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Effects of two different incubation media on hatching success, body mass, and length in *Caiman latirostris*

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Abstract

Since 1990, Proyecto Yacaré (Min. Prod./MUPCN) is developing new technologies and improving/adapting old ones to reduce the production costs for the ranching programs in South America. Previous work evaluated growth rates of *Caiman latirostris*, the effect of time of collection on hatching success, depredation and temperature effects on incubation, but there is no information about incubation media for the species. In this experiment, we test the effects of two incubation media, vermiculite and grasses ('nesting material'), on hatching success, and hatchling size. We collected nine nests (350 eggs) from natural *C. latirostris* populations in Santa Fe, Argentina soon after oviposition. In Proyecto Yacaré facilities, each nest was divided in two groups and each one received one of two incubation treatments (vermiculite or nesting material as incubation media). We found no difference in hatching success among the treatments (89% for vermiculite and 87% for nesting material). We observed, but did not measure, that incubation with vermiculite tended to be longer and that hatchlings from this treatment had more unabsorbed abdominal yolk in all the nests used in the experiment. Hatchlings from nesting material treatment were longer ($p<0.001$) than those incubated in vermiculite, but we found no differences between treatments in body mass. As we found similar hatching success in both treatments (but possibly overestimated in vermiculite), a larger size of hatchlings (which could enhance survivorship as was reported in lizards and turtles) and lower cost, we consider that there is no advantage to the use of vermiculite over nesting material. We recommended nesting material (natural grasses) as incubation media. Future investigations should address how incubation media might modify hatchling performance.

Keywords: Caiman latirostris; Incubation; Nesting material; Vermiculite; Hatching success

1. Introduction

In 1997, *Caiman latirostris* populations within Argentina were changed from CITES Appendix I to Appendix II under the Ranching resolution. In order to improve the emerging ranching programs,

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Proyecto Yacaré (Min. Prod./MUPCN) in Santa Fe Province is developing new technologies and improving/adapting old ones to reduce production costs. These programs in Argentina consist of the collection of eggs of *C. latirostris* for artificial incubation; a percentage of the hatchlings produced are reintroduced into the wild, and the remaining animals are used for commercial use of leather and meat.

Larriera (1995) evaluated the effect of time collection on hatching success, reporting that the best time for egg collection is at the beginning of the incubation process. Collecting eggs at that time reduces losses due to flooding or depredation (Larriera and Piña, 2000) and optimizes the harvesting in terms of number of viable eggs collected/hour of work.

Once the eggs are collected they are transported for artificial incubation. *C. latirostris* eggs can be handled with a minimum of expertise and tolerate horse transport and truck shipment with minimum losses. When programs such as Proyecto Yacaré harvest the eggs at the optimum time and do it in the proper manner, their future success would, in part, depend on efficient artificial incubation.

Information is available about growth rates of *C. latirostris* in captivity (Larriera and Aguinaga, 1990; Piña and Larriera, 2002; Piña et al., 1996), but only a few studies have been done about incubation (Larriera, 1995; Piña, 2002; Piña et al., 2003) which forms the base of the ranching programs in Argentina. There is no information about incubation media for the species. In this experiment, we tested the effects of two different materials widely used as incubation media, vermiculite and grasses ('nesting material'), on hatching success, total length (TL), snout–vent length (SVL), and body mass (BM) in order to provide information for the management programs of *C. latirostris* in Argentina.

2. Materials and methods

During summer 2001/2002, we collected nine nests (350 eggs, clutch size ranged from 28 to 40) from natural *C. latirostris* populations in Santa Fe, Argentina (29°58′S; 60°5′W), within 5 days after oviposition (based on Donayo et al., 2002). All the nests were collected on the same day and maintained under the same conditions from harvest to treatment assignment. Before harvesting, eggs were marked in the nest to maintain the natural upright position, placed in plastic containers and transported to Santa Fe city by road. Once in Proyecto Yacaré facilities in Santa Fe city, each nest was randomly divided in two groups and each half received one of two incubation treatments. Incubation treatments consisted of using vermiculite or nesting material as incubation media. Prior to be used in the incubation, nesting material was hydrated by submerging it in water for 12 h. Vermiculite was hydrated adding 100 ml of water to 100 g of vermiculite. During the experiment, both treatments measured over 92% relative humidity.

We placed each nest (both treatments) in the same plastic grid for artificial incubation in Proyecto Yacaré's incubator. A temperature of 31±1 °C was maintained during incubation. We followed the same procedure for the nine nests, producing nine independent groups for each treatment.

Following Larriera (1993), when hatchlings started to call within the eggs, the whole nest was removed from the incubation, and eggs were assisted with hatching. We recorded the number of hatchlings produced for each nest in each incubation treatment. The hatchlings were measured for total length (TL), snout–vent length (SVL), and body mass (BM) within 24 h after hatching.

We analyzed hatchling success with a Chi square test (χ^2) and ran a factorial ANOVA for each measurement (TL, SVL, and BM) where incubation treatment and nests of origin were the grouping factors. We used InfoStat for Windows (InfoStat., 1998) for the analysis.

Table 1
Mean size±standard error (TL, SVL, and body mass) of the nine nests incubated with vermiculite or nesting material and their respective hatching success (no. of hatchlings/no. of eggs)

	Total length (cm)	Snout–vent length (cm)	Body mass (g)	Hatching success
Vermiculite	24.87±0.06	11.84±0.03	48.25±0.23	(159/178) 0.89
Nesting material	25.49±0.06	12.08±0.03	47.84±0.24	(150/172) 0.87
P-value	<0.001	<0.001	=0.230	=0.8376

3. Results

In this experiment, we found no difference in hatching success among the treatments (89% for vermiculite and 87% for nesting material; χ^2=0.04; *df*=1; *P*=0.8376; Table 1). We observed, but did not measure, that incubation time with vermiculite tended to be longer and that hatchlings from this treatment had more unabsorbed abdominal yolk than incubation in nesting material in all the nests used in the experiment.

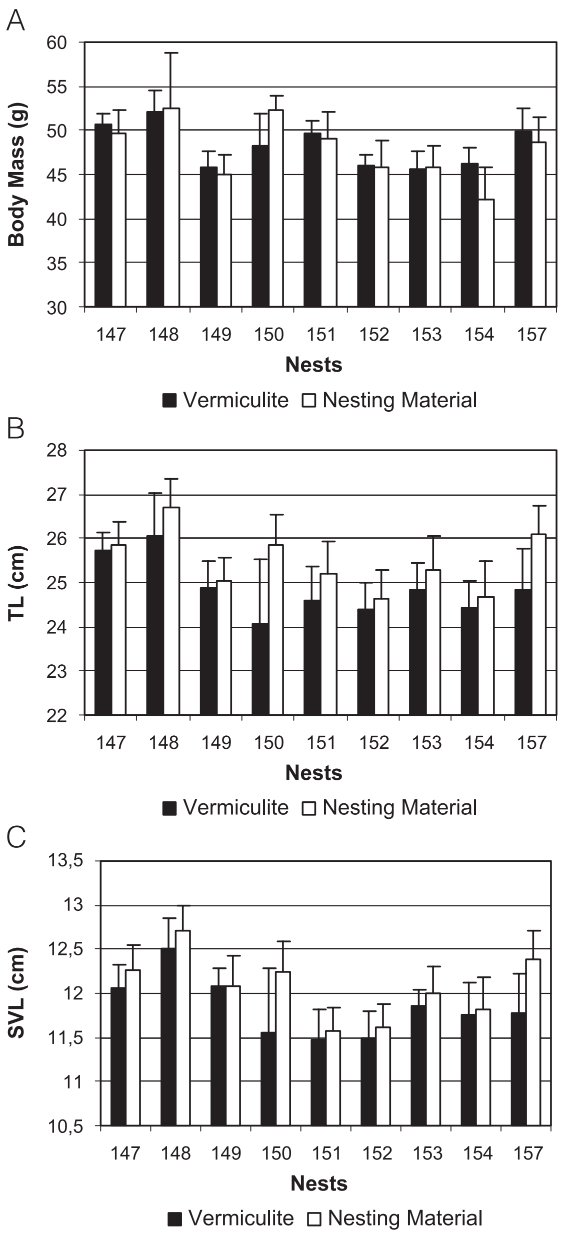

Fig. 1. Body mass (A), total length (B), and snout–vent length (C), and their associated standard deviation for the nine nests used in the experiment. Half of each nest was incubated in vermiculite, the rest in nesting material (grass dehydrated in direct sun and then hydrated in water).

We found that the incubation media affected hatchling length (TL and SVL; *P*<0.001) but not BM (*P*=0.230; Table 1). The nest was a significant source of variation for the three variables (*P*<0.001) and the size of the hatchlings was different among the nests; not all the nests responded in the same way to treatments since we found an interaction between nests and incubation treatments (*P*<0.001) in all the variables (Fig. 1A–C). Hatchlings from nesting material treatment were bigger than vermiculite in both length variables, but difference in total length within the same nest varied from 0.11 to 1.97 cm, whereas in SVL differences varied from no difference in nest 149 to 0.68 cm in nest 150 (Fig. 1B and C). Regarding BM, some of the vermiculite nests had large hatchlings as did some from the nesting material treatment (Fig. 1A).

4. Discussion

We present a simple experiment in which incubation with 'nesting material' was shown to produce the same hatching success but longer, not heavier hatchlings than incubation with vermiculite. It was reported that nest material serves many important functions: it cushions the eggs from shock and stress, insulates so that the temperature does not change dramatically, maintains egg moisture levels, and produces bacteria media for exterior shell degradation (Ferguson, 1981). Because of our procedure of assisting hatching, we could have underestimated differences between treatments in hatching success since smaller hatchlings from the vermiculite treatment could be less able to open eggs without assistance compared to longer hatchlings.

Joanen and McNease (1981) recommend using fresh natural nest material if it is suitable, their best hatching results were obtained using wire grass (*Spartina patens*), about 81% success. In addition, Chabreck (1971) indicated that organic materials are best for artificial incubation. Child (1987), on the other hand, recommended use of moistened vermiculite (an extremely light micaceus one) for incubation into styrofoam boxes. In this experiment, we had a mean hatching success of 88%.

In some reptiles, the mineral layer appears to degrade during incubation, which may enhance gas exchange (Ferguson, 1982). In reptilian eggs, variation in the structure and function of the eggshell during incubation may moderate the extent of hypoxia and hypercapnia that accompany increasing metabolic demands as embryos grow. Degradation can occur extrinsically by the withdrawal of solids from the eggshell (Jenkins, 1987) because of the influence of microorganisms and /or organic acids (Ferguson, 1981). This causes a weakening of the calcareous shell, making for an easy exit from the egg when incubation was complete. In addition, it was reported that failure to promote increasing porosity during incubation may cause hatchling mortality from asphyxia (Ferguson, 1981). Based on this scenario, we assume that we may have overestimated spontaneous hatching success in the vermiculite treatment, since hatchlings were smaller, and the eggshell perhaps stronger than in the nesting material treatment.

The indication of a longer incubation period in the vermiculite treatment could be due to the stronger buffering effect of vermiculite avoiding extreme high temperatures during the incubation compared to nesting material. Proyecto Yacaré's incubator functions with heat only and is unable to cool down temperatures during the incubation process. At the maximum temperatures during the day, the vermiculite might not be at the same rising temperatures as nesting material and thereby maintaining a lower metabolism than the other treatment, which produces an extended incubation period. We know that incubation temperature affects incubation period (Piña et al., 2003) and hatchling body size (Piña, 2002). In this experiment, some eggs (those from nests 148, 150, 151, 153, and 157) incubated with 'nesting material' produced longer hatchlings but of the same BM. In a previous study with *C. latirostris*, there was no difference in hatchlings BM incubated at different temperatures, but TL varied, producing longer animals at higher incubation temperatures (Piña, 2002), but this is not the case in *Crocodylus johnstoni*, where animals produced at lower temperatures were bigger (Webb et al., 1987). Moreover, there is evidence that nests interact with incubation temperature in TL and BM in both *C. latirostris* and in *Alligator mississippiensis* (Piña, 2002; Allsteadt and Lang, 1995), and this could be the explanation of hatchlings size and incubation temperature inconsistencies among species or works.

Based on our results, we assume that eggshell degradation in the 'nesting material' treatment improved O_2 and CO_2 diffusion. This scenario, added to the temperature buffering effect of vermiculite, allowed a higher metabolism in the nesting material treatment, and produced longer hatchlings and a reduced incubation period (Piña, 2002; Piña et al., 2003). As we found similar hatching success in both treatments (but possibly overestimated in vermiculite), a larger size of hatchlings on half the nests used in this experiment (which could enhance survivorship as was reported in lizards and turtles; Ferguson and Fox, 1984; Janzen et al., 2000; Du and Ji, 2003) and lower cost, nesting material is the recommended incubation media.

Future investigations should address how incubation media might modify hatchling performance. It would be particularly instructive to examine how feeding, physiology, behavior, growth, and survivorship is affected by incubation media during the first year.

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