PRE- AND POST-HATCHING FACTORS AFFECTING JUVENILE GROWTH RATES IN THE YELLOW-SPOTTED RIVER TURTLE *(Podocnemis unifilis)*

FACTORES PRE Y POSECLOSIÓN QUE AFECTAN LAS TASAS DE CRECIMIENTO DE JUVENILES DE LA TORTUGA TERICAYA *(Podocnemis unifilis)*

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Abstract

Podocnemis unifilis eggs obtained from the Cahuinarí National Park, Colombia were incubated artificially under constant temperatures during two nesting seasons. A sample of the hatchlings produced were sexed to document the pivotal temperature for this population (32 °C). It appears that during both nesting seasons, most nests on the study beach produced individuals of each sex. The remaining turtles obtained in the laboratory were reared for one year in order to study the effects of pre- and post-hatching factors on their growth rates. We documented maternal effects on egg sizes and initial hatchling sizes each year, but not on subsequent juvenile growth rates. Incubation condition influenced both initial hatchling sizes and growth rates, with turtles from the high temperature condition emerging at smaller initial sizes and then demonstrating higher growth rates. By the end of the first year, these individuals were significantly larger than their siblings that had experienced cooler incubation temperatures, which is consistent with predictions of the Charnov and Bull hypothesis concerning the adaptive value of temperature-dependent sex determination. We also examined the effects of diet, density, and basking opportunities on growth rates, and found that turtles that received meat in their diets grew faster than those feed only vegetable matter. Turtles in the low density condition attained larger sizes in one of the two years of the study, while access to direct sunlight for basking did not significantly alter growth rates.

Key words: Podocnemis unifilis, diet, growth, incubation, sex determination, temperature, Colombia.

Resumen

Durante dos años, incubamos artificialmente bajo temperaturas constantes huevos de *Podocnemis unifilis* obtenidos del Parque Nacional Natural Cahunarí, Colombia. Una muestra de los neonatos fueron sexados para documentar la temperatura pivotal de esta población (32 °C). Aparentemente, en ambas estaciones de anidación, la mayoría de los nidos en la playa de estudio produjeron individuos de ambos sexos. El remanente de las tortugas obtenidas de las incubadoras por estación reproductiva fue mantenido en cautiverio por un año con el propósito de estudiar los efectos de factores pre y poseclosión en las tasas de crecimiento. En ambos años, documentamos un efecto materno en el tamaño de los huevos y en el tamaño inicial de los neonatos, pero no en las tasas de crecimiento posteriores. Las condiciones de incubación afectaron el tamaño inicial de los neonatos y sus tasas de crecimiento. Los individuos incubados a temperaturas cálidas emergieron con tamaños iniciales menores y luego presentaron tasas de crecimiento mayores. Para el final del primer año, estos individuos fueron significativamente más grandes que sus hermanos incubados a temperaturas más frías, lo cual es consistente con las predicciones de la hipótesis de Charnov y Bull sobre el valor adaptativo de la determinación sexual dependiente de la temperatura. También examinamos los efectos del tipo de dieta, la densidad y las oportunidades de soleamiento en las tasas de crecimiento, y encontramos que las tortugas que recibieron carne en sus dietas crecieron más rápidamente que las que se alimentaron exclusivamente de material vegetal. Las tortugas en la condición de densidad baja alcanzaron tamaños mayores en uno de los dos años del estudio, mientras que el acceso directo a la luz solar para el soleamiento no afectó significativamente las tasas de crecimiento.

Palabras clave: Podocnemis unifilis, crecimiento, determinación sexual, dieta, incubación, temperatura, Colombia.

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INTRODUCTION

The yellow-spotted river turtle, *Podocnemis unifilis* (Chelonia; Podocnemididae), is an important component of the aquatic fauna of the Orinoco and Amazon river basins, both in terms of the ecological role the species probably plays and due to its economic importance as a source of protein for local communities. While various aspects of the natural history of this species have been studied (i.e.: nesting ecology, Escalona and Fa, 1998; Fachin, 1992, 1993, 1994; Foote, 1978; Medem, 1964; Páez, 1995; Páez and Bock, 1997, 1998; Souza and Vogt, 1994; Thorbjarnarson *et al.*, 1993; Vanzolini, 1977; female post-nesting behavior, Bock *et al.*, 1998; adult diet, de Almeida *et al.*, 1986; Fachin *et al.*, 1995), almost nothing is know of the basic biology of juveniles. As in most freshwater turtle species (Bury, 1979), the majority of hatchling *P. unifilis* probably fail to survive their first year of life, making detailed ecological studies in the field nearly impossible. However, appropriately designed captive studies of juvenile turtles may provide insights into the factors that are ecologically important to individuals in the field (Vogt, 1980). Given the presumably high predation rates that neonate turtles experience, it seems reasonable to assume that natural selection favors individuals that experience high initial growth rates, as their vulnerability to predators surely decreases as they attain larger sizes and develop harder carapaces. For this reason, we conducted a captive study of the pre- and post-hatching factors that influence early growth rates in juvenile *P. unifilis* in the Colombian Amazon.

One pre-hatching factor already known to be important in *P. unifilis* is the incubation temperature the eggs experience, which determines the sex of the juvenile turtles produced (Páez, 1995; Souza and Vogt, 1994). As in many species of turtles, high incubation temperatures in *P. unifilis* produce clutches that hatch as females, while lower incubation temperatures produce male individuals. Various hypotheses have been proposed to explain the existence of this unusual sex determination mechanism (Bull, 1985a; Burke, 1993; Ewert and Nelson, 1991; Janzen and Paukstis, 1991; Roosenburg, 1996; Shine, 1999; Standora and Spotila, 1985; Viets *et al.*, 1994; Wibbels *et al.*, 1994). The model that has gained the widest acceptance was first proposed by Charnov and Bull (1977), and argues that Temperature-dependent Sex Determination (TSD) should be favored over genetic sex determination where the fitness of individuals of one sex exceeds that of the other sex within a given environment. Specifically in turtles, the temperature-dependent differential fitness hypothesis states that hatchlings from hot nests may have a greater probability of maturing as superior females, because they emerge at larger sizes and/or grow more rapidly (Burke, 1993; Ewert and Nelson, 1991; Janzen and Paukstis, 1991; Shine, 1999; Vogt, 1980). This is important in terms of fitness for the great majority of turtle species (including *P. unifilis*, Medem, 1964), because adult females are larger than adult males. This presumably is because large size does not influence fecundity in males as much as it does in females (due to the constraint body size places on clutch size in females; Gibbons, 1982; Valenzuela, 2001; Vogt, 1980). The argument is that an egg that finds itself in a relatively warmer incubation environment is for some reason more likely to either emerge at a relatively larger size, or at least eventually grow faster, and thus become a more fit female. Eggs in cooler sites would do better to become males, where size exerts less of an influence on lifetime fecundity (Burke, 1993; Ewert and Nelson, 1991; Janzen and Paukstis, 1991; Shine, 1999; Vogt, 1980). But critics of the differential fitness hypothesis claim that it is difficult to imagine, in the case of turtles, how such small difference in initial sizes or growth rates could translate into adult size differences many years later.

Some studies on freshwater turtles have inspected for how maternal (nest) effects and incubation temperatures may influence various characteristics related to fitness, such as clutch size, egg size, neonate survivorship and juvenile growth rates over varying time periods. Most of these studies have documented positive correlations between female size and the size of the clutch, eggs and neonates produced (Congdon and Gibbons, 1987; Congdon *et al.*, 1983; Vogt, 1980). However, given the differences in the captive conditions and time scales of the different studies, associations between maternal effects and incubation temperature (= sex) on growth rates have not always been consistent (Bobyn and Brooks, 1994; McKnight and Gutzke, 1993; Valenzuela, 2001; Vogt, 1980).

In this study, we sacrificed a limited number of individuals during two nesting seasons obtained from differing incubation conditions, and reared the remaining individuals for one year to document the effects of this factor on growth rates. In turtles, most growth rate data have been obtained by means of mark-recapture studies (Brown *et al*., 1994; Dunham and Gibbons, 1990; Frazier *et al*., 1990; Gibbons, 1987; Wilbur, 1975), but such studies often require considerable time investment in order to obtain even limited results, and normally do not provide information concerning juvenile growth. Obviously, while studies of growth in captivity are of more limited value (given that they only indicate potential growth rates, and not necessarily those attained in the field), they can provide more precise data, especially when conducted utilizing designs that permit assessment of the influence of different factors on growth rates (Avery *et al*., 1993; Brooks *et al*., 1991; Rhen and Lang, 1995; Vogt, 1980).

Our study considered three different post-hatching factors known to be important determinants of growth rates in other turtle species. One factor was diet, as many species of turtles exhibit accelerated growth rates when they are provided with high quality diets in captivity (Avery *et al*., 1993; Bjorndal, 1985, 1991; Jackson *et al*., 1976), which suggests that they are limited by food in their natural habitats. In the field, *P. unifilis* appears to be largely herbivorous (De Almeida *et al*., 1986; Fachin *et al*., 1995). However, animal matter also has been found in stomach contents of individuals obtained from natural populations, and juvenile *P. unifilis* will eat substantial quantities of meat in captivity (Cole and Link, 1972; Fachin *et al*., 1992).

Another environmental factor known to affect juvenile turtle growth rates is environmental temperature (Avery *et al*., 1993; Parmenter, 1980; Vogt and Guzman, 1988), as metabolic rates and digestive efficiencies are greater when individuals are maintained at higher body temperatures (Avery *et al*., 1993; Parmenter, 1980, 1981; Spotila, 1995). One common mechanism for turtles to elevate their body temperature is by basking (Boyer, 1965; Janzen *et al*., 1992; Lindeman, 1993), especially in situations where exposure to direct solar radiation is possible. Finally, a third post-hatching factor that might influence growth rates is social behavior (although little is known about social interactions among juvenile turtles in the field). Some laboratory studies have demonstrated competition for food among juvenile turtles, with larger individuals obtaining greater access to available food (Froese and Burghardt, 1974; McNight and Gutzke, 1993). Other laboratory studies have found that turtles may grow more rapidly on average when they are maintained in social groups. Perhaps captive individuals are less stressed when they are held with other conspecifics, or they may begin to feed or bask more readily when in the presence of other individuals.

In summary, this study examined the effects of various factors on growth rates of juvenile *P. unifilis* during their first year of life, including pre-hatching factors (maternal or nest effects and incubation temperatures) and post-hatching factors (diet, density, and opportunities for basking). Incubation temperature effects on sex determination also were examined.

MATERIALS AND METHODS

Sex determination. Artificial incubation of *P. unifilis* eggs was conducted in the town of La Pedrera, located at 1° 18’ S and 69° 33’ W on the Caquetá River (Colombia). Eggs were obtained from the Cahuinarí National Park, located approximately 200 km to the west of La Pedrera, where we also conducted studies of natural nest incubation (described in Páez, 1995 and, Páez and Bock, 1997). In 1993, 58 eggs from each of two clutches were divided randomly among two incubators adjusted to maintain temperatures of approximately 27 and 32º C. In 1994, 67 eggs from each of three clutches were randomly divided among three incubators set to maintain temperatures of approximately 27, 30, and 32 ºC. Eggs from each nest were weighed to the nearest 0.1 g using an Ohaus triple-beam balance, before being placed in the incubators. The temperatures within each incubator were recorded three to four times daily and the thermostats adjusted when necessary to maintain the appropriate temperature.

In 1993, we monitored nest temperatures in nine natural nests on a beach on Cahuinarí Island (located in the Caquetá river adjacent to the park headquarters) and collected the hatchlings produced by the two nests that exhibited the highest and lowest mean incubation temperatures. Thirteen of these individuals were reared for several weeks in the laboratory facility in La Pedrera and then sacrificed for gonadal sex determination via histology. We also inspected the sex of 13 hatchlings obtained from the hot and cold artificial incubators this year; the remaining hatchlings were incorporated into the juvenile growth rate study (see below). Some of the turtles that died during the growth rate study also were inspected histologically for the sex determination analyses, as well as ten turtles obtained in 1994 from the intermediate temperature condition at the conclusion of the growth rate study that year (see below).

All of the individuals were sacrificed with chloroform, preserved with and stored in buffered 10% formalin solution until dissected for sexing. One gonad from each juvenile was removed, sectioned, and fixed with haematoxylin and eosine for histological inspection. Gonadal differentiation was based upon the criteria of Danni and Alho (1985) and, Yntema and Mrosovsky (1980).

Captive juvenile growth study design. All the remaining hatchling turtles obtained from the artificial incubators (32 in 1993 and 54 in 1994) were first measured on the day following their emergence from the egg (week 1) and individually marked with ink on their carapaces. Turtles were remeasured periodically during the remainder of the study (see below) and marks were reinforced when necessary. All turtles were measured for straight-line carapace length (CL) across the maximum distance between the anterior edge of the right first marginal scute to the posterior edge of the right eleventh marginal scute, using a Spi caliper to the nearest 0.1 mm. Following measurement, each turtle was weighed on an Ohaus triple-beam balance to the nearest 0.1 g. After the hatchlings had fully re-absorbed any protruding yolk, they were assigned to one of twelve captive rearing conditions resulting from the interaction of three variables (three diet, two density, and two basking opportunity conditions). We placed 12 plastic containers of 50 cm diameter on two long, parallel tables (figure 1). Each container contained 8 l of rainwater (to achieve a depth of 6 cm), as well as an inverted plastic bowl in the center to provide both a hiding refuge within and a basking platform above the water line, which the turtles accessed via a hole in one side of the bowl and a wooden ramp placed along the opposite side, respectively.

Of the 12 containers in the growth study, four were assigned to each of three different diet conditions. In 1993, the initial diets employed were: 1) vegetable matter: finely diced cabbage leaves and small pieces of local fruits, mainly plantain; 2) meat: finely diced pieces of fish viscera, mostly liver; and 3) mixed: a combination of the first two diets. After the first

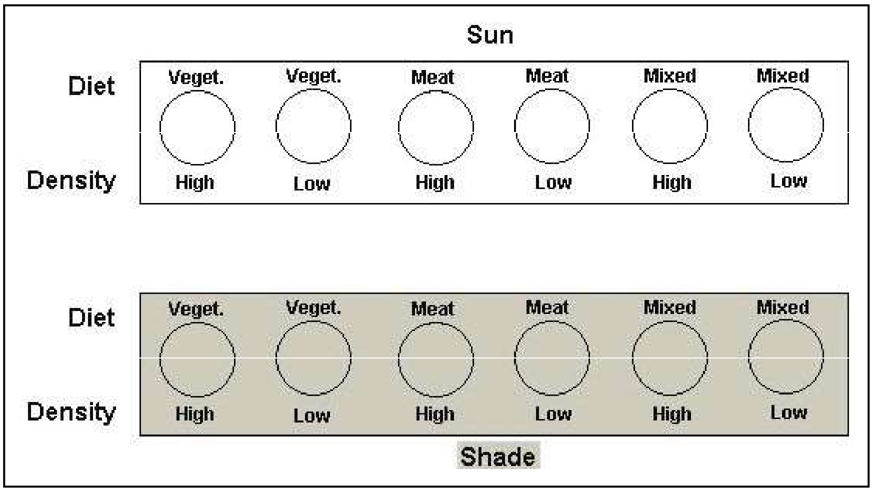

Figure 1. Diagram of the distribution of the 12 containers used in the juvenile growth rate study. The positions of the six containers on the shade table and six containers on the sun table were rotated at each cleaning

six months, these original diets were altered, based on recommendations offered by nutrition experts of the Wildlife Conservation Society. The diets were changed in the following manner. To diets 1 and 3 we included small pieces of leaves from several local plants, mainly Araceace. To diets 2 and 3 we replaced the fish viscera with grated pieces of commercially canned tuna (packed in water). Also, to all three diets we added approximately 0.5 g of a commercial veterinary vitamin and mineral supplement incorporated into a fish bone-meal powder base. In 1994, the modified diets were used throughout the entire study.

In both years, turtles were fed their respective diets every third day, immediately after cleaning the containers and changing the water. The food was presented *ad libitum* so that when the container was next cleaned and fresh food added, there still was some uneaten food present. Of the four containers in each diet condition, two were assigned to the low turtle density condition and two were assigned to the high turtle density condition. At the beginning of the study, each low density container contained three turtles (1 individual/655 cm^2 water), while each high density container contained nine individuals (1 individual/218 cm^2 water). When a turtle died, it was replaced with an individual of the same approximate age, and when possible, of the same nest and incubation condition. In 1993, after nine months of study there were no longer sufficient replacements available, so the number of turtles within each high density container was reduced to eight (1 individual/246 cm^2 water).

Finally, one of the two containers for each combination of diet and density was placed on the table located in the shade, while the other container with this combination of diet and density was placed on the other table that received direct exposure to sunlight from approximately 10:00 to 16:00 h daily, weather permitting. Thus, half of the turtles in the growth study were able to emerge from the water onto the platforms provided and dry off, but never had access to direct sunlight for basking. The remaining half of the turtles were able to thermoregulate through a broader range of temperatures by moving between the water and the platform above the water level, often in direct sunlight.

In this manner, the three environmental variables that the turtles experienced in the growth study were partitioned in a design that involved twelve distinct conditions (three diet conditions by two density conditions by two basking conditions). In 1993, when turtles from two artificially incubated nests were studied, turtles obtained from one of the natural nests from the Cahuinarí beach also were reared in the study in order to maintain the appropriate densities in each container, but these latter individuals were not included in the analyses of the effects of pre-hatching factors on juvenile growth rates. Turtles obtained from the two artificially incubated nests were assigned to the 12 growth containers so that the two pre-hatching experimental factors (nest and incubation condition) were balanced within each main environmental factor (diet, density, and basking conditions). In other words, the six low and high density containers held equal numbers of turtles from Nest 1 and Nest 2 and equal numbers of turtles from the hot and cold incubation conditions. This was also true for the six sunlight and six shade containers, and for the four containers for each diet condition.

In 1994, the design of the study was identical, except that the number of individuals obtained from each artificial incubator was too low to permit their assignment to the 12 growth conditions with the same density levels as in the preceding year. Therefore, some individuals obtained from a natural nest that emerged on the Cahuinarí Island were reared in the containers to maintain the appropriate density levels. In this manner, the six low density containers maintained equal numbers of turtles from each of the three artificially incubated nests (Nest A, Nest B, and Nest C) and three artificial incubation conditions (hot, intermediate, and cold). In the high density containers, two turtles from each artificial incubation condition were placed with three turtles obtained from the natural nest. In the container corresponding to the vegetable diet, high density, and shade condition, only one individual from Nest A was available, so a third individual from Nest C was substituted. Fortunately, of the turtles that died, only two were individuals from the laboratory incubators (in 1993) and individuals from the same nests and incubator conditions were available as replacements. All remaining deaths were of individuals from natural nests, which were immediately replaced by comparable individuals from the same or another natural nest.

Captive juvenile growth study analyses. The size and growth rate analyses (except where specifically noted) were based on comparisons of individuals of the same age, instead of between individuals measured on the same date, since not all turtles were incorporated into the growth study simultaneously, due to the approximately seven week period between the hatching date of the first and last artificially incubated eggs (Páez and Bock, 1997, 1998).

In 1993, all turtles were first measured on week 1 and then re-measured on weeks 3, 5, 7, 9, 11, 13, 15, 19, 23, 27, 31, 39 and 43 (based upon their actual ages). In 1994, all turtles were measured on week 1 and re-measured on weeks 2, 3, 18, 22, 42, and 46. Egg size and initial hatchling size data were compared using simple ANOVAs, as were comparisons of hatchling size data on the date that the emergence period ended and the date that the growth study ended. However, since re-measurement of the same individual does not constitute an independent event, the analysis employed for the overall growth data was a repeated-measures ANOVA. The study design permitted that the effects of the pre-hatching factors could be inspected independent of the effects of the post-hatching factors, using a two-way ANOVA model (Nest and Incubation condition main effects). Similarly, the study design also permitted an inspection of the effects of the three post-hatching factors independent of the effects of the Nest and Incubation condition factors, by conducting a three-way ANOVA (with Diet, Density, and Basking condition main effects). All analyses were conducting using the SAS statistical software package (SAS, 1989).

RESULTS

Temperature-dependent sex determination. The histological analysis of the gonads of hatchlings obtained from the two natural nests from the Cahuinarí beach in 1993 showed that the coldest nest produced 78% males, while the hottest nest produced all females (table 1). Hatchling sex ratios obtained from the artificial incubators in 1993 and 1994 also varied depending upon incubation temperature, with the cold incubator producing only males, the intermediate incubator producing 90% males, and the hot incubator producing 50% males (table 2).

We had initially intended to examine the effects of sex on juvenile growth rates, but although the hot artificial incubation temperature employed was higher than the mean incubation temperatures documented in the field (Páez and Bock, 1998), it only produced 50% females, while the remaining two incubators produced mostly males. Thus, there was not a comparable number of individuals of each sex in each of the growth conditions. Unfortunately, the responsible governmental regulatory agency denied us permission to sacrifice all of the individuals at the end of the growth study to determine their sexes and thereby include this variable in the analyses.

Initial hatchling sizes. In 1993, eggs from Nest 1 were significantly larger than those from Nest 2 (ANOVA, $F_{(1,30)} = 110.77$, $P < 0.001$; table 3). Because eggs from each clutch were assigned at random to the two artificial incubation conditions, there were no significant differences in egg sizes among

Table 1. Results of the histological analysis of gonads of the neonate turtles obtained in 1993 from the two nests with most extreme incubation temperatures on Cahuinarí Island. Mean diurnal incubation temperatures for each nest are indicated

	Number of males	Number of females	Sex ratio
Hot nest (31.5 °C)	0	4	100% ♀
Cold nest (29.5 °C)	7	2	78% ♂ : 22% ♀

Table 2. Results of the histological analysis of the gonads of turtles obtained in 1993 and 1994 from the artificial incubators, with the mean incubation temperature for each condition

	Number of males	Number of females	Sex ratio
Hot incubator (31.9 °C)	2	2	50% ♂ : 50% ♀
Intermediate incubator (30.2 °C)	9	1	90% ♂ : 10% ♀
Cold incubator (27.1 °C)	9	0	100% ♂

incubators (ANOVA, $F_{(1,30)} = 0.01$, $P > 0.05$; table 3). In 1994, there also were significant differences in egg size among the three nests (ANOVA, $F_{(2,51)} = 36.37$, $P < 0.001$; table 3), and, as in 1993, because eggs from each clutch were assigned at random to the three artificial incubation conditions, there were no significant differences in egg sizes among incubators (ANOVA, $F_{(2,51)} = 0.12$, $P > 0.05$; table 3). Egg size in 1994 was significantly smaller than in 1993 (ANOVA, $F_{(1,97)} = 1387.54$, $P < 0.001$).

Regression analyses were used to test whether egg weight could be used to predict initial hatchling weights, but in four of the five nests, there were no significant relationships between these two variables (Pearson r, $\alpha = 0.05$ criterion). In 1993, hatchlings produced from Nest 1 were significantly larger than those from Nest 2 (ANOVA, weight, $F_{(1,29)} = 16.49$, $P < 0.001$; ANOVA, CL, $F_{(1,29)} = 10.48$, $P < 0.005$; table 3) and hatchlings from the two artificial incubation conditions also differed in initial sizes (ANOVA, weight, $F_{(1,29)} = 7.06$, $P < 0.05$; ANOVA, CL, $F_{(1,29)} = 17.62$, $P < 0.001$; table 3), with no interaction effect among these two main effects (2-way ANOVA, weight interaction effect, $F = 0.04$, $P > 0.05$; 2-way ANOVA, CL interaction effect, $F = 0.68$, $P > 0.05$; table 3, figure 2). That is, hatchlings from Nest 1 were always the largest in each incubation condition.

In 1994, there were significant differences in initial hatchling sizes among the three nests in terms of both hatchling weights (ANOVA, $F_{(2,51)} = 12.91$, $P < 0.001$; table 3) and CL (ANOVA, $F_{(2,51)} = 23.66$, $P < 0.001$; table 3) and also among the three artificial incubation conditions in terms of hatchling weights (ANOVA, $F_{(2,51)} = 3.75$, $P < 0.05$; table 3). There were no significant interactions among the effects (2-way ANOVA, weight, $F = 0.70$, $P > 0.05$, table 3, figure 3). Consistent with the significant differences in egg weights between the two years, initial hatchling weights also differed, with 1993 hatchlings significantly larger (ANOVA, weight, $F_{(1,98)} = 15.81$, $P < 0.001$; CL, $F_{(1,98)} = 7.70$, $P < 0.001$).

Table 3. Egg weights and initial and final juvenile turtle sizes by incubation condition and nest

	Incubation condition		Nest	
1993	Hot (31.5 °C)	Cold (29.5 °C)	1	2
Mean egg weight (g)	26.5	26.6	28.3	24.7
Mean juvenile weight at hatching (g)	18.5	20.0	20.3	18.2
Mean juvenile CL at hatching (cm)	4.1	4.3	4.3	4.1
Mean juvenile weight at week 43 (g)	34.6	38.3	37.8	35.1
Mean juvenile CL at week 43 (cm)	5.7	5.8	5.8	5.6

	Incubation condition			Nest		
1994	Hot (31.9 °C)	Intermediate (30.2 °C)	Cold (27.1 °C)	A	B	C
Mean egg weight (g)	13.7	13.9	13.7	13.8	14.9	12.6
Mean juvenile weight at hatching (g)	17.8	18.8	18.4	18.5	19.1	17.5
Mean juvenile CL at hatching (cm)	4.1	4.2	4.1	4.2	4.1	4.0
Mean juvenile weight at week 46 (g)	53.0	34.4	35.4	34.5	49.3	40.2
Mean juvenile CL at week 46 (cm)	6.9	6.3	5.9	6.1	6.8	6.3

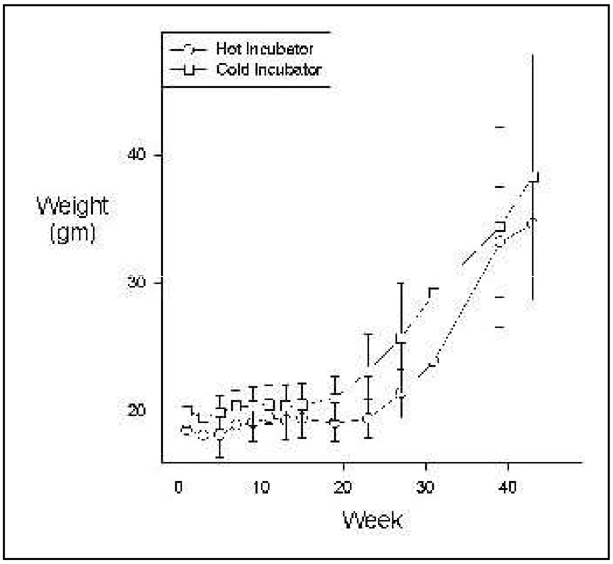

Figure 2. Growth of juvenile turtles obtained from the two incubation conditions in 1993

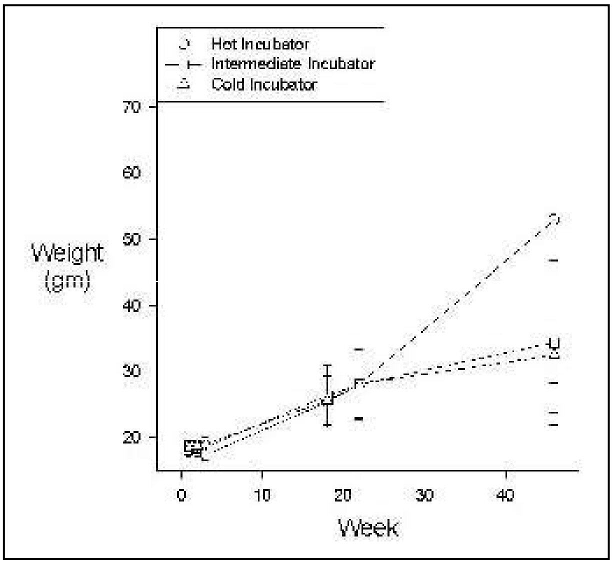

Figure 3. Growth of juvenile turtles obtained from the three incubation conditions in 1994

Mean hatchling sizes from natural nests studied on Cahuinarí Island were compared to the mean hatchling sizes obtained in the laboratory each year. In 1993, there were no significant differences between the two laboratory and 25 natural nests in terms of mean hatchling sizes at emergence (Mann-Whitney U test, $P > 0.05$), with hatchlings from the two laboratory nests on the large end of the continuum. In 1994, there also were no significant differences between the three laboratory and 17 natural nests in mean hatchling sizes (Mann-Whitney U test, $P > 0.05$), with hatchlings from the three laboratory nests in the center of the distribution.

Pre-emergence factors and juvenile growth rates. We compared juvenile turtles from the different nests and incubation conditions independent of date (that is, comparing the sizes of individuals of approximately equal ages), using a repeated measures ANOVA analysis for each year, and including all measurement data (14 measurements over 43 weeks in 1993 and 7 measurements over 46 weeks in 1994). In 1993, there was a significant time*nest interaction (weight, $F = 3.59$, $P < 0.05$; CL, $F = 2.78$, $P < 0.05$), with hatchlings from Nest 2 significantly smaller than those from Nest 1 at the time of hatching (see above), but not by the time they had attained 43 weeks of age (ANOVA, weight, $F_{(1,29)} = 1.17$, $P > 0.05$; ANOVA, CL, $F_{(1,29)} = 2.05$, $P > 0.05$; table 4). There also was a significant time*incubation condition interaction (weight, $F = 12.41$, $P < 0.001$; CL, $F = 15.24$, $P < 0.001$), with turtles from the hot incubator initially significantly smaller (see above), but not significantly different from those from the cold incubator condition by the time they had attained 43 weeks of age (ANOVA, weight, $F_{(1,29)} = 1.17$, $P > 0.05$; ANOVA, CL, $F_{(1,29)} = 0.40$, $P > 0.05$; table 4). There was not a significant time*nest*incubation condition interaction (weight, $F = 1.90$, $P > 0.05$; CL, $F = 0.85$, $P > 0.05$).

In 1994, there was no significant time*nest interaction (weight, $F = 0.83$, $P > 0.05$; CL, $F = 1.81$, $P > 0.05$), but again there was a significant time*incubation condition interaction (weight, $F = 2.87$, $P < 0.01$; CL, $F = 3.09$, $P < 0.01$), with turtles from the cold incubation condition initially larger at the time of emergence (see above) but smaller by the time they attained 46 weeks of age (ANOVA, weight, $F_{(2,40)} = 4.12$, $P < 0.05$; ANOVA, CL, $F_{(2,29)} = 4.47$, $P < 0.05$; table 4). In 1994 we also failed to detect a significant time*nest*incubation condition interaction (weight, $F = 0.63$, $P > 0.05$; carapace length, $F = 1.32$, $P > 0.05$). Thus, in both years turtles from the hot incubator condition emerged at smaller sizes, but then grew faster, so that by the end of their first year of life, they were equal to or greater in size than their siblings that had experienced cooler incubation temperatures.

Table 4. Size of juvenile turtles by incubation condition and nest (by calendar date rather than by age)

	Incubation condition		Nest	
1993	Hot (31.5 °C)	Cold (29.5 °C)	1	2
Mean juvenile weight at the end of hatching (g)	19.0	20.0	20.3	18.7
Mean juvenile CL at the end of hatching (cm)	4.6	4.7	4.7	4.5
Mean juvenile weight at the end of the study (g)	50.7	45.5	49.4	50.4
Mean juvenile CL at the end of the study (cm)	6.5	6.1	6.4	6.3

	Incubation condition			Nest		
1994	Hot (31.9 °C)	Intermediate (30.2 °C)	Cold (27.1 °C)	A	B	C
Mean juvenile weight at the end of hatching (g)	20.5	20.9	18.8	20.0	21.0	19.3
Mean juvenile CL at the end of hatching (cm)	4.7	4.7	4.3	4.7	4.6	4.5
Mean juvenile weight at the end of the study (g)	55.0	45.5	37.7	41.6	54.4	42.2
Mean juvenile CL at the end of the study (cm)	6.7	6.4	5.9	6.2	6.7	6.2

This difference was even more dramatic when we compared individuals by date rather than by age, since incubation temperature also strongly affected incubation period (Páez, 1995; Páez and Bock, 1997, 1998), with turtles from the hot incubation condition emerging approximately seven weeks before those incubated in the cold condition.

The next question was whether the turtles from the hot incubator condition were still smaller than those from the cold incubator condition by the time emergence had finished in the cold condition, and also at the time the study concluded. In 1993, by the date at which emergence in the laboratory had ended, the turtles from the hot incubation condition were significantly lighter than those from the cold incubation condition (ANOVA, $F_{(1,30)} = 4.33$, $P < 0.05$), but were comparable to them in terms of CL (ANOVA, $F_{(1,30)} = 0.54$, $P > 0.05$; table 4). In 1994, by the date at which emergence in the laboratory had ended, the turtles from the hot and intermediate incubation conditions had grown sufficiently to be significantly larger than the recently emerged individuals from the cold incubation condition (ANOVA, weight, $F_{(2,50)} = 11.50$, $P < 0.001$; ANOVA, CL, $F_{(2,50)} = 42.53$, $P < 0.001$; table 4).

In 1993 at the conclusion of the study (when some individuals from the hot incubator were 54 weeks old and others from the cold incubator condition were 47 weeks old), turtles from both incubation conditions had comparable sizes (ANOVA, weight, $F_{(1,29)} = 1.79$, $P > 0.05$; ANOVA, CL, $F_{(1,29)} = 3.38$, $P > 0.05$; table 4). In 1994, the differences among turtles from the hot, intermediate, and cold incubator conditions persisted until the end of the study (with turtles ranging from 47-54 weeks of age; ANOVA, weight, $F_{(2,38)} = 3.75$, $P < 0.05$; ANOVA, CL, $F_{(2,38)} = 4.01$, $P < 0.05$; table 4), with the turtles from the hot incubation condition the largest and those from the cold incubation condition the smallest.

Post-emergence factors and juvenile growth rates. The study design permitted consideration of the effects of three post-emergence factors (diet, density, and basking opportunities) on juvenile growth rates, independent of the influence of pre-emergence variables (nest and incubation condition). A comparison of juvenile turtles from the different rearing conditions independent of date (that is, comparing the sizes of individuals of approximately equal ages) was accomplished using a repeated measures ANOVA analysis for each year. In 1993, there was a significant time*diet interaction (weight, $F = 2.15$, $P < 0.05$; CL, $F = 2.81$, $P < 0.01$), with individuals receiving meat in their diet attaining significantly larger weights at 43 weeks of age than those turtles which only received vegetable material (ANOVA, $F_{(2,28)} = 5.32$, $P < 0.05$; table 5). In 1994, the results were comparable to 1993 (with a significant time*diet interaction; weight, $F = 5.65$, $P < 0.001$; CL, $F = 2.47$, $P < 0.05$), with individuals receiving meat in their diet attaining significantly larger sizes (ANOVA, weight, $F_{(2,39)} = 5.77$, $P < 0.01$; ANOVA, CL, $F_{(2,29)} = 7.53$, $P < 0.01$; table 5). In 1994, turtles in the low density conditions also achieved greater weights by the time they had attained 46 weeks of age (ANOVA, weight, $F_{(1,40)} = 9.37$, $P < 0.01$). There were no significant time*diet*density interactions in either year (table 5).

Table 5. Initial and final juvenile turtle sizes by diet, density, and basking condition

Diet	**Vegetable**	**Meat**	**Mixed**
1993			
Mean juvenile weight at week 43 (g)	31.3	40.3	38.1
Mean juvenile CL at week 43 (cm)	5.6	5.9	5.7
1994			
Mean juvenile weight at week 46 (g)	30.5	38.9	53.5
Mean juvenile CL at week 46 (cm)	5.6	6.7	6.8

Density	**Low**	**High**
1993		
Mean juvenile weight at week 43 (g)	37.7	35.9
Mean juvenile CL at week 43 (cm)	5.8	5.7
1994		
Mean juvenile weight at week 46 (g)	56.0	36.5
Mean juvenile CL at week 46 (cm)	6.8	6.2

Basking condition	**Shade**	**Sun**
1993		
Mean juvenile weight at week 43 (g)	34.5	38.2
Mean juvenile CL at week 43 (cm)	5.7	5.8
1994		
Mean juvenile weight at week 46 (g)	40.6	44.0
Mean juvenile CL at week 46 (cm)	6.3	6.6

DISCUSSION

Souza and Vogt (1994) first documented the ocurrence of TSD in *P. unifilis* in Brazil, using constant incubation temperatures in the laboratory, demonstrating a threshold temperature for this population of 32 °C. The laboratory results in this study agree with those from Brazil, as the artificial incubator set at 32 °C produced equal numbers of males and females, while eggs incubated in the two cooler conditions produced an excess or exclusively males (table 2). The results obtained from the two natural nests from Cahuinarí Island also were consistent with the hypothesis that both populations have the same pivotal temperature of 32 °C, with the cool nest producing mostly males and the hot nest producing exclusively females (table 1). While both of these nests produced more females than one would predict based upon their mean incubation temperatures (compared to the sex ratios obtained in the laboratory incubators), many studies have shown that mean nest temperature is a poor predictor of hatchling sex ratios when nest temperatures fluctuate widely (Bull, 1985b; Bull and Vogt, 1979; Doody, 1999; Georges, 1989; Georges *et al.*, 1994; Marcovaldi *et al.*, 1997; Paukstis *et al.*, 1984; Pieau, 1984; Schwartzkopf and Brooks, 1985; Thompson, 1988; Vogt and Bull, 1984; Valenzuela *et al.*, 1997; Wood and Wood, 1982).

Georges (1989) argued that the proportion of the development period that the embryos spend above the pivotal temperature is what actually determines the sex ratio in a given nest. Although our field incubation temperature data were not sufficient to permit an accurate estimation of the proportion of time each nest spent above 32 °C, we could inspect for how often the daytime temperatures we recorded exceeded this value. In the hotest natural nest (100% females), 43.7% of all daytime readings exceeded the pivotal value, compared to 33.2% of all readings obtained for the hot artificial incubator condition in the laboratory (50% females). Similarly, in the coldest natural nest (22% females), 26.3% of all daytime readings exceeded the pivotal value, as compared to only 2% of all readings for the intermediate artificial incubation condition in the laboratory (10% females). Thus, in both comparisons, the proportion of readings above the presumed pivotal temperature better predicted sex ratios than did the mean of all of the temperatures we recorded from these nests or incubators. Our data suggest that during these years, the majority of natural nests produced a mix of the two sexes.

Despite the relatively low incubation temperatures that characterize nesting beaches in this area of Colombia (Bock and Páez, 1998), there was no evidence that the pivotal temperature for this population differed from that reported in Brazil (Souza and Vogt, 1994). This does not mean that such differences do not exist within the range of the species, but merely argues for the need for more information on interpopulational variation in pivotal temperatures in turtles, especially using comparable techniques and controlling more for the effects of fluctuating incubation temperatures (i.e.: Georges, 1989, Georges *et al*., 1994). In those temperate zone species for which the pivotal temperature has been documented for more than one population, evidence for interpopulational variation in pivotal temperatures has been documented (Bull *et al.*, 1982; Passmore and Brooks, 1997; Tucker and Warner, 1999; Vogt and Flores, 1992). To our knowledge, only a few studies have attempted to inspect for differences in pivotal temperatures among populations of tropical freshwater turtles (Vogt and Flores, 1992), in contrast to what has been documented for tropical sea turtles (Baptitotte *et al*., 1999; Binckley *et al*., 1998; Limpus *et al*., 1985; Mrosovsky, 1994).

This study also demonstrated an incubation temperature effect on initial hatchling sizes irrespective of sex or initial egg size, with the hot incubation condition producing the smallest hatchlings. This is contrary to what Souza and Vogt (1994) found for this species in Brazil. However, this pattern is consistent with results from comparable studies on other reptile species, including turtles (Bobyn and Brooks, 1994; Brooks *et al*., 1991; Cagle *et al*., 1993; Gutzke and Packard, 1987; Gutzke *et al*., 1987; McKnight and Gutzke, 1993; Packard *et al*., 1988, 1989; Valenzuela, 2001).

We also documented strong maternal effects on egg and juvenile sizes in this species, with egg size varying substantially between nests and this variation leading to significant differences among hatchlings in terms of their initial sizes. However, within nests it usually was not possible to predict hatchling size from information on egg size. Parental investment theory predicts that natural selection should favor an optimal egg (or offspring) size in any given species (Brockelman, 1975; Parker and Begon, 1986; Smith and Fretwell, 1974). This optimal amount of parental investment should occur at the point where investing more yolk in a particular offspring yields less benefit to the parent than that obtained by producing another egg (Congdon and Gibbons, 1985, 1987; Sargeant and Gross, 1985; Smith and Fretwell, 1974). That is, the prediction is that most of the variation in reproductive investment should be expressed in terms of the number of eggs produced, rather than in variation in the sizes of the eggs themselves. Nevertheless, this does not seem to be the case in turtles, given that there is frequently significant variation in egg size within populations (Congdon and Gibbons, 1990; Congdon *et al*., 1999; Valenzuela, 2001). In this study, variation among nests in egg sizes was much greater than variation among nests in hatchling sizes, raising the question of which would be the better measure of parental investment for turtle species (Brooks *et al*., 1991; Rowe, 1995; Valenzuela, 2001).

In both years of this study, individuals from the different nests exhibited comparable growth rates, suggesting that the maternal effect on egg and initial hatchling sizes resulted largely from differences in the amounts of yolk invested by each female in the eggs, rather than any underlying genetic developmental differences among the individuals they produced. This is in contrast to several studies that have documented a continued maternal effect on juvenile turtle growth rates (Bobyn and Brooks, 1994; Brooks *et al.*, 1991; McKnight and Gutzke, 1993; Rhen and Lang, 1995; Roosenburg and Kelly, 1996). However, in both years of this study there was a significant incubation condition effect on juvenile growth rates. Although turtles obtained from the hot incubation condition emerged at smaller sizes, they experienced sufficient growth while the eggs in the cold incubation condition were still incubating that they had attained comparable or greater body sizes by the time the latter turtles finally emerged (table 4). In 1994, turtles from the warmer incubation conditions continued to grow faster than their siblings incubated under cooler temperatures, attaining significantly larger body sizes by the time they reached 46 weeks of age (table 3). This effect was even more pronounced when turtles were compared based on calander date at the end of the study (table 4) rather than simply comparing individuals of similar ages. These results are consistent with those obtained by Bobyn and Brooks (1994) and Valenzuela (2001), although the latter study only quantified growth rates during the initial months of life and did not conduct comparisons based on the ages of the individuals (however, see Vogt, 1980).

In a review of the literature on TSD in turtles, Ewert, *et al.* (1994) found that in those species known to have both TSD and larger adult female sizes, most exhibit one threshold temperature with females being produced at higher incubation temperatures, as predicted by the differential fitness model of Charnov and Bull (see introduction; Burke, 1993; Burke *et al.*, 1996; Charnov and Bull, 1977; Ewert and Nelson, 1991; Janzen and Paukstis, 1991; Shine, 1999). *Podocnemis unifilis* exhibits adult sexual dimorphism, with females being the larger sex (Medem, 1964) and this study demonstrated that eggs incubated in warmer temperatures produced individuals which attained larger sizes by the end of the first year of life, consistent with the Charnov and Bull model. Had we not studied growth rates of these individuals, our study might have come to the opposite conclusion, since the individuals produced in the warmer incubation conditions were actually smaller than those from the cooler conditions at the time of emergence. Studies of the TSD phenomenon in turtles need to be aware that the potential effects of incubation temperature on traits related to fitness may be unapparent at hatching, arguing for the need to include longer-term rearing studies in such studies, rather than sacrificing all individuals obtained from the incubators for sexing at the time of hatching, as is commonly done (Vogt, 1980).

We complicated the design of this study by also including a consideration of three post-hatching variables on juvenile turtle growth rates, in an effort to obtain as much information as possible from these individuals. The fact that turtles from the different nests and incubation conditions were raised under different environmental conditions increased the variances for these main effects in the analyses of the effects of pre-hatching factors on growth rates, and viceversa. Nevertheless, we succeeded in documenting significant pre- and post-hatching effects. Our analysis of the effects of differing diets on growth rates confirmed other reports that juvenile *P. unifilis* readily consume meat (Cole and Link, 1972; Fachin, *et al.*, 1995), and demonstrated that they grow faster when they do. The effects of adding vitamin supplements to the diet in 1993 also was conspicuous (figure 2). In 1994, turtles in the low density condition also grew faster, but exposure to direct sunlight did not significantly increase growth rates in either year. Turtles in both shade and sun conditions frequently emerged to bask, but perhaps given their small body sizes, individuals in both conditions were probably able to rapidly elevate their body temperatures, irregardless of whether they had access to direct sunlight or not.

Turtle captive rearing projects, whether motivated by economic or conservation purposes, have been heavily criticized in the literature on a variety of grounds (Dodd, 1982; Ehrenfeld, 1974; Frazer, 1992, 1997). Opponents claim that commercial farming ventures merely expand the demand for products obtained from already over-exploited species while providing very little protein to needy people. Headstarting programs also have been criticized because eggs are usually taken from natural nests rather than produced via captive breeding, and because data are often lacking to support claims that such programs actually benefit the natural populations into which captive-reared individuals are released. Our study

was not a pilot head-starting project, but rather an attempt to learn about the ecology of juvenile *P. unifilis*, given that conducting studies of this kind in the field is extremely complicated. The results of our study suggest that in the field, juveniles of this species are opportunistic omnivores, but probably are not limited by competition for food except under unusual circumstances where they attain high densities. Basking also appears to be a common behavior, but apparently does not need to occur in areas that receive direct sunlight, with a thermal benefit accruing even to individuals that receive only reflected ambient radiation. Nevertheless, even under the optimal laboratory conditions, overall growth rates in the juvenile *P. unifilis* were relatively low, suggesting that captive rearing of this species for economic purposes does not hold much promise. Although the juveniles exhibited high survivorships in captivity, any repopulation project would probably have to continue captive rearing of individuals for at least one to two more years before they would attain sizes that are less vulnerable to predators in the field.

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REFERENCES

Avery HW, Spotila JR, Congdon JD, Fisher RUJr., Standora EA, Avery SB. 1993. Roles of diet protein and temperature in the growth and nutritional energetics of juvenile slider turtles, *Trachemys scripta*. *Physio Zool* 66:902-925.

Baptistotte C, Scalfoni JT, Mrosovsky N. 1999. Male-producing thermal ecology of southern loggerhead turtle nesting beach in Brazil: implications for conservation. *Anim Conserv* 2:9-13.

Binckley CA, Spotila JR, Wilson KS, Paladino FV. 1998. Sex determination and sex ratios of Pacific leatherback turtles, *Dermochelys coriacea*. *Copeia* 1998:291-300.

Bjorndal KA. 1985. Nutritional ecology of sea turtles. *Copeia* 1985:736-751.

Bjorndal KA. 1991. Diet mixing: nonadditive interactions of diet items in an omnivorous freshwater turtle. *Ecology* 72:1234-1241.

Bobyn ML, Brooks RJ. 1994. Interclutch and interpopulation variation in the effects of incubation condition on sex, survival, and growth of hatchling turtles (*Chelydra serpentina*). *J Zool* 233:233-257.

Bock BC, Páez VP, Pérez NF. 1998. Estudio preliminar con radiotelemetría sobre los desplazamientos de hembras de la tortuga *Podocnemis unifilis* en el río Caquetá, Amazonas, Colombia. *Actual Biol* 20:29-36.

Boyer DR. 1965. Ecology of the basking habit in turtles. *Ecology* 46:99-118.

Brockelman WY. 1975. Competition, the fitness of offspring, and optimal clutch size. *Amer Nat* 109:677-699.

Brooks RJ, Bobyn ML, Galbraith DA, Layfield JA, Nancekivell EG. 1991. Maternal and environmental influences on growth and survival of embryonic and hatchling snapping turtles (*Chelydra serpentina*). *Can J Zool* 69:2667-2676.

Brown GP, Bishop CA, Brooks RJ. 1994. Growth rate, reproductive output, and temperature selection of snapping turtles in habitats of different productivities. *J Herpetol* 28:405-410.

Bull JJ. 1985a. Sex determining mechanisms: an evolutionary perspective. *Experientia* 41:1285-1296.

Bull JJ. 1985b. Sex ratio and nest temperature in turtles: comparing field and laboratory data. *Ecology* 66:1115-1122.

Bull JJ, Vogt RC. 1979. Temperature-dependent sex determination in turtles. *Science* 206:1186-1188.

Bull JJ, Vogt RC, McCoy CJ. 1982. Sex determining temperatures in turtles: a geographic comparison. *Evolution* 36:326-332.

Burke RL. 1993. Adaptive value of sex determination mode and hatchling sex ratio bias in reptiles. *Copeia* 1993:854-859.

Burke RL, Ewert MA, McLemore JB, Jackson DR. 1996. Temperature-dependent sex determination and hatching success in the tortoise (*Gopherus polyphemus*). *Chelon Conserv Biol* 2:86-88.

Bury RB. 1979. Population ecology of freshwater turtles. *En:* Harless M, Morlock H (eds.). *Turtles: perspectives and research*. New York, USA, John Wiley and Sons, pp. 571-602.

Cagle KD, Packard GC, Miller K, Packard MJ. 1993. Effects of the microclimate in natural nests on development of embryonic painted turtles, *Chrysemys picta*. *Funct Ecol* 7:653-660.

Charnov EL, Bull JJ. 1977. When is sex environmentally determined? *Nature* 266:828-830.

Cole M, Link B. 1972. *Podocnemis* in captivity. *Int Turtle Tortoise Soc J* 6:12-14.

Congdon JD, Gibbons JW. 1985. Egg components and reproductive characteristics of turtles: relationships to body size. *Herpetologica* 41:194-205.

Congdon JD, Gibbons JW. 1987. Morphological constraint on egg size: A challenge to optimal egg size theory? *Proc Natl Acad Sci USA*, 84:4145-4147.

Congdon JD, Gibbons JW. 1990. Turtle eggs: their ecology and evolution. *En:* Gibbons JW (ed.). *Life history and ecology of the slider turtle*. Washington, D.C. USA, Smithsonian Institution Press, pp. 109-123.

Congdon JD, Gibbons JW, Greene JL. 1983. Parental investment in the chicken turtle (*Deirochelys reticularia*). *Ecology* 64:419-425.

Congdon JD, Nagle RD, Dunham AE, Beck CW, Kinney OW, Yeomans SR. 1999. The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentina*): an evaluation of the "bigger is better" hypothesis. *Oecologia* 121:224-235.

Danni TMS, Alho CJR. 1985. Estudio histológico da diferenciação sexual em tartarugas recém-eclodidas (*Podocnemis expansa*, Pelomedusidae). *Rev Brasil Biol* 45:365-368.

De Almeida SS, Sa PGS, García A. 1986. Vegetais utiizados como alimento por *Podocnemis* (Chelonia) na região do Baixo Rio Xingu (Brasil-Pará). *Bol Mu Par Emílio Goeldi Se Bot* 2:199-211.

Dodd CK Jr. 1982. Does sea turtle aquaculture benefit conservation? *En:* Bjorndal KA (ed.). *Biology and conservation of sea turtles*. Washington, D.C. USA, Smithsonian Institution Press, pp. 473-480.

Doody JS. 1999. A test of the comparative influences of constant and fluctuating incubation temperatures on phenotypes of hatchling turtles. *Chelon Conserv Biol* 3:529-531.

Dunham AE, Gibbons JW. 1990. Growth in the slider turtle. *En:* Gibbons JW (ed.). *Life history and ecology of the slider turtle.* Washington D.C. USA, Smithsonian Institution Press, pp. 135-145.

Ehrenfeld DW. 1974. Conserving the edible sea turtle: Can mariculture help? *Amer Sci* 62:23-31.

Escalona T, Fa JE. 1998. Survival of nests of the terecay turtle (*Podocnemis unifilis*) in the Nichare-Tawadu rivers, Venezuela. *J Zool Lond* 244:303-312.

Ewert MA, Nelson CE. 1991. Sex determination in turtles: diverse patterns and some plausible adaptive values. *Copeia* 1991:50-68.

Ewert MA, Jackson DR, Nelson CE. 1994. Patterns of temperature-dependent sex determination in turtles. *J Exper Zool* 270:3-15.

Fachin-T A. 1992. Desove y uso de playas para nidificación de taricaya (*Podocnemis unifilis*) en el río Samiria, Loreto, Perú. *Bol Lima* 79:65-75.

Fachin-T A. 1993. Características de *Podocnemis unifilis* (Reptilia, Testudines) en el río Samiria, Loreto. *Bol Lima*, 87:69-74.

Fachin-T A. 1994. Depredación de la taricaya *Podocnemis unifilis* en la Reserva Nacional Pacaya-Samiria, Loreto. *Bol Lima*, 91-96:417-423.

Fachin-T A, Acosta-D A, Vilchez-R I. 1992. Tortugas *Podocnemis* mantenidas en cautiverio en los alrededores de Iquitos, Loreto, Perú. *Bol Lima*, 84:79-88.

Fachin-T A, Vogt RC, Soares-G MF. 1995. Food habits of an assemblage of five species of turtles in the Rio Guapore, Rondonia, Brazil. *J Herpetol* 29:536-547.

Foote RW. 1978. Nesting of *Podocnemis unifilis* (Testudines: Pelomedusidae) in the Colombian Amazon. *Herpetologica* 34:333-339.

Frazer NB. 1992. Sea turtle conservation and halfway technology. *Conserv Biol* 6:179-184.

Frazer NB. 1997. Turtle conservation and half-way technology: What is the problem? *En:* Van Abbema J (ed.). *Proceedings: Conservation, restoration, and management of tortoises and turtles. An international conference.* New York, USA, New York Turtle and Tortoise Society, pp. 422-425.

Frazer NB, Gibbons JW, Greene JL. 1990. Exploring Fabens' growth interval model with data on a long-lived vertebrate, *Trachemys scripta* (Reptilia, Testudinata). *Copeia* 1990:112-118.

Froese AD, Burghardt GM. 1974. Food competition in captive juvenile snapping turtles, *Chelydra serpentina*. *Anim Behav* 22:735-740.

Georges A. 1989. Female turtles from hot nests: Is it duration or proportion of development at high temperatures that matters? *Oecologia* 81:323-328.

Georges A, Limpus C, Stoutjesdijk R. 1994. Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *J Exper Zool* 270:432-444.

Gibbons JW. 1967. Variation in growth rates in three populations of the painted turtle, *Chrysemys picta*. *Herpetologica* 23:296-303.

Gibbons JW. 1982. Reproductive patterns in freshwater turtles. *Herpetologica* 38:222-227.

Gibbons JW, Greene JL, Patterson KK. 1982. Variation in reproductive characteristics in aquatic turtles. *Copeia* 1982:776-784.

Gutzke WH, Packard GC, Packard MJ, Boardman TJ. 1987. Influence of the hydric and thermal environments on eggs and hatchlings of painted turtles (*Chrysemys picta*). *Herpetologica* 43:393-404.

Gutzke WHN, Packard GC. 1987. The influence of temperature on eggs and hatchlings of Blanding's turtles, *Emydoidea blandingii*. *J Herpetol* 21:161-163.

Jackson CG Jr, Trotter JA, Trotter TH, Trotter MW. 1976. Accelerated growth rate and early maturity in *Gopherus agassizi* (Reptilia: Testudines). *Herpetologica* 32:139-145.

Janzen FJ, Paukstis GL. 1991. Environmental sex determination in reptiles: ecology, evolution and experimental design. *Quart Rev Biol* 66:149-179.

Janzen FL, Paukstis GL, Brodie EJ III. 1992. Observations on basking behavior of hatchling turtles in the wild. *J Herpetol* 26:217-219.

Limpus CJ, Reed PC, Miller JD. 1985. Temperature-dependent sex determination in Queensland sea turtles: intraspecific variation in *Caretta caretta*. *En:* Grigg G, Shine R, Ehmann H (eds.). *Biology of Australian frogs and reptiles.* New South Wales, Australia, Royal Zool Soc, pp. 342-351.

Lindeman PV. 1993. Aerial basking by hatchling freshwater turtles. *Herpetol Rev* 24:84-87.

Marcovaldi MA, Godfrey MH, Mrosovsky N. 1997. Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation durations. *Can J Zool* 75:755-770.

McKnight CJ, Gutzke WHN. 1993. Effects of the embryonic environment and of hatchling housing conditions on growth of young snapping turtles (*Chelydra serpentina*). *Copeia* 1993:475-482.

Medem F. 1964. Morphologie, Öcologie und Verbreitund der Schildkröte *Podocnemis unifilis* in Kolumbien (Testudinata, Pelomedusidae). *Senckenb Biol* 45:353-368.

Mrosovsky N. 1994. Sex ratios of sea turtles. *J Exper Zool* 270:16-27.

Packard GC, Packard MJ, Birchard GF. 1989. Sexual differentiation and hatching success by painted turtles incubating in different thermal and hydric environments. *Herpetologica* 45:385-392.

Packard GC, Packard MJ, Miller K, Boardman TJ. 1988. Effects of temperature and moisture during incubation on carcass composition of hatchling snapping turtles (*Chelydra serpentina*). *J Comp Physiol* B158:117-125.

Páez VP. 1995. *The conservation and nesting ecology of the endangered yellow-spotted Amazonian turtle, Podocnemis unifilis*. Doctoral dissertation, Department of Biological Sciences. Ohio University, Athens, Ohio, USA.

Páez VP, Bock BC. 1997. Nesting ecology of the yellow-spotted river turtle in the Colombian Amazon. *En:* Van Abbema J (ed.*). Proceedings: Conservation, restoration, and management of tortoises and turtles. An international conference.* New York, USA, New York Turtle and Tortoise Society, pp. 219-224.

Páez VP, Bock BC. 1998. Temperature effect on incubation period in the yellow-spotted river turtle, *Podocnemis unifilis*, in the Colombian Amazon. *Chelon Conserv Biol* 3:31-36.

Parker GA, Begon M. 1986. Optimal egg size and clutch size: effects of environment and maternal phenotype. *Amer Nat* 128:573-592.

Parmenter RR. 1981. Digestive turnover rates in freshwater turtles: the influence of temperature and body size. *Comp Biochem Physiol* 70A:235-238.

Passmore HL, Brooks RJ. 1997. Effects of geographic origin and incubation temperature on hatchling snapping turtles, *Chelydra serpentina*: implications for conservation practices across the species range. *En:* Van Abbema J (ed.). *Proceedings: Conservation, restoration, and management of tortoises and turtles. An international conference.* New York, USA, New York Turtle and Tortoise Society, pp. 195-202.

Paukstis GL, Gutzke WHN, Packard GC. 1984. Effects of substrate water potential and fluctuating temperatures on sex ratios of hatchling painted turtles (*Chrysemys picta*). *Can J Zool* 62:1491-1494.

Pieau C. 1982. Modalities of the action of temperature on sexual differentiation in field-developing embryos of the European pond turtle *Emys orbicularis* (Emydidae). *J Exp Zool* 220:353-360.

Rhen T, Lang JW. 1995. Phenotypic plasticity for growth in the common snapping turlte: effects of incubation temperature, clutch, and their interactions. *Amer Nat* 146:726-747.

Roosenburg WM. 1996. Maternal condition and nest site choice: An alternative for the mantenance of environmental sex determination? *Amer Zool* 36:157-168.

Roosenburg WM, Kelley KC. 1996. The effect of egg size and incubation temperature on growth in the turtle, *Malaclemys terrapin*. *J Herpetol* 30:198-204.

Rowe JW. 1995. Hatchling size in the turtle *Chrysemys picta bellii* from Western Nebraska: relationship to egg and maternal body size. *J Herpetol* 29:73-79.

Sargent RC, Gross MR. 1985. Parental investment decision rules and the Concorde fallacy. *Behav Ecol Sociobiol* 17:43-45.

SAS Institute Inc. 1989. *SAS/STAT Users Guide, Version 6*. SAS Institute Inc., Cary, North Carolina. USA.

Schwartzkopf L, Brooks RJ. 1985. Sex determination in northern painted turtles: effect of incubation at constant and fluctuating temperatures. *Can J Zool* 63:2543-2547.

Shine R. 1999. Why is sex determined by nest temperature in many reptiles? *Tree* 14:186-189.

Smith CC, Fretwell SD. 1974. The optimal balance between size and number of offspring. *Amer Nat* 108:499-506.

Souza RR, Vogt RC. 1994. Incubation temperature influences sex and hatchling size in the neotropical turtle *Podocnemis unifilis*. *J Herpetol* 28:453-464.

Spotila JR. 1995. Metabolism, physiology, and thermoregulation. *En:* Bjorndal KA (ed.). *Biology and conservation of sea turtles*. Washington, D.C. USA, Smithsonian Institution Press, pp. 591-592.

Spotila JR, Zimmerman LC, Binckley CA, Grumbles JS, Rostal DC, Lest A Jr, Berger EC, Phillips KM, Kemp SJ. 1994. Effects on incubation conditions on sex determination, hatching success and growth of hatching desert tortoises *Gopherus agassizii*. *Herpetol Monog* 8:103-116.

Standora EA, Spotila JR. 1985. Temperature dependent sex determination in sea turtles. *Copeia* 1985:711-722.

Thorbjarnarson JB, Pérez N, Escalona T. 1993. Nesting of *Podocnemis unifilis* in the Capanaporo River, Venezuela. *J Herpetol* 27:344-347.

Thompson MB. 1988. Influence of incubation temperature and water potential on sex determination in *Emydura macquarii* (Testudines: Pleurodira). *Herpetologica* 44:86-90.

Tucker JK, Warner DA. 1999. Microgeographic variation in response of red-eared slider (*Trachemys scripta elegans*) embryos to similar incubation environments. *J Herpetol* 33:549-557.

Valenzuela N, Botero R, Martínez M. 1997. Field study of sex determination in *Podocnemis expansa* from Colombian Amazonia. *Herpetologica* 53:390-398.

Valenzuela N. 2001. Maternal effects on life-history traits in the Amazonian giant river turtle *Podocnemis expansa. J Herpetol* 35:368-378.

Vanzolini PE. 1977. A brief biometerical note on the reproductive biology of some South American *Podocnemis* (Testudines, Pelomedusidae). *Pap Avulsos Zool Sao Paulo* 31:79-102.

Viets BE, Ewert MA, Talent LG, Nelson CE. 1994. Sex-determining mechanisms in squamate reptiles. *J Exper Zool* 270:45-56.

Vogt RC. 1980. Natural history of the map turtles *Gratemys pseudogeografica* and *G. ouachitensis* in Wisconsin. *Tulane Stud Zool Bot* 22:17-48.

Vogt RC, Bull JJ. 1984. Ecology of hatchling sex ratio in map turtles. *Ecology* 65:582-587.

Vogt RC, Guzmán-S S. 1988. Food partitioning in a neotropical freshwater turtle community. *Copeia* 1988:37-46.

Vogt RC, Flores-V O. 1992. Effects of incubation temperature on sex determination in a community of neotropical freshwater turtles in southern Mexico. *Herpetologica* 48:265-270.

Wibbels ST, Bull JJ, Crews D. 1994. Temperature-dependent sex determination: a mechanistic approach. *J Exper Zool* 270:71-78.

Wilbur HM. 1975. A growth model for the turtle *Chrysemys picta*. *Copeia* 1975:337-343.

Wood FE, Wood JR. 1982. Sex ratios in captive-reared green turtles, *Chelonia mydas*. *Copeia* 1982:482-485.

Yntema CL, Mrosovsky N. 1980. Sexual differentiation in hatchling loggerheads (*Caretta caretta*) incubated at different controlled temperatures. *Herpetologica* 36:33-36.