South African Journal for Science and Technology
ISSN: (Online) 2222-4173, (Print) 0254-3486

Egg attributes and hatchling mass as predictors of hatchling growth on a Nile crocodile (*Crocodylus niloticus*) farm

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Dates:
Received: 02/03/21
Accepted: 11/08/21
Published: 10/12/21

How to cite this article:
Johan O Nöthling, Riaan Crafford, Jaco Theron, Johan A Nöthling, Egg attributes and hatchling mass as predictors of hatchling growth on a Nile crocodile (*Crocodylus niloticus*) farm, *Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie* 40(1) (2021). https://doi.org/10.36303/SATNT.2021.40.1.846

'n Afrikaanse vertaling van die manuskrip is aanlyn beskikbaar by http://www.satnt.ac.za/index.php/satnt/article/view/846

Rapid growth of Nile crocodile (*Crocodylus niloticus*) hatchlings on farms shortens the time to slaughter for skins, thus reducing costs and improving farm efficiency. The aims with the project were to assess the utility of various egg attributes (length, width, volume, mass and density) as predictors of hatchling mass ($HM_{initial}$) for 269 eggs from 15 clutches and to assess these egg attributes, $HM_{initial}$ and egg productivity as predictors of the mass of the hatchlings 68–75 days after hatching (HM_{end}). Hatchlings heavier than 61,5 g were individually marked and assigned to five pens according to $HM_{initial}$ until the HM_{end} of 129 hatchlings was determined. Strong and meaningful positive, linear relationships existed between $HM_{initial}$ and all egg attributes except egg density, where the relationship was strong and negative ($P < 0{,}001$). HM_{end} varied widely across as well as within clutches. It is most compatible with the data that $HM_{initial}$ ($P = 0{,}08$) and egg productivity ($P = 0{,}02$) have meaningful negative relationships with HM_{end} when all five pens are included in the model. Considering pens separately, the direction of the relationship between $HM_{initial}$ and HM_{end} was negative for three pens and neutral for two. The direction of the relationship between egg productivity and HM_{end} was negative for four pens and positive for one. The models that each included one predictor as well as pen only explained 8–14% of the variation in HM_{end}, suggesting that factors other than those studied largely affected growth.

Keywords: crocodile, egg, hatchling, growth

Eierattribute en broeilingmassa as voorspellers van broeilinggroei op 'n Nylkrokodilplaas (*Crocodylus niloticus*-plaas): Nylkrokodilbroeilinge (*Crocodylus niloticus*-broeilinge) wat vinniger groei op plase kan vroeër geslag word vir hulle velle, wat koste verminder en plaasdoeltreffendheid verhoog. Die projek se doel was om die nut van verskeie eierattribute (lengte, wydte, volume, massa en digtheid) as voorspellers van broeilingmassa (BM_{begin}) vir 269 eiers van 15 broeisels te bepaal, en om hierdie eierattribute, BM_{begin} en eierproduktiwiteit as voorspellers van die massa van die broeilinge 68–75 dae ná uitbroeiing (BM_{einde}) te beoordeel. Broeilinge swaarder as 61,5 g is individueel gemerk en volgens BM_{begin} aan vyf hokke toegewys, totdat die BM_{einde} van 129 broeilinge bepaal is. Sterk, betekenisvolle, positiewe, lineêre verbande bestaan tussen BM_{begin} en al die eierattribute behalwe eierdigtheid, waar die verband sterk en negatief was ($P < 0{,}001$). BM_{einde} het baie tussen en binne broeisels gevarieer. Dit is die beste versoenbaar met die data dat BM_{begin} ($P = 0{,}08$) en eierproduktiwiteit ($P = 0{,}02$) betekenisvolle negatiewe verbande met BM_{einde} het wanneer al vyf hokke by die model ingesluit is. Vir hokke wat afsonderlik beoordeel is, was die rigting van die verband tussen BM_{begin} en BM_{einde} negatief vir drie hokke en neutraal vir twee. Die rigting van die verband tussen eierproduktiwiteit en BM_{einde} was negatief vir vier hokke en positief vir een. Die modelle wat elk slegs een voorspeller en hok as koveranderlike ingesluit het, verklaar 8–14% van die variansie in BM_{einde}, wat aandui dat ander faktore as dié wat bestudeer is groei grootliks beïnvloed.

Sleutelwoorde: krokodil, eier, broeiling, groei

Introduction

Nile crocodile (*Crocodylus niloticus*) farming in South Africa is an important source of foreign exchange. Nile crocodile leather is globally sought-after by affluent people. Hatchling growth is important for successful crocodile farming. Rapid hatchling growth shortens the time to slaughter and harvesting the skin, rendering the production more cost-effective (Isberg et al., 2005).

Saltwater crocodile (*Crocodylus porosus*) (Garnett and Murray, 1986; Brien et al., 2014) and Australian fresh water crocodile (*Crocodylus johnstoni*) (Webb et al., 1983a) growth vary widely. Runting – an extreme form of restricted growth of crocodile hatchlings and juveniles – is characterised by very slow growth or a lack of growth, while some individuals fail to start eating and starve to death (Garnett and Murray, 1986; Buenviaje et al., 1994; Brien et al., 2014; Huchzermeyer, 2003). Early growth in some crocodilians relates positively to growth when they are older (Eme et al., 2010; Brien et al., 2014). Runtism (Brien et al., 2014) and growth are strongly affected by clutch (Garnet and Murray, 1986; Riese et al., 1991; Brien et al., 2014).

It is easy to weigh hatchlings upon removal from the incubator. Hatchling mass is, therefore, a candidate attribute for the prediction of hatchling growth. Webb et al. (1983a) found no significant relationship between the size (SVL) of Australian freshwater crocodile (*Crocodylus johnstoni*) hatchlings and growth rate to the age of six months. The relationship between hatchling size and growth in the saltwater crocodile is uncertain; Brien et al. (2014) and Brien et al. (2016) found a negative relationship between hatchling mass and growth rate to the age of 24 days and Riese (1991) found a positive relationship between hatchling size, measured as SVL, and growth rate to the age of 48 days. Although Hutton (1987) did weigh and measure the length of Nile crocodile hatchlings and repeated the measurements at three months, he did not relate initial weight or length to those at three months. There is a need to study the relationship between hatchling size and growth in the Nile crocodile.

Studies exist on the relationship between the size of crocodilian hatchlings and various egg attributes such as width (Webb et al., 1983b), length (Webb et al., 1983b; Brien et al., 2014), mass (Deitz and Hines, 1980; Webb et al., 1983b; Garnett and Murray, 1986; Webb and Cooper-Preston, 1989; Brien et al., 2014; Eme et al., 2019) and volume estimated from hatched shells (Nöthling et al., 2019b). However, the relationships between these egg attributes and crocodilian hatchling growth remains unstudied.

Using intact, infertile Nile crocodile eggs after the other eggs in the clutch have hatched, Nöthling et al. (2019a) developed a computer program that allowed them to calculate the volume of an intact egg from a photograph thereof (EV_{photo}). On average, EV_{photo} was 1,49% higher than the water displacement volume (EV_{wd}). The authors suspected that water may have entered the egg through the porous shells (Ferguson, 1982; Grigg, 1986) and filled air pockets that may have formed during incubation (Manolis et al., 1986), which might have caused an erroneously low measurement of EV_{wd}. It is of interest to determine whether measuring EV_{wd} shortly after laying, before air pockets develop underneath the shell, would still yield volumes that are lower than EV_{photo}. It is also of interest to determine the precision of estimating hatchling mass from EV_{photo} and to determine whether egg volume relates to hatchling growth.

Nöthling et al. (2019b) estimated the volume of Nile crocodile eggs from the hatched shells. Based on these estimated volumes, they reported that hatchling mass as a fraction of egg volume – which they referred to as the egg productivity – varied from 0,57 g/mL to 0,82 g/mL (mean 0,70 g/mL). There is a need to determine whether egg productivity, based on an accurately determined egg volume, relates to hatchling growth.

Clutch significantly affects egg volume, egg productivity (Nöthling et al., 2019b), hatchling size (Isberg et al. 2005, Brien et al. 2014, Nöthling et al., 2019b) and growth of crocodiles (Garnett Murray 1986; Isberg et al., 2005; Brien et al., 2014).

The density of Nile crocodile eggs is unknown. Brown et al. (2019) showed that the mass fraction of the various components of Nile crocodile eggs varies. It is likely that these components have different densities (shell, for example, may have a higher density than yolk), making it feasible that, for a particular egg volume, egg mass might vary. Yet, egg mass may be easier to measure than egg volume, raising the question whether egg mass may be used as a substitute for egg volume to express egg productivity. There is also a need to determine the relationships among egg density, hatchling mass and hatchling growth.

The first aim of this study was to assess the utility of various attributes of Nile crocodile eggs as predictors of hatchling mass on a commercial Nile crocodile farm. The second was to assess the utility of the same egg attributes, as well as hatchling mass and hatchling mass expressed as a fraction of egg volume or egg mass as predictors of the growth of hatchlings on the same farm.

Materials and methods

The Animal Ethics Committee of the University of Pretoria approved the study (Project number REC084-19). The director of the farm gave written consent to use the animals and facilities for the study. Data were collected on a farm in the North West Province in South Africa between October 2019 and March 2020.

Eggs laid during the night were collected in the morning and placed on moist vermiculite in polystyrene boxes, which were placed in a pre-incubator at 32 °C and 90% air moisture. A day or two later, eggs were trans-illuminated and those with an opaque band developing at the equator identified (Ferguson, 1982). Eggs with an opaque band were deemed fertile and those without infertile.

Two days after laying, 267 infertile eggs from 53 clutches were labelled and weighed to the nearest 0,01 g. Each egg was photographed with its long axis perpendicular to the optic axis of the camera at a distance of one meter between its horizon and the camera sensor, with a Vernier next to the egg, in the plane of its horizon (Figures 1 and 2 in the supplementary file). Once photographed, the volume of each egg was determined by water displacement (EV_{wd}). Later, the EV_{photo} of each egg was derived from its photograph by means of a computer program as described by Nöthling et al. (2019a).

Two days after laying, 18 fertile eggs from each of 14 clutches and 17 from one clutch laid during three consecutive nights (4–6 October 2019) were used for the study. Without turning it, each egg was carefully cleaned with paper towel, labelled (clutch number and egg number) and photographed as described for infertile eggs (Figure 2 in the supplementary file). EV_{photo}, egg length (EL) and egg width (EW) were derived from the photograph, as described by Nöthling et al. (2019a). Thirty polystyrene boxes (inner dimensions 548 mm × 353 mm, depth 121 mm) were divided into nine equal-sized compartments (three rows by three columns) by means of interlocking Perspex strips, each with several 12-mm holes through it (Figure 3 in the supplementary file). The eggs of each clutch were placed in the compartments of two boxes – one egg per compartment on a few-centimetres thick layer of moist vermiculite (Figure 3 of the supplementary file). Each box was covered with a clear 4-mm thick Perspex sheet and the box as well as the lid labelled with the clutch number. The corner of the box and the lid at the compartment of the first row and the first column was also labelled.

Hatching occurred from 18 to 25 December 2019 after incubation periods of 75–80 d (mean 77,6 d, SD 1,72 d). Once hatched, the hatchling and eggshell were removed from their compartment (Figure 4 in the supplementary file). Each shell (labelled with its box, the row by column coordinates of its compartment, clutch n umber and egg number) was photographed as described for the whole eggs and the photos used to estimate the volume of the eggs as described by Nöthling et al. (2019a, 2019b) (Figure 2 in the supplementary file). Hatchlings were carefully cleaned of loose vermiculite and dab-dried with paper towel, identified by clipping tail scutes and weighed (hatchling mass at the beginning of the growth period or $HM_{initial}$). The clipped tail scutes represented a unique three-digit code, which, via a master list, linked the hatchling to its clutch and egg of origin. According to normal farm practice hatchlings were classified according to $HM_{initial}$ as large (≥ 72,5 g), medium (≥ 67,5 g, < 72,5 g), small (≥ 61,5 g, < 67,5 g) or underweight (< 61,5 g).

Normal farm practice is to keep large, medium and small hatchlings – except those that are potbellied, which are deemed to have poor absorption of the yolk sac – for growth and transfer them to hatchling pens in a grower house for 9–10 months and then to larger growing pens for the full duration of the growth period. Complying with farm practice, experimental hatchlings of different mass classes were placed in separate, neighbouring pens. Pens 1 and 2 received small hatchlings, pen 3 medium hatchlings and pens 4 and 5 large hatchlings. The grower house had a plastic roof and walls and its temperature was maintained at 28–34 °C by rolling up or lowering the eastern and western curtain walls. Each pen was approximately 8 m^2 in surface area and had a one-metre high wall around, with a smooth cement floor gently sloping inwards into a water pool occupying the central third of the pen. The depth of the pool increased to about 25 cm at its centre.

Hatchlings received a ration every afternoon that consisted of 50% chicken and 50% of a specially formulated and balanced meal. The amount fed depended on the amount of waste food collected. Waste equal to 10–15% of the ration supplied was considered acceptable. The ration was increased once the percentage of waste decreased below 10%. Every morning waste food was collected, the pens cleaned with degreaser (Sparkle, Glenchem, Kempton Park, South Africa) and disinfectant (F10, Health and Hygiene (Pty) LTD, Florida Hills, South Africa), and the water in the ponds replaced. According to managerial practice on the farm, runts and weak hatchlings were removed. Brien et al. (2014) and Riese (1991), both studying growth in saltwater crocodile (*Crocodylus porosus*) hatchlings, respectively found large variations in growth as early as 24 days and 35–55 days. Based on these studies, the remaining hatchlings were weighed on March 2, 2020, when they were 68–75 d old, to determine their body mass at the end of the growth period (HM_{end}).

Data analysis

For each variable, the proportions of the total variance that occurred within- and between clutches were determined using a simple model with the variable as the outcome variable, without any covariates, and clutch as random grouping variable (Rabe-Hesketh and Skrondal, 2012).

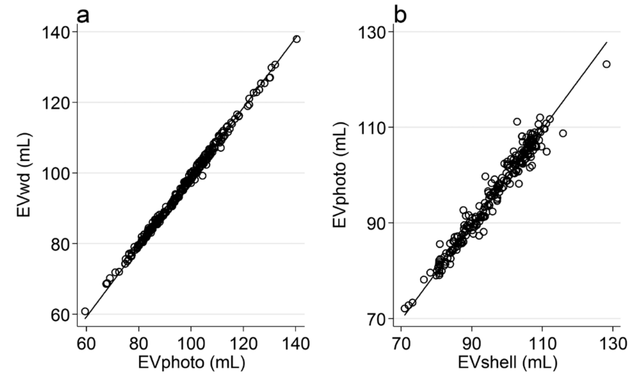
In model 1 a two-level linear regression, with clutch as second-level random grouping variable was used to determine the association between $HM_{initial}$ (outcome variable) and an egg attribute (predictor variable). Egg attribute was any one of EW, EL, EV_{photo}, EM or ED_{photo} (calculated as EM/EV_{photo}), as well as EV_{shell}. Model 1 was $y_{ij} = \beta_0 + \beta_1 x_{1ij} + u_j + \varepsilon_{ij}$.

y_{ij} Is the $HM_{initial}$ from the ith egg from the jth clutch. $\beta_0 + \beta_1 x_{1ij}$ defines the linear regression line of the fixed component of the model with β_0 as intercept and β_1 as slope. $\beta_1 x_{1ij}$ Estimates the effect of the attribute of the ith hatched egg from the jth clutch on the mass of the ith hatchling from the jth clutch. u_j Estimates the combined effect of the unmeasured characteristics associated with the jth clutch on the mass of the ith hatchling from the jth clutch. ε_{ij} Estimates the combined effect of the unmeasured characteristics associated with the ith hatchling from the jth clutch on its mass.

In model 2 a two-level linear regression, with clutch as random, second-level grouping variable was used to determine the association between HM_{end} and an egg attribute or $HM_{initial}$, as well as clutch size and hatchling age at final weighing. Because hatchling pens (pens) received hatchlings according to their initial mass, pen was included in the model as confounder. Egg attribute was any one of EW, EL, EV_{photo}, EM, ED_{photo}, Epm or Epv. The equation for model 2 was as follows:

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

y_{ij} Is the end mass of the hatchling from the ith egg from the jth clutch. $\beta_0 + \beta_1 x_{1ij} + \beta_2 x_{2j} + \beta_3 x_{3ij} + \beta_4 x_{4ij}$ defines the linear regression line of the fixed component of the model, with β_0 as intercept. $\beta_1 x_{1ij}$ Estimates the effect of the attribute of the ith hatched egg or the initial mass of the ith hatchling from the jth clutch on the end mass of the ith hatchling from the jth clutch. $\beta_2 x_{2j}$ Estimates the effect of the size of the jth clutch on the end mass of the hatchlings in the clutch. $\beta_3 x_{3ij}$ Estimates the effect of the age of the hatchling from the ith egg from the jth clutch on the hatchling's end mass. $\beta_4 x_{4ij}$ Estimates the effect of the pen on the end mass of the ith hatchling from the jth clutch. u_j Estimates the combined effect of unmeasured characteristics associated with the jth clutch on the end mass of the ith hatchling from the jth clutch. ε_{ij} Estimates the combined effect of the unmeasured characteristics associated with the ith hatchling from the jth clutch on its end mass. Clutch size and age of hatchlings were removed stepwise from the model if their effect on end mass did not significantly deviate from zero ($P > 0,05$).



EVwd = 0,9872 × EVphoto (95% CI 0,986, 0,988; t = 1839; P< 0,001; R^2 0,999; n 267).
EVphoto = 0,996 × EVshell (95% CI 0,994, 0,999; t = 783; P< 0,001; R^2 0,999; n 233).

FIGURE 1: Graph a shows the volumes of infertile Nile crocodile (*Crocodylus niloticus*) eggs determined by water displacement (EV_{wd}), plotted against their volumes calculated from photographs (EV_{photo}); Graph b shows the volumes of fertile eggs calculated from photographs of the whole eggs prior to incubation (EV_{photo}), plotted against their volumes estimated from photographs of the hatched shells (EV_{shell})

If model 2 indicated that a predictor variable had a meaningful association with HM_{end}, model 3 was used to assess the association between the predictor and HM_{end} for each pen separately, thereby avoiding any confounding by pen (Hernán et al., 2002). Model 3 is given by the equation $y_{ij} = \beta_0 + \beta_1 x_{1ij} + \beta_2 x_{2ij} + \beta_3 x_{3ij} + u_j + \varepsilon_{ij}$, with the terms meaning the same as those in model 2. Using model 3 to assess pens separately reduced the number of observations to the extent that the associations between a predictor and HM_{end} were not statistically significant, but the direction of the associations in individual pens was compared to that of the association found when all pens were included in the analysis.

The coefficient of determination (R^2) indicates the percentage of the variance in the outcome variable that is explained by the variation in the predictor variables within and between clutches (Rabe-Hesketh and Skrondal, 2012). The distribution of data was described as their mean (point estimate) with its 95% compatibility interval (95% CI) and the distribution of coefficients resulting from a regression analysis as the expected coefficient (point estimate) with its 95% compatibility interval (Amrhein et al., 2019). Values falling within the limits of a 95% compatibility interval were deemed compatible with the data (Amrhein et al., 2019).

Results

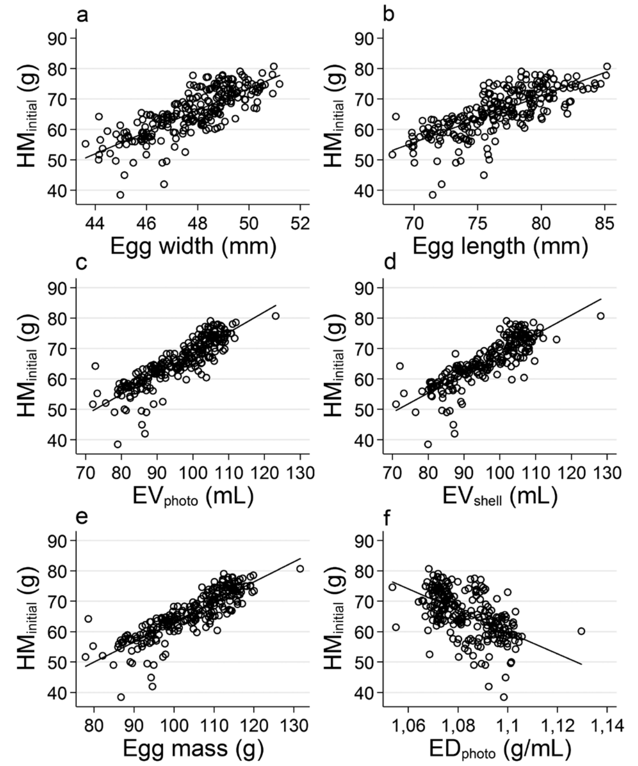
A summary of egg attribute values and initial hatchling mass appears in Table I. Of the 269 fertile eggs 248 (92%) hatched, with 233 shells remaining sufficiently intact to permit estimating egg volume from a photograph of the hatched shell.

There were moderate clutch effects on EL, EV_{shell}, EM and $HM_{initial}$, strong clutch effects on EW and ED, and very weak clutch effects on Epv and Epm (Table II). Unlike $HM_{initial}$, where 63% of the variance is expected to occur between clutches, only 33% of the variance in HM_{end} is expected to occur between clutches (Table II).

There was a strong positive linear relationship between EV_{photo} and EV_{wd} of infertile eggs, and EV_{shell} and EV_{photo} of fertile eggs (Figure 1). Confirming an earlier observation by Nöthling et al. (2019a), EV_{photo} was 1,3% higher than EW_{wd} (Figure 1).

Predicting initial hatchling mass

Strong positive, linear relationships existed between $HM_{initial}$ and all egg attributes except egg density, where the relationship was negative (Figure 2). The regression equations by which to predict $HM_{initial}$ from various egg attributes appear in Table III. It is most compatible with the data that $HM_{initial}$ increased by about 0,61 g for each one-millilitre increase in EV_{photo}, although increases from 0,55 g to 0,68 g are also compatible therewith. Most compatible with the data is that $HM_{initial}$ increased about 0,58 g for each one-gram increase in EM, although increases from 0,52 g to 0,65 g are also compatible therewith. The expected effect of EV_{shell} on $HM_{initial}$ is an increase by about 0,56 g for each one-millilitre increase in EV_{shell}, although increases from 0,49 g to 0,63 g are also compatible with the data. It is most compatible with the data that $HM_{initial}$ would increase by about 3,5 g for each one-millimetre increase in EW, although increases by 3,0 or 4,0 g are also compatible with the data. The expected effect of EL on $HM_{initial}$ is an increase by 1,27 g for each one-millimetre increase in EL, although increases by 1,08 g or 1,46 g are also compatible with the data. The higher values of z and R^2 in Table III show that EV_{photo} and EM, as well as EV_{shell}, more precisely predicted $HM_{initial}$ than EL and EW and much more than ED_{photo}.



Observations = 233 for graph d and 248 for all others.
Abbreviations defined near the end of the article.

FIGURE 2: Linear relationship between the initial mass of Nile crocodile (*Crocodylus niloticus*) hatchlings ($HM_{initial}$) and various egg attributes

TABLE I: Summary statistics of Nile crocodile (*Crocodylus niloticus*) egg attributes and initial mass of hatchlings

	Data across clutches				Clutch means	
	Mean	**SD**	**Minimum**	**Maximum**	**Minimum**	**Maximum**
Infertile eggs (267 eggs from 53 clutches, average 5,0 eggs per clutch)						
EL (egg length in mm)	76,79	4,62	63,69	92,17	67,58	87,69
EW (egg width in mm)	47,71	2,16	41,66	52,84	42,99	52,27
EM (egg mass in g)	104,36	14,26	65,0	151,66	75,33	140,53
EV_{wd} (egg volume measured by water displacement in mL)	95,13	12,92	60,78	137,87	68,73	127,88
EV_{photo} (egg volume calculated from photo in mL)	96,31	13,44	59,53	140,50	67,80	129,45
ED_{wd} (egg density based on EV_{wd} in g/mL)	1,0968	0,0085	1,056	1,1426	1,0809	1,1137
ED_{photo} (egg density based on EV_{photo} in g/mL)	1,0840	0,0129	1,0429	1,1236	1,0637	1,1177
Fertile eggs (269 eggs from 15 clutches, average 17,9 per clutch)						
EL	76,54	3,62	68,29	85,18	71,52	82,00
EW	47,80	1,63	43,62	51,20	45,13	50,13
EM	103,65	9,80	77,88	131,58	90,90	113,35
EV_{photo}	95,74	9,64	72,09	123,16	83,10	105,56
ED_{photo}	1,0833	0,0117	1,0537	1,1297	1,0703	1,1002
Hatchlings (248 hatchlings from 15 clutches, average 16,5 per clutch)						
$HM_{initial}$ (mass at the time of hatching in grams)	65,82	7,46	38,43	80,65	56,64	75,26
Egg productivity (n = 248)						
Epm ($HM_{initial}$/EM in g/g)	0,633	0,039	0,443	0,817	0,597	0,670
Epv ($HM_{initial}$/EV_{photo} in g/mL)	0,685	0,041	0,484	0,882	0,649	0,719
Hatched eggshells (n = 233 from 15 clutches, average 15,5 per clutch)						
EV_{shell} (egg volume estimated from a photo in mL)	96,46	9,68	70,99	128,23	82,25	105,47

TABLE II: Percentage of total variance in egg attribute values, initial hatchling mass ($HM_{initial}$) and hatchling mass at the end of a growth period of 68–75 days (HM_{end}) occurring within and between clutches

	Percentage of variance				
Variable	**Within clutches**	**Between clutches**	**n**	**n clutches**	**n Per clutches**
EL	40 (24–59)[a]	60 (41–76)[a]	269	15	17,9 (17–18)[b]
EW	27 (15–44)	73 (56–85)	269	15	17,9 (17–18)
EV_{photo}	33 (19–52)	67 (48–81)	269	15	17,9 (17–18)
EM	36 (21–54)	64 (46–79)	269	15	17,9 (17–18)
ED_{photo}	20 (11–35)	80 (65–89)	269	15	17,9 (17–18)
$BM_{initial}$	37 (22–56)	63 (44–78)	248	15	16,5 (14–18)
EV_{shell}	36 (21–55)	64 (45–79)	233	15	15,5 (13–17)
Epv	81 (63–92)	19 (8–37)	248	15	16,5 (14–18)
Epm	77 (57–89)	23 (11–43)	248	15	16,5 (14–18)
BM_{end}	67 (40–86)	33 (14–60)	129	14	9,2 (1–14)

[a] Expected percentage with its 95% compatibility interval between parentheses.
[b] Mean, with the minimum and maximum between parentheses.

TABLE III: Regression equations by which to predict the initial mass of Nile crocodile (*Crocodylus niloticus*) hatchlings from egg attributes

Predictor variable	Regression equation	$95\%\text{-}VI_{coëff}$	z	P	R^2
EW[a]	−102,32 + 3,5118 × EW	3,0235 tot 4,0001[b]	14,10	< 0,001	0,61
EL[a]	−31,43 + 1,2683 × EL	1,0770 tot 1,4596	12,99	< 0,001	0,54
EV_{photo}[a]	6,80 + 0,6139 × EV	0,5486 tot 0,6791	18,45	< 0,001	0,74
EM[a]	5,02 + 0,5841 × EM	0,5205 tot 0,6477	18,0	< 0,001	0,73
ED_{photo}[a]	318,64 − 233,4991× ED_{photo}	−334,83 tot −132,17	−4,64	< 0,001	0,27
EV_{shell}[c]	12,09 + 0,5577 × EV_{shell}	0,4883 tot 0,6270	15,76	< 0,001	0,69

[a] There were 248 eggs from 15 clutches (14–18 per clutch, with a mean of 16,5).
[b] Column 95% CI_{coeff} indicates the upper and lower limits of the 95% compatibility interval for the coefficient of the regression equation.
[c] There were 233 hatched egg shells from 15 clutches (13–17 per clutch, with a mean of 15,5).

Predictors of hatchling growth

Table IV shows the number of hatchlings from each clutch and size category that entered the growth period, and the numbers that remained throughout the growth period. The initial- and end masses of the 129 hatchlings that were retained to the end of the growth period appear in Table V. End mass varied widely in each pen (Table V, Figures 3–6). HM_{end} also varied widely within clutches, with the coefficients of variation in the 13 clutches for which at least two HM_{end} were measured varying from 8% to 37% (mean 19,6%).

The effects of egg attributes and $HM_{initial}$ on HM_{end} appear in Table VI. Age at final weighing and clutch size was removed from all models because it did not meaningfully alter the association between any predictor and HM_{end} ($P \geq 0{,}26$). The R^2 values in Table VI show that the covariates (an egg attribute or $HM_{initial}$) and pen only explained 8–14% of the variance in HM_{end}. The effects of each egg attribute and $HM_{initial}$ on growth appear below (Sections 4.2.1 to 4.2.7).

TABLE IV: Numbers of Nile crocodile (*Crocodylus niloticus*) hatchlings from each clutch and each size category that entered the hatchling pens at the beginning of the growth period and the numbers remaining there until the end of the growth period

	Hatchlings in pens at the beginning of the growth period				Hatchlings in pens at the end of the growth period			
Clutch	**Small**	**Medium**	**Large**	**Total**	**Small**	**Medium**	**Large**	**Total**
62	10	2	0	12	6	2	0	8
64	7	0	0	7	6	0	0	6
72	2	0	0	2	1	0	0	1
73	0	4	14	18	0	4	10	14
74	5	0	0	5	5	0	0	5
75	1	1	14	16	0	1	12	13
76	2	2	4	8	2	2	4	8
77	4	2	0	6	1	2	0	3
78	12	1	1	14	12	1	1	14
79	3	0	0	3	0	0	0	0
82	5	3	0	8	4	3	0	7
83	1	4	11	16	1	4	7	12
84	1	6	11	18	1	4	9	14
92	12	4	0	16	8	4	0	12
93	6	8	3	17	4	5	3	12
Total	71	37	58	166	51	32	46	129

TABLE V: Mass of Nile crocodile (*Crocodylus niloticus*) hatchlings upon hatching and at the end of the growth period of 68–75 days

	Initial mass			End mass				
Pen	**Mass class**	**Mean**	**SD**	**Mean**	**SD**	**Min**	**Max**	**n**
1	Small	64,7	1,46	318,6	66,14	217	478	25
2	Small	65,7	3,15	312,5	91,32	190	563	26
3	Medium	69,7	1,73	353,4	92,71	191	545	32
4	Large	74,3	1,25	296,9	68,20	164	424	15
5	Large	75,2	1,85	276,2	74,51	143	415	31

TABLE VI: Effect of predictor variables on the end mass of 129 Nile crocodile (*Crocodylus niloticus*) hatchlings after a growth period of 68–75 days

Predictor variable[a]	Coefficient[b]	95%-CI$_{coeff}$[c]	P$_{predvar}$[d]	P$_{pen}$[e]	R^2
EW	−7,538	−25,048, 9,972	0,40	0,17	0,107
EL	6,334	−0,738, 13,406	0,08	0,02	0,139
EM	0,903	−2,625, 4,431	0,62	0,08	0,103
EV$_{photo}$	0,941	−2,739, 4,621	0,62	0,08	0,108
ED$_{photo}$	−168,287	−2 415,067, 2 078,493	0,88	0,08	0,108
BM$_{initial}$	−5,747	−12,178, 0,684	0,08	0,07	0,084
Epv	−802,006	−1 452,418, −151,594	0,02	0,17	0,121
Epm	−911,509	−1 628,660, −194,358	0,01	0,14	0,089

[a] Each predictor variable was included in the model on its own, with all five pens, while clutch was included as random grouping variable.
[b, c] The coefficient for the effect of the predictor variable on end mass, with the limits of its 95% compatibility interval.
[d] The P-value for the effect of the predictor variable on end mass.
[e] The lowest P-value for the effect of any pen on end mass when all five pens were included in the model together with the predictor variable shown on the left.

Egg width

The coefficient (Table VI) suggests that end mass is expected to decrease by 7,5 g for each one-millimetre increase in EW. The 95% compatibility interval of the coefficient indicates that an increase of about 10 g in end mass for each mm increase in EW, as well as a decrease of about 25 g, are both also compatible with the data, suggesting substantial uncertainty about the true relationship between EW and end mass.

Egg length

Over all 129 observations the coefficient and its 95% CI suggest that an increase by about 6 g in HM$_{end}$ for each one-millimetre increase in EL is most compatible with the data, although an increase as large as 13,4 g or a decrease as small as 0,7 g are also compatible (Table VI). Relative to the limits of the 95% CI, the coefficient is quite far above zero. Although the true coefficient is unknown, values closer to the expected coefficient are more compatible with the data than values further away, implying that HM$_{end}$ is likely to increase meaningfully with an increase in EL (Amrhein et al., 2019). Regressing HM$_{end}$ on EL for pens individually showed a positive relationship between EL and HM$_{end}$ for pens that received small or medium hatchlings and a negative relationship for pens that received large hatchlings (Figure 3).

Egg mass and egg volume

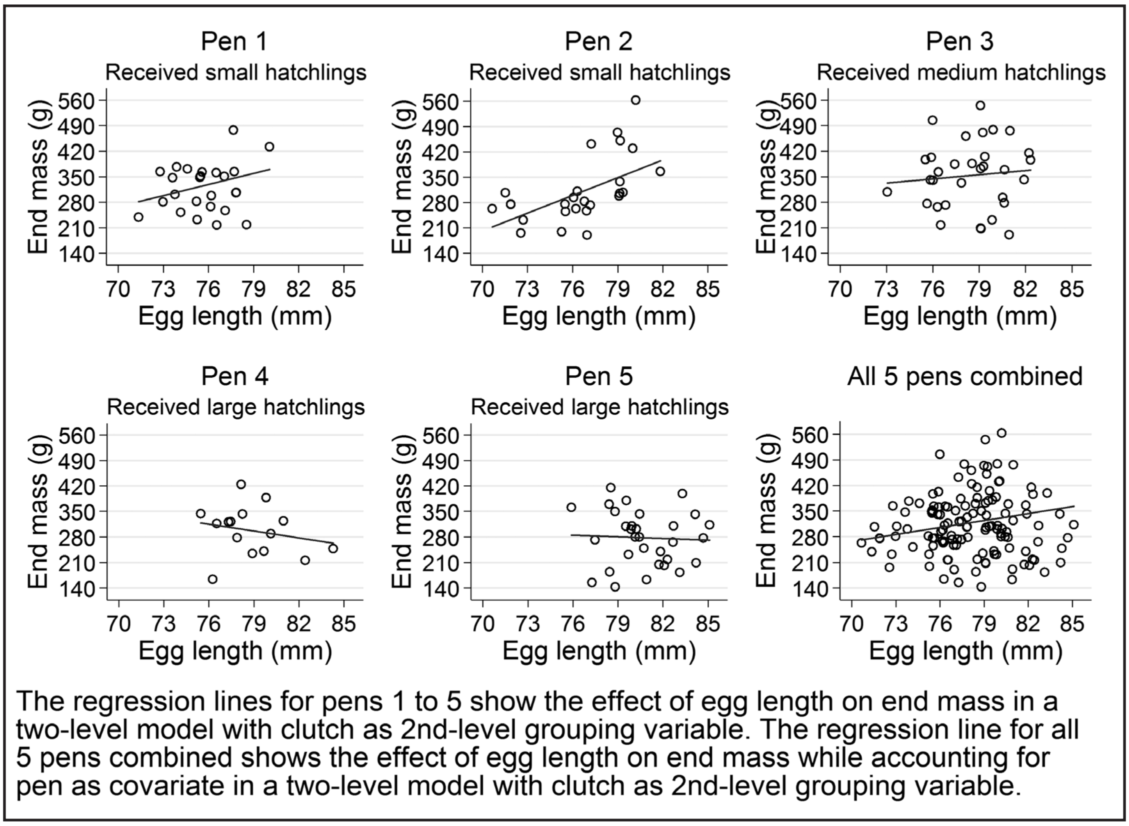
According to Table VI, EM and EV have similar coefficients and 95% CIs for their associations with HM$_{end}$. Because EM is easier to measure than EV the focus remains on EM: The coefficient and its 95% CI show that HM$_{end}$ is expected to increase by about 0,9 g for each one-gram increase in EM, although an increase by as much as 4,4 g or a decrease by as much as 2,6 g is also compatible with the data. Relative to the width of the 95% CI, the proximity of the expected coefficient to zero suggests considerable uncertainty whether end mass would increase or decrease with an increase in EM.

Egg density

The 95% CI for the coefficient of the association between ED$_{photo}$ and HM$_{end}$ is quite symmetrical around zero (Table VI). A decrease as large as 24 g in HM$_{end}$ or an increase as large as 21 g for each increase by 0,01 g/mL in ED$_{photo}$ are both compatible with the data, suggesting considerable uncertainty in what the true relationship between ED$_{photo}$ and HM$_{end}$ would be. The coefficient is relatively close to zero, indicating an expected decrease of 1,68 g in HM$_{end}$ for each increase by 0,01g/mL in ED$_{photo}$. Although the true coefficient is unknown, our data suggest that a change in ED is likely to be associated with a small and uncertain change in HM$_{end}$.

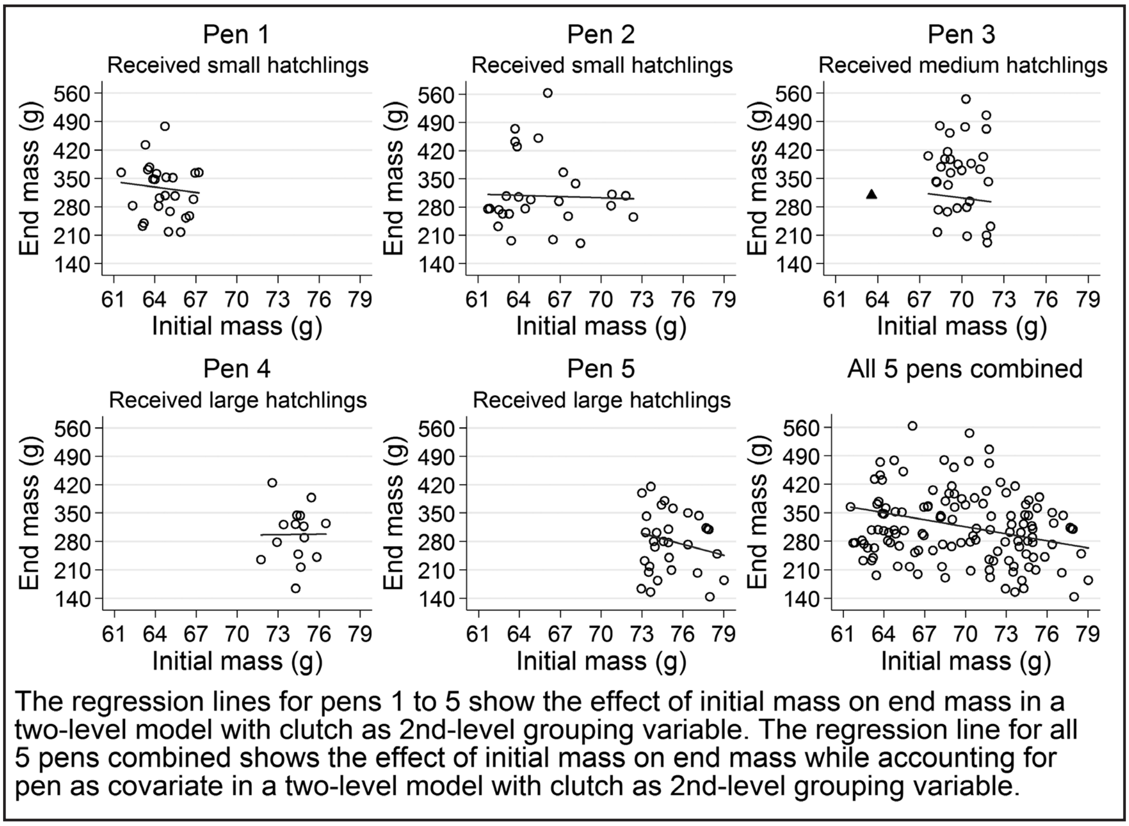
Initial hatchling mass

With all five pens included in the model, an increase by one gram in initial mass was compatible with a decline of as much as 12,2 g in end mass or a relatively minor increase of no more than 0,7 g therein, while the coefficient indicates an expected decrease by 5,75 g (Table VI). Although the actual coefficient is unknown, values closer to the expected coefficient are more compatible with the data than values further away from it. Considering pens separately, the direction of the relationship between HM$_{initial}$ and HM$_{end}$ was negative for pens 1, 3 and 5 and neutral in pens 2 and 4 (Figure 4). It is most compatible with the data that HM$_{initial}$ has a meaningful negative relationship with HM$_{end}$.



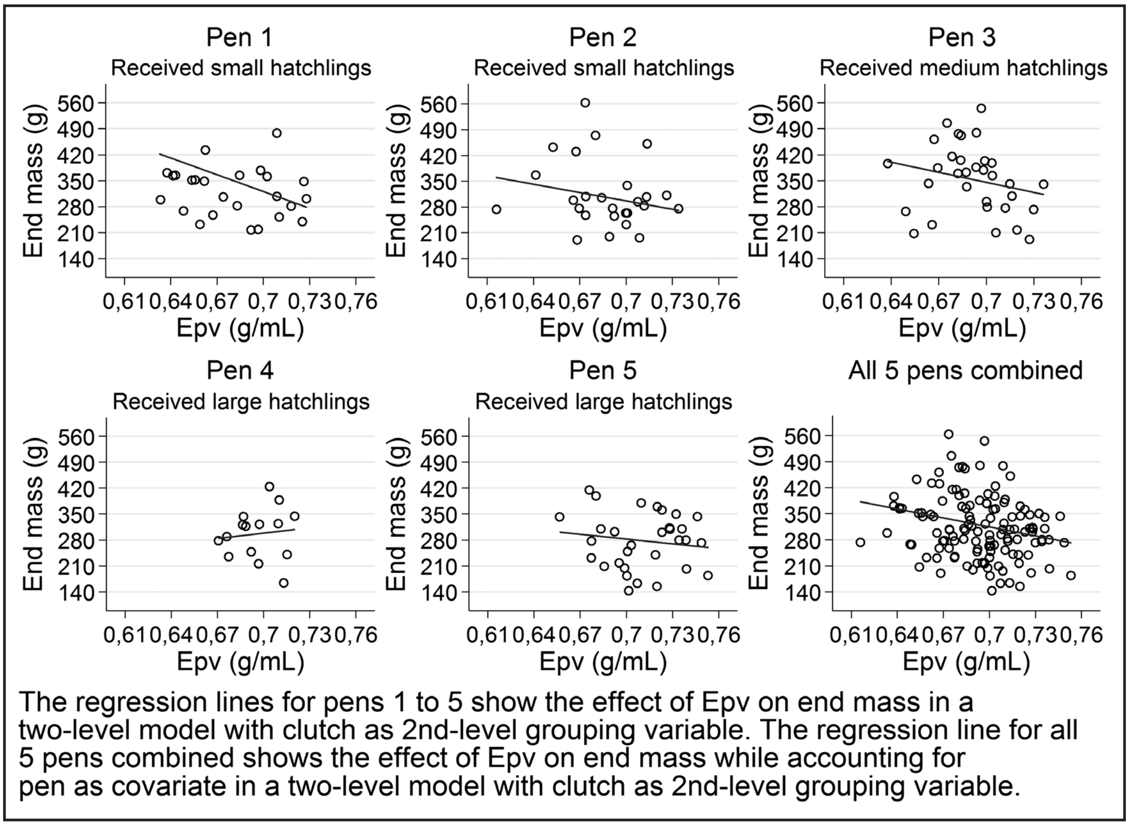
The regression lines for pens 1 to 5 show the effect of egg length on end mass in a two-level model with clutch as 2nd-level grouping variable. The regression line for all 5 pens combined shows the effect of egg length on end mass while accounting for pen as covariate in a two-level model with clutch as 2nd-level grouping variable.

FIGURE 3: Relationship between the length of 129 Nile crocodile (*Crocodylus niloticus*) eggs and the mass of the hatchlings that they yielded when they were 68–75 days old (end mass)



The regression lines for pens 1 to 5 show the effect of initial mass on end mass in a two-level model with clutch as 2nd-level grouping variable. The regression line for all 5 pens combined shows the effect of initial mass on end mass while accounting for pen as covariate in a two-level model with clutch as 2nd-level grouping variable.

FIGURE 4: Relationship between the initial mass of 129 Nile crocodile (*Crocodylus niloticus*) hatchlings and their mass when they were 68–75 days old (end mass)


Pen 1
Received small hatchlings

Pen 2
Received small hatchlings

Pen 3
Received medium hatchlings

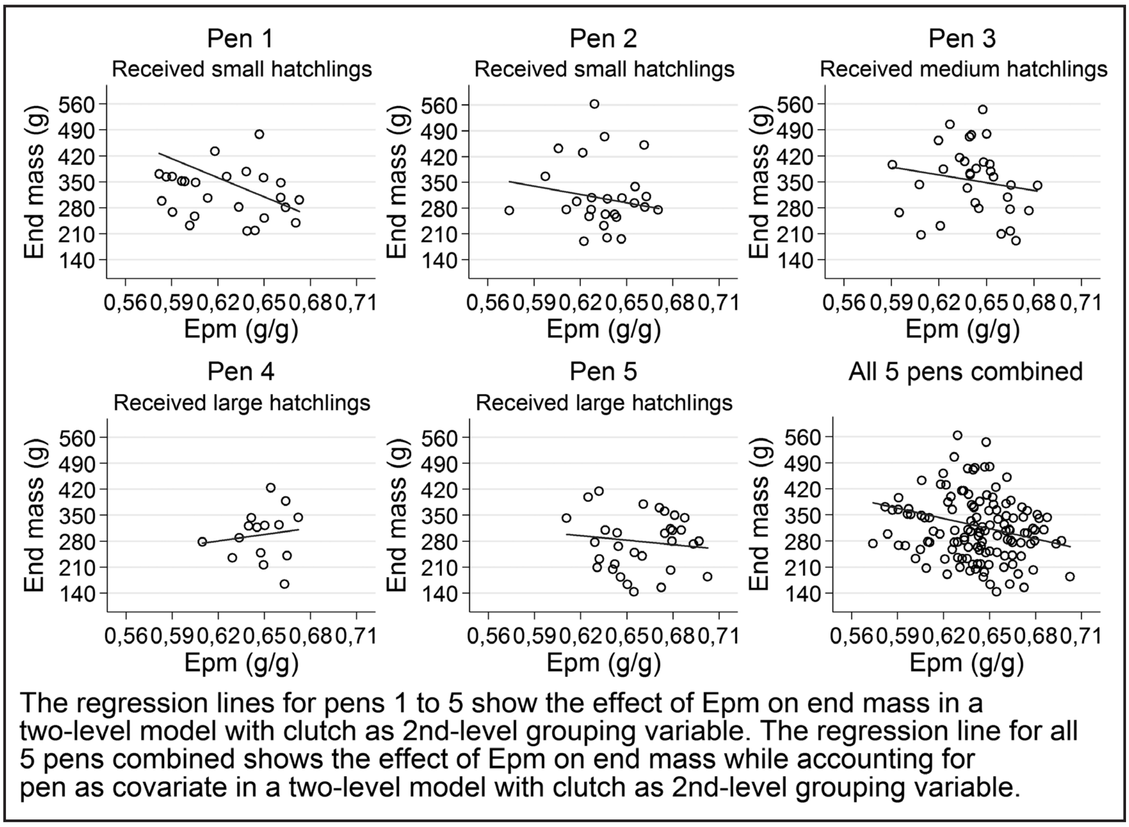
Pen 4
Received large hatchlings

Pen 5
Received large hatchlings

All 5 pens combined

The regression lines for pens 1 to 5 show the effect of Epv on end mass in a two-level model with clutch as 2nd-level grouping variable. The regression line for all 5 pens combined shows the effect of Epv on end mass while accounting for pen as covariate in a two-level model with clutch as 2nd-level grouping variable.

FIGURE 5: Relationship between the productivity of 129 Nile crocodile (*Crocodylus niloticus*) eggs based on their volume (Epv) – calculated as the initial mass of the hatchling that each egg yielded divided by the volume of the egg – and the mass of the hatchlings when they were 68–75 days old (end mass)


Pen 1
Received small hatchlings

Pen 2
Received small hatchlings

Pen 3
Received medium hatchlings

Pen 4
Received large hatchlings

Pen 5
Received large hatchlings

All 5 pens combined

The regression lines for pens 1 to 5 show the effect of Epm on end mass in a two-level model with clutch as 2nd-level grouping variable. The regression line for all 5 pens combined shows the effect of Epm on end mass while accounting for pen as covariate in a two-level model with clutch as 2nd-level grouping variable.

FIGURE 6: Relationship between the productivity of 129 Nile crocodile (*Crocodylus niloticus*) eggs based on their mass (Epm) – calculated as the initial mass of the hatchling that each egg yielded divided by the mass of the egg – and the mass of the hatchlings when they were 68–75 days old (end mass)

Egg productivity based on egg volume

With all five pens included in the model, an increase by 0,01 g/mL in Epv is compatible with a decrease by as much as 14,5 g in end mass or with a decrease as small as 1,5 g, while the expected coefficient indicates that it is expected to decrease substantially by 8,0 g (Table VI). Considering pens separately, the relationship between Epv and HM_{end} was negative for all pens, except pen 4 (Figure 5). It is most compatible with the data that a meaningful negative relationship exists between Epv and HM_{end}.

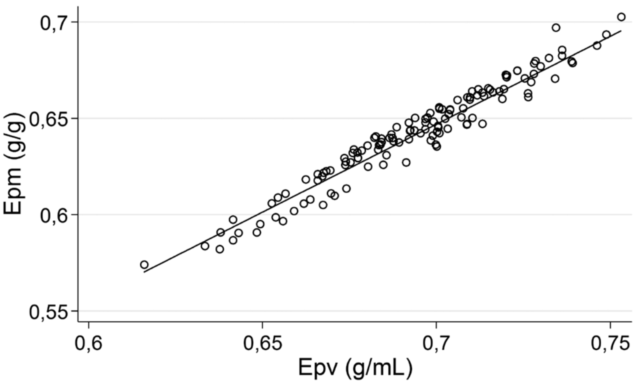
Egg productivity based on egg mass

EM and initial mass of hatchlings, which are required to calculate Epm, are easy to determine. With all five pens included in the model, an increase by 0,01 g/g in Epm is compatible with a decrease by as much as 16,3 g in HM_{end} or with a decrease as small as 1,9 g, while the coefficient indicates that it is expected to decrease substantially by 9,1 g (Table VI). Considering pens separately, the relationship between Epm and HM_{end} was negative for all pens, except pen 4 (Figure 6). It is most compatible with the data that Epm has a meaningful negative relationship with HM_{end}.

The association between Epm and HM_{end} is very similar to that between Epv and HM_{end} (Table VI, Figures 5 and 6) because there exists a strong linear relationship between Epm and Epv (Figure 7).

Discussion

This study supports the conclusion of Nöthling et al. (2019a) that a photograph of a whole egg allows estimation of its volume (EV_{photo}) to within 3,6% of its actual volume as determined by water displacement (EV_{wd}), and that EV_{wd} is usually slightly lower than EV_{photo}. Having measured EV_{wd} prior to incubation in the current study, it does not support the suspicion of Nöthling et al. (2019a) that the EV_{wd} they reported was erroneously low due to water that filled air pockets underneath the shell that had formed during incubation.



The regression line shows the effect of Epv on Epm in a two-level model with clutch as 2nd-level grouping variable. Epm = 0,00831 + 0,91235 × Epv (z = 70,6, P < 0,001, R^2 0,937, n = 129).

FIGURE 7: Strong linear relationship between the productivity of 129 Nile crocodile (*Crocodylus niloticus*) eggs based on their mass (Epm) and the productivity based on their volume (Epv). Epm = initial hatchling mass/egg mass. Epv = initial hatchling mass/egg volume.

A photograph of a hatched shell allows estimation of the egg's volume to within 4% of its actual volume. This method is worthy of investigation as a means of estimating egg size from the remains of nests in the wild.

Some crocodile farmers prefer larger hatchlings, believing that they grow better. For such farmers it would be beneficial if they could already select eggs for incubation that are likely to yield hatchlings of the desired size. The first aim of this study was to assess the utility of Nile crocodile egg attributes as predictors of $HM_{initial}$. The study shows that EW, EL, EM, EV_{photo} and EV_{shell} have strong positive relationships with $HM_{initial}$ whereas ED_{photo} has a strong negative relationship therewith. The current study shows that EV_{photo} and EM, as well as EV_{shell}, more precisely predict $HM_{initial}$ than EL and EW and much more precisely than ED_{photo}. $HM_{initial}$ increases by about 0,6 g for each one-millilitre increase in egg volume or each one-gram increase in egg mass. Although slightly less precise than EV_{photo}, EM and EV_{shell}, EW and EL are also meaningful predictors of than $HM_{initial}$.

EV_{photo}, EM, EV_{shell}, EW and EL are not only meaningful predictors of $HM_{initial}$ but they also show moderate to strong clutch effects. The indiscriminate use of these predictors as sole means of selecting eggs for incubation may therefore result in mostly selecting eggs from certain clutches while discriminating against other clutches. Such indiscriminate selection of eggs may affect other economically important characteristics such as skin quality and it may decrease genetic variation of future breeding stock selected from growers.

Our findings support others that crocodilian hatchling mass increases with egg width and length (Webb et al., 1983b), egg length (Brien et al., 2014), egg mass (Deitz and Hines, 1980; Webb et al., 1983b; Garnett and Murray, 1986; Webb and Cooper-Preston, 1989; Brien et al., 2014; Eme et al., 2019) and egg volume estimated from hatched shells (Nöthling et al., 2019b). The findings of Brien et al. (2014) that the body mass of *C. porosus* hatchlings is expected to increase by 0,54 g or 1,4 g for each one-gram increase in EM or each one-millimetre increase in EL are remarkably similar to the 0,58 g and the 1,27 g of the current study for *C. niloticus*. In accordance with the current study, Webb et al. (1983b) also showed that hatchling mass can be more precisely estimated from egg mass or egg width than from egg length.

The second aim of the study was to assess the utility of EW, EL, EV_{photo}, EV_{shell} and ED_{photo} as well as $HM_{initial}$, Epv and Epm as predictors of hatchling growth, as measured by HM_{end}. In line with the studies by Brien et al. (2014) and Riese (1991), the large variation in HM_{end} in the current study confirms that the growth period of 68–75 days was sufficient to identify the effect of predictors on early growth. The models that each included one of the predictors together with pen only explained 8–14% of the variation in HM_{end}, suggesting that growth was largely affected by factors other than those studied. About two thirds of the variation in growth occurred within clutches, suggesting that the factors affecting growth after hatching have their effects largely across clutches. Brien et al. (2014) found a large variation in growth rate among saltwater crocodile (*Crocodylus porosus*) siblings, which supports the current finding of a large variation in HM_{end} that mostly occurs within clutches. The large variation in early growth observed in the current study should be taken into account when sample sizes are derived for future studies on factors affecting early growth in Nile crocodile hatchlings.

Incubation temperature affects the growth of hatchlings of the Nile crocodile (Hutton, 1987), American alligator (Joanen and McNease, 1989) and the saltwater crocodile (Webb and Cooper-Preston, 1989). The eggs in the current study were incubated at the same temperature in an attempt to limit any effect of incubation temperature on foetal development and growth of hatchlings. Yet, we cannot rule out the possibility that the incubation temperature used in the current study had different effects on different clutches.

No prior study on the relationship between egg attributes and hatchling growth exists. The current study showed that neither EW nor EM nor EV nor ED_{photo} serve as meaningful predictors of HM_{end} because there is considerable uncertainty whether HM_{end} would increase or decrease with an increase in any of these predictors. A complex relationship existed between EL and HM_{end}. Over all five pens and hatchling sizes, there was a meaningful positive relationship between EL and HM_{end}, but this relationship appeared strongest in pens that received small hatchlings and weaker in the pen that received medium hatchlings whereas, in the pens that received large hatchlings, EL may have a negative relationship with HM_{end}. Seeing that EL is easy to measure and, therefore, attractive to consider as a criterion by which to select eggs for incubation on farms, there is a need for further research to more precisely define its relationship with the growth of hatchlings.

The direction of the association between $HM_{initial}$ and HM_{end} was negative in one pen that received small hatchlings, one that received medium hatchlings and one that received large hatchlings, whereas the direction was neutral in one pen that received small hatchings and one that received large hatchlings. Considering all five pens together while accounting for the effects of clutch and pen, it was most compatible with the data that $HM_{initial}$ had a meaningful negative association with growth. This association was evident in spite of the large variation in HM_{end} in each pen. This negative association supports those of Brien et al. (2014) and Brien et al. (2016), who also found a negative relationship between hatchling mass and growth to the age of 24 days in the saltwater crocodile, with lighter hatchlings growing faster than heavier ones. Although Riese (1991) did not weigh the hatchlings but expressed their size in terms of their SVL, he found that larger saltwater crocodile hatchlings grew faster than smaller ones, which is in contrast to our findings. Webb et al. (1983a), who also measured SVL, found no significant relationship between the size of Australian fresh water crocodile hatchlings and growth. The cause of the negative association between $HM_{initial}$ and HM_{end} is unknown. Poletta et al. (2008) and Brien et al. (2016) respectively found that stocking density has a negative effect on growth of broad-snouted caiman (*Caiman latirostris*) hatchlings and salt-water crocodile hatchlings. Best practice guidelines recommend at least 0,07 m^2 per individual for Nile crocodile hatchlings during the first few months (Manolis and Webb, 2016). It is unlikely that stocking density affected growth in the current study as the stocking densities in the five pens were far below those that Poletta et al. (2008) and Brien et al. (2016) used and those recommended by Manolis and Webb (2016).

No prior study on the effect of egg productivity on crocodilian growth exists. In spite of the variance in growth that was largely unexplained, Epm and Epv had meaningful negative associations with growth when considering all five pens together. The direction of the associations was also negative for four of the five pens. In the current study, Epv varied from 0,48 to 0,88 g/mL, with a mean of 0,69 g/mL, which seems similar to the variation from 0,57 to 0,82 g/mL around a mean of 0,70 g/mL reported by Nöthling et al. (2019b). The cause of the variation in egg productivity is unknown. A likely cause is a variation in yolk size relative to egg size (Brown et al., 2019). The cause of the negative association between egg productivity and HM_{end} is unknown. Epm is easier to determine than Epv, because the latter requires that the volume of fertile eggs be determined. There exists a very strong linear relationship between Epm and Epv, resulting in them having very similar associations with HM_{end}. Future studies may employ Epm as a proxy for Epv.

Hatchlings were assigned to pens according to $HM_{initial}$. This was done to avoid the perceived risk of larger hatchlings dominating smaller ones, reducing their growth and survival (Riese, 1991; Brien et al., 2013) which may weaken the latter and cause them to become ill and serve as sources of infection to all other hatchlings in the pen and grower house (Huchzermeyer, 2003). In the current study, pen may have confounded the effect of the predictor variables on HM_{end}. For those predictors that have a meaningful association with HM_{end}, namely EL, $HM_{initial}$, Epv and Epm, the relationship was assessed for each pen on its own. This prevented pen from confounding the association between a predictor and HM_{end} but it also restricted the numbers of hatchlings in the regression to those of a single pen, which rendered the associations statistically non-significant.

This study is the first to describe the density (ED_{photo}) of Nile crocodile eggs and shows that it varies slightly, between 1,04 and 1,13, with a mean of 1,083. Egg density is unsuitable as a predictor of $HM_{initial}$ or HM_{end}.

There is a need for further research to clarify the relationships of EL, $HM_{initial}$ and Epm on the growth of Nile crocodile hatchlings. The design of such studies should be such that it would avoid hatchlings of higher $HM_{initial}$ dominating those of lower $HM_{initial}$, as well as pen confounding the effects of predictor variables on HM_{end}. In the light of the large variation in HM_{end} that is not explained by the predictors considered in this study, further research is also needed to identify the causes of that variation and to find ways to mitigate them.

List of abbreviations

ED_{photo}: egg density in g/mL, calculated as egg mass/ EV_{photo}
ED_{wd}: egg density in g/mL, calculated as egg mass/ EV_{wd}
EL: egg length in mm
EM: egg mass in g
Epv: egg productivity based on egg volume (calculated as hatchling mass in g/egg volume in mL)
Epm: egg productivity based on egg mass (calculated as hatchling mass in g/egg mass in g)
EV_{shell}: egg volume in mL, as determined from a photograph of the hatched shell
EV_{photo}: egg volume in mL, as determined from a photograph of the egg
EV_{wd}: egg volume in mL, as determined by water displacement
EW: egg width in mm
$HM_{initial}$: mass of hatchling once it has hatched in g
HM_{end}: mass of hatchling in grams after a growth period of 68–75 days
SVL: snout to vent length

Acknowledgements

The authors acknowledge Le Croc Estate for the permission to collect data, the National Research Foundation as the incentive fund for rated researchers (grant number 119162) allocated to J. O. Nöthling partially funded the study, Martha M. Nöthling who assisted with data collection and the valuable suggestions of the reviewers.

Author contributions

JON designed the study, wrote the protocol, participated in data collection, performed the data analysis and wrote the manuscript. RC and JT assisted greatly with data collection. JAN adapted the computer program and the method of taking the photographs of eggs to streamline the determination of egg volume.

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SUPPLEMENT

Photos of selected aspects of the study reported in the article "Egg attributes and hatchling mass as predictors of hatchling growth on a Nile crocodile (*Crocodylus niloticus*) farm" by Johan O. Nöthling, Riaan Crafford, Jaco Theron, Johan A. Nöthling

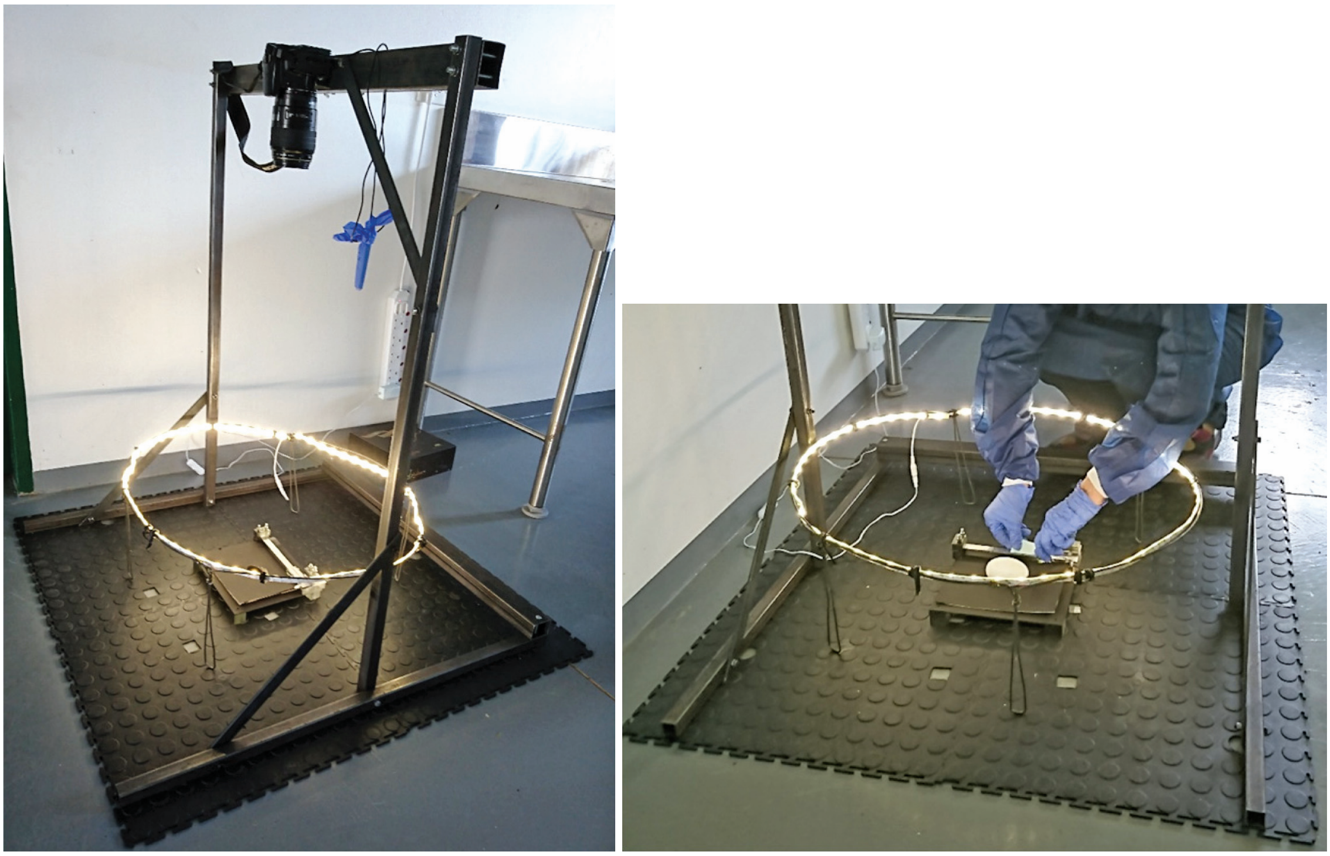

FIGURE 1: Stable steel frame supporting camera to photograph Nile crocodile eggs (left). An egg being placed on the matt black background and labelled with a sticker being stuck onto the Vernier (right). Note the homemade ring light to provide optimal illumination of the egg without shadows around it.

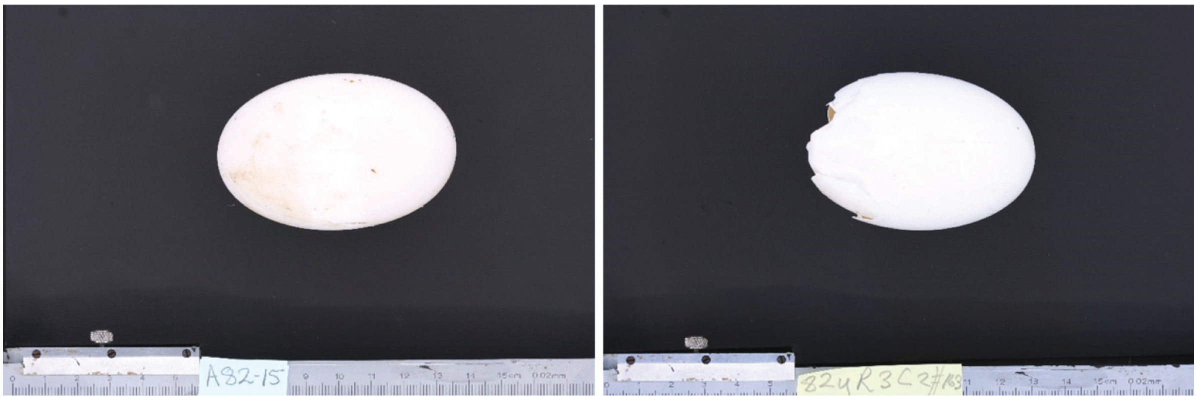

FIGURE 2: Egg 15 of Clutch 82 before it was placed into a compartmentalised polystyrene box for incubation (left). The shell of the same egg after the egg has hatched (right). Note the clutch number (82), box identifier (y), and compartment identifier (Row 3, Column 2), and the three-digit code of the hatchling (163) on the label stuck to the Vernier (right). Note the black background and that the egg and shell casted no shadows.

FIGURE 3: Oblique top view of a polystyrene box divided into nine equal-sized compartments (three rows by three columns) with interdigitating Perspex sheets, with one egg on moist vermiculite in each compartment. Note the holes through the Perspex sheets. The box is now ready to cover with a solid Perspex sheet that will serve as cover throughout the incubation period.

FIGURE 4: In order to individually remove hatchlings with their shells, the solid Perspex cover is slid off the polystyrene box after a lid with nine subdivisions that each cover a compartment has been placed on top thereof (left). The middle photograph shows one of the nine lids turned side-ways for demonstration. One compartment has been opened to allow the hatchling and shell therein to be removed without risk of hatchlings from different eggs commingling (right).