

The relationship between hatchling mass and egg volume in the Nile crocodile (*Crocodylus niloticus*): The productivity of eggs varies

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The survival and vitality of Nile crocodile (*Crocodylus niloticus*) hatchlings is important for the success of commercial crocodile farming and the maintenance of wild populations.

In this study we describe the relationship between the mass of Nile crocodile hatchlings and the estimated volume of the eggs from which they hatched and, following that, we describe the variation in the ratio between the mass of individual hatchlings and the egg volume from which each hatched (the productivity of the egg) within and among clutches. The volumes of 316 Nile crocodile eggs from 51 clutches were estimated by means of a purpose-made algorithm that uses key dimensions of eggs obtained from photos of the hatched shells. The hatchling from each egg was weighed immediately after hatching.

Neither the duration of incubation prior to hatching ($P = 0.88$) nor the year in which clutches were laid ($P = 0.35$), nor the number of eggs per clutch ($P = 0.57$) affected hatchling mass. There exists a strong, linear, positive relationship between the mass of Nile crocodile hatchlings and the estimated volume of the eggs from which they hatched ($r = 0.88$, $P < 0.001$, $n = 316$). Without considering estimated egg volume, 86.9% of the variation in hatchling mass occurs among clutches and 13.1% within clutches. Taking estimated egg volume into account, 59% of the variation in hatchling mass occurs among clutches and 41% within clutches. Egg productivity varied from 0.57 g/ml to 0.81 g/ml, with 45.4% of the variation occurring among clutches and 54.6% within clutches.

Further research is required to investigate the relationship between hatchling mass and the growth of hatchlings, as well as between egg productivity and the growth of hatchlings.

Keywords: Nile crocodile, crocodile, egg, hatchling, volume

Die oorlewing en lewenskragtigheid van Nylkrokodil (*Crocodylus niloticus*) broeilinge is belangrik vir die sukses van kommersiële krokodilboerdery en die instandhouding van populasies in die natuur.

In hierdie studie beskryf ons eers die verband tussen die massa van Nylkrokodilbroeilinge en die geskatte volume van die eier waaruit elkeen gebroei het. Daarna beskryf ons die variasie in die verhouding tussen die massa van individuele broeilinge en die volume van die eier waaruit elk gebroei het (die produktiwiteit van die eier) binne en tussen broeisels. Die volumes van 316 Nylkrokodileiers uit 51 broeisels is met 'n doelgemaakte algoritme geskat op grond van sleutelafmetings van die eiers wat op foto's van die uitgebroeide eierdoppe gemaak is. Die broeiling wat uit elke eier gebroei het is direk na uitbroei geweeg.

Nóg die duur van inkubering tot uitbroei ($P = 0.88$) nóg die jaar waarin broeisels gelê is ($P = 0.35$) nóg die aantal eiers in die broeisel ($P = 0.57$) het broeilingmassa beïnvloed. Daar bestaan 'n sterk, lineêre positiewe verband tussen die uitbroeimassa van Nylkrokodilbroeilinge en die geskatte volume van die eiers waaruit hulle gebroei het $r = 0.88$, $P < 0.001$, $n = 316$). Sonder inagneming van geskatte eiervolume is 86.9% van die variasie in broeilingmassa die gevolg van variasie tussen broeisels en 13.1% die gevolg van variasie binne broeisels. Indien geskatte eiervolume in berekening gebring word is 59% van die variasie in broeilingmassa vir 'n bepaalde geskatte eiervolume die gevolg van variasie tussen broeisels en 41% die gevolg van variasie binne broeisels. Die produktiwiteit van eiers het van 0.57 g/ml tot 0.81 g/ml gevarieer, met 45.4% van die variasie wat tussen broeisels voorgekom het en 54.6% binne broeisels.

Verdere navorsing is nodig om die verband tussen broeilingmassa en die groei van broeilinge, sowel as die verband tussen eierproduktiwiteit en die groei van broeilinge te ondersoek.

Sleutelwoorde: Nylkrokodil, krokodil, eier, broeiling, volume

Introduction

Wild Nile crocodiles have for long been recognised as being of great ecological importance in rivers and other inland waters in large parts of Africa (Pooley, 1973). By the beginning of the 20th century Nile crocodiles occurred from as far north as the Nile delta (Brito et al. 2011) to as far south as the eastern Cape (Feely 2010). Today, Nile crocodiles naturally occur from as far north as southern Egypt, Mauritania in West Africa and Chad (Brito et al. 2011) and further south throughout Africa (Fergusson 2010) to as far south as Lake St Lucia in Kwazulu-Natal (Combrink et al. 2013). Various Nile crocodile populations are declining (Behangana et al. 2017; Botha et al. 2011; Calverley and Downs 2014) or have recently suffered severe mortality (Ferreira and Pienaar 2011).

Breeding Nile crocodiles (*Crocodylus niloticus*) is of commercial importance in southern Africa because their leather is highly sought-after (Fergusson 2010; Tosun 2013). The survival and growth of hatchlings affect the number and size of crocodiles that are slaughtered for their skins and, therefore, the production of leather and the economy thereof (Isberg et al. 2005). On South African commercial crocodile farms, hatchlings are produced in captivity. Nile crocodiles mate in the water during the second half of winter and females lay their eggs during spring in holes they dig in sand next to the water (Kofron 1990). On average there are about 40 eggs per clutch but clutch size varies a lot (Khoza 2012). On farms, eggs are removed from the nests the morning after they were laid and are then incubated at a constant temperature and air humidity.

Garnet and Murray (1986) found that the average hatchling mass of eight clutches of the salt water crocodile (*Crocodylus porosus*) varied from 40 grams to 80 grams. The standard deviations they reported show that the mass of hatchlings from the same clutch often varied by about 16 grams and sometimes even by as much as about 20 grams.

The mean hatchling mass of American alligator (*Alligator mississippiensis*) clutches show a strong linear relationship with their mean egg masses (Deitz and Hines 1980). Similarly, the data of Garnet and Murray (1986) and Stoker et al. (2013) respectively show a strong positive relationship between the mean hatchling mass and the mean egg mass of *Crocodylus porosus* and broad-snouted caiman (*Caiman latirostris*) clutches. Webb et al. (1983a) showed that hatchling mass in the Australian fresh water crocodile (*Crocodylus johnstoni*) is expected to increase by 0.62 g for each one-gram increase in egg mass, and that egg mass explains 89% of hatchling mass. Webb et al. (1983b) showed that the mass of *Crocodylus porosus* hatchlings is expected to increase by 0.64 g for each one-gram increase in egg mass, and that egg mass explains 79% of the variation in hatchling mass.

Deitz and Hines (1980) and Garnett and Murray (1986) respectively showed that the mean hatchling mass of *Alligator mississippiensis* and *Crocodylus porosus* clutches varied more than the mass of individual eggs within clutches. However, Stoker et al. (2013) reported that the variation in egg mass within clutches was larger than the variation among the mean egg masses of clutches for *Caiman latirostris*.

Clutch has a significant effect on the growth of *Crocodylus porosus* hatchlings (Brien et al. 2014; Garnett and Murray 1986) and the occurrence of hatchlings that fail to thrive (Brien et al. 2014).

The results of Garnet and Murray (1986) show that, in the majority of the eight *Crocodylus porosus* clutches in their study, the mass of individual hatchlings varied more than the mass of individual eggs in the same clutches. This may indicate that the mass of hatchlings yielded by eggs of a particular mass might vary.

Larger Nile crocodile females lay heavier eggs than smaller ones (Huchzermeyer 2003; Swanepoel et al. 2000). The data of Deitz and Hines (1980) suggested that larger American alligator females laid heavier eggs than smaller ones although their sample was too small to show a significant effect.

Various studies mentioned above related hatchling mass to egg mass in crocodilians but none have related hatchling mass to egg volume. The mass of crocodile eggs is easier to determine than their volume, provided that the intact eggs are available for weighing. Such conditions prevail on commercial farms where eggs are routinely removed from the nests and during studies where wild nests are opened before the eggs hatch (Swanepoel et al. 2000). Although more cumbersome, the volume of both intact and hatched eggs may be determined (Nöthling et al. 2019). If the relationship between egg mass and egg volume – egg density – was constant, the relationship between hatchling mass and egg volume would provide no information that would not also be provided by the relationship between hatchling mass and egg mass, so that the one could be substituted with the other. The density of Nile crocodile eggs, as well as the variation therein are, however, unknown. The ability to estimate the volume of Nile crocodile eggs from the shells after the eggs have hatched enables one to relate egg size to hatchling size on farms where egg size has not been determined prior to hatching. Further, estimating the volume of eggs from the hatched shells collected from wild nests after the hatchlings have reached the safety of the water may also enable one to estimate the likely size and age of the female that laid the eggs and the likely sizes of the hatchlings produced from a nest in the wild. Nöthling et al. (2019) showed that the volume of a Nile crocodile egg can be estimated to within 4.57 ml of its actual volume from a photo of its hatched shell.

No study has, to our knowledge, been done to describe the relationship between the size of individual eggs (being it their mass or their volume) and the mass of hatchlings yielded by each, and the variation of this relationship within and among clutches.

The aim of this study was to describe the relationship between the mass of Nile crocodile hatchlings and the estimated volume of the eggs from which they hatched, as well as the variation in the ratio between the mass of hatchlings and the volume of the egg from which each hatched (the productivity of the egg) within and among clutches.

Materials and methods

The research was approved by the Animal Ethics Committee of the University of Pretoria (project V071-14, V078-14).

Collection of egg shells and hatchlings

Nile crocodile hatchlings with the shells of the eggs from which they hatched were collected during two hatching seasons on a single commercial farm in the North West Province of South Africa. Three hundred and eighty one hatchlings with their egg shells were collected from 52 clutches (2–18 per clutch), while they were busy hatching or directly after hatching, while the hatchlings were still connected by their umbilical cords to their shells.

Weighing of the hatchlings

Each hatchling was weighed to the nearest 0.1 gram once its umbilical cord was broken.

Photos of the egg shells

Within five minutes after each hatchling had hatched and its umbilical cord had broken, the largest undamaged part of the egg shell was photographed as described by Nöthling et al. (2019), with the restriction that the pole through which the hatchling left the shell was towards the top of the photo.

Estimating the volume of hatched eggs

The photo of each egg shell was prepared, following which the threshold value for the identification of egg pixels and the plotted edge of the egg image were determined as described by Nöthling et al. (2019). The position of the polar axis was then determined as described by Nöthling et al. (2019) for masked egg images before the position of the girth (the largest transverse diameter, perpendicular to the polar axis) was determined. Following this, various diameters perpendicular to the polar axis were measured as described by Nöthling et al. (2019) for the masked images of whole eggs.

For each egg shell image where diameters perpendicular to the polar axis could be measured to a height of at least 1.3 times the distance from the bottom pole to the girth (that is, egg shell images with a scope of at least 1.3), the polarity was determined as described by Nöthling et al. (2019). Egg polarity was calculated as the sum of the lengths of the diameters at heights 1.05, 1.1, 1.15, 1.2, 1.25 and 1.3 times the distance from the bottom pole to the girth minus the sum of the lengths of the diameters at heights 0.7, 0.8, 0.85, 0.9 and 0.95 times the distance from the bottom pole to the girth. An egg had a polarity of minus one if this difference was minus one millimetre or less, zero if it was between minus one millimetre and one millimetre and one if it was one millimetre or more. Depending on its scope and polarity, the volume of each egg was estimated using the appropriate model (Restricted Akaike, Full Akaike or Full, as defined in Nöthling et al. (2019)). Henceforth these volumes will be referred to as the estimated volumes of hatched eggs.

Data analysis

Data summary

Data with a normal distribution are summarised as the mean, with its standard deviation. Data with a skew distribution are summarised as percentiles. The initial capture and some preparation of the data were done in Excel (Microsoft Office 2013), whereas most of the data preparation and all analyses were done using STATA version 14 (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

The relationship between hatchling mass and estimated egg volume while considering the effects of clutch and other covariates

Incubation period was included as covariate in the model to determine the effect thereof on hatchling mass. Data were collected during two breeding seasons. The year in which each clutch was laid may, therefore, have confounded the effect of estimated egg volume on hatchling mass and year was included in the model. The effect of the number of eggs in a clutch (clutch size) could also confound that of estimated egg volume on hatchling mass and clutch size was therefore also included as a confounding covariate in the model. Because mother may have affected hatchling mass as well as the effect of estimated egg volume on hatchling mass, the effect of mother was controlled by the inclusion of clutch as a stochastic grouping variable in the model.

Multiple mixed-effect regression was used to estimate the effect of the estimated volume of hatched eggs on hatchling mass. The complete model for the estimation of hatchling mass is given by equation 1:

$$\text{Equation 1: } y_{ij} = \beta_0 + \beta_1 x_{1ij} + \beta_2 x_{2j} + \beta_3 x_{3j} + \beta_4 x_{4j} + u_j + \varepsilon_{ij}$$

y_{ij} Is the hatchling mass of the i th egg from the j th clutch. $\beta_0 + \beta_1 x_{1ij} + \beta_2 x_{2j} + \beta_3 x_{3j} + \beta_4 x_{4j}$ defines the linear regression line of the fixed component of the model with β_0 the y intercept thereof. $\beta_1 x_{1ij}$ estimates the effect of the estimated volume of the i th hatched egg from the j th clutch on the mass of the i th hatchling from the j th clutch, $\beta_2 x_{2j}$ the effect of year thereon, $\beta_3 x_{3j}$ the effect of clutch size thereon and $\beta_4 x_{4j}$ the effect of the incubation period of the j th clutch thereon. u_j Estimates the combined effect of unmeasured effects associated with the j th clutch on the mass of the i th hatchling from the j th clutch. ε_{ij} Estimates the combined effect of unmeasured characteristics associated with the i th hatchling from the j th clutch on the mass thereof. The final model is given by Equation 2. The final model is the same as the one given by Equation 1, except that all covariates that did not have a significant effect on hatchling mass were excluded from the model.

The fitted hatchling mass included the fixed effect of the final model, namely the linear regression of the actual hatchling mass on the estimated volume of the hatched egg, as well as the contribution of the estimated stochastic effect of clutch in the final model. The difference between the actual hatchling mass and the fitted hatchling mass was expressed as a percentage of the fitted hatchling mass (percentage error in the fitted hatchling mass).

Comparison of the percentage errors in the fitted volume of unhatched, whole eggs to the percentage errors in the fitted hatchling mass

Nöthling et al. (2019) calculated the volume of 138 whole Nile crocodile eggs that did not hatch by integration of measurements obtained by means of a computer programme from a digital photo image of each egg. Henceforth, these eggs are referred to as eggs with known volume. They then masked the photo image of each of these 138 eggs to varying extents by hiding 15–30% of the upper part of the egg image to simulate the situation when photo images of hatched egg shells would be measured. Nöthling et al. (2019) then estimated the egg volume from each of the resulting 524 masked photo images (1–4 from each of 138 eggs).

The egg volume estimated from 487 of these masked egg images fell in the same range as the estimated volumes of the 316 hatched eggs in the current study for which the hatchling mass was known. These 487 estimated volumes include 1–4 estimates of the volume of 127 eggs with known volume from 35 clutches, with 1–5 eggs per clutch. A three-level, mixed effect regression, with the actual volume of these 127 eggs as outcome variable, their 487 estimated volumes as fixed effect, egg identity as second-level stochastic effect and clutch as third-level stochastic effect was used to determine the fitted volume for each estimated volume (Rabe-Hesketh and Skrondal 2012). These 487 fitted volumes of the eggs with known volume included the fixed effect, namely the linear regression of their actual volumes on their estimated volumes, as well as the contribution of the stochastic effects of egg and clutch. The difference between the actual volume of each egg and its fitted volume was expressed as a percentage of the fitted volume (percentage error in the fitted volume of whole eggs with known volume).

Nöthling et al. (2019) showed that the precision with which the volume of an egg is estimated increases as the scope of the egg shell increases. Wilcoxon's rank sum test was used to compare the scope of the 316 hatched egg shells with those of the 487 masked egg images.

If the error in the fitted hatchling mass were solely due to erroneous estimation of the volume of the hatched eggs, the variance in the percentage error in the fitted hatchling mass and the variance of the percentage error in the fitted volume of eggs with known volume would have been the same. The variances of the two sets of percentage errors were compared by means of Levene's test to determine whether the percentage error in the fitted hatchling mass was larger than the variance in the percentage error in the fitted volume of whole eggs with known volume.

Determining the variation in the estimated volume of hatched eggs and hatchling mass within and among clutches

The variation in the estimated volume of hatched eggs within clutches relative to the variation thereof among clutches was determined by calculating the intraclass correlation coefficient of the estimated egg volume in a model including estimated egg volume as outcome variable and clutch as stochastic group variable, without any covariates (Rabe-Hesketh and Skrondal 2012). In the same way the variation in hatchling mass within and among clutches was determined by calculating the intraclass correlation coefficient of hatchling mass in a model including hatchling mass as outcome variable and clutch as stochastic group variable, without any covariates. Both these intraclass correlation coefficients were calculated using only the 270 estimated egg volumes and the 270 hatchling masses from the 34 clutches that each had at least five data for each of the two variables.

Determining the productivity of eggs and the variation thereof within and among clutches

The estimated mass of hatchling (in grams) per millilitre of egg (the productivity of an egg) was calculated by dividing the mass of a hatchling by the estimated volume of the egg from which it hatched. Egg productivity was summarised and demonstrated graphically. The intraclass correlation coefficient for egg productivity was determined using a model with egg productivity as outcome variable and clutch as stochastic group variable, without any covariates.

Results

Summary of hatched shells and estimated egg volume, and hatchling mass

Of the 381 hatchlings for which it was known from which egg each hatched, and that were weighed, the shells were sufficiently intact to yield reliable measurements to above the girth, so that the position of the girth line and its point of intersection with the polar axis could be determined. Of these, 316 from 51 clutches were sufficiently intact to yield reliable measurements to a height of at least 1.3 times the distance from the bottom pole to the girth and for which, therefore, the egg volume could be estimated (Nöthling et al. 2019). One to 14 of these 316 observations came from the same clutch (mean 6.2, SD 3.35). The polarity of the 316 egg shell images was -1 ($n = 83$), 0 ($n = 121$) and 1 ($n = 112$).

The distribution of the estimated volume of the 316 hatched eggs was negatively skewed (minimum 57.1 ml, lower quartile 92.6 ml, median 99.9 ml, upper quartile 106.1 ml, maximum 121.7 ml). The distribution of the 316 hatchling masses was also negatively skewed (minimum 41.1 g, lower quartile 64.0 g, median 69.9 g, upper quartile 76.0 g, maximum 86.2 g).

The relationship between hatchling mass and the estimated volume of the egg from which each hatched

Figure 1 shows that a positive relationship existed between hatchling mass and estimated egg volume in most clutches that had at least five estimated egg volumes with their paired hatchling masses. Figure 1 also shows that those clutches that had quite low estimated egg volumes also had quite low hatchling masses, while the clutches with quite high estimated egg volumes also had quite high hatchling masses. Showing the combined data of all 51 clutches, Figure 2 shows a strong, positive, linear relationship between hatchling mass and estimated egg volume (Spearman’s rank correlation coefficient 0.88, $n = 316$, $P < 0.001$). Figure 2 also shows that, when the clutches to which eggs belong are not considered, considerable variation in hatchling mass occurs for eggs of a particular estimated volume.

Incubation period did not significantly affect hatchling mass ($P = 0.88$) and was therefore removed from the final model for estimating hatchling mass. Estimated egg volume had a highly significant effect on hatchling mass whereas neither the year in which clutches were laid nor clutch size had a significant effect thereon (Table 1). The coefficients of the intercept and estimated egg volume changed very little when year and clutch size were left out of the model (Table 1). Hence, year and clutch size were excluded from the final model, which is described in equation 2:

$$\text{Equation 2: } y_{ij} = \beta_0 + \beta_1 x_{1ij} + u_j + \varepsilon_{ij}$$

with the meaning of each symbol as defined for equation 1.

The final model explained 84.6% of the variation in hatchling mass (Wald $\chi^2 = 566$, with one degree of freedom).

The regression equation to estimate hatchling mass from estimated egg volume is as follows: Hatchling mass = $8.82 + 0.61 \times$ estimated egg volume (S.E. of the coefficient was 0.026 and the 95% CI 0.56–0.66, $z = 23.8$, $P < 0.001$).

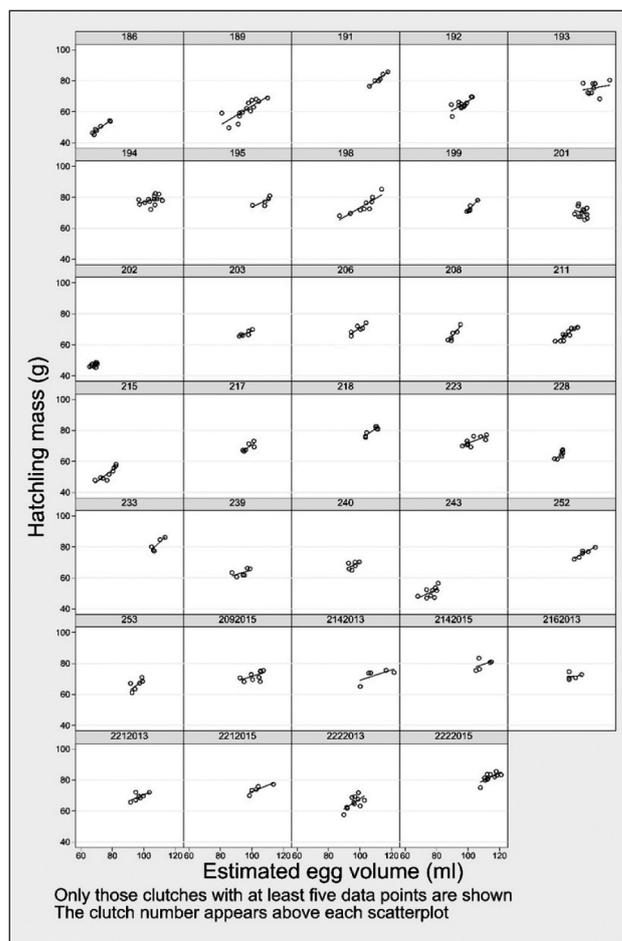


FIGURE 1: The relationship between mass of hatchlings upon hatching and estimated egg volume in 34 Nile crocodile clutches

Using the final model, which includes the effect of estimated egg volume, 59.2% of the variation in hatchling mass occurred among clutches and 40.8% within clutches. Clutch had a highly significant effect on hatchling mass in the final model ($\chi^2 = 128$, 1 degree of freedom, $P < 0.001$, 316 hatchling masses in 51 clutches).

TABLE 1: The coefficients of the fixed component of the model for the estimation of hatchling mass

Variable	Identity ^a	Regression coefficient		
		Expected value	95% CI ^b	P
Intercept	β_0	8.83 (8.82c)	2.20, 15.46 (3.79, 13.85)	0.01 (0.001)
Estimated egg volume	β_1	0.60 (0.61)	0.55, 0.66 (0.56, 0.66)	< 0.001 (< 0.001)
Year	β_2	-0.90	-2.79, 0.99	0.35
Clutch size	β_3	0.04	-0.09, 0.17	0.57

^a The identity of the coefficients as they appear in equations 1 and 2

^b The limits of the 95% confidence interval

^c All values between parentheses apply to the final model, which excludes year and clutch size as they had no significant effect on hatchling mass

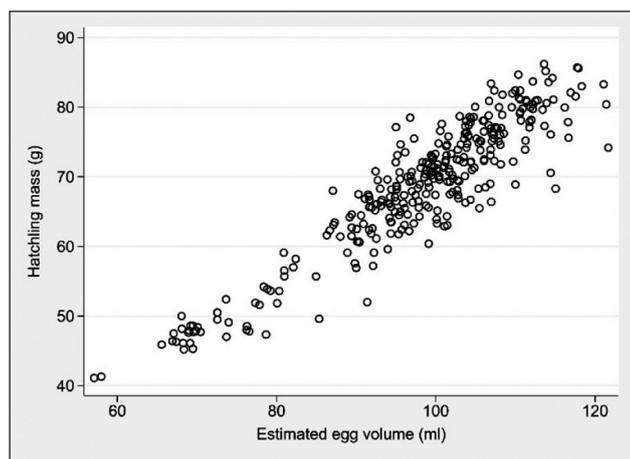


FIGURE 2: There exists a strong, positive, linear relationship between the mass of hatchlings and the estimated volume of the eggs from which they hatched (R -squared 0.85, $n = 316$), although considerable variation in hatching mass occurs for a particular estimated egg volume

The percentage errors in fitted volumes of whole eggs with known volume, and the percentage errors in fitted hatchling masses

The scope of the 316 hatched egg shell images varied from 1.3 to 1.8 (median 1.6, lower quartile 1.5, upper quartile 1.7), which was higher than the scope of the 487 masked images of whole eggs (median 1.5, lower quartile 1.4, upper quartile 1.6), $z = 8.84$, $P < 0.001$.

Figure 3a shows that 50% of the percentage errors in fitting the actual volumes of whole eggs on their estimated volumes fell within a band from -0.37% to 0.39% of the fitted volume and 95% within a band from -1.44% to 1.47% thereof. Figure 3b shows that 50% of the percentage error in fitting the masses of hatchlings on the estimated volumes of the eggs from which they hatched fell within a band from -1.83% to 2.07% of the fitted hatchling mass and 95% within a band from -7.99% to 6.80% . The range between the 2.5th and 97.5th percentiles in the percentage errors in fitting the masses of hatchlings on the estimated volumes of their eggs of origin was 5.1 times as wide as the corresponding range in the percentage errors in fitting the actual volumes of whole eggs on their estimated volumes. The variance of the 316 percentage errors in fitting the masses of hatchlings on the estimated volumes of their eggs of origin is larger than the variance among the 487 percentage errors in fitting the actual volumes of whole eggs on their estimated volumes (Levene's test statistic $W_0 = 392.5$ (F-distributed with 1, 801 degrees of freedom), $P < 0.001$).

The variation in the estimated volume of hatched eggs and hatchling mass within and among clutches

The intraclass correlation coefficient for the estimated volume of hatched eggs, without considering any covariates, was 0.849, indicating that 84.9% of the variation in estimated volume occurs among clutches and 15.1%

within clutches. Clutch had a highly significant effect on estimated egg volume ($\chi^2 = 391.7$ (1 degree of freedom), $P < 0.001$, 270 estimated egg volumes in 34 clutches that each had at least five estimated egg volumes). Figure 4a provides a visual impression of these variations: although estimated egg volume varied most among clutches, considerable variation also occurred within clutches.

The intraclass correlation coefficient for hatchling mass, without considering any covariates, was 0.869, indicating that 86.9% of the variation in hatchling mass occurred among clutches and 13.1% within clutches. Clutch had a highly significant effect on hatchling mass ($\chi^2 = 426.9$ (1 degree of freedom), $P < 0.001$, 270 hatchling masses in 34 clutches that each had at least five hatchling masses). Figure 4b shows that, as for estimated egg volume in Figure 4a, hatchling mass mostly varied among clutches, although considerable variation also occurred within clutches. Figures 4a and 4b also show that hatchling mass and estimated egg volume increase in a similar way among clutches when the clutches are sorted in order of their mean estimated egg volumes.

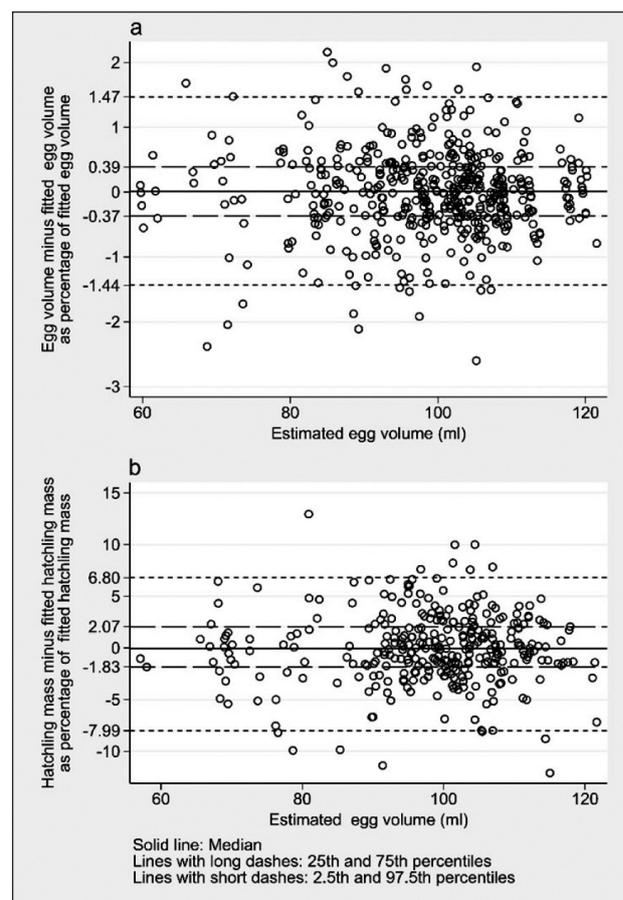


FIGURE 3: Percentage error in fitting the volumes of 127 eggs on the estimates of their volumes derived from measurements on 487 masked photo images of the same eggs (graph a), and the percentage error in fitting the masses of 316 hatchlings on the estimated volumes of their eggs of origin derived from measurements on photo images of the egg shells (graph b)

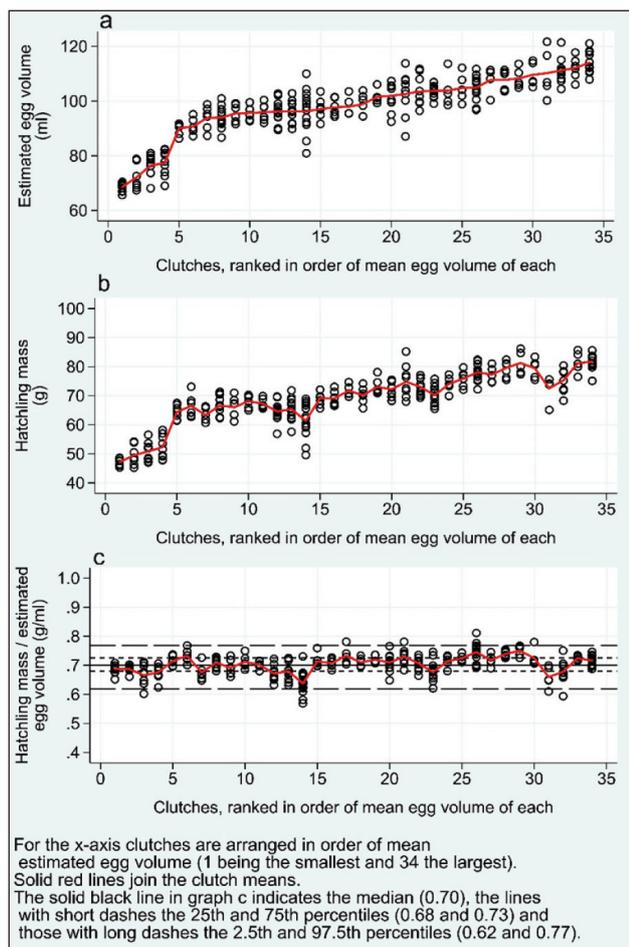


FIGURE 4: Variation in estimated egg volume (graph a), hatchling mass (graph b) and hatchling mass divided by estimated egg volume (egg productivity, graph c) between, as well as within 34 Nile crocodile clutches that each had at least five estimated egg volumes with their respective hatchling masses ($n = 270$)

The productivity of eggs

Different to hatchling mass, which increases as the mean estimated egg volume of clutches increases as shown in Figure 4b, Figure 4c shows that egg productivity varies in a band between approximately 0.6 and 0.8, irrespective of the mean estimated egg volumes of clutches.

Figure 5 shows a histogram of the productivity of all 316 eggs in 51 clutches. The productivity of eggs varied from 0.57 g/ml to 0.81 g/ml with an interquartile range of 0.05 g/ml and a range of 0.15 g/ml between the 2.5th and 97.5th percentiles. These results were the same as when only the 34 clutches, of which each had at least five estimated egg volumes, with their hatchling masses were considered (Figure 4c).

The intraclass correlation coefficient for productivity, without considering any covariate, was 0.454, indicating that 45.4% of the variation in productivity occurred between clutches and 54.6% within. The effect of clutch on productivity was highly significant ($\chi^2 = 111.3$ with one degree of freedom, $P < 0.001$, 270 productivity values in 34 clutches that had at least five productivity values each).

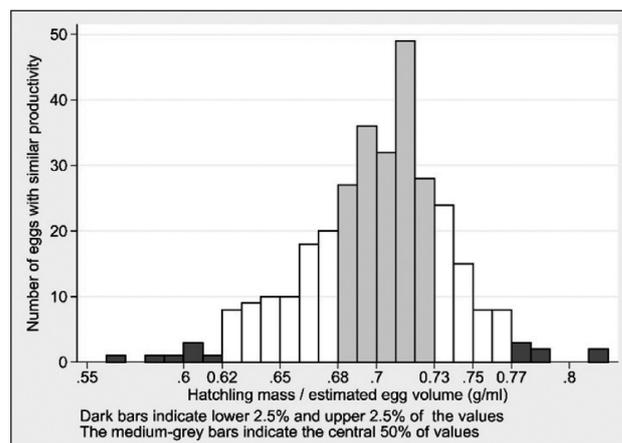


FIGURE 5: The productivity (hatchling mass / estimated egg volume) of 316 Nile crocodile eggs from 51 clutches

Discussion

This study not only shows a strong linear relationship between estimated egg volume and hatchling mass but also that hatchling mass for a particular estimated egg volume varies. This study also shows that the ratio between the mass of the hatchling and the estimated volume of the egg from which it hatched (the productivity of the egg) varies among eggs.

Egg volume as well as hatchling mass mostly vary among clutches, with about 85% and 87% of the variation in these two variables occurring among clutches and only about 15% and 13% among eggs of the same clutch. By contrast, egg productivity mostly varies within clutches, with 55% of the variation occurring among the eggs of the same clutch and 45% among clutches.

The growth rate of crocodiles is important on commercial crocodile farms (Isberg et al., 2005). Research on *Crocodylus porosus* hatchlings yielded conflicting results. Brien et al. (2014) found no significant effect of the mass of *C. porosus* hatchlings and their growth, whereas Brien et al. (2016) showed that *C. porosus* hatchlings of lower mass grew faster than those that were heavier. It is not known whether hatchling mass of the Nile crocodile relates to growth rate after hatching. In the light of the different patterns of variation in egg volume, hatchling mass and egg productivity, research is indicated to determine the relationship between each of these variables and growth rate after hatching in the Nile crocodile.

The cause of the variation in egg productivity is unknown. A Nile crocodile female lays her eggs in the same hole during the same night and, on farms, they are all collected the next morning and incubated together, in the same container at the same temperature and humidity level, and for a period that seldom differs by more than 2–3% among the eggs. The similarity in handling eggs of the same clutch makes it unlikely that the environment to which the eggs are exposed after laying would cause the variation in egg

productivity. Nelson et al. (2010) showed that the mass of American alligator (*Alligator mississippiensis*) foetuses increases by one gram for each one gram decrease in yolk mass, which suggests that yolk is an important determinant of the mass of foetuses and, eventually, hatchlings. Brown et al. (2019) showed that the yolk of Nile crocodile eggs constitutes from 29% to 62% (mean 43%, SD 5.6%) of their mass. Variation in yolk size in eggs of the same size may thus have caused the variation in egg productivity that occurred in the current study among and within clutches.

The volume of the eggs that yielded the hatchlings in the current study was estimated from their shells after they had hatched. The method of estimating the egg volume from the hatched shells was the same as the one Nöthling et al. (2019) used to estimate the volume of whole eggs of known volume from measurements done on masked photo images of the eggs. The precision with which the actual volume of eggs was fitted on their estimated volume is significantly better than the precision with which hatchling mass could be fitted on the estimated volume of the eggs from which they hatched. Actually, the interval between the 2.5th and the 97.5th percentiles of the percentage error between the fitted volume and the actual volume of eggs was only one fifth as wide as the interval between the 2.5th and the 97.5th percentiles of the percentage error between the fitted hatchling mass and the actual hatchling mass. The variation in egg productivity was therefore not solely due to erroneous estimation of the volumes of the hatched eggs. Still, further research is necessary to precisely calculate the actual volume of eggs prior to incubation, while the eggs are intact, by means of the methods described by Nöthling et al. (2019) and weigh the hatchling that each eventually yields and, from these, precisely quantify the variation in egg productivity. Research is necessary to determine the extent to which egg mass, which is easier to measure on crocodile farms than egg volume, can replace egg volume as a means of determining egg productivity.

This study may be of value in the conservation setting: Hatched shells from wild nests may be used to estimate the volume of the eggs and, from that, the most likely sizes of the hatchlings yielded by the eggs. Older, larger crocodiles lay larger eggs than younger, smaller ones (Huchzermeyer 2003; Swanepoel et al. 2000). Estimating the volume of eggs from the hatched shells from wild nests may be of use to estimate the size of the female that laid the eggs.

Conclusion

There exists a strong, linear, positive relationship between the mass of Nile crocodile hatchlings at the time of hatching and the estimated volume of the eggs from which they hatched. Without considering any covariate, 84.9% of the variation in estimated egg volume and 86.9% of the variation in hatchling mass are due to variation among clutches. The ratio between the mass of a hatchling and the estimated volume of the egg from which it hatched (the

productivity of the egg) varies among eggs, with 54.6% of the variation being as a result of variation within clutches.

Authors' contributions

JGM assisted JON during one of the two years to collect hatchlings and their egg shells as they hatched, and to photograph the eggs and weigh the hatchlings, whereas JON did this alone during the second year. JAN refined the computer program he had written earlier (Nöthling et al. 2019) in order to ensure its suitability for use on egg shells after hatching. JAN and JON prepared the photographs for measuring the egg shell images with the computer program and estimating the egg volume. JON performed the data analysis. JON wrote the manuscript in Afrikaans and then translated it into English.

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