

A model by which to estimate the volume of Nile crocodile eggs after they have hatched

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The value of their leather stimulates commercial farming with several crocodilian species. The survival and growth of crocodile hatchlings depends on their birth mass. There exists a positive relationship between the hatchling mass and egg size of several crocodilian species. The size of crocodilian eggs is often not measured before hatching. The aim of this study was to create a model whereby the volume of individual Nile crocodile eggs can be estimated after they have hatched and the shell been broken, so that the relationship between egg volume and hatchling mass can be studied on an individual basis without the size of the eggs having been measured prior to hatching.

Infertile eggs were photographed in side view, with one pole towards the bottom and the other towards the top and a scale in the focal plane. Their volumes were measured by water displacement (displacement volume). A custom-written computer program was used to measure the photo images. The program turned the image of the egg upright and the position of the polar axis was determined without considering the position of the upper pole. Various transverse diameters (perpendicular to the polar axis) were measured, and the volume of the egg calculated by integrating the function describing the horizon of the egg on the polar axis (calculated volume). The best models for estimating the calculated volume were determined by using information limited to various distances from the bottom pole of the egg's image towards its upper pole, and for eggs of different polarity. The models were then used to estimate the volumes of 138 eggs of which 15%, 20%, 25% and 30% of the image of each egg were masked from the upper pole towards the bottom pole.

Volumes of 60–135 ml derived from analysis of the masked photographic images of eggs permitted estimation of the eggs' volumes to within 4.57 ml of their actual volumes with 95% confidence. This study makes it possible to estimate the volume of eggs from the shells of hatched eggs and identify a variation larger than 7.72% in the birth mass of Nile crocodile hatchlings from eggs with an estimated volume of 60 ml, with the precision increasing to identify a variation larger than 3.40% for hatchlings from eggs with an estimated volume of 135 ml with 95% confidence.

Keywords: Nile crocodile, crocodile, egg, volume

'n Model om Nylkrokodileiers se volumes mee te skat nadat hulle uitbroei het: Die waarde van hul leer stimuleer kommersiële boerdery met verskeie spesies van die Crocodilia. Oorlewings- en groeivermoë van krokodilbroeilinge hou verband met hul geboortemassa. Daar bestaan 'n positiewe verband tussen broeilinggrootte en eiergrootte van verskeie spesies van die Crocodilia. Krokodileiers se grootte word dikwels nie gemeet voordat hulle uitbroei nie. Die doel van hierdie studie was om 'n model te skep waarmee die volume van individuele Nylkrokodileiers geskat kan word nadat hulle uitbroei het en die dop gebreek is, sodat die verband tussen eiervolume en broeilingemassa op 'n individuele basis bestudeer kan word sonder dat die grootte van eiers gemeet is voordat hulle uitbroei.

Onvrugbare eiers is in sy-aansig gefotografeer, met een pool na onder en die ander na bo en 'n skaal op die vlak van fokus. Hul volume is met waterverplasing gemeet (verplasingvolume). 'n Rekenaarprogram wat vir die doel geskryf is, is gebruik om die fotobeelde te meet. Die program het die eierbeeld regop gedraai en die posisie van die poolas bepaal sonder om die boonste pool se posisie in ag te neem. Die lengte van verskeie dwarsdeursnitte (loodreg op die poolas) is gemeet, en die volume van die eier bereken deur die funksie wat die horison van die eier op die poolas beskryf te integreer (berekende volume). Die beste modelle is bepaal om die berekende volume te skat deur gebruik te maak van inligting beperk tot verskillende afstande van die onderste pool in die rigting van die boonste te gebruik, en vir eiers met verskillende polariteit. Die modelle is daarna gebruik om die volumes van 138 eiers waarvan 15%, 20%, 25% en 30% van elke eierbeeld, van die boonste pool in die rigting van die onderste, gemaskeer is.

Deur analise van die gemaskeerde fotobeelde kan die volume van eiers met volumes van 60 ml tot 135 ml met 95% vertroue binne 4.57 ml van die werklike voorspel word. Hierdie studie skep die moontlikheid om die volume van uitgebroeide Nylkrokodileiers te skat. Variasie in Nylkrokodille se geboortemassa van groter as 7.72% kan met 95% vertroue geïdentifiseer word vir broeilinge uit eiers met 'n geskatte volume van 60 ml, terwyl die presisie verbeter tot 'n variasie groter as 3.40% vir broeilinge uit eiers met 'n geskatte volume van 135 ml.

Sleutelwoorde: Nylkrokodil, krokodil, eier, volume

Introduction

The leather of several crocodylian species endemic to various parts of the globe is highly sought after, which stimulates commercial farming with such species. The Nile crocodile (*Crocodylus niloticus*), which has a wide distribution in Africa, is the only species used for farming in South Africa. The survival and growth of hatchlings is important for the success of crocodile farming. At birth a hatchling consists of its body tissues, which determines its body size, and the yolk that remains after prenatal development, which the hatchling would have drawn into its abdomen shortly prior to birth, and which serves as a post-natal source of nutrients for the hatchling (Huchzermeyer 2003). Both its body size and the mass of the yolk determine the survival and growth of the hatchling (Hutton 1987). Birth mass is therefore a meaningful indicator of the ability of crocodile hatchlings to survive and grow.

Various studies have shown a positive relationship between hatchling size and egg size in various crocodylian species. Total hatchling mass increases with egg mass (Brien et al. 2014; Deitz and Hines 1980; Garnett and Murray 1986; Webb and Cooper-Preston 1989), egg length (Brien et al. 2014; Garnett and Murray 1986; Gómez-González et al. 2017) and egg width (Garnett and Murray 1986). The yolk-free body mass of hatchlings is also positively related to egg size (Whitehead 1987). Hatchling length depends on egg length (Isberg et al. 2005). Xia et al. (2008) states that “larger eggs seem to be more likely to produce successful hatchlings” in the Chinese alligator (*Alligator chinensis*).

Large variation in egg size occurs among clutches of several crocodylian species, although substantial variation may also occur within clutches (Deitz and Hines 1980; Garnett and Murray 1986; Stoker et al. 2013). Crocodylian eggs are considered ellipsoid in shape (Marzola et al. 2015; Stoker et al. 2013).

When hatching, the hatchling breaks and opens the egg shell at one of its two poles. Egg mass and egg length can only be measured on whole eggs. In practice, the size of eggs is often not measured before they hatch in order to minimise the handling of fertile eggs. The aim of this study is to create a model whereby the volume of individual Nile crocodile eggs can be estimated after they have hatched, so that the relationship between egg volume and hatchling mass can be studied on an individual basis without having to measure the eggs prior to hatching.

Materials and methods

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

Eggs

Nile crocodile eggs were collected on a single commercial farm in the North West Province in South Africa during

two breeding seasons. All hatchlings on the farm were from artificially-incubated eggs produced from breeding animals in captivity.

Once the last hatchling of each clutch had hatched, 497 whole, infertile eggs from 62 clutches were collected (1–27 per clutch, mean 7.8). The number of the clutch and egg was written in black on the egg. Figure 1 demonstrates the variation in shape and size of hatched Nile crocodile eggs (eggs of proven fertility) that were collected at the same time and on the same farm as the infertile eggs used in this study.

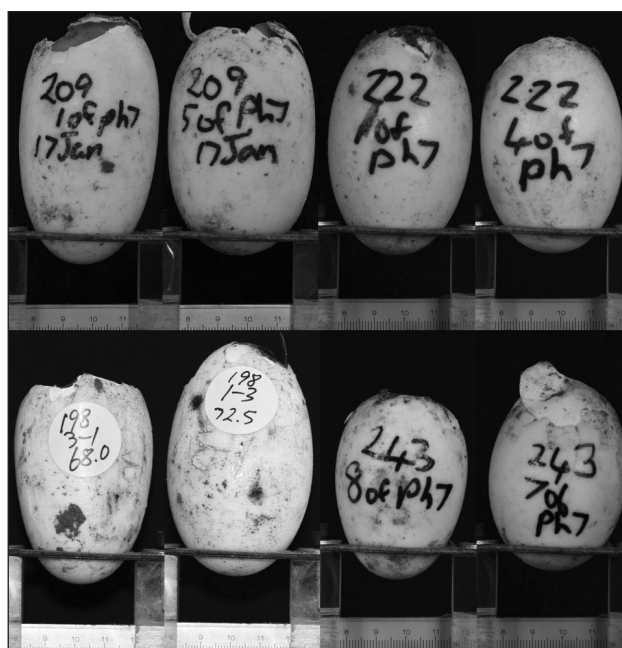


FIGURE 1: Two hatched eggs from each of four clutches (209, 222, 198 and 243) demonstrate the variation in size and shape of eggs within and among clutches.

Photography

Each egg was photographed at a distance of exactly 1 m together with the measurement arm (marked with 1 mm increments) of a vernier caliper, using a single reflex camera with a 100 mm fixed focal distance lens. The photographs were saved in jpg format, using the photo number that the camera assigned as filename. Each photo showed the perimeter of the egg's image and the marks on the measurement arm sharply in focus, as well as the numbers of the clutch and egg. These are the unprepared photos. The clutch and egg number of each egg was stored against the file name of the photo in an Excel spreadsheet.

Naming egg measurements

Figure 2 explains the measurements on the photographs that were recorded. The length of the polar axis, equator and the equatorial quarter lines could be measured on paper prints of the photographs (paper photos), whereas all the measurements could be made on the photograph images in digital format.

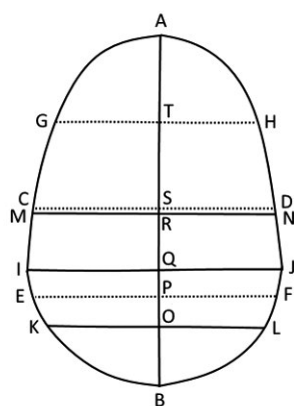


FIGURE 2: Schematic representation of a Nile crocodile egg explaining the various diameters. AB = Polar axis, CD = Equator diameter [AS = BS], EF = Lower equatorial quarter line [BP = PS], GH = Upper equatorial quarter line [AT = TS], IJ = Girth diameter (Diameter at height 1.0, with the “1.0” indicating that diameter IJ lies one index unit above the lower pole), BQ = Height 1.0 (lower pole to girth; with the “1.0” indicating that height BQ has a length of one index unit, measured from the lower pole), KL = Diameter at height 0.5 [BO = OQ], BO = Height 0.5 (0.5 × lower pole to girth), MN = Diameter at height 1.5 [QR = QO], BR = Height 1.5 (1.5 × lower pole to girth).

Measuring egg volume by water displacement

Two wide-mouthed glass bottles (Consol Glass, Johannesburg, South-Africa), each with a volume of about 500 ml and a glass lid with two holes of 5 mm in diameter at its highest point, were used. Having secured the lid on a bottle, a beaker filled with water and standing on a weight scale was connected to one of the holes in the lid via a thin plastic tube, which was sealed with a stop cock once the tube was completely filled with water from the beaker. The mass of the beaker with water was recorded, following which the bottle was filled until all air in the bottle had escaped and the meniscus (5 mm in diameter) was in the second hole in the lid. The mass of the beaker with the water remaining in it was measured again and the mass subtracted from the initial mass to yield the volume of the bottle, assuming that the volume of the water required to fill the bottle was exactly one millilitre per gram. The bottle was then emptied and dried. The process was repeated 10 times for each bottle and its mean volume calculated.

Each egg was placed in one of the dry bottles and the bottle filled with water as described above. The volume of the egg was calculated by subtracting the volume of water required to fill the bottle with the egg inside it from the average volume of the bottle. This egg volume is henceforth referred to as the displacement volume.

Measurements on paper photos

A full-page print of each unprepared photo image was made on A4 paper. The polar axis was drawn onto the image after its position was estimated with the eye. The bisecting perpendicular to the polar axis was drawn from one edge of the egg’s image to the opposite and considered the equator. The bisecting perpendiculars to the top and bottom halves of the polar axis were considered the respective equatorial quarter lines. The length of each line,

as well as the length of 140 mm indicated by the marks on the image of the measurement arm of the vernier caliper were measured to two decimals of a millimetre, using a vernier caliper held by hand. The actual lengths of the polar axis, equator and equatorial quarter lines of the egg were then calculated.

Selection of eggs for photo measurement (extended set)

As many as possible, to a maximum of five eggs, were selected from each of 62 clutches for measurement and calculation of their volume on the computer (this yielded photographic images of 224 eggs, with five from each of 26 clutches, four from each of nine, three from each of nine, two from each of 13 and one from each of five).

Preparation of photographs

The program Paint.net version 4.0.21 (www.getpaint.net) was used on each unprepared digital photo to cover the background to near the edge of the egg’s image with black and all dark markings, as well as the numbers of the clutch and egg on the egg’s image, with white. Each prepared photo was saved with the same name as its unprepared counterpart, but as a .png file instead of a .jpg file.

Program

A program (henceforth “the program”) was written in Python (www.python.org) with which measurements and volume calculations could be done on the prepared photographs.

The program was first used to select a threshold value for each prepared photographic image so that as near as possible every background pixel was identified as black and as near as possible every pixel forming part of the egg image as white.

Parametric linear interpolation between neighbouring egg pixels on the edge of the egg’s image was used to reduce the faulty identification of the edge of the egg’s image resulting from pixels actually belonging to the egg’s image, but which were classified as black. This interpolation also yielded a higher density and more uniform spacing of points on the edge of the egg’s image. The interpolation was done by plotting along the edge of the egg’s image a number of equally spaced interpolation points equal to five times the number of photo pixels on the edge of the egg’s image. Henceforth, the edge of the egg’s image defined by these plotted points is referred to as the plotted edge of the egg’s image, and the surface it encloses as the enclosed egg’s image.

The program then generated a virtual frame with edges parallel to each other and to the vertical axis of the cartesian coordinate system of the photo, and only touching those points that are furthest to the left and furthest to the right on the plotted edge of the egg’s image. The heights of these left and right points of contact were compared and the egg’s

image rotated until the points of contact were at the same height. (While rotating the egg's image, the width of the virtual frame was continuously decreased, so that the left and right edges of the frame remained in contact with the plotted edge of the egg's image). At this stage of rotation it was assumed that the egg's image was upright with respect to the frame and the cartesian coordinate system (henceforth the upright egg's image).

An egg was not necessarily a perfect ellipsoid, and the plotted edge of its image not a perfect ellipse. Therefore, the line between the left and right points of contact between the virtual frame and the edge of the upright egg's image did not necessarily identify the girth diameter of the egg's image and the bisecting perpendicular thereto not necessarily its perfect polar axis. In the same way the lowermost and uppermost points on the plotted edge of the upright egg's image did not necessarily exactly identify the poles. The polar axis and the girth diameter were determined by the lowermost and uppermost points of the plotted edge of the upright egg's image provisionally dividing it into left and right plotted edges of the egg's image. Approximately 10 pixels away from the lowest point on the left plotted edge of the egg's image an interpolation point was then identified, followed by an interpolation point on the right plotted edge of the egg's image as near as possible on the same height as the first. The midpoint of the line joining these points was identified. In the same way the midpoint of the line joining each pair of interpolation points on the left and right plotted edges above the first pair was identified, until about 10 pixels away from the uppermost point of the plotted edge of the egg's image. The best fit line over these midpoints identified the polar axis and its inclination, while its lower and higher points of contact with the plotted edge of the upright egg's image identified the respective poles. The largest diameter of the enclosed egg's image perpendicular to the polar axis identified its girth diameter.

Measurements and volume calculations of complete eggs with the computer program

First, the number of photo pixels representing one millimetre real distance on an egg (not the egg's image) was determined. To do this, the width of the photo image on the computer screen was first calculated. Using the program "Measure" (Baron Roberts, <http://www.thing.com/Meazure.asp>), the number of screen pixels respectively underlying the width of the unprepared photo and 140 mm on the image of the measurement arm of the vernier caliper were determined. The width of the photo image in millimetre was calculated as 140 mm multiplied by the number of screen pixels underlying the width of the photo divided by the number of screen pixels underlying 140 mm on the scale. The number of photo pixels over the width of the corresponding prepared photograph was determined with the program written in Python. Knowing the width of the photo image in millimetres, the number of photo pixels per millimetre on the egg image was calculated and used in

the program to convert distances in photo pixels on the egg image to distances in millimetres on the real egg.

With the polar axis known, the lengths of the diameters perpendicular thereto and 0.25, 0.5 and 0.75 times the distance between the poles, from the lower pole towards the upper pole, were calculated as the lower equatorial quarter line, equator and upper equatorial quarter line, respectively, and recorded.

That part of the plotted egg's edge to the left of the poles was considered the final left plotted edge of the egg's image. Integration was used to calculate the volume of the egg as

$$V = \pi \int_{\mathcal{D}}^{\mathcal{B}} [f(x)]^2 dx, \quad (1)$$

Where $f(x)$ describes the egg's edge as a function of perpendicular intercept on the polar axis, x , and the coordinates of the lower and upper poles measured on the polar axis are given by \mathcal{D} and \mathcal{B} . The final volume was calculated as the average of the calculation by integration for the left and right plotted edges of the egg's image as $f(x)$. This volume is henceforth known as the calculated egg volume.

The repeatability of determining the calculated egg volume was assessed by determining it twice by independent measurement of the same prepared photograph of each of 55 randomly chosen eggs from the group of 224. The second determination of the calculated volume of each egg was done using a new threshold, which was determined without any knowledge about the threshold used to determine the calculated volume the first time.

It is impossible to identify the equator on egg images of which the upper part is masked, or eventually on those of hatched eggs. The lengths of transverse diameters (perpendicular to the polar axis) were therefore measured at various heights from the lower pole relative to the girth and recorded. The height of a measurement is the distance from the bottom pole in the direction of the upper pole, measured on the polar axis and expressed as a fraction of the distance between the bottom pole and the girth diameter. Transverse diameters were measured at heights 0.2 and 0.3, and then at height increments of 0.05 from height 0.35 to height 1.7 and finally at height 1.8. For brevity, these transverse diameters are referred to as diameter at height 0.2, diameter at height 0.3, etc. to diameter at height 1.8, with the girth diameter as diameter at height 1.0. The volume of the egg below each of these diameters from the girth diameter and those above were determined using equation 1 (with \mathcal{B} now being the polar axis coordinate of the chosen cut-off diameter).

Generating the model

The final set of eggs for generating the model

The final set of eggs for generating the model were those 167 (from 58 clutches; four with five eggs, 18 with four, 11

with three, 17 with two and eight with one) from the 224 of which the displacement volume and the lengths of the polar axis, equator and equatorial quarter lines on paper photos differed by two percent or less from the corresponding lengths measured with the program.

Calculating the polarity

Some eggs were distinctly asymmetrical, with the girth closer to one pole than the other. For each egg in the final set the sum of the lengths of the diameters at heights 0.7, 0.75, 0.8, 0.85, 0.9 and 0.95 was subtracted from the sum of the length of the diameters at heights 1.05, 1.1, 1.15, 1.2, 1.25 and 1.3. The polarity of an egg was 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. This classification yielded three similar sized groups of eggs. Eggs with a polarity of minus one mostly had their girth closer to the upper pole than the bottom pole, those with a polarity of one had theirs closer to the bottom pole than the upper pole, while the girths of eggs with a polarity of zero were generally spread more closely around the midpoint of the polar axis.

Determining models with a restricted initial set of predictor variables, reduced to the set yielding the lowest Akaike information criterion (shortened to "Restricted Akaike")

Each egg in the final set with a polarity of minus one was included in a multiple linear regression of the volume of the whole egg (calculated by integration) as the outcome variable, and the distance from the bottom pole to the girth as well as each measured diameter from that at height 0.2 to that at height 1.3 and the calculated volume below the diameter at height 1.3 were included in the initial set as predictor variables. The predictor variables were then reduced to the final set yielding the lowest Akaike information criterion. The intercept and coefficients of the predictor variables of this final set were stored, so that it could be used to estimate the volume of eggs during the phase of model assessment, when information above the diameter at height 1.3 would not be available. This process was repeated for eggs with a polarity of zero and then for those with a polarity of one.

The above processes were repeated for the diameter at height 1.35, with the addition of the diameter at height 1.35 to the initial set and the replacement of the calculated volume below the diameter at height 1.3 with the calculated volume below the diameter at height 1.35. In the same way the procedures were repeated for each diameter above the diameter at height 1.35 and up to the diameter at height 1.8. As only the calculated volume below the highest diameter of each height by polarity combination was included, before the predictors were reduced to minimise the Akaike information criterion, these models are referred to as the models with restricted initial predictors, reduced to the minimum Akaike information criterion, shortened to "Restricted Akaike".

Determining models with full initial predictors, reduced to minimise Akaike's information criterion (shortened to "Full Akaike")

The above processes were repeated for each height by polarity combination, with the only difference being that not only the calculated volume below the highest diameter was included in the initial set of predictors but the calculated volume below each diameter, from the diameter at height 1.0 to the highest for each height by polarity combination. As for the Restricted Akaike models, the distance from the bottom pole to the diameter at height 1.0 as well as each diameter from the diameter at height 0.2 to the highest diameter of each particular height by polarity combination were also included in the initial set as predictor variables, following which the predictors were reduced until Akaike's information criterion reached a minimum. These models are known as those with full initial predictors, reduced to minimum Akaike's information criterion, shortened to "Full Akaike".

Determining models containing the full set of predictor variables (shortened to "Full")

Apart from the Restricted Akaike and Full Akaike models, a full model was determined for each height by polarity combination. The process was the same as described for Full Akaike models, except that the full set of predictors was retained in the model. These models are referred to as the full models, shortened to "Full".

Regression of estimated egg volume on calculated egg volume

In order to demonstrate the linear nature of the relationship between the estimated egg volume and the calculated egg volume, the predictor variables with their coefficients and the intercepts as determined above for models based on information restricted to each height from the diameter at height 1.3 and those at heights above, were used to estimate the volume of each egg in the final set of 167 based on information of eggs at each height. Thereafter these volumes, estimated with the models based on information restricted to the respective heights, were regressed on the calculated volumes of the same eggs to determine the intercept, coefficient of the calculated volume with its 95% confidence interval and the coefficient of determination.

Testing the models

Eggs in the test set

All the eggs not among the 224 initially considered for model generation, to a maximum of five per clutch, were used for model testing. So, 138 eggs from 36 clutches (five from each of 18 clutches, four from each of seven and one to three from the remaining 11 clutches) were selected to test the models.

Assessment of the precision of measurement on the eggs of the test set and their unmasked images

As for model generation the lengths of the polar axis, equator and equatorial quarter lines of the eggs of the

test set were measured on photographs on paper and on digital photographs. Their volumes were measured by water displacement and calculated by integration using the program. The lengths measured and the volumes calculated with the program were expressed as percentages of the corresponding measurements done by hand and compared to the percentages for the 224 eggs from which the final set for model generation was selected.

Masking of egg images and measuring the masked images

Fifteen percent of the Cartesian height of each of the 138 egg images was masked on the prepared photograph, before it was rotated in the virtual frame. The polar axis was then determined as described for model generation, but only using measurements below the line of masking, without using any information above the line of masking. With the position of the polar axis known, the girth line was identified (provided that it was not above the line of masking). Following that, the distance from the bottom pole to the girth line and each transverse diameter below the line of masking were measured as described under model generation. The volume of the egg below the girth diameter and each other transverse diameter above the girth up to the highest transverse diameter below the line of masking were determined using equation 1 (with B being the polar axis coordinate of the particular diameter).

The above process was repeated for each of the 138 eggs, but with 20%, 25% and 30% of the upper end of each egg masked. Together, the 138 eggs thus yielded 552 masked egg images. The same threshold was used for the measurements on the unmasked egg image and the image after each of the four levels of masking.

Determining the scope and polarity of masked egg images

The scope of a masked egg image was the height along the polar axis, measured from the bottom pole in the direction of the upper pole, to the highest measured transverse diameter below the line of masking, expressed as a fraction of the distance from the bottom pole to the girth line. Each of the four masked images of each egg (except for six eggs; see below) had a different scope, with different amounts of information on which the position of the polar axis had to be determined, as well as different numbers of measured transverse diameters and different highest transverse diameters below which the volume had to be calculated. Two of the four masked images of each of the six eggs had the same scope. The image with the lowest degree of masking from each of these six pairs was excluded from further analysis. Only those masked egg images with a scope of at least 1.3 were used for model testing.

The polarity of each masked egg image with a scope of 1.3 or higher was calculated as described for the images of whole eggs used for model generation.

Estimating egg volumes by applying the regression formulas of the created models on masked egg images

Knowing the scope and polarity of an egg in the test set, the intercept and coefficients of the predictor variables in the Restricted Akaike, Full Akaike and Full models as identified during model generation for the height equal to the scope of the test set egg image and for eggs with a polarity equal to that of the test set egg were used to estimate the volume of the whole test set egg (estimated volume).

Comparing the precision of volume estimation with masked egg images differing in polarity and scope

The concordance correlation coefficient (rc) with its z-transformed 95% confidence interval (Lin 1989) was used to describe the agreement between the estimated and calculated egg volumes for each polarity and scope. According to Lin rc will be equal to one only when the precision and accuracy of the estimation is perfect; that is when Pearson's correlation coefficient is one and the means of the estimated and calculated volumes as well as their standard deviations are equal. With $rc = 1$ the points of a scatterplot of the estimated volume against the calculated volume will lie on a straight line with a slope of 45° through the origin if the axes have the same scale.

The precision of estimation was expressed as the limits of agreement according to Bland and Altman (1986) and the relative limit of agreement, which was calculated as the estimated volume expressed as a percentage of the calculated volume of each test set egg.

Only the model (Restricted Akaike or Full Akaike or Full) that yielded the highest rc and the smallest interval between the upper and lower limits of agreement are reported under Results.

The width of the 95% confidence interval with which the calculated volume of an egg in the test set can be forecast from its masked egg image was determined (Steyn et al. 1998). In the same way we determined the 95% confidence interval with which the calculated volume of an egg from the test set, as well as an egg from either the test set or the model generation set can be forecast from the volume derived by applying the formula for an ellipsoid. Wilcoxon's rank sum test was used to compare the precision with which the estimated volumes produced by the models from measurements on masked egg images predicted the calculated egg volumes in the test set to the precision attained by using the ellipsoid volumes derived from measurements on unmasked eggs instead.

Analyses were done using STATA version 14 (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA) and Excel.

Results

The precision of measurement

Table 1 shows the high degree of agreement among 10 measurements of the volume of each of two bottles, with the maximum exceeding the minimum by 0.4% and 0.24% percent.

The displacement volumes of four eggs used for model generation and one for model testing were obviously wrong. Their displacement volumes were each compared to those of two other eggs with similar dimensions (10 control eggs) and they differed by 5.4% to 13.4% (mean 9.4%, SD 3.1%). These five wrong displacement volumes also differed by 6.6% to 13.2% (mean 9.4%, SD 2.7%) from their respective calculated volumes, whereas the latter agreed closely (0.0% to 2.9%, mean 1.6%, SD 0.9%) with the displacement volumes of the 10 control eggs. These five wrong displacement volumes were excluded from the comparison to calculated egg volume. The precision of diameters measured with the computer program relative to those measured by hand on paper photographs, and the calculated egg volumes relative to the displacement volumes did not differ between eggs used for model generation and those used for model testing ($p > 0.2$). Table 2 shows that, on average, measurements done with the computer program differed by no more than 0.41% from measurements done by hand on photographic images on paper and that any measurement by the program did, with a 95% confidence, not differ by more than 1.27% from the corresponding measurement by hand.

Table 2 shows that, on average, the calculated volume of eggs was 1.49% higher than the displacement volume and the calculated volume of any egg was, with a 95% confidence, between 0.49% below and 3.48% above its displacement volume.

TABLE 1: Summary of 10 measurements of the volume of each of the two bottles that were used to measure the volumes of Nile crocodile eggs

	Bottle 1	Bottle 2
Minimum (ml)	535.20	540.80
Maximum (ml)	537.36	542.08
Mean (ml)	535.96	541.22
Standard deviation (ml)	0.645	0.399
Coefficient of variation (%)	0.120	0.074
Maximum as percentage of the minimum	100.40	100.24

TABLE 2: Calculated volume and measurements done with the computer program, expressed as percentages of the displacement volume and measurement done by hand on photographic images on paper for eggs considered for model generation as well as those used for model testing

	Mean	Lower boundary ^a	Upper boundary ^b	Number of eggs
Egg volume	101.49	99.51	103.48	357
Polar diameter	99.97	99.36	100.59	362
Equatorial diameter	100.34	99.52	101.16	362
Lower equatorial quarter line	100.26	99.36	101.15	362
Upper equatorial quarter line	100.41	99.54	101.27	362

^a 1.96 standard deviations below the mean; ^b 1.96 standard deviations above the mean

On average the calculated volume of eggs was 1.46 ml higher than their displacement volume (95% limits of agreement -0.394 ml to 3.32 ml). The calculated volume was lower than the displacement volume in only 15 (4%) of the 357 eggs.

The determination of the calculated egg volume was highly repeatable: Figure 3 shows that a second, independent analysis on each of the 55 prepared photographs — but using a new threshold level that was determined independently of the first — yielded a calculated volume that agreed closely with the first. The concordance correlation coefficient was 1.000 and the mean difference between the first and second calculated volume of each egg -0.142 ml (95% limits of agreement -0.548 ml and 0.263 ml). On average, the second calculated volume was 0.15% higher than the first, with a 95% probability of falling between 0.27% below and 0.57% above the first.

The regression equation for the model (Restricted Akaike, Full Akaike or Full) that estimated the calculated egg volumes of eggs in the test set for each level of scope from 1.3 to 1.8 combined with each of the three levels of polarity with the greatest precision appears in the supplementary file named “Regression equations”. Both, the predictor

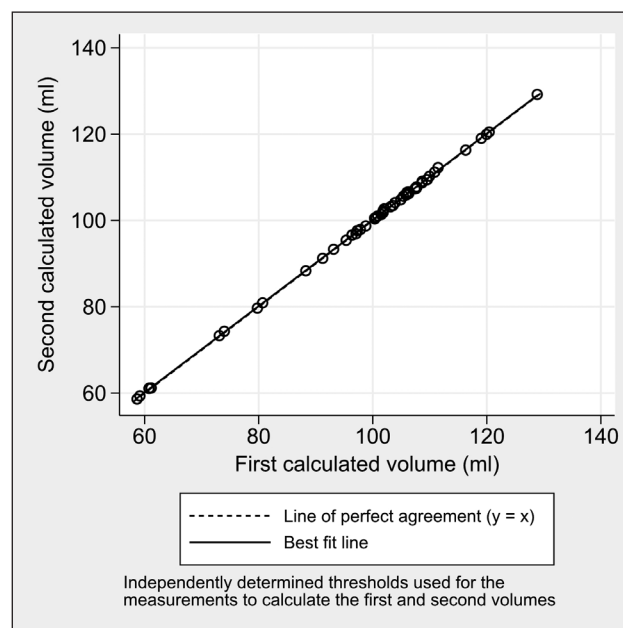


FIGURE 3: Close agreement between two independent calculations of the volume of each of 55 Nile crocodile eggs by integration of measurements done by a computer program using an independently determined threshold for each set of measurements on each egg image

variables and their coefficients, differ for each of the three polarity levels at a particular level of scope, as well as at different levels of scope.

The intercepts near zero and the slopes (coefficients) near one and the smallness of the 95% confidence intervals for the slopes in Table 3 show that the egg volumes, as estimated with the regression equations in the supplementary file, closely agree with the calculated egg volumes. Although the 95% confidence intervals were quite small relative to the slopes, they were somewhat wider at lower heights than at higher heights, presumably because the volumes at lower heights were estimated on less information than those at higher heights. Those regression equations that are, for the sake of brevity, not shown in Table 3 were similar to those that are shown.

Model testing

The effect of masking on the amount of information retained on an egg

The scope of 22 of the 552 masked egg images was below 1.3 (two after 25% masking and 20 after 30%), the scope of 12 only was 1.8 (nine after 15% masking and two after 20%) and those of 48 were 1.7 (40 were 15% masked, six 20% and two 25%). Each of the 138 eggs in the test set yielded one or more masked image that was suitable for model testing.

The precision and accuracy of estimating egg volume

Table 4 shows high rc values (≥ 0.95) with narrow 95% confidence intervals, confirming high accuracy and precision of estimating egg volume. The bottom row of Table 4 shows that, with 95% confidence, the estimated egg volumes were no more than 2.26 ml higher and no more

than 2.29 ml lower than the calculated volume. Figure 4 shows that the estimated volumes of eggs were on average equal to 100% of their respective calculated volumes, with 95% of the estimated volumes falling within 2.3% of their respective calculated volumes.

Table 4 shows that the limits of agreement and relative agreement, as well as the widths of the intervals between them varied among the scope-by-polarity combinations. The widths of the intervals between the limits of relative agreement of the various scope-by-polarity combinations decreased with an increase in scope (Spearman's rank correlation coefficient 0.66, $p < 0.001$, $n = 28$). Figure 5 shows the number of scope-by-polarity combinations for which the egg volume could be estimated within the respective limits of relative agreement. Figure 5 shows that

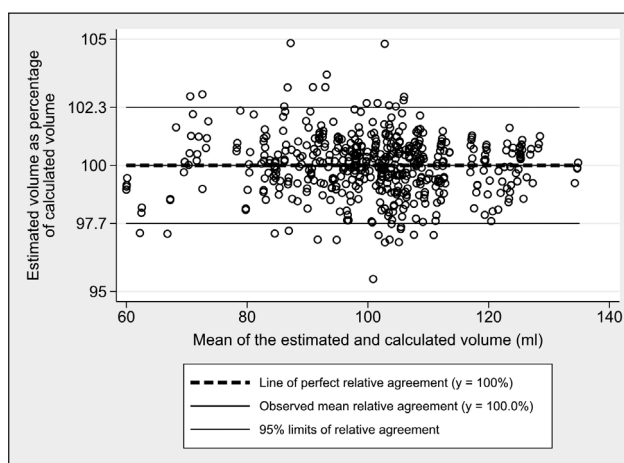


FIGURE 4: The limits of relative agreement between the egg volumes estimated from 524 masked egg images and the egg volumes calculated by integration of measurements on the same images before they were masked

TABLE 3. Regression equations of estimated egg volume (e.e.v.) on the calculated egg volume (c.e.v.) at selected heights above the lower pole of the Nile crocodile eggs in the final model generation set

Height ^a	Polarity ^b	Regression of estimated egg volume (e.e.v.) on calculated egg volume (c.e.v.)	95% CI. ^c	R ²	n
1.3	-1	e.e.v. = 0.767 ml + 0.9923 × c.e.v. (ml)	0.9671–1.0174	0.992	51
1.3	0	e.e.v. = 0.624 ml + 0.9938 × c.e.v. (ml)	0.9728–1.0148	0.994	58
1.3	1	e.e.v. = 0.642 ml + 0.9938 × c.e.v. (ml)	0.9727–1.0148	0.994	58
1.4	-1	e.e.v. = 0.607 ml + 0.9939 × c.e.v. (ml)	0.9715–1.0163	0.994	51
1.4	0	e.e.v. = 0.279 ml + 0.9972 × c.e.v. (ml)	0.9832–1.0113	0.997	58
1.4	1	e.e.v. = 0.459 ml + 0.9955 × c.e.v. (ml)	0.9777–1.0134	0.996	58
1.5	-1	e.e.v. = 0.503 ml + 0.9949 × c.e.v. (ml)	0.9745–1.0153	0.995	51
1.5	0	e.e.v. = 0.206 ml + 0.9980 × c.e.v. (ml)	0.9859–1.0100	0.998	58
1.5	1	e.e.v. = 0.290 ml + 0.9972 × c.e.v. (ml)	0.9830–1.0114	0.997	58
1.6	-1	e.e.v. = 0.347 ml + 0.9965 × c.e.v. (ml)	0.9796–1.0135	0.997	51
1.6	0	e.e.v. = 0.209 ml + 0.9979 × c.e.v. (ml)	0.9858–1.0100	0.998	58
1.6	1	e.e.v. = 0.282 ml + 0.9973 × c.e.v. (ml)	0.9833–1.0113	0.997	58
1.7	-1	e.e.v. = 0.017 ml + 0.9998 × c.e.v. (ml)	0.9961–1.0036	1.000	51
1.7	0	e.e.v. = 0.244 ml + 0.9976 × c.e.v. (ml)	0.9844–1.0107	0.998	58
1.7	1	e.e.v. = 0.237 ml + 0.9977 × c.e.v. (ml)	0.9848–1.0105	0.998	58
1.8	-1	e.e.v. = 0.126 ml + 0.9987 × c.e.v. (ml)	0.9885–1.0090	0.999	51
1.8	0	e.e.v. = 0.062 ml + 0.9994 × c.e.v. (ml)	0.9928–1.0060	0.999	58
1.8	1	e.e.v. = 0.061 ml + 0.9994 × c.e.v. (ml)	0.9929–1.0059	0.999	58

^a The distance from the bottom pole in the direction of the upper as a fraction of the distance between the bottom pole and the girth.

^b Minus one if the difference between the sum of the lengths of six equally-spaced transverse diameters of an egg at heights 1.05 to 1.3 and those of six equally-spaced transverse diameters at heights 0.7 to 0.95 were -1 mm or less, one if the difference was 1 mm or more and zero if the difference was between -1 mm and 1 mm.

^c The 95% confidence interval of the coefficient.

TABLE 4: Measures of agreement between the estimated volumes of Nile crocodile eggs and their volumes as calculated by integration

Scope	Polarity	Concordance correlation			Agreement (ml)			Relative agreement (%)			Model
		r_c^a	95% CI ^b	\bar{d}^c	$\bar{d} - 1.96s_{\bar{d}}$	$\bar{d} + 1.96s_{\bar{d}}$	\bar{p}^e	$\bar{p} - 1.96s_{\bar{p}}$	$\bar{p} + 1.96s_{\bar{p}}$	n	
1.3	-1	0.992	0.978-0.997	0.346	-2.277, 2.968 (5.245)	100.3	97.8, 102.9 (5.1)	18	Restricted Akaike		
1.3	0	0.995	0.986-0.998	0.171	-2.531, 2.873 (5.404)	100.3	97.2, 103.4 (6.2)	16	Restricted Akaike		
1.3	1	0.998	0.992-0.999	-0.108	-2.270, 2.055 (4.325)	100.0	97.7, 102.3 (4.6)	11	Restricted Akaike		
1.35	-1	0.995	0.984-0.998	0.541	-1.909, 2.991 (4.900)	100.5	98.0, 103.1 (5.1)	13	Restricted Akaike		
1.35	0	0.996	0.991-0.998	-0.387	-1.889, 2.662 (4.551)	100.4	98.0, 102.8 (4.8)	24	Full Akaike		
1.35	1	0.989	0.969-0.996	-0.064	-3.562, 3.435 (6.997)	100.0	96.6, 103.4 (6.8)	17	Restricted Akaike		
1.4	-1	0.994	0.986-0.997	-0.525	-2.828, 1.778 (4.606)	99.5	97.5, 101.6 (4.2)	20	Full Akaike		
1.4	0	0.996	0.992-0.998	-0.610	-2.148, 2.025 (4.173)	100.0	97.7, 102.2 (4.5)	25	Restricted Akaike		
1.4	1	0.998	0.996-0.999	-0.130	-2.164, 1.905 (4.069)	99.8	97.6, 102.0 (4.4)	28	Restricted Akaike		
1.45	-1	0.996	0.987-0.999	0.715	-1.611, 3.040 (4.651)	100.7	98.1, 103.3 (5.2)	14	Full Akaike		
1.45	0	0.996	0.990-0.998	-0.271	-2.360, 1.817 (4.177)	99.8	97.7, 101.8 (4.1)	26	Restricted Akaike		
1.45	1	0.996	0.990-0.998	-0.034	-2.518, 2.449 (4.967)	100.0	97.5, 102.6 (5.1)	21	Restricted Akaike		
1.5	-1	0.991	0.977-0.997	-0.286	-3.076, 2.504 (5.580)	99.7	97.1, 102.4 (5.3)	18	Full Akaike		
1.5	0	0.999	0.997-0.999	0.053	-1.422, 1.529 (2.951)	100.1	98.6, 101.6 (3.0)	26	Restricted Akaike		
1.5	1	0.998	0.996-0.999	0.067	-1.914, 2.048 (3.962)	100.0	98.1, 101.9 (3.8)	27	Full Akaike		
1.55	-1	0.983	0.951-0.994	1.302	-1.861, 4.465 (6.330)	101.4	98.0, 104.8 (6.8)	11	Restricted Akaike		
1.55	0	0.995	0.988-0.998	-0.210	-2.536, 2.116 (4.652)	99.8	97.4, 102.2 (4.8)	22	Restricted Akaike		
1.55	1	0.996	0.991-0.998	-0.231	-2.347, 1.886 (4.233)	99.8	97.8, 101.8 (4.0)	22	Restricted Akaike		
1.6	-1	0.995	0.988-0.998	0.129	-1.965, 2.223 (4.188)	100.2	98.1, 102.2 (4.1)	17	Restricted Akaike		
1.6	0	0.999	0.998-1.000	0.200	-0.961, 1.361 (2.322)	100.2	99.1, 101.4 (2.3)	28	Restricted Akaike		
1.6	1	0.997	0.995-0.999	-0.207	-2.549, 2.136 (4.685)	99.8	97.4, 102.1 (4.7)	31	Restricted Akaike		
1.65	-1	0.992	0.963-0.998	-0.442	-1.457, 2.302 (3.759)	100.4	98.7, 102.2 (3.5)	4	Restricted Akaike		
1.65	0	0.990	0.968-0.997	0.144	-1.908, 2.197 (4.105)	100.2	98.1, 102.2 (4.1)	12	Restricted Akaike		
1.65	1	0.997	0.993-0.999	0.123	-1.609, 1.854 (3.463)	100.1	98.5, 101.7 (3.2)	17	Restricted Akaike		
1.7	-1	0.981	0.751-0.999	-1.408	-2.993, 0.177 (3.170)	98.7	97.0, 100.4 (3.4)	3	Full		
1.7	0	0.998	0.994-0.999	-0.617	-1.784, 0.550 (2.334)	99.4	98.3, 100.5 (2.2)	14	Restricted Akaike		
1.7	1	0.997	0.994-0.999	-0.208	-2.430, 2.015 (4.445)	99.8	97.7, 101.9 (4.2)	30	Full Akaike		
1.8	-1							0			
1.8	0							1			
1.8	1	0.958	0.840-0.990	-0.844	-2.188, 0.499 (2.687)	99.2	97.9, 100.5 (2.6)	8	Full Akaike		
All estimates of egg volumes as calculated by integration											
		0.996	0.996-0.997	-0.013	-2.287, 2.261 (4.548)	100.0	97.7, 102.3 (4.6)	524			

^a Concordance correlation coefficient; ^b 95% confidence interval for r_c ; ^c Mean difference between the estimated volume and calculated volume; ^d 95% limits of agreement (1.96 standard deviations below and above the mean) according to Bland and Altman (1986), followed by the width of the interval between parentheses; ^e Mean of the estimated volume as a percentage of the calculated volume; ^f 95% limits of the relative agreement (1.96 standard deviations below and above the mean) followed by the width of the interval between parentheses.

the models estimated the volumes of eggs in four of the 28 scope-by-polarity combinations to within two to three percent of their calculated volumes and those of eggs in 20 combinations to within two to five percent. The volumes of eggs in five of the remaining eight combinations were estimated to within five to six percent of their calculated volumes and those of eggs in the remaining three to within six to seven percent.

Forecasting the volume of an egg from its estimated or ellipsoid volume

The height of the 95% confidence interval for forecasting the volume of an individual egg shown in Figure 6 is 4.572 ml on average (varying from 4.567 ml to 4.607 ml). Given

this small variation for an egg with an estimated volume between 60 ml and 135 ml, it is reasonable to conclude that its estimated volume predicts its calculated volume with a 95% confidence to within 4.57 ml.

The ellipsoid volume of an egg from the test set predicted its calculated volume with a 95% confidence to within 7.01 ml (varying from 6.99 to 7.20 ml, n = 138) and that of an egg from either the model generation set or the test set to within 7.02 ml (7.01–7.09 ml, n = 357).

The width of the 95% confidence interval for forecasting the volume of an individual egg varied only slightly over

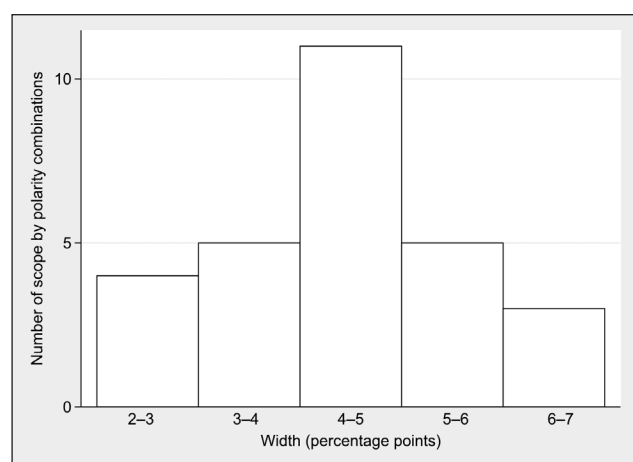


FIGURE 5: The number of scope-by-polarity combinations for which egg volume could be estimated within the respective limits of relative agreement

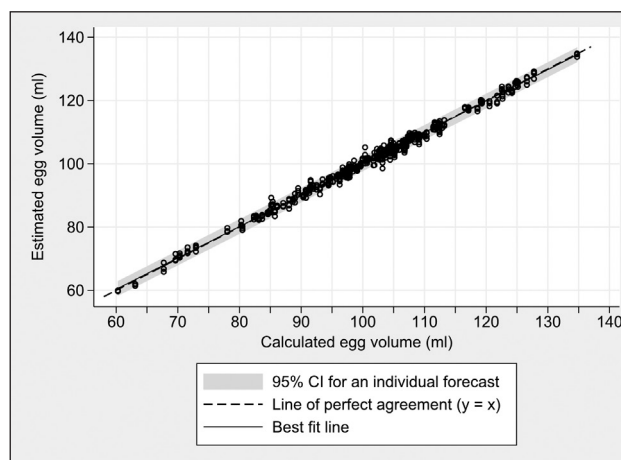


FIGURE 6: The volumes of 138 Nile crocodile eggs calculated by integration of measurements on an image of each against 1–4 estimated volumes for each, produced from the same images after they were masked to varying extents (524 masked images in total)

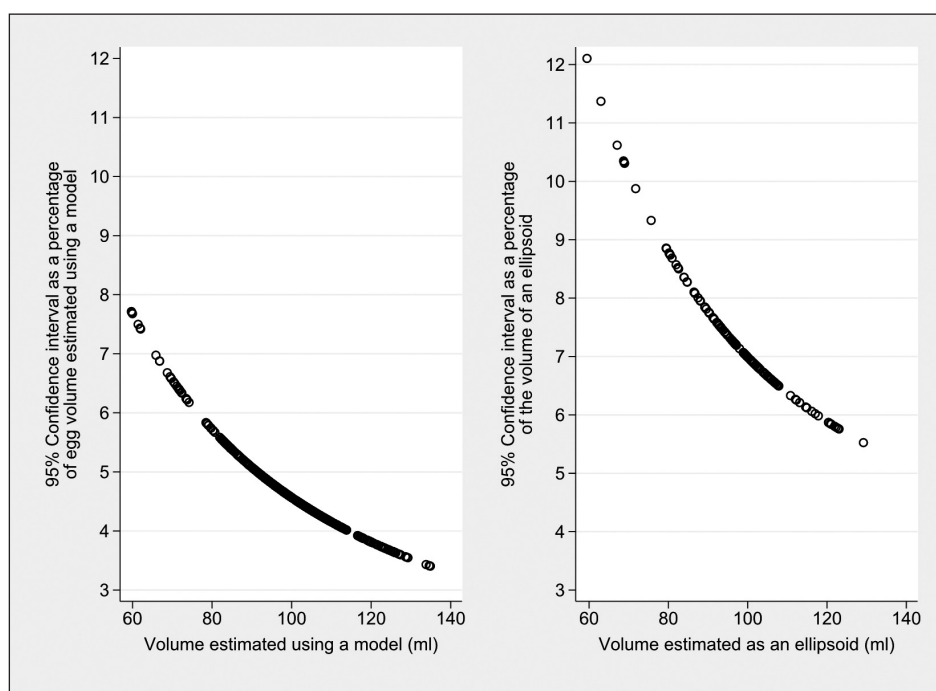


FIGURE 7: The precision of forecasting the volume of an individual Nile crocodile egg, shown as the width of the 95% confidence interval (CI) for the forecast expressed as a percentage of the estimated volume of the egg. Egg volume was estimated using a model on each of 524 masked images of 138 eggs (left) and applying the formula of an ellipsoid on measurements made on an unmasked image of each of the same 138 eggs (right).

the range of estimated egg volumes derived using the models on masked egg images or by applying the formula of an ellipsoid on measurements made on unmasked egg images. Yet, the width of the confidence interval, expressed as a percentage of estimated egg volume decreased with an increase in estimated egg volume (Figure 7). In line with the graph on the left (Figure 7), the smallest estimated volume in the test set (59.7 ml) predicted the calculated volume of the corresponding egg with a 95% confidence to within 7.72% whereas the largest estimated egg volume (134.9 ml) did so within 3.40%. The ellipsoid volume derived from measurements on an unmasked egg image of each of the 138 eggs in the test set predicted the calculated volume less precisely than the volumes estimated using the models on the 524 masked images of the same 138 eggs did ($P < 0.001$).

Discussion

The models in this study underestimated or overestimated the volume of Nile crocodile eggs from measurements on masked images of the eggs with a scope of at least 1.3 (images on which at least 1.3 times the length from the bottom pole to the girth along the polar axis, in the direction of the upper pole, was retained) by no more than 2.3% with a 95% level of confidence. The study shows that, for eggs with estimated volumes in the range of 60–135 ml, as used in this study, the volume of a single egg will be predicted to within 4.57 ml of its actual volume with a 95% level of confidence. An estimated volume of 60 ml will predict the actual volume of the egg with 95% confidence to within 7.7% of the actual volume, and this precision improves with increasing estimated volume to within 3.4% for an egg with an estimated volume of 135 ml. The models in this study render it possible to identify variation larger than 7.7% in the birth mass of hatchlings from eggs with estimated volumes of 60 ml. This level of identifiable variation decreases with an increase in estimated egg volume to 3.4% for hatchlings from eggs with estimated volumes of 135 ml. Variation in birth mass exceeding these levels would indicate the existence of factors affecting the relationship between hatchling mass and egg volume, which could then be investigated.

Apart from the fact that neither the equatorial diameter nor the length of the polar axis (or the distance between the intact pole and the equator) can be determined on masked egg images or hatched egg shells, this study shows that the models estimated egg volumes from masked egg images (and consequentially from hatched egg shells) with greater precision than did the ellipsoid volume.

The study shows that the precision with which egg volume can be estimated varies among scope-by-polarity combinations, with the precision increasing with an increase in scope. This increase in precision is likely due to various factors. First, the amount of information with which the program estimates the position of the polar axis increases with scope. Second, the number of transverse diameters available for inclusion in the model increases

with scope. Third, the number of calculated volumes below specific transverse diameters that could be included in the Full or Full Akaike models increases with scope. Last, the calculation of the volume below highest transverse diameter within the scope, which was included in all models, is based on a larger fraction of the total egg volume.

Even though each egg image in the test set was masked to the same extents, it resulted in a large variation in the number of masked egg images in each scope-by-polarity combination. The extent to which the frequencies of egg images in the respective scope-by-polarity combinations represent those of hatched eggs is unknown.

In this study the calculated egg volume was used and not the displacement volume. On average, the calculated volume of 96% of the eggs was 1.49% higher than their displacement volumes, with 95% of the differences being between 0.49% lower and 3.48% higher. Repeated measurements of the displacement volume of each of two bottles differed very little as did the repeated calculation of the volumes of 55 eggs. The mean difference between the displacement volumes and their calculated volumes, as well as the magnitude of variation of these differences among eggs were substantially higher than the differences among repeated measurements of the volumes of the bottles and the repeated calculation of the volumes of 55 eggs. Further, the measurements done by the computer program agreed closely with those done by hand on photographs on paper, suggesting that the measurements on which the computer based its calculation of egg volume by integration were precise and reliable. Measuring egg volume by water displacement may therefore have yielded erroneous results that led to the difference between the displacement volume and the calculated volume and the variation in magnitude of this difference. One possible mechanism that may have led to erroneous displacement volumes is that the porous shell (Ferguson 1982; Grigg 1986), possibly with air spaces below the shell as well (Manolis et al. 1986), became impregnated with water while the bottle with the egg therein was filled with water to measure the displacement volume. This would have created the impression that a larger volume of water was needed to fill the space around the egg than was really the case, which, in turn, would have yielded a spuriously low displacement volume.

Calculating the egg volume by integration was based on the assumption that all cross sections (perpendicular to the polar axis) through the egg were circular in outline. If an egg was not circular in cross section its calculated volume would have been wrong. It seems unlikely that the common observation that calculated egg volume was larger than the displacement volume was due to eggs with non-circular cross-sectional shape as that would require photos of such eggs to have been taken with their largest transverse diameters closer to perpendicular to the optical axis of the camera rather than closer to parallel thereto.

We attempted to position eggs vertically for photography, yet they were mostly positioned skew. The program corrected for the extent to which an egg was tilted to the left or right. It is also possible that eggs were leaning forward, with their upper pole towards the camera, or backwards, for photography. The program did not correct for such tilting. The more an egg leaned forward or backward, the less slender and more circular the egg image would be, which may have affected the calculated volume. It is unlikely that forward or backward tilting of eggs on the photos would cause the calculated volume to exceed the displacement volume, as such leaning would not change the girth diameter, while the apparent length of the polar axis would decrease, yielding a false low calculated volume.

Each scope-by-polarity combination required a unique model to estimate egg volume most precisely. In this study no attempt was made to decide which had to be the upper and which the lower pole of an egg for photography. An attempt to orientate the poles of an egg may have affected its polarity. Seeing that it is unknown whether hatching occurs at a specific pole relative to the asymmetry of an egg, any attempt to orientate the poles in this study was contra-indicated.

The results of this study may be useful in the nature conservation setting, provided that Nile crocodile egg shells can be collected in a sufficiently intact state from nests in the wild once they have hatched.

Conclusion

This study renders it possible to estimate the volume of Nile crocodile eggs after they have hatched to within 7.7% of their actual volume for eggs with estimated volumes of 60 ml, decreasing to within 3.4% for eggs with estimated volumes of 135 ml, with 95% confidence. The results of this study also enable one to study the relationship between egg volume and hatchling mass at the level of individual eggs and their hatchlings.

Author contributions

JGM and JON photographed the eggs. JAN wrote the computer program and taught JON to use it. JAN and JON prepared the photographs for measurement by the computer program and determining the calculated volumes. JON measured the egg volumes by water displacement, took the measurements on the photographs on paper and performed the data analysis. JON wrote the manuscript in Afrikaans and then translated it into English.

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