Effects of Egg Mass, Hatchling Size, and Clutch on Body Masses and Lengths of Female American Alligators (Alligator mississippiensis)

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Introduction

American alligators (*Alligator mississippiensis*) are a long-lived reptile (~50→75 years) native to marshes in southeastern North America, and they are a member of the order Crocodilia. There are 23 extant species of Crocodilia, an order whose paleontological record stretches back one quarter of a billion years on Earth. American alligators have a single, annual breeding period in summer, with one clutch of eggs laid by a female (Lance, 1989). Eggs incubate for 2→2.5 months, and clutch size typically varies between 20→50 (Lance, 1989). Environmental conditions and genetic background are important determinants of organismal growth and fitness. Interactions between environmental and genetic factors have been widely studied across different animal clades including turtles (Rhen and Lang 1995), birds (Haywood and Perrins 1992), and crocodilians (Bagatto et al. 2012). Clutch effects explain variations among crocodilian hatchling size (Allsteadt and Lang 1995), but it remains unexplored how variation in hatchling size within and between clutches affects overall mass and length of young crocodilians (Eme et al. 2019).

In this study, we examined effects of egg mass, hatchling size and clutch on the size of young female American alligators (hatching to 6 months old) in each of three distinct years. We hypothesized that initial hatchling size would significantly affect 6-month old alligators body masses and lengths, and that the strength of this effect would vary with clutch. American alligators are archosaurs, a clade of animals that includes extant birds and extinct dinosaurs, and this study can therefore be used in comparative analyses within this notable clade.

Methods and Materials

American alligator eggs (N=117 total animals) were obtained from Rockefeller Wildlife Refuge in Grand Chenier, LA, for three distinct years (hatched summer 2017, 2018, and 2019). Eggs from 5 different clutches (labeled A, B, C, D, or E) were numbered (clutch and unique number) and weighed. This initial egg mass (±0.1g; Mettler Toledo, SB8001, Columbus, OH) was taken within the first 2 weeks post laying for each clutch. Eggs were distributed into 2.1L plastic containers (N=5-9 eggs per container) containing a bed of vermiculite: water mixed in 1:1 ratio. Eggs were candled using a flashlight to note embryonic development inside the egg. Containers were weighed 1-3 times per week, and water was added to maintain the appropriate ratio. Containers were placed in 76L Ziploc "Big Bags" (SC Johnson, Racine, WI, USA), attached to a 1.9 L/min aquarium air pump (Fusion 400, J.W. Pet Company, Inc., China). The pump was connected to 0.5 L water bubbler to maintain a high humidity within the bag. Bags were placed in an incubator (567L; Thermo Scientific™ Precision™, Model 815, Marietta, OH) at 30°C for ~70 days until hatching (late August→ early September 2017, 2018, and 2019). Eggs were incubated at 30°C to ensure that all hatchlings developed as females, as alligators have temperature-dependent sex determination. Each container was photographed weekly to record egg location and orientation.

Upon hatching, alligators' dorsal sides were photographed for identification of hatchling's unique markings. Photographs allowed for matching individual animal's egg and hatchling data with growth. Within the first month post-hatching, alligators were given a unique scute-clip, by cutting a small part of their tail, and photographed again. Hatchlings' initial wet body mass (±0.1g; SB8001), snout-to-vent length (SVL±1mm; metric ruler) and head length (HL±0.1mm; digital calipers) were measured within 4 days of hatching, prior to yolk absorption. SVL is the distance from the snout's tip to the anterior edge of the cloaca, and HL is the distance from the snout's tip to the poster dorsal edge of the occiput. Animals were group housed in tanks with access to water-filled and dry areas. Water (24-32°C) from each tank was replaced daily, and the rooms ambient temperature was 25-30°C. Animals were fed *ad libitum* 56% protein alligator food (LoneStar Nacogdoches, TX) once a day, 2-7 times per week; alligators were fed substantially more in 2017 (4-7 times per week) and 2018 (2-4 times per week), compared to 2019 (2 times per week).

Matching diet, husbandry, and housing conditions within each year allow for an analysis of variance (ANOVA) and simple linear regression (SLR) to examine effects of egg mass on hatchling body mass, SVL, and HL for all three years combined (2017, 2018, 2019). Separate 2-way ANOVAs and SLRs examined effects of egg mass, clutch, and the interaction of clutch and egg mass on hatchling and 6-month old alligator body mass, SVL, and HL within each year. For all combined clutches within each year, a 1-way ANOVA and SLRs examined the effect of hatchling body mass on 6-month old body mass, SVL, and HL. For all combined clutches within each year, separate 1-way ANOVAs and SLRs examined the effect of hatchling SVL and HL on 6-month old SVL and HL, respectively. Within each year, a 2-way ANOVA and SLRs was used to examine the effect of hatchling body mass, clutch, and the interaction of clutch and hatchling body mass on 6-month old alligator body mass, SVL, and HL; significant effects of hatchling body mass, clutch, or their interaction was followed by a Tukey honest significant difference test (Tukey HSD). JMP version 15.2.1 (SAS Institute Inc., Cary, NC) was utilize for statistical analyses.

Results

Alligators grew rapidly each year for their first 6 months, increasing in average body mass from 50±0.05→439±0.16g, 52±0.7→362±0.15g, and 51±0.05→215±0.6g, average SVL from 11±0.05→23±0.3cm, 11±0.09→22±0.3cm, and 12±0.03→19±0.2cm, and average HL from 37±0.02→65±0.7mm, 36±0.2→60±0.7mm, and 37±0.8→56±0.4mm (mean ± standard error of the mean) for 2017, 2018, and 2019 respectively. Clutches differed in hatchling body masses and lengths (Table 1, 2 and 3). Egg mass is a good predictor of hatchling body mass (SLR, r^2=0.69), SVL (SLR, r^2=0.26), and HL (SLR, r^2=0.13; 1-way ANOVA, $F_{1,115}$>18.4, P<0.001) for all three years combined (Figure 1). Egg mass and clutch did not consistently interact each year to affect hatchling body masses and lengths. In 2017, there was a significant effect of clutch (F_4=5.87, P<0.01) and egg mass on hatchling body mass (F_1=47.09, P<0.001), but not their interaction. In 2017, there was no significant effect of clutch, egg mass or their interaction on hatchling SVL or HL. In 2018, there was a significant effect of egg mass on hatchling body mass (F_1=9.75, P<0.004), but not clutch or their interaction. In 2018, there were no significant effects of clutch, egg mass or their interaction on hatchling SVL or HL. In 2019, there was a significant effect of clutch (F_4=4.27, P=0.0055) and egg mass on hatchling body mass (F_1=12.01, P=0.0013), but not their interaction. In 2019, there was a significant effect of clutch (F_4=3.77, P=0.01) and egg mass on hatchling SVL (F_1=4.7, P=0.035), but not their interaction. In 2019, there was a significant effect of clutch on hatchling HL (F_4=7.35, P<0.001), but not egg mass or their interaction. Egg mass was not a consistent predictor of body mass, SVL, or HL for 6-month old alligators within each year, with only SVL showing a significant effect of egg mass in 2017 (2-way ANOVA, $F_{1,24}$=7.4, P=0.0116).

For all clutches combined within years, hatchling body mass significantly predicted some 6-month old masses and lengths in 2018 and 2019, but not 2017 (Figure 2). In 2017, hatchling body mass did not significantly predict 6-month old body mass, SVL, or HL (1-way ANOVAs, $F_{1,24}$<2.9, P>0.09). Hatchling body mass did significantly predict 6-month old body mass in 2018 and 2019 and HL in 2019 (1-way ANOVAs, $F_{1,24}$>4.1, P<0.05), but not SVL in 2018 and 2019 or HL in 2018 ($F_{1,24}$<1.7, P>0.20). For all clutches combined within years, hatchling SVL (Fig. 3) and HL (Fig. 4) were not consistent predictors for 6-month old alligators. Hatchling SVL did not significantly predict 6-month old SVL for any year (1-way ANOVA, $F_{1,24}<3.1$, $P>0.08$). Hatchling HL did not significantly predict 6-month old HL for 2017 and 2018 (1-way ANOVAs, $F_{1,24}<1.9$, $P>0.16$), but did for 2019 ($F_{1,24}=13.5$, $P<0.001$). Hatchling body mass was a consistent predictor of 6-month old body masses and lengths in 2018 and 2019, and we examined the interaction of clutch and hatchling body mass further. Within years, hatchling body mass and clutch did not interact to predict 6-months old masses or lengths (2-way ANOVAs, $F_4<0.86$, $P>0.49$). In 2017, clutch significantly predicted 6-month old body mass, SVL, and HL with only one identical clutch consistently being the smallest. In 2018, clutch did not significantly predict 6-month old alligator body mass, SVL, or HL. In 2019, clutch significantly predicted 6-month old body mass, SVL, and HL with only one identical clutch consistently being the smallest.

Discussion

Alligator egg mass and clutch contribute to variation in alligator hatchling size and predict hatchling body mass well, but the metrics did not interact to affect hatchling size. This result is similar to a previous study (Allsteadt and Lang 1995) that found significant effects of clutch on SVL and HL. However, in our study clutch did not consistently predict hatchling SVL or HL. In 2017 and 2018, there was no significant effect of clutch on hatchling SVL or HL, but in 2019, there was a significant effect of clutch on hatchling SV and HL. Clutch effects have shown significant effects on the growth of 1 year old alligators raised in common environmental conditions (Bagatto et al. 2012), but the current study supports findings that hatchling body mass, rather than clutch or egg mass, is the best indicator of juvenile alligator growth (Eme et al. 2010). Initial hatchling size can be predicted by egg mass, and it is this hatchling size that best then predicted 6-month old alligator size. These data can be used to support relationships between the size of fossilized, small archosaurs and predictions of their growth and juvenile size.

Appendices

References

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Tables

Table 1. Sample size and mean values (±standard deviation) for initial egg mass, initial hatchlings wet body mass, SVL and HL for five alligator clutches in 2017

Clutch (n)	Initial egg mass (g)	Initial hatchling wet body mass (g)	Initial hatchling SVL (cm)	Initial hatchling HL (mm)
A (5)	75.9±4.4	52.2±2.6	11.5±0.3	37.3±0.6
B (5)	75.1±4.2	48.1±3.1	11.2±0.3	37.1±0.7
C (7)	69.0±2.2	48.6±1.5	11.2±0.2	36.7±0.8
D (4)	70.5±1.4	48.3±1.7	11.4±0.2	36.5±0.6
E (5)	76.1±4.4	50.5±3.4	11.4±0.3	38.0±0.8

Table 2. Sample size and mean values (±standard deviation) for initial egg mass, initial hatchlings wet body mass, SVL and HL for five alligator clutches in 2018

Clutch (n)	Initial egg mass (g)	Initial hatchling wet body mass (g)	Initial hatchling SVL (cm)	Initial hatchling HL (mm)
A (10)	72.4±3.7	48.9±2.5	11.2±0.4	36.0±1.0
B (9)	77.4±4.1	52.0±2.4	11.5±0.2	36.0±0.4
C(7)	73.1±1.4	47.4±3.0	10.9±0.7	35.5±1.5
D (6)	80.2±4.0	54.4±3.2	11.2±0.4	35.7±0.9
E(8)	87.1±4.8	58.4±2.7	12.1±0.3	37.7±0.5

Table 3. Sample size and mean values (±standard deviation) for initial egg mass, initial hatchlings wet body mass, SV,L and HL for five alligator clutches in 2019.

Clutch (n)	Initial egg mass (g)	Initial hatchling wet body mass (g)	Initial hatchling SVL (cm)	Initial hatchling HL (mm)
A (11)	76.0±4.0	50.0±2.6	11.6±0.2	36.7±0.3
B (11)	80.1±2.8	54.0±1.8	11.8±0.2	37.8±0.3
C (9)	79.6±1.7	53.1±1.6	11.7±0.2	37.1±0.3
D (10)	78.1±4.4	49.7±2.7	11.7±0.2	37.6±0.5
E (10)	73.42±3.9	48.6±3.5	11.7±0.2	37.1±0.8

Figures

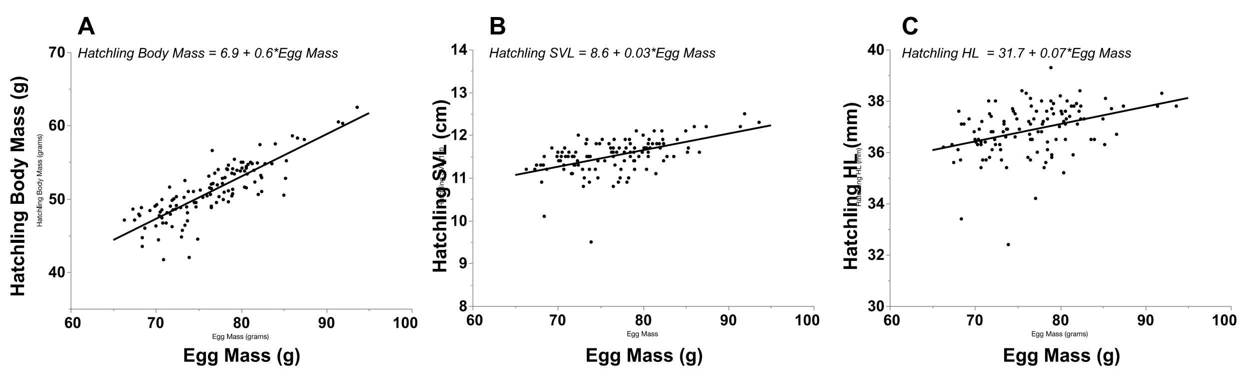

Fig. 1. Linear regressions of egg mass with values for hatchling body mass (A), SVL (B), and HL (C) for all three years combined (N=117 total animals). Regression line is shown as y=mx+b, where y is hatchling body mass, SVL, or HL (A, B, or C, respectively), and x is the egg mass. Significant ANOVAs (P<0.05) occurred for each growth metric.

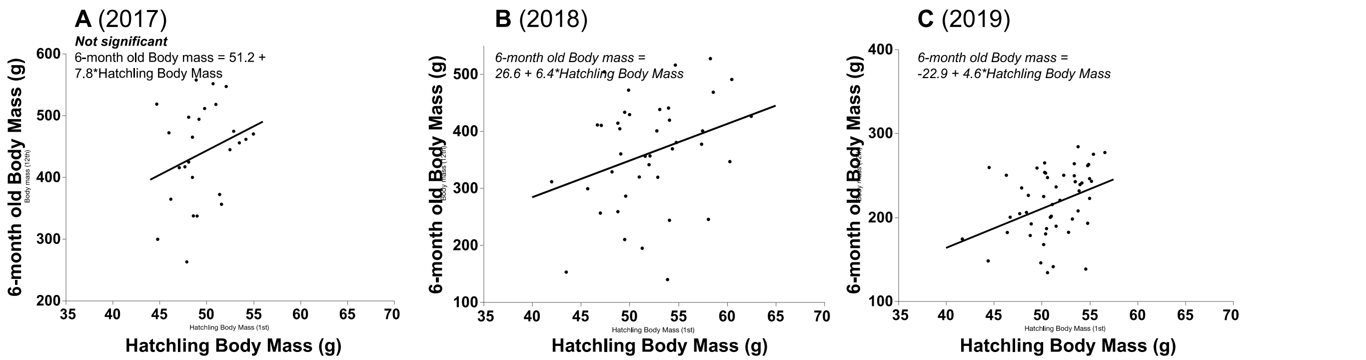

Fig. 2. Linear regressions of hatchling body mass (g) with 6 month-old body mass (g) values (N=117 total animals). Regression line is shown as y=mx+b, where y is 6-month old body mass, and x is hatchling body mass. ANOVAs for the SLRs are italicized if significant (P<0.05), otherwise it is noted as Not significant.

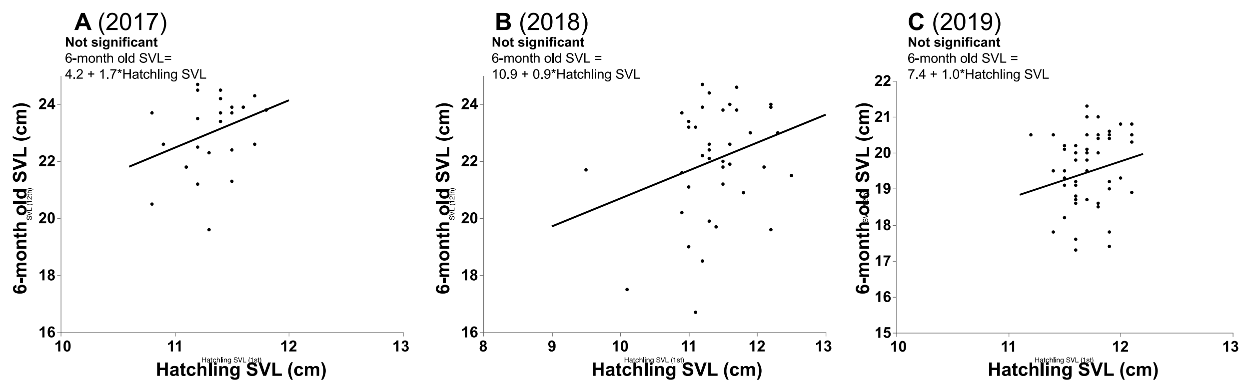

Fig. 3. Linear regression of hatching SVL (cm) on 6-month old SVL (cm) within each year (N=117 total animals). Regression line is shown as y=mx+b, where y is 6-month old SVL, and x is hatchling SVL. ANOVAs for the SLRs are italicized if significant (P<0.05), otherwise it is noted as Not significant.

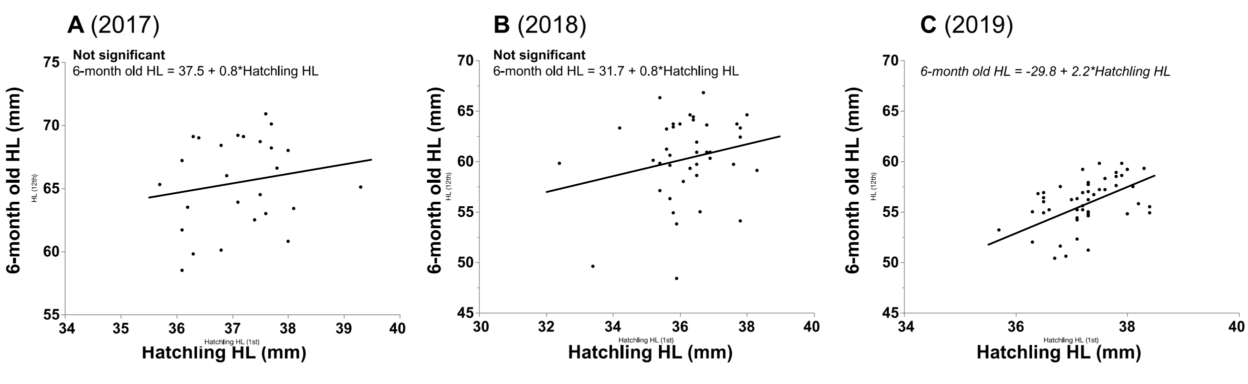

Fig 4. Linear regression of hatchling HL (cm) on 6-month old HL (mm) within each year (N=117 total animals). Regression line is shown as y=mx+b, where y=6-month old HL, and x is hatchling HL. ANOVAs for the SLRs are italicized if significant (P<0.05), otherwise it is noted as Not significant.