - $21.75 \pm 1.08\%$ and $12.40 \pm 1.02\%$ - $16.78 \pm 1.02\%$. There was no statistical difference ($p > 0.05$) between *P. sinuatus* and *C. sulcata* for REM and RCM respectively. But there was significant difference ($p < 0.05$) between *T. marginata* and *P. castaneus* for REM and RCM respectively. The REL for *T. marginata* was superior over the three species but the *P. castaneus* had the lowest value which was statistically significant ($p < 0.05$) from the values obtained on the other three species (Figure 2). There was remarkable similarity in the REW recorded in the *T. marginata*, *P. sinuatus* and *C. sulcata* species which not statistically different ($p > 0.05$) but

the values obtained for *P. castaneus* was significantly ($p<0.05$) lower than values obtained in the three species (Table 2).

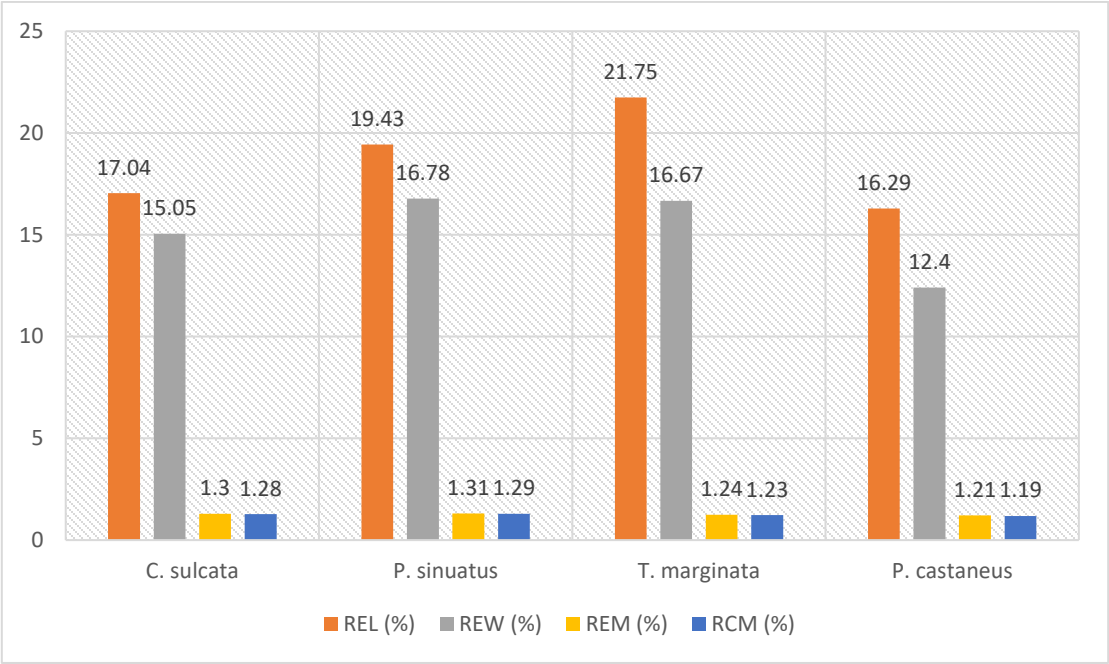


Figure 2: Bar chart representation of REL, REW, REM and RCM of Tortoise Species

Discussion

The only significant difference due to species observed in term of CLUTS in *P. castaneus* specie against the other three species (*P. sinuatus*, *T. marginata* and *C. sulcata*) as a result of the fact that, the other three species were in the same lowland forest zone origin, the *P. castaneus* specie evolved in the freshwater swamp and was developed more for SCL (morphometric trait) than for reproductive trait.

Also, variation in the genetic make-up of the different species of tortoises is expected to influence the performances of the tortoises even when reared under similar environmental conditions (Sadeghi and Torki, 2012 and Werner *et al.*, 2016).

These experimental animals originated from the wild stock which were sourced from different ecological zones and managed intensively through selection process and used for breeding. This explain the similarity in the total of eggs recorded in *P. sinuatus*, *T. marginata* and *C. sulcata* species. However, the *P. castaneus* specie was managed to perform better in the stressful environment of the freshwater swamp zone and has not evolved to the stage of the other species in terms of reproductive performance (Krüger, 2007). This observation confirms earlier reports (sources) on the consequence of specie

or breed on tortoise reproductive parameters. The ELT, EWD, EV, EM, and ESI varied among tortoise species with vary CLUTS. The highest CLUTS value of 8 eggs was obtained and is larger than the reports in several studies but the mean CLUTS value of 6 was higher than other researchers studies (Thompson and Speake, 2002; Lapid *et al.*, 2004; Barje *et al.*, 2005; Daiz-Paniagua *et al.*, 2006; Krüger , 2007; Sadeghi and Torki, 2012; Werner *et al.*, 2016).

P. castaneus species had the largest CLUTS, EWD, EM and EV were lower than the other three species. The mean EWD was shorter than the other three species, while the mean ESI was superior than *C. sulcata*, *P. sinuatus* and *T. marginata*. The most elongated eggs were found in *P. castaneus* as reported by Sadeghi and Torki, 2012 and Werner *et al.*, 2016. ESI indicated that the eggs in the largest clutch were narrower and elongated as reported in this study.

It was discovered that *P. sinuatus* female tortoise lay smaller and narrower eggs than the other three species. However, the female tortoise *C. sulcata* species laid the largest CLUTS of 9 eggs (Sadeghi and Torki, 2012). It was observed that female *P. castaneus* species possess smaller narrower-more elongated eggs, while the narrowest eggs give rise to highest ESI and the lowest RELs, REWs, REMs, and RCMs were found in a medium size *P. castaneus* (Werner *et al.*, 2016).

Nevertheless, the largest clutch value of 24 eggs with the smaller female SCL (177.01 mm) was recorded lower in *T. marginata* than other three species (Fritz, 2004). The width of pelvic aperture and the **posterior shell opening would determine the size of EWD and ESI in laying female tortoise (Gastro-Gonzaliz and Perez-Gillromo, 2011).**

P. sinuatus recorded the highest EM (29.47 g) in this study, while the lowest EM is found in *T. marginata* as reported by Sadeghi and Torki, 2012. The mean ELT (38.50 mm) was longer in *T. marginata* than the other three species but researchers reported 33.09 mm (Hichami *et al.*, 2016). This finding is similar to the reports of Werner *et al.*, 2016. The average EWD (31.21 mm) was longer in *P. sinuatus* than other three species, which is in line with Sadeghi and Torki, 2012 and Fritz, 2004 reports. This revealed that the eggs were oval and sub-spherical in shape rather than *P. sinuatus* whose eggs is large clutch. The EV (21.26 cm³) was higher in *C. sulcata* than other three species as recorded except for *P. sinuatus* that had lower value (18.22 cm³) from lowland rainforest zone as reported by (Kobler, 2006).

The mean SCL (208.03 mm) was higher in *P. castaneus* than other species in the study except for *T. marginata*. with lower value (177.01 mm) which is higher as reported by Bellairs and Osmond, 2014. The mean REL (21.75%) obtained was lower in this study compared to the reports of Bertolero *et al.*, 2011 and Werner *et al.*, 2016. The mean REW value 16.78% obtained was higher than the reports of Werner *et al.*, 2016 and similar to

(Miller and Dinkelacker, 2008 and Werner *et al.*, 2016). The mean REM value 1.31 % and RCM 1.29 %) obtained in the present study were comparatively lower than the reports of (Blanvillain *et al.*, 2011 and Prajanban *et al.*, 2012)

Conclusion

Differences in clutch frequency of the four species studied was substantial source of variation in clutch frequency, CLUTS and total eggs produce. It is only the *P. castaneus* that exhibited significantly different values in all parameters studied. The findings described the extend of our knowledge on egg properties of tortoise species in Delta State ecological zones. It is essential to obtain information on egg parameters of tortoise species in order to develop measures of conserving *P. castaneus* species from endangered.

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