Hatching Success of American Alligator Eggs When Subjected to Simulated Collection Trauma

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Abstract: Hatching success of crocodilian eggs can be influenced by the age of the embryo at time of collection. We conducted an experiment to determine the amount of care necessary during egg collection to optimize embryo survival when alligators eggs at different stages of development were subjected to simulated field conditions of choppy vs. smooth water and cushioned vs. non-cushioned support. We also compared hatching success of rolled vs. non-rolled eggs at different stages of development. We found that transporting eggs in choppy water reduced hatch rates in the absence of cushioning ($P < 0.001$) and cushioning improved the likelihood of eggs hatching ($P = 0.071$). The hatch rate probability of eggs with 7–8 days of embryonic development was less than those of 12–14 or 17–18 days ($P < 0.010$). Rolling did not significantly affect the probability of eggs hatching ($P > 0.89$) for the ages 13–24 days. This study indicated that precautionary measures can reduce egg mortality. We suggest that a simple cushioning system be used to minimize egg mortality when transporting eggs in choppy water conditions or over rough terrain.

Key words: American alligator, *Alligator mississippiensis*, hatching success, trauma, egg collection, incubation

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The collection of wild American alligator *(Alligator mississippiensis)* eggs for incubation has become an important component for alligator management (Chabreck 1978, Joanen and McNease 1987, Woodward et al. 1989). However, crocodilian embryos can be damaged or killed if eggs are not handled properly during collection and incubation (Ferguson 1985, Webb et al. 1987).

The chorion, inner and outer layers of the allantois, and the amnion (yolk sac membrane) fuse to form an attachment of the amnion to the eggshell membrane at day 2 of development (Ferguson 1982, 1985). In the early stages of embryonic development this attachment is fragile because of a fusion of the chorion with the outer layer of the allantois by a network of blood vessels (Ferguson 1982). After the embryo attaches to the eggshell membrane, agitation or inversion of the egg, which may occur during handling or field transport, can cause increased embryonic mortality by shearing the vascular membrane (Chabreck 1978, Ferguson 1985, Webb et al. 1987).

Egg collection experiments have shown evidence that hatching success of crocodilian eggs is influenced by age of the embryo at time of collection (Chabreck 1978, Blake 1992, Joanen and McNease 1987). Chabreck (1978) found that eggs collected during an early stage of incubation (25 June) were less likely to hatch than eggs collected in later stages of incubation (19 July, 2 August, 19 August). Joanen and McNease (1987) reported that the most sensitive period for handling alligator eggs was between days 9 and 16 of development due to their susceptibility to injury at that stage. However, neither of these studies tested the effectiveness of transportation and handling measures that might reduce collection-related mortality. Moses (1989) reported no difference in the hatching success of alligator eggs collected at two-week intervals throughout incubation, although the exact age of embryos at the onset of collections in his experiment is not known.

Eggs collected by different collection groups have been handled with varying amounts of care, and the extent of necessary caution in collections is unknown. Moses (1989) and Moses and Chabreck (1990) examined the effects of cushioning eggs with 10-cm foam rubber padding during transport and found no significant difference in survival between the cushioned and non-cushioned eggs, but the exact age of embryos was unknown. Woodward et al. (1989) found no difference in hatch rates of eggs collected at different ages when handled carefully and transported on foam cushions, but they did not test this procedure against non-cushioned eggs.

Another source of field trauma occurs when the eggs are transferred from the nest into a transportation container. During this process, it is possible for eggs to roll because of the precarious position of some eggs within the nest cavity. Although egg turning is an important aspect in the development in birds, crocodilians do not turn their eggs. In some cases, rotation of the eggs in crocodilians and many turtles can cause mortality (Chabreck 1978, Limpus et al. 1979, Ferguson 1985, Deeming and Ferguson 1991).

Alligator research crews at the Florida Fish and Wildlife Conservation Commission (FFWCC) have used a special protocol for egg collections and padded transport vehicles to minimize trauma during field transport (Woodward et al. 1989). Because this protocol requires extra planning and materials, there is a need to determine whether these precautions are necessary. In addition, commercial egg collection crews in Florida typically do not use cushioning additional to nest material for eggs during transport. We questioned whether embryo mortality would increase significantly when eggs were transported without additional cushioning. The objectives of this study were to determine the amount of care necessary in the collection of American alligator eggs to optimize embryo survival and determine whether age of the embryo at collection affected hatch rates. We compared hatch rates among eggs of different ages subjected to different levels of field transportation trauma. The conditions tested were choppy vs. smooth water conditions and cushioning vs. non-cushioning of eggs. In a separate experiment, we compared the hatching success of rolled vs. non-rolled eggs at different stages of development.

Methods

Collection

Nine alligator egg clutches were collected on Orange Lake (5,254 ha), located in the Orange Creek drainage of the St Johns River watershed in north central Florida (Alachua County) during 27–28 June 1998. Clutches were collected using the protocol established by Woodward et al. (1989). Nests were probed and excavated until the top part of the clutch cavity was exposed. Exposed eggs were immediately recovered and a thermometer inserted into the top portion of the clutch cavity to determine temperature. Clutch cavity temperatures ranged between 31 and 34 C. Eggs were uncovered and marked on the upper surface with a permanent marker. Eggs were carefully removed from the nest cavity and placed in a plastic egg pan on top of nest material. The clutch was then covered with nesting material, and the pan was hand-held during transport on an airboat to an egg transportation boat. The cushioning system on the egg transport boat included a layer of inflated inner tubes, 0.9-cm plywood, 5-cm foam rubber, then a layer of egg pans. If another layer was necessary, we placed 0.9-cm thick plywood on top of the first layer to assist in distributing weight, then another 5-cm layer of foam was added. At the boat ramp, egg pans were carefully transferred to a pickup truck with a covered bed. During road transport, clutches were placed on top of a layer of 5-cm foam, which was laid on the covered bed of a pickup truck. The inner sides of the truck were cushioned with 5-cm foam to reduce side-to-side movement. If double layering was necessary, the same cushioning system of plywood and foam was applied as in the double layering of the boat.

Incubation

Eggs were candled at the FFWCC Wildlife Research Laboratory incubation facility. One egg from each clutch was sacrificed to determine the stage of development of the embryos and age of the clutch (Ferguson 1985). Developmental stage of embryos is roughly equivalent to age in days up to approximately 20 days for incubation temperatures ranging from 30–33 C (Smith and Joss 1994). Therefore, we assigned an age in days to eggs based on embryo developmental stages identified by Ferguson (1985). Using this standard, the ages of 20–24 day eggs in the rolling experiment may have been slightly over-estimated. Fertilization status (banded or non-banded) and embryo viability (alive or dead) was determined by transillumination (Woodward et al. 1989). Each clutch was transferred to a pan with sphagnum moss as the nesting medium, and the dead and non-banded eggs were removed and discarded.

Incubation, hatchling care, and housing met Florida Fish and Wildlife Conservation Commission regulations (FLFWCC 1998). We incubated eggs in a 7.3 x 3.6-m portable insulated storage building with a heating, cooling, and humidifying system. The target incubation temperature was 32 C. Nest temperatures within the incubator typically fluctuated between 31.5 and 33 C but occasionally ranged from 30 to 34.5 C due to workers opening the incubator door. Average nest temperature within the incubator was 32.1 C. Incubator humidity was maintained at an average of 93.9%.

Experimental Treatments

Cushioning/Waves Treatment.—Sixteen banded-live eggs were randomly selected from each of eight clutches and distributed among treatments (four eggs per clutch in each treatment; $N = 128$ eggs); waves/cushioned, waves/not cushioned, no waves/cushioned, and no waves/not cushioned. The estimated ages of embryos ranged from 7–18.5 days. They were then grouped into age classes based on their estimated ages (Table 1). One egg from each clutch was placed in sphagnum moss in a container, representing one replicate of each treatment, for a total of 16 boxes (eight eggs per box).

On 1 July 1998, we transported eggs back to Orange Lake for treatments, using the methods described above. Cushioned versus non-cushioned treatments consisted of setting egg boxes on the bottom of the boat on either 5-cm foam pads (cushion) or directly on the floor of the airboat (no cushion). Choppy vs. smooth water treatments consisted of running the airboat at approximately 25 km/h along the shore in shallow water among emergent vegetation (smooth) or at the same speed in open water in waves ranging from 0.3 to 1.3 m in height (choppy) for 30 minutes. We attempted to apply the same relative amount of shock to all trips for choppy conditions, although wave conditions varied (standard waves ranged from 0.3 to 1.3m). If the lake lacked sufficiently choppy conditions, we simulated waves by creating wakes with the boat, then running perpendicular to those wakes in order to impact the created waves. All smooth condition trips were conducted along the edge of the lake on the lee side from the wind, often through vegetation with minimal to no wave action.

This variability in wave height among replicates occurs naturally during collections due to weather, water depth, waterbody size, vegetation, varying travel distances, and drivers. Extremely rough wave conditions (>1 m), such as would realistically occur with an impending thunderstorm, resulted in eggs from both treatments (cushioned and non-cushioned) being repositioned in their clutch boxes from the force of the jarring. Following trials, we transported eggs back to the incubator in a truck as previously described. Eggs were checked twice for mortality during incubation. Any non-viable eggs were opened to determine age of death of the embryo.

The cushioning/waves treatment experimental design was a split-split-plot (Table 1). The main-plot treatment was WAVE (yes or no), and the main-plot experimental unit was TRIP (30-minute trial). The sub-plot treatment was CUSHION (yes or no), and the sub-plot experimental unit was BOX (each experimental container).

Table 1. Frequencies of alligator eggs included in the cushioning/waves treatment split-split-plot experimental design. Age class is specified in days of embryonic development.

		Wave																
		No Trip								Yes Trip								
		1		3		5		7		2		4		6		8		
		Cushion		Cushion		Cushion		Cushion		Cushion		Cushion		Cushion		Cushion		
		No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	
		Box 2	Box 3	Box 16	Box 14	Box 13	Box 6	Box 8	Box 15	Box 4	Box 5	Box 11	Box 12	Box 10	Box 9	Box 7	Box 1	Total
Ageclass	Clutch ID																	
7–8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
	9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
	24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
12–14	27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
	31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
17–18	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
	25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
	26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
Total		8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	128

The sub-sub-plot treatment was AGECLASS, and the sub-subplot experimental unit was the egg. Also, CLUTCH (AGECLASS) (read as "CLUTCH within AGECLASS") was crossed with BOX.

The generalized linear mixed model (GLMM) methodology of Wolfinger and O'Connell (1993), as implemented in the GLIMMIX macro (Littell et al. 1996), was used. The binomial error distribution and the logit link were specified. Fixed effects were specified as the full factorial arrangement of the factors WAVE, CUSHION, and AGECLASS. TRIP (WAVE), CUSHION x TRIP (WAVE), AGECLASS x TRIP (WAVE x CUSHION), and CLUTCH (AGECLASS) were specified as random effects. Model selection for random effects was performed as follows. The full factorial of fixed effects was specified. In addition to the model containing all random effects, all possible reduced models formed by dropping one or more random effects, while maintaining a hierarchical structure, were fitted. Models were then compared using Akaike's Information Criterion (AIC) (Littell et al. 1996). Values of AIC closer to zero indicate a better fit. Using the model for random effects determined to be best according to the above procedure, an iterative model selection procedure for fixed effects was performed. In an iteration, consistent with maintaining a hierarchical structure, the highest order term with the largest non-significant *P*-value for the Type III Wald test was dropped from the model, and the reduced model was refitted. Iteration ceased when all higher order terms in the model were significant.

The WAVE x CUSHION interaction was explored by examining WAVE and CUSHION simple effects (averaged over AGECLASS levels). Simple WAVE effects in the logit scale were the log of the odds ratios, where $odds_{i,j}$ was the odds of hatching for WAVE = *i* and CUSHION = *j:*

$$odds_{i,j} = \pi_{i,j} / 1 - \pi_{i,j}$$

where $\pi_{i,j}$ = the probability of hatching for WAVE = *i* and CUSHION = *j*. Similarly, simple CUSHION effects were the log of the odds ratios.

All statistical computations were performed using SAS (SAS 1999).

Roll Treatment.—The roll experiment was initiated six days after the cushioning/wave experiment and, therefore, the estimated ages of embryos ranged from 13 to 24.5 days. Eight banded-live eggs from each of nine clutches (N = 72 eggs) were randomly selected for the roll experiment (Table 2). Most eggs originated from the same eight clutches as previously selected eggs, but were not used in the previous treatments. Eight eggs from a ninth clutch were added to this experiment to increase sample size.

Four of eight eggs from each of the nine clutches were randomly selected to be rotated 360 degrees along their long axis on a flat surface. The other four banded-live eggs were used as controls for the rolling experiment. These 72 eggs were then set in sphagnum moss and incubated in the FFWCC incubation facility. Eggs were checked twice for mortality before the estimated hatch dates, and non-viable eggs were opened to determine the age of death of the embryo.

The experimental design for the roll treatment was a randomized complete block design (Table 2). GLMM methodology, as implemented in the NLMIXED procedure (SAS 1999) and based on theory and computational methods presented in

Table 2. Frequencies of alligator eggs included in the roll treatment randomized complete block experimental design. Age class is specified in days of embryonic development.

		Treatment		
Ageclass	Clutch ID	Control	Roll	Total
13	24	4	4	8
14	1	4	4	8
14	9	4	4	8
18.5	26	4	4	8
20.5	31	4	4	8
22	10	4	4	8
23	6	4	4	8
24	27	4	4	8
24.5	25	4	4	8
Total		36	36	72

Pinheiro and Bates (1995), was used in analysis. Random clutch effects and a fixed treatment (rolled, control) effect were specified in the model. A logit link was used, and the binomial distribution was specified. A likelihood ratio test and a *t*-test of the log (odds ratio) parameter were used to test for a treatment effect. Clutch effects, age class, and whether rolling affected hatching were investigated in the analyses. All statistical computations were performed using SAS (SAS 1999).

Results

Cushioning/Wave Treatment

The model with no random effects had the best fit (AIC = 749.2). AIC scores for other models with random effects were >765.4. No overdispersion was indicated based on the fit of the no random effects model. CUSHION x AGECLASS, WAVE x AGECLASS, and WAVE x CUSHION x AGECLASS were not significant ($P > 0.38$ for each term, Table 3) and were dropped from the model. This means that the WAVE x CUSHION interaction did not depend on AGECLASS, and AGE effects did not depend on WAVE or CUSHION (df 2/116, $F = 0.970$, $P = 0.3820$). The final model contained the WAVE x CUSHION interaction, and the AGECLASS main effect (Fig. 1).

The results of the Type III Wald test of fixed effects of waves, cushioning, and embryo age in the final model for the probability of alligator eggs hatching following transportation on an airboat under different wave and cushioning conditions were as follows: WAVE (df 1/122, $F = 13.598$, $P = 0.0003$), CUSHION (df 1/122, $F = 0.752$, $P = 0.3874$), WAVE x CUSHION (df 1/122, $F = 1.956$, $P = 0.1645$), AGECLASS (df 2/122, $F = 5.567$, $P = 0.0049$).

Based on the reduced model for fixed effects, a Wald test of the WAVE simple

Table 3. The full factorial of fixed effects for the split-split-plot design of the waves/cushioning treatments of alligator eggs. Results are based on the fit of no random effects model.

	Degrees of freedom			
Source	Numerator	Denominator	*F*	*P*
	Type I Hypothesis Test			
Wave	1	116	4.4598	0.0368
Cushion	1	116	0.2735	0.6020
Wave x cushion	1	116	0.8673	0.3536
Ageclass	2	116	2.9198	0.0579
Wave x ageclass	2	116	0.3215	0.7257
Cushion x ageclass	2	116	0.4642	0.6298
Wave x cushion x Ageclass	2	116	0.9704	0.3820
	Type III hypothesis tests			
Wave	1	116	0.0044	0.9473
Cushion	1	116	0.0000	0.9950
Wave x cushion	1	116	0.0001	0.9937
Ageclass	2	116	3.3550	0.0383
Wave x ageclass	2	116	0.6872	0.5050
Cushion x ageclass	2	116	0.4902	0.6138
Wave x cushion x ageclass	2	116	0.9704	0.3820

effect was highly significant in the absence of cushioning (logit scale: $\hat{\mu} = 2.3191$, SE = 0.6548; odds ratio scale: $\hat{\mu} = 10.1661$, SE = 6.6942; $P = 0.0006$), but was less significant in the presence of cushioning (logit scale: $\hat{\mu} = 1.0676$, SE = 0.6233; odds ratio scale: $\hat{\mu} = 2.9084$, SE = 1.8128; $P = 0.0893$). In the absence of waves, cushioning had no significant effect (logit scale: $\hat{\mu} = 0.2384$, SE = 0.6996; odds ratio scale: $\hat{\mu} = 1.2692$, SE = 0.8879; $P = 0.7339$). However, in the presence of waves, cushioning improved the likelihood of eggs hatching (logit scale: $\hat{\mu} = -1.0131$, SE = 0.5565; odds ratio scale: $\hat{\mu} = 0.3631$, SE = 0.2021; $P = 0.0712$). There was no significant difference ($P = 0.4871$) in hatchability between eggs of age classes 12–14 days ($\hat{\mu} = 0.849$, SE = 0.0647) and 17–18 days ($\hat{\mu} = 0.786$, SE = 0.0636), but the hatch rate probability of eggs of age class 7–8 days ($\hat{\mu} = 0.494$, SE = 0.0806) was less than the other two age classes ($P < 0.010$ for each of the two comparisons, Fig. 1).

The ratio of the odds of hatching for eggs not cushioned to the odds of hatching for cushioned eggs was 0.3631 for eggs transported under rough conditions, based on the final model.

Roll Treatment

Based on the fit of the GLMM, the expected hatch rate was the same ($\hat{\mu} = 0.924$, SE = 0.0534) for both rolled and control eggs. Thus, egg rolling did not affect the probability of hatching ($\chi^2 = 0.1863$, DF = 1, $P = 0.8914$) for the ages of eggs in our experiment (13–24 days). Variability in hatching probability among clutches was not

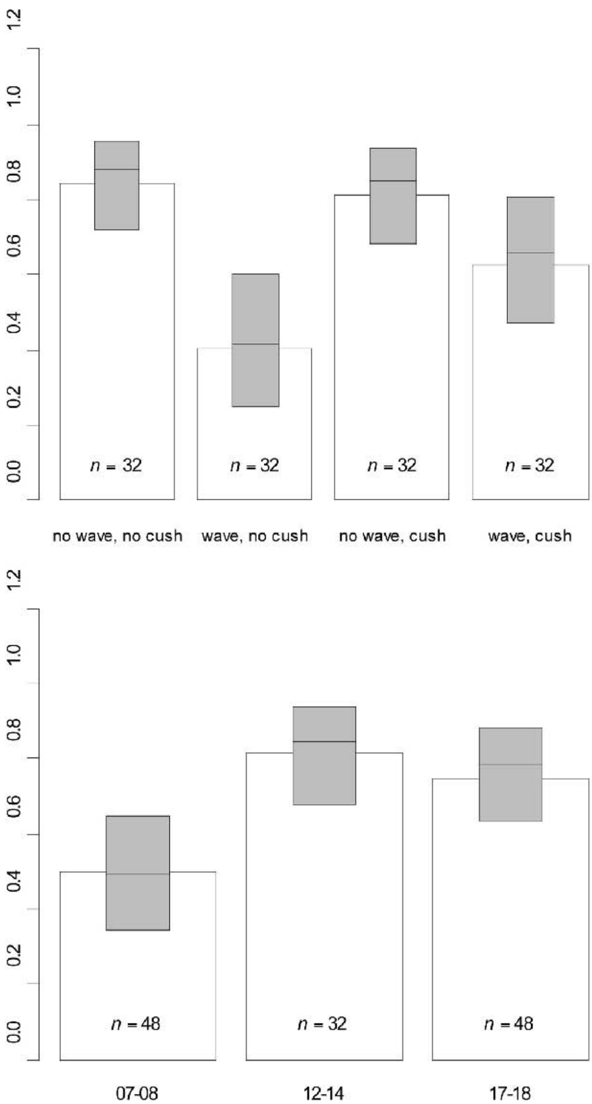

Figure 1. Proportion of sample eggs that hatched (hatch probability) for alligator eggs exposed to various combinations of wave-cushion and age class conditions separately. Shaded bars and horizontal lines indicate back-transformed 95% confidence intervals for expected values, and horizontal lines within the shaded bars indicate back-transformed expected values, based on the final model. The top of open bars indicate the observed mean hatch probabilities for this experiment.

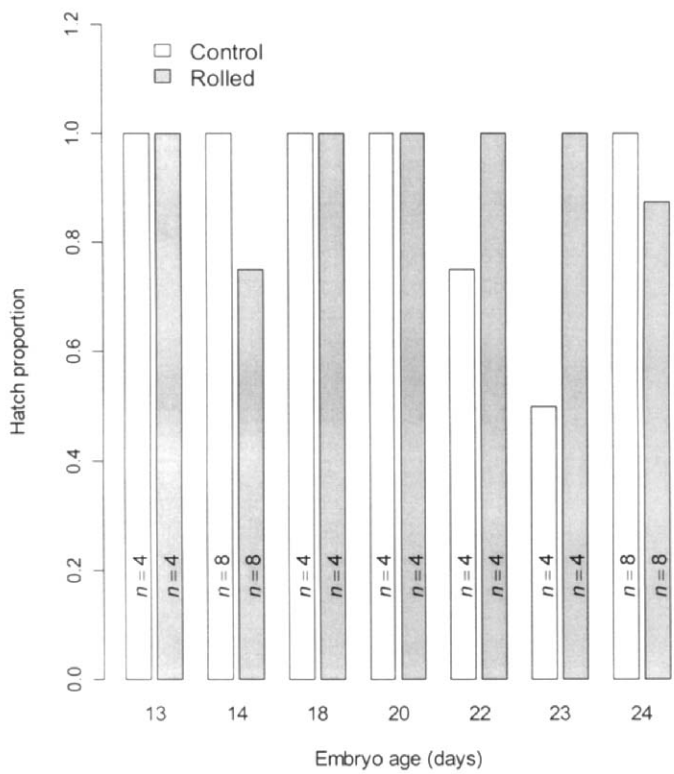

Figure 2. Proportion of sample alligator eggs that hatched (hatch proportion) for eggs exposed to a 360-degree roll (rolled) and eggs not rolled (control).

significant (t = 0.4604, P = 0.6575) (Fig. 2). This implied that age did not affect hatchability, given that all but two of the clutches differed in age. However, there was a wide confidence interval for the ratio (odds of hatching for control eggs)/(odds of hatching for rolled eggs), which indicated relatively poor power for detecting rolling effects on hatchability (90% CI for odds ratio was (0.20, 4.95)).

Discussion

Previous studies cautioned against collecting alligator eggs during certain stages of early incubation to avoid trauma-related mortality (Chabreck 1978, Ferguson 1985, Joanen and McNease 1987). However, it is generally advantageous to collect eggs as early as possible to preclude predation and flooding losses. In Florida, the optimum nest collection time, in terms of nest availability and risk of nest loss, appears to be near the end of oviposition (approximately 5–15 July). However, the age of embryos at this point may range from 1–40 days. We conducted experiments to determine if handling and transportation precautions would reduce choppy water-related mortality during this collection period.

Overall, we found that eggs in the 7–8 day age range were more susceptible to trauma-related mortality than older (12–14, 17–18 days) age classes, but this susceptibility depended on the level of trauma. This confirms reports from other studies that have found young eggs to be sensitive to collection trauma (Chabreck 1978, Ferguson 1985, Joanen and McNease 1987, Blake 1992). It is thought that increased care reduces trauma impact (Woodward et al. 1989) and findings from our study supported that notion. In our experiment, cushioning with 5-cm foam in the presence of choppy water significantly improved hatching success. Cushioning did not affect the hatching success of eggs when there was smooth water. Based on the final model, for eggs transported under rough conditions the ratio of the odds of hatching for eggs not cushioned to the odds of hatching for cushioned eggs was 0.3631. This translates to a reduction of hatch rates of 26%–51% for eggs transported under rough conditions without cushioning.

Because the experiment was conducted in the field, the WAVE treatment was inconsistently applied. We attempted to apply the same net amount of jarring to the eggs on all TRIPS with choppy waves and same lack of shock to the TRIPS with smooth water conditions. Although it can be argued that these are not true replicates, the experiment was conducted under authentic field conditions and still yields valid inferences in regards to whether ages are affected by trauma and whether cushioning is beneficial.

We saw no negative effects of eggs rolling 360 degrees for eggs of age 13 days and greater, though younger embryos may be more susceptible to rolling trauma, as they were in the wave-related study. We advise egg collectors to take precautions to prevent eggs from rolling to minimize the possibility of shearing the vascular membrane and increasing embryo mortality (Chabreck 1978, Ferguson 1985, Deeming and Ferguson 1991) in eggs of unknown developmental stage. Our study indicates that hatch rates of eggs aged over 13 days are not significantly affected by a single 360-degree roll. However, the power of our analysis was adequate to detect only very wide differences in hatch rates due to rolling. Our findings indicate that rolling does not inevitably result in the death of the embryo, and that rolled eggs of the ages we tested have a relatively high chance of surviving. We were unable to test eggs with embryos younger than 13 days, which may be more affected by rolling. Therefore, we would advise caution when handling young eggs.

It is difficult to determine the age of crocodilian eggs in the field, and it would be economically and logistically difficult to collect eggs during the first 24 hours after oviposition as suggested by Ferguson (1985). Further, weather and terrain conditions cannot be controlled, and situations might arise where eggs may be jostled during transport. A simple cushion system such as the layer of 5-cm thick foam padding we used in these experiments can significantly reduce mortality. Therefore, if alligator eggs are collected in June or early July, as most eggs are, we recommend using a system similar to this one for protecting against shock-related trauma.

Management Implications

Alligator embryos are vulnerable to shock-related trauma. This necessitates precautions to minimize trauma related mortality during egg collections. The roll experiment demonstrated that older eggs (>13 days) can survive a 360-degree roll. However, younger eggs may not fare as well. Because a wide age range is handled during egg collections, we recommend that commercial collectors and researchers take precautions to prevent any eggs from rolling.

It is best to avoid waves or rough terrain, if possible, while transporting alligator eggs. Because this cannot always be done, we recommend cushioning egg containers with a 5-cm thick layer of foam. This simple precautionary measure can significantly reduce mechanical trauma to eggs and, thereby, increase hatch rates. We recommend additional cushioning as a standard procedure for alligator egg collections in Florida, where eggs are frequently collected on large lakes and streams where wave conditions are variable and choppy water is sometimes difficult to avoid. We suspect that our findings would also apply to transporting eggs through dry or semi-wet marshes were rough terrain might result in mechanical trauma to eggs.

Our experiments would have benefited from the inclusion of more eggs from younger clutches. It would be advantageous if further trauma experiments were conducted under more controlled conditions. Because this experiment was conducted in the field under realistic conditions, it was difficult to control the amount of shock during each TRIP. This can be more accurately regulated within a laboratory. We expect laboratory experiments would compliment our findings and would provide more precise information on the amount of shock required to damage embryos.

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Literature Cited

Blake, D. K. 1992. A basic method for the collection and incubation of crocodilian eggs. Journal of the Herpetological Association of Africa 40:97–100.

Chabreck, R. H. 1978. Collection of American alligator eggs for artificial incubation. Wildlife Society Bulletin 6:253–256.

Deeming, D. C. and M. W. J. Ferguson. 1991. Egg turning during incubation has no effect upon the growth of embryos of *Alligator mississippiensis*. Acta Zoologica 72:125–128.

Ferguson, M. W. J. 1982. The structure and composition of the eggshell and embryonic membranes of *Alligator mississippiensis*. Transactions of the Zoological Society of London 36: 99–152.

______. 1985. Reproductive biology and embryology of the crocodilians. Pages 329–491 *in* C.

Gans, F. Billett, and P. Maderson, eds. Biology of the Reptilia. Vol. 14. John Wiley and Sons, New York, New York.

Florida Fish and Wildlife Conservation Commission (FLFWCC). 1998. Regulations Governing the Operation of Alligator Farms. 68A-25.004 FLFWCC. *http://www.wildflorida.org/gators/farming/regulations.htm*

Joanen, T. and L. McNease. 1987. Alligator farming research in Louisiana. Pages 329–340 *in* G. J. W. Webb, S. C. Manolis, and P. J. Whitehead, eds. Wildlife management: crocodiles and alligators. Surrey Beatty and Sons, Chipping Norton, New South Wales, Australia.

Limpus, C. J., V. Baker, and J. D. Miller. 1979. Movement Induced Mortality of Loggerhead Eggs. Herpetologica 35(4):335–338.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Institute Inc., Cary, North Carolina.

Moses, R. D. 1989. Effect of time of collection, transportation, and incubation materials on hatching success of artificially incubated American alligator eggs. Thesis, Louisiana State University, Baton Rouge.

______ and R. H. Chabreck. 1990. Transportation and artificial incubation of American alligator eggs. Crocodiles Vol 2: Proceedings of the 10th working meeting of the Crocodile Specialist Group, IUCN - The World Conservation Union, Gland, Switzerland.

Pinheiro, J. C. and D. M. Bates. 1995. Approximations to the log-likelihood function in the nonlinear mixed-effects model. Journal of Computational and Graphical Statistics 4:12–35.

SAS. 1999. *SAS* Online *Doc, Version 7-1.* SAS Institute Inc., Cary, North Carolina.

Smith, C. A. and J. M. P. Joss. 1994. Sertoli cell differentiation and gonadogenesis in *Alligator mississippiensis.* Journal of Experimental Zoology 270:57–70.

Webb, G. J. W., S. C. Manolis, P. J. Whitehead, and K. Dempsey. 1987. The possible relationship between embryo orientation, opaque banding, and the dehydration of albumen in crocodile eggs. Copeia 1987:252–257.

Wolfinger, R. and M. O'Connell. 1993. Generalized linear mixed models: A pseudo-likelihood approach. Journal of Statistical Computation and Simulation 48:233–243.

Woodward, A. R., M. J. Jennings, and H. F. Percival. 1989. Egg collecting and hatch rates of American Alligator eggs in Florida. Wildlife Society Bulletin 17:124–130.