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Effect of Translocation on Egg Viability of the Giant Amazon River Turtle, *Podocnemis expansa*

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The giant Amazon river turtle (*Podocnemis expansa*) is the largest living South American freshwater turtle. Adult females have a flattened carapace up to 107 cm long (Ernst and Barbour, 1989). Its distribution includes the Orinoco and Essequibo to Amazon river drainages of Colombia, Venezuela, Guyana, eastern Ecuador, northeastern Peru, northern Brazil, and northern Bolivia (Iverson, 1992).

The eggs and meat of the species have been traditionally used by local people in the Brazilian Amazon, being socially important for the upper classes and economically important for the lower classes, which led the species to various levels of population decline in many parts of the Amazon (Pritchard and Trebbau, 1984; Alho, 1985). This decline stimulated the Brazilian government to establish in 1979 a ranching program of the species based on egg collection and captive raising of young for commercial purposes on a biologically sustainable basis (CENAQUA, 1989).

The species has a nesting behavior similar to that of some marine turtles with a markedly concentrated nesting period (Moll, 1979) which has been erroneously assumed to be related to low water levels (Alho and Pádua, 1982a; 1982b) and subsequent formation of sand beaches, known locally as *tabuleiros* (Alfinito, 1976). Average nesting female body mass can vary from 15.7 to 33.0 kg even in the

same population and as many as some thousands may nest simultaneously (Ojasti, 1967) which is locally called an *arribada* in Spanish (Carr, 1967) or *arribação* in Portuguese (Alfinito, 1976). Females may possibly store sperm after copulation (van Tienhoven, 1983) and multiple paternity has been described (Valenzuela, 2000).

Although successfully used for caiman in Brazil (Verdade, 1985; Verdade et al., 1992) artificial incubation has been discarded as a management tool for the *P. expansa* ranching program because of the immense number of nests that would have to be housed (CENAQUA, 1989). However, a high rate of unhatched eggs have been reported in some *tabuleiros*, and the usual occurrence of unseasonal short-term flooding in the middle of the nesting period — known locally as *repiquete* (Pádua and Alho, 1984) — generally causes massive egg mortality (CENAQUA, 1985).

In order to minimize egg loss caused by this flooding without having to invest in artificial incubators, translocation of eggs has been proposed (Alho, 1985). However, its possible influence on egg viability has never been determined. This was the main goal of the present study.

Methods. — Eggs of *P. expansa* were collected from September to December 2000 in the following *tabuleiros* of Rio Araguaia: 04GO (Praia 04 GO: 13°21'57.6"S; 50°39'05.7"W) and 06MT (13°23'32.3"S; 50°40'12.8"W), on the border between Goiás (GO) and Mato Grosso (MT) states, central Brazil. We tested four different translocation periods for the eggs, moving them at age 1, 14, 28, and 42 days since egg-laying.

We used eight nests per treatment (i.e., translocation period) with a total of 32 nests. The eggs were collected early in the morning (0700 to 0930 hrs) or late in the afternoon (1600 to 1830 hrs). Clutch size and the number of unviable eggs (damaged, decomposing, or no embryonic development) were recorded for each nest. Clutches were transported in separate sand-filled plastic boxes with the eggs placed in layers, reversed in relation to their original position in the nest (i.e., top layers placed in the bottom and vice-versa). The top of each egg was pencil-marked and eggs positions were maintained unaltered (i.e., eggs were not turned, so as to prevent egg mortality by respiratory or excretory dysfunction as suggested by Webb et al., 1987, for crocodile eggs).

All clutches were transported to a sand beach (18GO: 13°30'12.4"S; 50°44'12.4"W), a few kilometers from the *tabuleiros* of origin and less likely to be flooded. Careful transportation was by boat with the egg boxes eggs placed on a thick layer of foam and shaded by a layer of local soft weed (usually "*macela*", *Gnaphalium purpureum*) to prevent desiccation and overheating.

Artificial nests 50 cm deep and 25 cm wide were manually excavated one meter apart. The eggs were placed in the artificial nests in their original layer relationship. Local sand was gently spread over the eggs in order to fill up the nest without harming the eggs. Nests were individually identified by numbered stakes. A plastic fence (100 cm high, 15 cm buried in sand) was placed around the nests forming

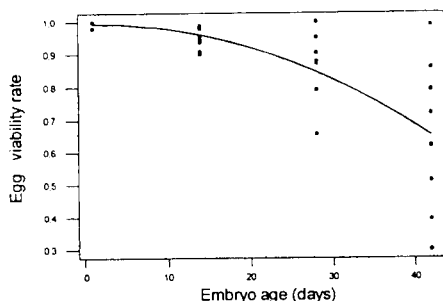


Figure 1. The relationship between egg viability rate on the day of translocation (y_1) and the age of embryos in days (x).

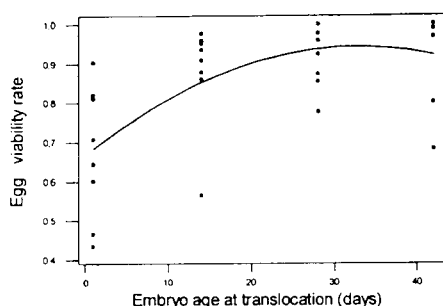


Figure 2. The relationship between egg viability rate at the end of incubation (y_2) and the age of embryos in days at translocation (x).

a "nursery" of 56 m² (8 x 7 m) in order to prevent egg predation and hatchling escape.

Nests were manually opened at day 45 of incubation (counting from laying date, not translocation date). The number of viable and unviable eggs (damaged, decomposing, or no embryonic development) was then recorded.

We used Analysis of Variance (ANOVA) to evaluate the effect of translocation on egg viability and Tukey Test to compare treatments. We also established regression models in order to assess embryo losses related to embryo age and egg translocation.

Results. — There was a significant relationship between egg viability rate and both age of embryo (Fig. 1) and age of embryo at translocation (Fig. 2). Translocation at day 1 of the incubation period resulted in a significantly higher embryo mortality than at days 14, 28, and 42 (Tukey Test, $\alpha = 0.05$), but there was no significant difference among these later three periods of translocation.

The relationship between egg viability rate on the day of translocation (y_1) and the age of embryos in days (x) can be expressed by the following regression equation (Fig. 1): $y_1 = 0.993647 + 0.000461x - 0.0002050x^2$; ($p < 0.0001$; $r^2 = 0.542$).

The relationship between egg viability rate at the end of incubation (y_2) and the age of embryos in days at translocation (x) can be expressed by the following regression equation (Fig. 2): $y_2 = 0.666665 + 0.0168995x - 0.0002581x^2$; ($p < 0.001$; $r^2 = 0.403$).

Discussion. — Natural mortality of embryos during the incubation period can be associated with intrinsic (both genetic and phenotypic) characteristics of eggs and embryos

as well as with the physical conditions of incubation. Low fertility rate of females may be the main cause for infertile eggs in crocodylians (Cardeilhac, 1989, 1990; Verdade et al., 1993) as well as in turtles (CENAQUA, 1985). Egg size (Gutzke and Packard, 1985), nesting site (Campos, 1993), and clutch volume (Schulte and Chabreck, 1990) may be associated with embryonic mortality in reptiles. However, temperature (Yntema, 1960; Ewert, 1985; Plummer et al., 1994), humidity (Grigg, 1987; Kam, 1994) and their interaction (Bustard, 1971) are possibly the main physical conditions related to embryonic mortality in reptiles.

Malvasio (2001) reported a temperature range in *P. expansa* nests in the northern Rio Araguaia from 25.8 to 39.1°C, with the upper limit, if sustained, possibly causing significant embryonic mortality. Although Malvasio worked at a lower latitude, a similar pattern can be expected for the *tabuleiros* in the present study. Eventual exposure to temperatures above or below a certain level usually causes embryonic mortality (Ewert, 1985; Plummer et al., 1994; Tucker and Warner, 1999). However, in such cases, a high embryonic mortality usually occurs at early stages of incubation, which did not occur in the present study.

Humidity can influence clutch size in turtles in that smaller clutches are usually associated with drier substrates (Zug, 1993). A dry substrate can cause embryonic death by desiccation, whereas a soaked substrate can result in suffocation of the embryos (Fenwick, 1992). Even a brief submergence of nests can result in high embryonic mortality (Plummer, 1976; Joanen et al., 1977; Magnusson, 1982). The distribution of nests on a *tabuleiro* can possibly result in significantly different humidity among the nests (Zwinnick and Young, 1990) because humidity in sand can vary significantly even on a small temporal-spatial scale (Sanchez, 1981). This would result in a cumulative downward curve of mortality along the incubation period (Zwinnick and Young, 1990). The curve of egg viability found in the present study (Fig. 1) can possibly be explained by these reasons.

Parasitism can also result in high embryonic mortality. Lopes (1982) identified *Eumacronychia sternalis* (Diptera, Sarcophagidae) larvae living on eggs and hatchlings of the east Pacific green turtle (*Chelonia mydas*). Broderick and Hancock (1997) report insect (four species of fly and one species of wasp) infestation on eggs of Mediterranean marine turtles (*Chelonia mydas* and *Caretta caretta*). Vogt (1981) reported a low eclosion rate in 39 out of 236 nests of *Graptemys* spp. and *Chrysemys picta* in Wisconsin, USA, due to phorid fly larvae infestation. In the present study, some nests contained fly larvae, that may have contributed to embryo mortality.

The temperature of incubation determines sex in *P. expansa* (Valenzuela, 2001) and other turtles (Bull, 1980; Mrosovsky and Yntema, 1980; Ewert et al., 1994; Shine, 1999). Embryonic development (Packard et al., 1981; McGehee, 1990; Whitehead et al., 1990), post-hatching growth (Joaen et al., 1987; Webb and Cooper-Preston, 1989), and skin pigmentation (Deeming and Ferguson, 1989) are also influenced by the incubation environment in rep-

tiles. These factors are possibly related to individual fitness, and their careless manipulation for management purposes, even when well intentioned, might result in deleterious consequences for the species. For these reasons, they should be carefully considered by ranching program managers.

In the present study, early (first third of incubation) translocation was associated with higher embryonic mortality, similar to the pattern found in sea turtles, unless eggs are moved during the first six hours after laying (Frazer, 1994). This period does not coincide with the thermosensitive period for sex determination in turtles, which occurs in the middle third of incubation (Mrosovsky and Yntema, 1980; Bull and Vogt, 1979; Gutzke and Chymiy, 1988; Wibbles et al., 1991; Pieau et al., 1994).

Although the factors above should be considered, egg translocation after the first third of the incubation period can be an effective tool for ranching programs of *P. expansa* in regions where short-term flooding (i.e., *repiquete*) is common during the incubation period. Although there are good ranching programs based on egg collection and captive rearing of crocodylians (Joanen and McNease, 1987; Larriera et al., 1996; Velasco and De Sola, 1997), freshwater turtles (CENAQUA, 1989), and sea turtles (Reichert, 1982), its effectiveness for the conservation of those species is controversial (Frazer, 1992; Meylan and Ehrenfeld, 2000). However, either for conservation or management purposes, further research on the possible influences of egg translocation on sex determination of embryos and fitness of young is urged. In particular, the shape of the translocation mortality curve during the first third (1 to 14 days) of the incubation period should be determined.

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