

## Monitoring an Endangered Freshwater Turtle Management Program: Effects of Nest Relocation on Growth and Locomotive Performance of the Giant South American Turtle (*Podocnemis expansa*, Podocnemididae)

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**ABSTRACT.** – Since 1993 the Venezuelan government has implemented an intensive management program aiming to increase the population size of the endangered giant South American turtle (*Podocnemis expansa*). Including nest relocation and a year of captive rearing of hatchlings, the program affects the main Venezuelan *P. expansa* population, located in the middle Orinoco River. Although the program seems to be increasing recruitment of new individuals into the population, the effects of such nest relocation on long-term fitness-correlated parameters have not been addressed. Here we measured posthatching mortality, presence of morphological abnormalities, first-year body growth rate, and locomotive performance of juvenile turtles subjected to this management program. Nest relocation had a profound influence on juvenile *P. expansa* turtles, affecting their mortality, morphology, body growth rate, and locomotive performance. Transplanted clutches showed higher hatchling mortality and higher incidence of morphological abnormalities, though higher growth rates than nontransplanted clutches. Likewise, juvenile turtles coming from nontransplanted clutches appeared to run faster than those coming from transplanted nests. Clutch manipulation seemed to be responsible for the occurrence of morphological abnormalities and the increased mortality. Higher temperatures, on the other hand, may be the cause for the observed higher body growth rates in transplanted nests. Our results suggest that future management efforts minimizing the effects of clutch manipulation will not only prevent clutch losses but also enhance the long-term survival of neonates.

**KEY WORDS.** – Reptilia; Testudines; Podocnemididae; *Podocnemis expansa*; turtle; endangered species; management programs; nest relocation; turtle conservation; turtle growth rate

Turtle conservation is a subject of great international interest (Swingland and Klemens 1989; Lutcavage et al. 1997; Eckert et al. 2000; Townsend et al. 2005). Turtles are long-lived animals, requiring a long time to reach sexual maturity due to their slow growth rates (Gibbons 1994). Even small species do not reproduce before 7 or 8 years of age (Pough et al. 1999). Because of this, turtle populations generally have low recruitment rates, and their population dynamics are very susceptible to adult mortality due to hunting or habitat perturbations (Gibbons 1990; Congdon et al. 1993, 1994). Many conservation efforts have been oriented precisely on trying to boost low recruitment rates of endangered turtle populations (Lutcavage et al. 1997; Eckert et al. 2000; Townsend et al. 2005) by enhancing survival and fecundity of easily accessible life stages: nesting females, eggs, and hatchlings.

In species that lay nests in suboptimal locations (e.g., with high predation pressures, high contamination levels, or under flood risk) some management programs have been implemented in order to relocate clutches to safer areas, such as protected nature reserves or captive breeding

facilities. However, although egg survival is greatly improved in most cases, variations in the incubation environment (temperature, humidity, and gas concentration) coupled with the egg manipulation, are known to affect turtle embryonic development, posthatching growth, and physical condition of hatchlings (Janzen and Paukstis 1991; Sinervo and Doughty 1996; Ackerman 1997; Wilson 1998; Packard and Packard 2001). Hence, studies quantifying the impact of clutch manipulation over the first life stage of hatchlings are needed in order to determine the impact of these manipulations on the population's recruitment rates.

In Venezuela, an intensive nest relocation program with the endangered giant South American turtle, *Podocnemis expansa* (Schweigger 1812) has been ongoing for almost 15 years. With adult females reaching a straight carapace length (SCL) of 90 cm and weighing more than 50 kg, *P. expansa* represents the largest freshwater turtle in the Neotropics. Basically herbivorous and mostly aquatic, it inhabits slow-flowing rivers, lakes, and swamps occurring in the Amazon, Orinoco, and Essequibo basins in South America (Carazo et al. 1997).

Nesting females concentrate once a year close to seasonal sand banks that are exposed during the dry season. At night they emerge onto the beaches and lay one clutch of approximately 90 eggs in the sand. After 42–68 days of incubation, hatchling turtles run towards the water line where they immediately submerge. Both eggs and neonates are known to suffer from high natural predation, from insects, fish, toads, crocodiles, lizards, raptor birds, and carnivorous mammals (Carazo et al. 1997). Moreover, some nests that are laid close to the waterline or have unusually long incubation periods, are flooded by rising river waters during the onset of the rainy season, losing all their eggs (Ojasti 1971; Alho and Pádua 1982; Vogt 1990; Licata 1994; Carazo et al. 1997).

Until the mid-20th century, *P. expansa* was one of the most valuable human food resources in the Amazon and Orinoco basins (von Hildebrand 1985; von Hildebrand et al. 1997). However, in spite of having been extremely abundant in these regions, overexploitation of eggs and adults has led to a drastic decline in their populations, restricting them to relatively isolated areas. Today, *P. expansa* is Critically Endangered in Venezuela (Rodríguez and Rojas-Suárez 1998), of Lower Risk, though Conservation Dependent, according to the IUCN Red List (IUCN 2006), and is included in CITES Appendix II (CITES 1995).

Following Carazo et al. (1997), the main threats currently affecting population growth of *P. expansa* are capture of adult and subadult individuals for human consumption, natural predation of hatchlings and juveniles, flooding of nests, and exploitation of eggs. Taking this into account, in 1993 the Venezuelan Ministry of Environment (MINAMB, formerly MARN) implemented an intensive nest relocation program in the main nesting beaches within the Arrau Turtle Wildlife Refuge (middle Orinoco River), coupled with a captive rearing program of hatchlings. Every nest located in areas susceptible to flooding is relocated to safer areas within the same beach and identified with its oviposition date. After hatching, juvenile turtles are transported to rearing facilities at Puerto Ayacucho where they are maintained for a year. Finally, turtles are released back into the Refuge.

Survival of transplanted nests and recapture of reintroduced juvenile turtles raised in captivity suggest the program might indeed be promoting recruitment of new individuals into the population (Venezuelan Ministry of Environment, unpubl. data 2006; Hernández 2003; Hernandez and Espín 2006). However, the effect of nest relocation on long-term fitness-correlated parameters has not been addressed so far. Since current management affects Venezuela's main turtle population (Pritchard and Trebbau 1984), it is very important to quantify its influence, if any, on survival and reproduction of these turtles.

In this study, we measured posthatching mortality, presence of morphological abnormalities, first-year growth rate, and locomotive performance in juvenile *P. expansa*

turtles subjected to the nest relocation program, comparing them to turtles reared in naturally incubated clutches. We discuss the implications of nest relocation on hatchling survival, providing basic guidelines to help enhance the efficiency of this management program.

## METHODS

The study was carried out in the Arrau Turtle Wildlife Refuge, located in the middle Orinoco River, Apure and Bolívar States, Venezuela (lat 06°33'37"N, long 67°07'36"W). All nests employed were found in the western side of the main nesting sand beach of the refuge (the natural area). Relocated nests were transported to the "transplant area," located 1.5 km upstream in the same sand beach. Before collecting the hatchling turtles, we measured sand temperature in 15 nests from the natural area and 15 nests from the transplant area. These nests were chosen at random in order to obtain the general temperature profiles of both incubation areas. A total of 30 Ibutton® temperature data loggers were placed at 0.6-m depth (average nest depth for this population), recording sand temperature to the nearest 0.5°C every hour from 9 March to 23 April 2003, during the incubation period. Once we retrieved the sensors, data was downloaded directly to the computer for later analysis.

*Sample.* — Between 8 and 23 February 2003, 6 clutches located near the shore were excavated and relocated into the transplant area. The nest relocation was performed the morning following the oviposition night or within the next day, following the same procedures employed in the MINAMB's nest relocation program. In order to quantify the effects of egg manipulation, we did not consider initial egg size, since this would have implied manipulating the control, e.g., the naturally incubated clutches. From 19 to 22 April, we collected the hatchling turtles from these 6 transplanted clutches and 4 other naturally incubated ones. All nonhatched eggs found in the nests were registered in order to estimate hatching success as the percentage of hatched eggs. We then chose 230 individuals, randomly selecting up to 28 individuals per nest, and excluding those with deformities that affected locomotion. Nonselected individuals were released back into the river. Selected hatchlings were disinfected for 15 minutes in chlorinated water, marked by clipping their shell, and kept in plastic crates for 4 days until they were transported to laboratory growth tanks in Puerto Ayacucho, Amazonas State, run by the Foundation for the Development of Physical and Natural Sciences (FUDECI). To avoid dehydration while kept in the refuge, turtles were put in water for 30 minutes every day. Turtles were disinfected again once they arrived at FUDECI and later kept in 1/16 of a 12-m diameter × 0.9-m-high Australian holding tank. After waiting 2 weeks for complete yolk absorption, young turtles were fed every other day with 2% body weight worth of high-protein fish

meal (28% protein). All turtles were later released as yearlings on the nesting beach where they were collected.

*Parameters Assessed.* — Soon after releasing the turtles in the Australian holding tank all individuals were measured, and if present, morphological abnormalities were classified into 3 groups: irregular marginal scutes, irregular plastral scutes, and supernumerary scutes. We checked the tank daily and removed any dead individuals. Mortality was calculated as the number of dead individuals after the entire year of captivity.

Growth rate was determined by taking 5 body measurements each week during the first 3 months and then again every 3 months from June 2003 to May 2004. On measurement days, we collected turtles in 2 groups (morning and afternoon), keeping them out of the tanks no more than 4 hours in order to minimize dehydration. Turtles were placed in plastic crates with absorbent towels until measured and then released back into the tank. Length measurements were taken with a digital caliper to the nearest 0.01 mm following Bolten (2000): SCL, straight plastral length (SPL), straight carapace width (SCW), and straight carapace depth (SCD). Body weight was quantified using an electronic balance with 0.01-g precision. Growth rate was defined as the difference in size and weight through time (millimeters per day and grams per day, respectively).

Running speed was used as the indicator for locomotive performance. Turtles were put on a 1-m-long  $\times$  0.11-m-wide wooden platform placed above the tank, and then hand-stimulated to run towards the water. We measured the time needed to cross the platform twice for each turtle, controlling the water temperature each 30 minutes. To evaluate the influence of water temperature on locomotive performance, we compared running speed in a group of 30 turtles kept first in a water pond at the observed minimal temperature (27°C) and then at the observed maximal one (32°C). Given all neonates with major morphological abnormalities were discarded (see above), we did not consider the effect of abnormalities on running speed (nonsignificant in all cases). Finally, since locomotive performance in reptiles is known to be less repeatable throughout time (Austin and Shaffer 1992), we only carried out 3 tests, at 1, 2, and 3 months of age. Some individuals refused to run during the locomotive performance essays (in particular during the first month measurement), and therefore sample sizes were different at each measurement.

*Statistical Analyses.* — Based on the temperature data provided by the 15 sensors located in each incubation area, we calculated the overall mean hourly temperature for each incubation area. These means were then compared using a paired *t*-test.

In order to compare mortality and presence of abnormalities between treatments (naturally incubated vs. transplanted), we performed Fisher  $2 \times 2$  contingency tests. The interclutch variation of the ratios normal:abnormal and dead:alive was assessed through a chi-

square test for heterogeneity, given that it is relatively insensitive to low expected frequencies in some cells, and it allows the partitioned  $\chi^2$  values from different colonies to be pooled in an overall analysis (Sokal and Rohlf 1994). Between-treatment comparisons of clutch size and hatching rate were achieved through Mann-Whitney U-tests.

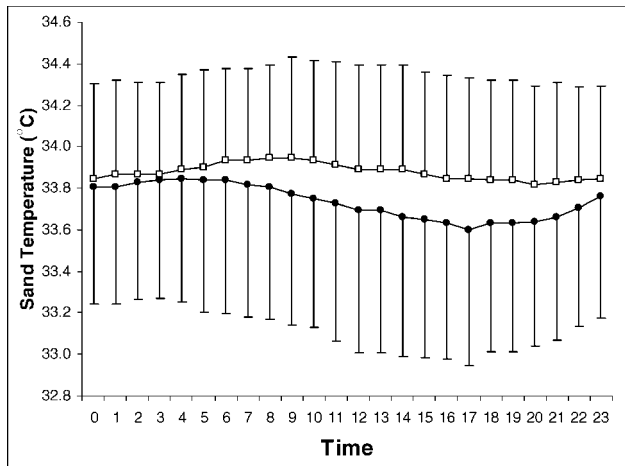
We performed linear regressions to describe the increase in body size and weight throughout the study period, employing the slopes of these regressions (namely mSCL, mSPL, mSCW, mSCD, and mWeight) as the growth rate estimates for each individual. In order to reduce the number of variables describing body length growth, we carried out a principal component analysis excluding mWeight. Since the first principal component explained 95.6% of the total variance (Eigenvalue = 3.8), showing that mSCL, mSPL, mSCW, and mSCD were strongly correlated, we decided to employ only mSCL together with mWeight in all subsequent growth rate analyses.

To evaluate the variation of body size at hatching and first-year growth rate between the 2 incubation treatments and between the different nests, we performed an unbalanced 2-way analysis of variance (ANOVA), setting treatment and nest as fixed factors. Similarly, mean running speed variations between treatments and nests were assessed through an analysis of covariance (ANCOVA), setting incubation treatment and nest as fixed factors and the SCL of the turtles at each locomotive performance measurement as a covariate. Aiming to test whether abnormal individuals had different growth rates than normal ones, we performed a 3-way ANOVA, setting treatment, nest, and presence/absence of any kind of abnormalities as fixed factors. Finally, the relationships between body size at hatching and growth rate and between running speed and growth rate were analyzed through Spearman rank correlations.

## RESULTS

Figure 1 shows the sand temperature profile of the natural and transplant areas in the Arrau Turtle Wildlife Refuge. Mean hourly temperature differed between areas, being higher in the transplant area (overall mean  $\pm$  SD =  $33.88 \pm 0.47^\circ\text{C}$ ) than in the natural one ( $33.73 \pm 0.62^\circ\text{C}$ ) ( $t_{1103} = 15.73$ ;  $p < 0.0001$ ) with differences observed during the day. Minimum temperature was registered towards the end of the nesting period (15 April) in both areas ( $31.5^\circ\text{C}$  in the natural area and  $32.5^\circ\text{C}$  in the transplant one). Maximum temperature was  $34.5^\circ\text{C}$  recorded at 3 different times (18 March, 4–12 and 26–27 April) in both areas.

*Mortality and Morphological Abnormalities.* — Clutch size varied between 66 and 110 eggs, while the hatching rate ranged from 54% to 98%, being similar for both natural and transplanted clutches ( $Z = -0.96$  and  $0.43$ , respectively,  $p > 0.1$ ). Mortality was higher during the third week of acclimation in the tank (when 4.5% of

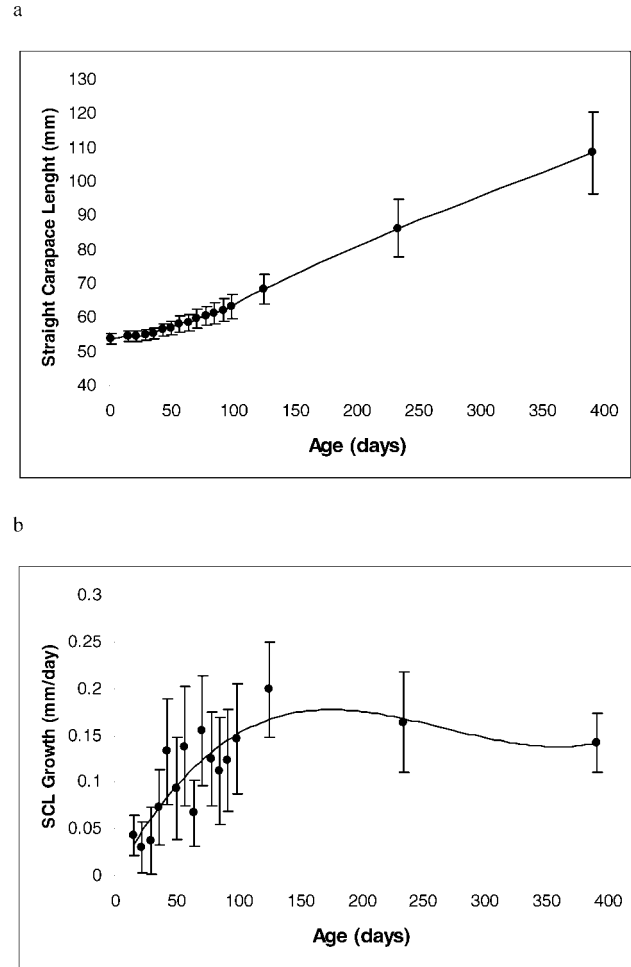


**Figure 1.** Mean hourly sand temperatures registered in the natural (filled dots) and the transplant nesting areas (open squares) between March and April 2003 ( $n = 1104$  in both cases). Points and whiskers represent the mean and the standard deviation of temperature records for each hour during the whole measurement period.

the whole sample died). Overall first-year mortality varied significantly between treatments, being higher in transplanted (12%) than in naturally incubated clutches (0.9%) (Table 1). Likewise, *P. expansa* hatchlings from transplanted clutches had more abnormalities in marginal (74%) and plastral scutes (77%) than did those from naturally incubated clutches (19% and 33%, respectively). The number of individuals with supernumerary scutes did not vary between treatments. Dead individuals did not exhibit a higher incidence of abnormalities (of any type) than surviving ones (Fisher exact test  $\chi^2 = 0.12$ ;  $p = 0.73$ ). Likewise, mortality did not show a significant interclutch variation either in the naturally incubated or the transplanted nests ( $\chi^2 = 2.76$  and  $10.10$ ;  $df = 3$  and  $5$ , respectively,  $p > 0.1$  in both cases). The incidence of morphological abnormalities (of any type) did show a significant interclutch variation among naturally incubated clutches but not between transplanted ones ( $\chi^2 = 34.11$  and  $8.69$ ;  $df = 3$  and  $5$ ;  $p < 0.001$  and  $p > 0.1$ , respectively).

**Table 1.** Comparison of first-year mortality and presence of morphological abnormalities between *P. expansa* turtles from naturally incubated and transplanted clutches (Fisher  $2 \times 2$  contingency tables). The number of dead and abnormal individuals from each category is given followed by the total sample sizes in parentheses.

Response variable	Naturally incubated	Transplanted	$\chi^2$	$p$
First-year mortality	1 (112)	13 (108)	10.10	0.002
Marginal scute abnormalities	18 (96)	62 (84)	21.39	< 0.001
Plastral scute abnormalities	32 (96)	65 (84)	10.50	< 0.001
Supernumerary scutes	5 (96)	3 (84)	0.26	0.60



**Figure 2.** Mean straight carapace length (SCL) (a) and mean SCL daily growth rate (mSCL) (b) during the first year of *P. expansa* turtles collected from 4 naturally incubated and 6 transplanted clutches at the Arrau Turtle Wildlife Refuge, Orinoco River, Venezuela (mean  $n = 178$ ). Points and whiskers represent the mean and the standard deviation of all measurements taken on each date.

*Post-Hatching Growth.* — During the first year, *P. expansa* juveniles showed a linear increase in their SCL ( $B = 0.147$ ;  $R^2 = 0.90$ ;  $F_{1,3027} = 26844.21$ ;  $p < 0.001$ ) (Fig. 2a). SCL daily growth rate (mSCL) varied between 0.03 and 0.20 mm/d during the first 4 months, after which it stabilized around 0.15 mm/d (Fig. 2b). SCL, SPL, SCW, SCD, and weight at hatching differed significantly between clutches. Hatchlings incubated in transplanted nests had longer SCL and SCD and were heavier than those coming from naturally incubated ones (Tables 2 and 3). The opposite was true for the SPL and SCW. Body length growth (expressed by mSCL) and body mass growth (mWeight) also showed a strong interclutch variation (Table 3), but in spite of this variation mSCL and mWeight were significantly higher in transplanted than in naturally incubated clutches (Tables 2 and 3). Abnormal individuals did not show different growth rates from normal ones, either between treatments or nests ( $F_{1,162}$  for mSCL = 0.33,  $F_{1,162}$  for mWeight = 0.03;

**Table 2.** Body size and weight at hatching and first-year growth rate in *P. expansa* turtles from 4 naturally incubated and 6 transplanted clutches (sample sizes are shown in parentheses), SCL at hatching, SPL at hatching, SCW at hatching, SCD at hatching, first-year length growth rate (mSCL), and first-year mass growth rate (mWeight) are all given as means ± SD.

Treatment	Nest (n)	SCL (mm)	SPL (mm)	SCW (mm)	SCD (mm)	Weight (g)	mSCL (mm/d)	mWeight (g/d)
Naturally incubated	1 (28)	55.23 ± 0.94	49.63 ± 0.76	50.72 ± 1.02	23.18 ± 0.62	26.40 ± 0.89	0.16 ± 0.03	0.43 ± 0.13
	2 (25)	53.74 ± 0.73	48.72 ± 0.72	50.27 ± 0.71	22.82 ± 0.68	25.12 ± 0.89	0.12 ± 0.03	0.28 ± 0.08
	3 (24)	51.68 ± 0.87	46.70 ± 0.93	48.50 ± 1.67	23.27 ± 0.54	23.66 ± 0.69	0.13 ± 0.02	0.27 ± 0.08
	4 (19)	53.84 ± 0.97	48.71 ± 0.58	50.98 ± 0.15	23.14 ± 0.42	24.47 ± 0.73	0.14 ± 0.03	0.34 ± 0.09
	Overall	53.68 ± 1.57	48.48 ± 1.34	50.10 ± 1.51	23.10 ± 0.60	25.00 ± 1.31	0.14 ± 0.03	0.33 ± 0.12
Transplanted	1 (22)	55.06 ± 0.92	49.42 ± 0.96	50.88 ± 1.78	24.65 ± 0.72	28.28 ± 0.74	0.16 ± 0.03	0.44 ± 0.13
	2 (6)	54.89 ± 1.21	48.96 ± 0.43	50.03 ± 2.10	25.30 ± 0.20	27.82 ± 0.88	0.19 ± 0.03	0.56 ± 0.14
	3 (4)	52.67 ± 1.21	47.48 ± 0.29	47.44 ± 2.28	22.80 ± 1.23	23.99 ± 1.07	0.16 ± 0.03	0.40 ± 0.13
	4 (23)	52.87 ± 1.56	47.12 ± 1.42	48.19 ± 1.97	23.55 ± 1.06	24.88 ± 1.02	0.14 ± 0.02	0.31 ± 0.07
	5 (18)	54.56 ± 0.86	47.77 ± 0.91	49.61 ± 1.33	23.71 ± 0.43	26.03 ± 0.66	0.15 ± 0.02	0.38 ± 0.09
	6 (11)	54.94 ± 1.44	48.65 ± 0.75	48.78 ± 1.71	23.55 ± 0.71	27.11 ± 1.11	0.17 ± 0.03	0.47 ± 0.14
Overall	54.21 ± 1.53	48.21 ± 1.37	49.38 ± 2.07	23.96 ± 0.99	26.48 ± 1.68	0.16 ± 0.03	0.40 ± 0.13	

$p > 0.5$  in both cases; no significant interactions were detected).

When first-year growth rate was correlated to neonate body size (analysis performed exclusively in natural nests), the heavier and larger hatchlings proved to have the highest growth rates in length and weight (correlations SCL–mSCL and SCL–mWeight,  $R = 0.28$  and  $0.33$ , respectively;  $p < 0.01$ ; correlations Weight–mSCL and Weight–mWeight,  $R = 0.22$  and  $0.29$ , respectively;  $p < 0.05$ ) (Fig. 3). Employing the growth rates observed during the first 2 months of age erased the correlation SCL–mSCL ( $R = 0.11$ ,  $p > 0.1$ ) but not SCL–mWeight ( $R = 0.24$ ,  $p < 0.05$ ).

*Locomotive Performance.* — Mean running speed (MRS) proved a reliable index of individual physical condition, since no significant variation between running speed replicas was found (paired  $t$ -test:  $t_{511} = 1.83$ ;  $p = 0.07$ ). Furthermore, locomotive performance was not affected by water temperature ( $t_{56} = 0.58$ ;  $p > 0.6$ ). MRS increased progressively throughout the first 3 months, varying by more than 10 cm/s (Table 4). Naturally incubated and transplanted turtles showed no difference in locomotive performance during the first and third month of age, when a significant interclutch variation in MRS was detected (Table 5). However, at 2 months of age, naturally incubated turtles showed higher MRS than transplanted ones. This effect remained significant after applying a Bonferroni correction for multiple tests ( $\alpha = 0.017$ ). On the other hand, growth rate was only correlated to MRS during the third month (correlation mSCL–MRS:  $R = 0.15$ ;  $p = 0.043$ ;  $n = 178$ ); although, significance disappeared after correcting for multiple tests.

## DISCUSSION

Our results show that clutch relocation affected hatchling turtles in several ways. Transplanted clutches showed higher posthatching mortality and higher incidence of morphological abnormalities than naturally incubated clutches. One out of three tests for locomotive performance suggested that young turtles coming from naturally incubated clutches can run faster than those coming from transplanted ones. On the other hand, hatchling turtles coming from transplanted nests were larger and heavier and showed higher body growth rates than those from nontransplanted nests.

The direct attribution of clutch relocation as the main cause explaining the observed physiological differences is supported by 3 reasons. First, the strong influence of parental effects, reflected by the interclutch variation of body size at hatching, occurrence of abnormalities, growth rate, and running speed, are sources of stochastic variations, likely to hinder the detection of any environmental effects over these variables. Detecting them in spite of this high stochasticity hence suggests a profound influence of the incubation environment over turtle development. Second, *P. expansa* is known to have

**Table 3.** Analysis of variance (ANOVA) of body size and weight at hatching and first-year growth rate in *P. expansa* turtles from 4 naturally incubated and 6 transplanted clutches. The table shows the *F* values obtained when comparing treatments, nests, and the interaction between these 2 factors. Degrees of freedom (df) are given for each effect and its error, respectively, while significance is highlighted by \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

Factor	df	Response variable						
		SCL	SPL	SCW	SCD	Weight	mSCL	mWeight
Treatment	1170	11.59***	3.94*	10.56**	70.31***	135.52***	19.06***	19.93***
Nest	5170	40.46***	40.44***	12.59***	5.11***	70.03***	6.13***	9.14***
Interaction	3170	5.88***	8.80***	7.88***	17.16***	11.62***	10.42***	11.86***

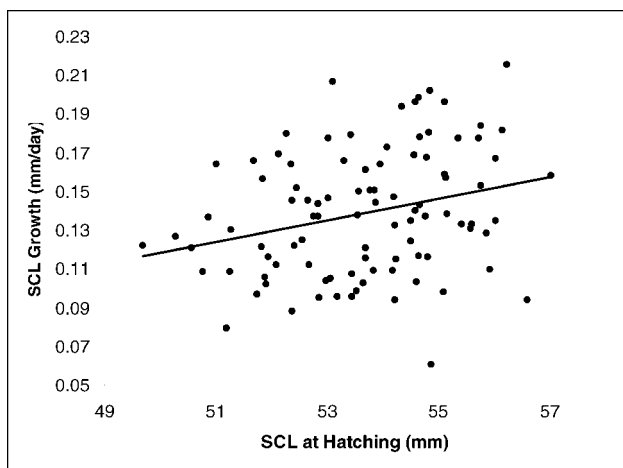
temperature-dependent sex determination, with higher temperatures producing a larger proportion of females (Valenzuela et al. 1997). The pivotal temperature for this species is known to range between 31°C and 33°C (Valenzuela 2001a), and though this may vary among localities, it is very likely that our clutches consisted primarily of females due to the high temperatures herein recorded (see Fig. 1). Furthermore, a previous study on the same turtle population (Peñaloza et al., unpubl. data 2004) quantified a sex ratio of 1:250 males:females. Hence the physiological differences exhibited by the turtles incubated in naturally incubated and transplanted clutches cannot be attributed to sex proportion variation, rather pointing to the incubation environment and egg handling as possible causes. Finally, although the confinement of turtles into artificial rearing facilities (with a restricted but constant diet, no predators, etc.) is certainly likely to have influenced their physiology in different ways, the fact that all individuals were exposed to the same rearing conditions during their first year, allow us to exclude captive rearing as a cause explaining the observed differences between naturally incubated and transplanted turtles.

*Mortality and Morphological Abnormalities.* — Both parental effects (genetic factors and maternal condition) and incubation environment, are known to influence egg

and hatchling mortality (Brooks et al. 1991; McKnight and Gutzke 1993; Rhen and Lang 1995; Wilson 1998; Packard et al. 1999). Given that clutch size and hatching success showed similar values to those reported for other *P. expansa* populations (Vanzolini 2003), differences in posthatching mortality rates could be mostly explained by the incubation conditions. Among these, incubation temperature, humidity, and egg manipulation seem to be the main determinants of posthatching mortality.

The water potential inside natural and transplanted clutches may have had a similar influence over nest performance (Rimkus et al. 2002), since it was found to be the same in both incubation areas ( $\Psi = 0$ ; Peñaloza et al., unpubl. data 2004). On the other hand, although the mean incubation temperature approached 34°C in both the natural and the transplant areas, the daily distribution of incubation temperatures during the whole incubation period (approximately 45 days), showed that transplanted nests had significantly higher temperatures throughout the day. Brooks et al. (1991) and Packard (1999) found a high-temperature linked mortality in *Chelydra serpentina*, presumably due to excessive water loss towards the environment or exposure to temperatures above the thermal tolerance threshold. For instance, during very warm years, temperatures in our transplant area may surpass the maximum survival thresholds, causing high mortality (N. Valenzuela, *pers. comm.* May 2006). In this case, however, incubation temperatures ranging well below known temperature values tolerated by this species (45°C), coupled with high humidity levels, rather suggest the egg manipulation as the main factor responsible of turtle mortality.

Egg manipulation is also known to affect embryo development. Turtle eggs are laid at a late gastrula stage (Ewert 1985). From 3 hours to 21 days after oviposition (depending on the species), the embryo attaches to the egg shell to continue development. Once this occurs, rough movement of the egg may detach the embryo or tear its connection to the egg shell, negatively affecting development or causing death (Eckert et al. 2000). For instance, the higher incidence of marginal and plastral scute abnormalities in turtles coming from transplanted clutches reflect abnormal embryonic development (Carr 1967; Gilbert 2000). Since the actual egg handling took place during the day after oviposition, it is not possible to



**Figure 3.** Relationship between straight carapace length (SCL) at hatching and first-year length growth rate (mSCL) in turtles from naturally incubated clutches. The line shows a significantly positive correlation ( $R = 0.28$ ;  $p < 0.01$ ;  $n = 96$ ).

**Table 4.** MRS at 1, 2, and 3 months of age in *P. expansa* turtles from 4 naturally incubated and 6 transplanted clutches. All values are given as means ± SD.

Treatment	Nest	MRS at 1 month (cm/s)	MRS at 2 months (cm/s)	MRS at 3 months (cm/s)
Naturally incubated	1	39.80 ± 11.29	51.27 ± 12.01	53.89 ± 12.04
	2	37.73 ± 8.5	47.41 ± 10.79	47.69 ± 9.59
	3	43.36 ± 8.17	54.75 ± 9.53	53.52 ± 8.89
	4	41.11 ± 10.94	52.84 ± 9.27	54.86 ± 10.87
	Overall	40.73 ± 9.48	51.53 ± 10.73	52.44 ± 10.68
Transplanted	1	43.83 ± 8.84	49.24 ± 12.04	50.64 ± 14.63
	2	46.20 ± 12.29	55.43 ± 12.64	51.54 ± 8.83
	3	41.74 ± 2.95	43.02 ± 3.87	56.53 ± 7.13
	4	35.84 ± 8.12	43.56 ± 7.72	53.01 ± 9.25
	5	37.30 ± 11.40	48.91 ± 13.77	50.20 ± 11.98
	6	28.83 ± 4.98	46.71 ± 5.84	56.65 ± 9.75
	Overall	38.77 ± 10.05	47.39 ± 10.81	52.35 ± 11.39

attribute the occurrence of these abnormalities to late egg manipulation alone (though the precise moment of the embryo’s attachment to the egg shell is still unknown for this species). Alternatively, momentary exposure of eggs to open air conditions, together with their relocation into a human-built nest, may also have contributed to abnormal embryonic development and the higher posthatching mortality found in transplanted clutches. Of particular interest is the higher first-year mortality found in transplanted clutches (12%) as compared with 0.9% in naturally incubated ones. Although these figures should not raise any major concern (similar experiences considered successful in Perú revealed mortalities ranging from 12% to 30% for *P. unifilis*; Martinez 2006), they point to egg manipulation as the main cause of mortality.

*Post-Hatching Growth.* — Animals with indeterminate growth such as fish and reptiles show a logistic-like growth rate curve, exponentially increasing in early life stages, progressively decreasing until reaching sexual maturity and finally falling asymptotically towards zero in final life stages (Galbraith et al. 1989). Figure 2b shows the first part of this curve, where 0.15 mm/d is the presumed postexponential growth rate. A similar pattern of growth was found in another *P. expansa* study (Augusto et al. 2004).

According to our results, and provided growth rate does not exhibit a drastic reduction until reaching sexual maturity, the turtles should approach the minimal size of laying females (Alho and Pádua 1982; Valenzuela 2001b) in no less than 10 years. This estimate is, however, an underestimation, since 1) growth rate will progressively decay when approaching sexual maturity, and 2) turtle growth in the wild probably differs from that observed in artificially reared individuals, fed on large quantities of highly proteic nourishment, without any predation pressures, and maintained relatively free of diseases and parasites. For instance, a recent study based on mark-recaptured individuals of the same Venezuelan population estimated the age of first reproduction at 17 years (Hernández and Espín 2006). These results suggests that only long-term management efforts, guaranteeing turtle survival well over a decade, could expect a real increase in the population’s recruitment rates.

The higher growth rates found in transplanted nests, though indicative of enhanced fitness (Stearns and Koella 1986; Galbraith et al. 1989; Janzen 1993; Congdon et al. 1999), should be taken with caution as other variables suggest that egg manipulation negatively affected turtle survival: mortality and presence of morphological abnormalities could also be considered tokens of fitness. Hence, the higher growth rates of transplanted clutches may not necessarily imply fitness benefits. For instance, they could be a consequence of the higher incubation temperatures registered in the transplant area, enhancing embryonic development and posthatching growth.

Growth is known to be influenced not only by parental but also by environmental factors (Brooks et al. 1991; McKnight and Gutzke 1993; Bobyn and Brooks 1994; Packard and Packard 2001; Valenzuela 2001b; Augusto et al. 2004). Several studies have found that only low and moderate temperatures tend to enhance embryonic growth in turtles (Brooks et al. 1991; McKnight and Gutzke 1993; Bobyn and Brooks 1994; Rhen and Lang 1995); although, there is also a report of larger hatchlings found in warmer clutches of the congener species *P. unifilis* (Souza and Vogt 1994). Valenzuela (2001b) showed that *P. expansa* hatchlings incubated at 30.5°C

**Table 5.** Analysis of covariance (ANCOVA) of the MRS at 1, 2, and 3 months of age in *P. expansa* turtles from 4 naturally incubated and 6 transplanted clutches. The table shows the *F* values obtained when comparing treatments, nests, the interaction between these 2 factors, and the effect of the SCL as a covariate. Degrees of freedom (df) are given for each effect and its error respectively, while significance is highlighted by \**p* < 0.05; \*\**p* < 0.01; and \*\*\**p* < 0.001.

Factor	Response variable (df)		
	MRS at 1 month	MRS at 2 months	MRS at 3 months
SCL (covariate)	0.91 <sub>(1,97)</sub>	0.02 <sub>(1,166)</sub>	6.43 <sub>(1,167)</sub> *
Treatment	1.17 <sub>(1,97)</sub>	6.25 <sub>(1,166)</sub> *	0.31 <sub>(1,167)</sub>
Nest	3.80 <sub>(5,97)</sub> **	0.95 <sub>(5,166)</sub>	2.77 <sub>(5,167)</sub> *
Interaction	1.29 <sub>(3,97)</sub>	3.47 <sub>(3,166)</sub> *	0.10 <sub>(3,167)</sub>

were heavier than those incubated at 32.5°C, 33.5°C, and 34.5°C; while no significant differences were detected in hatchling length. However, the same study reported a negative correlation between neonate body size and 2 months' posthatching growth, contradicting our findings: our larger hatchlings also exhibited the higher growth rates (Fig. 3). Since we exclusively considered natural clutches, this relationship could not have been a by-product of clutch manipulation. Moreover, when employing the growth rates observed during the first 2 months of age the correlation SCL at hatching–mSCL disappeared, suggesting that the inclusion of growth rates based on body size measurements beyond the initial exponential growth (after the fourth month in our study) may be the reason making our results differ from those of Valenzuela (2001b). Hence, although the relationship between incubation temperature and growth does not seem to follow a simple and general rule across taxa, we cannot exclude temperature as an environmental factor enhancing turtle development in this case.

*Locomotive Performance.* — Running speed reflects physical condition, which in turn can determine the ability of an individual to reach the water after hatching, acquire resources, and escape from predators (Miller et al. 1987; Austin and Shaffer 1992; Janzen 1995). Hence, locomotive performance could also be indirectly related to fitness, allowing testing if faster-growing transplanted turtles have a better physical condition than that of natural ones.

Finding a significantly higher MRS in naturally incubated clutches in one of the locomotive performance tests provides support for the idea that the higher growth rates observed in transplanted clutches do not necessarily translate into higher fitness advantages. Environmental factors present during incubation, such as temperature and humidity, are indeed known to affect running and swimming speed together with behavioral aspects of some reptiles (Gutzke and Crews 1988; Janzen 1995; Shine et al. 1995). Supporting our findings, Janzen (1995) also reported a negative influence of high incubation temperatures on the locomotive performance of juvenile *Chelydra serpentina* turtles.

In conclusion, clutch manipulation was found to have profound effects on juvenile *P. expansa* turtles, influencing their mortality, presence of abnormalities, growth rate, and locomotive performance. It is worth mentioning, however, that this management program of transplanting nests avoids losing complete nests due to flooding. Hence our aim here is not to diminish the program's value but rather to enhance its efficiency, not only preventing the clutch losses but also increasing the hatchlings' long-term survival. Future turtle conservation programs should be particularly careful when initiating this kind of management, trying to minimize the effect of egg manipulation on developing embryos as well as maximizing the similarity of conditions between transplanted and natural nests. Additionally, our results suggest that only management efforts guaranteeing *P. expansa* survival well over a

decade could expect a significant effect on the population's recruitment rates.

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#### RESUMEN

Desde 1993 el gobierno Venezolano implementó un programa intensivo de manejo dirigido a incrementar el tamaño poblacional de la amenazada Tortuga Gigante Sudamericana (*Podocnemis expansa*). Mediante el transplante de nidos y un año de cría en cautiverio de los tortuguillos, el programa afecta a la principal población Venezolana de *P. expansa*, ubicada en la porción media del río Orinoco. A pesar de que el programa pareciera estar incrementando el reclutamiento de nuevos individuos a la población, los efectos del mencionado transplante de nidos sobre parámetros correlacionados con la adecuación de los tortuguillos a largo plazo no han sido estudiados hasta la fecha. En el presente trabajo medimos la mortalidad postnatal, presencia de anomalías morfológicas, tasa de crecimiento corporal durante el primer año y capacidad física en tortuguillos sujetos a dicho programa de manejo. El transplante de nidos influyó profundamente a los tortuguillos de *P. expansa*, afectando su mortalidad, morfología, tasa de crecimiento corporal y capacidad física. Los nidos transplantados mostraron una mayor mortalidad de tortuguillos y una mayor incidencia de anomalías morfológicas, pero también una mayor tasa de crecimiento que los nidos no transplantados. Asimismo, los tortuguillos provenientes de nidos no transplantados tendieron a correr más rápido que aquellos incubados en nidos transplantados. La manipulación de nidos en particular, fue encontrada responsable de la incrementada incidencia de anomalías morfológicas y la mayor mortalidad. Por otra parte, las temperaturas más altas encontradas en los nidos transplantados pudieran ser la causa de las más altas tasas de crecimiento corporal.

Nuestros resultados sugieren que futuros esfuerzos de manejo que traten de minimizar los efectos de la manipulación de nidos, no solo prevendrán la pérdida de nidadas sino también aumentarán la supervivencia de los tortuguillos en el largo plazo.

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