

## REPRODUCTIVE BIOLOGY AND NOTES ON NATURAL HISTORY OF THE SIDE-NECKED TURTLE *ACANTHOCHELYS RADIOLATA* (MIKAN, 1820) IN CAPTIVITY (TESTUDINES: CHELIDAE)

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**ABSTRACT.** Sixteen adult specimens (six males and ten females) of *Acanthochelys radiolata* from different regions of the state of Rio de Janeiro, Brazil, were monitored in captivity from 1996 to 2000 in order to obtain data on species reproductive biology including breeding season, egg laying, egg incubation, and hatchlings. Mating behaviour was observed between November and March and showed a regular pattern constituted by three conspicuous phases: approximation, persecution, and copulation. Sixteen clutches were found between March and July and twelve eggs were found in natural conditions. Hatches occurred between September and November. Hatchlings showed gular region, anterior and posterior members, tail, and edges of carapace and plastron with reddish blotches resting on a black background. This pattern was modified to an orange tonality in the third month of life.

**KEYWORDS.** *Acanthochelys radiolata*, Reproductive biology, Conservation.

### INTRODUCTION

*Acanthochelys radiolata* (Mikan, 1820) is a freshwater side-necked turtle endemic to regions of Brazilian Atlantic rainforest, with a geographical distribution ranging from the State of Alagoas south to the State of São Paulo (Iverson, 1986). The species inhabits slow moving waters, oxbows, and impoundments with a soft bottom where it can bury itself (Bonin *et al.*, 2006), feeding on earthworms, amphibians, insects, mollusks, and fishes (Ernst and Barbour, 1989). Adults have an uniform olive-green, gray or black carapace and a yellow or black mottled plastron; head and neck are creamish white dorsally, and yellowish laterally and ventrally; two conspicuous yellowish barbels with sensorial function (Murphy and Lamoreaux, 1978) are present in the anterior portion of gular region.

*Acanthochelys radiolata* is considered the most threatened chelonian species in the state of Rio de Janeiro (Rocha e Silva and Kischlat, 1992), since the Atlantic Forest within this state was reduced to 20% of the original coverage and the coastal lowlands are under severe anthropic pressure (Tanizaki-Fonseca *et al.*, 2000). The zoo of the City of Rio de Janeiro still receives sporadic donations of specimens of *A. radiolata* from lagoons situated in different regions of the state (M. A. Mocelin, pers. obs.). Fernandes and Gan-

dolfi (2000) considered this species endangered in the municipality of Rio de Janeiro, while Rocha *et al.* (2000) argue that available data for this species are insufficient, classifying this taxon as probably threatened in the same state.

Studies focusing on reproductive biology as well as different aspects of the natural history of *A. radiolata* are very scarce (Molina, 1998; Bonin *et al.*, 2006). The present study reports data on reproductive behavior, ontogenetic variation of color pattern, and growth rate of neonates of *A. radiolata* in captivity. Furthermore, we describe clutches of this turtle found in natural conditions. The aim of this work is to provide information about the biology of *A. radiolata* in order to reinforce the importance of conserving the Atlantic Rainforest biome as well as this endangered species.

### MATERIAL AND METHODS

Sixteen adult specimens (six males and ten females) of *A. radiolata* from different regions of the State of Rio de Janeiro were monitored from 1996 to 2000 in order to obtain data on breeding season, egg laying, egg incubation, and hatchlings. Three hatchlings donated to the Fundação Jardim Zoológico da Cidade do Rio de Janeiro (RIOZOO), from the la-

goon of Geribá, Municipality of Búzios, State of Rio de Janeiro, had their development monitored to analyze aspects of growth rate and ontogenetic variation. Specimens examined were housed at RIOZOO and adults were maintained at least for four years in captivity, in the same enclosure, before the present study was performed. This enclosure (code S02V14) is situated in the public visitation area of RIOZOO, being occupied exclusively by specimens of *A. radiolata*. The total area corresponds to 21.90 m<sup>2</sup>, consisting of a 8.75 m<sup>2</sup> reservoir with 0.60 m depth and a dry area of 13.75 m<sup>2</sup>. Water was completely replaced every week.

All specimens were fed three times a week. Adults received bovine mince on Mondays; dog ration (Frollic®) containing bovine meat, vitamins A, B1, B12, D, and E with maximum levels of calcium (2.5%), and minimum of phosphorous (1%) on Wednesdays; and sardines on Fridays. Young specimens were fed on Mondays with bovine mince; Wednesdays with chelonian specific ration (Tetra Reptomin®) containing fish and meat flour, vitamins A, C, D3, and E, with maximum levels of calcium (3.5%), and minimum of phosphorous (1.2%), and rough protein (39%); and with sardine mince on Fridays. These items were supplemented with calcium carbonate and spread on water for both adults and young.

Sex of adult specimens was determined through the presence of a concavity in the plastron and longer tail in males. Measurements of carapace length (CL: from the cranial portion of nuchal scute to the caudal portion of supracaudal scute) were taken with a dial caliper to the nearest 0.1 cm for adult specimens in order to verify the existence of sexual dimorphism. Young specimens were weighted with an electronic balance Ohaus® L 200 to the nearest 0.1 g, and measured with a digital dial caliper Starrett® to the nearest 0.01 mm. The following measurements were taken every month until young specimens completed ten months and a last measure was taken after one year and four months: carapace length (CL); plastron length (PL: from the cranial portion of intergular scute to the caudal portion of cloacal scutes), and plastron width (PW: taken on the level of suture between pectoral and abdominal scutes).

Behavioral observations of adult specimens were carried out throughout the year during intervals of fifteen minutes, twice a day (morning and afternoon), according to the method *ad libitum* of Martin and Bateson (1986). Observation site allowed inferences about the activities of specimens on both the dry area and the bottom of water reservoir. During this period

any behavior that could be associated with reproductive activity was registered, such as different kinds of interactions between two or more specimens (Molina, 1998). Young specimens were observed each day to assert the period of ontogenetic change of color pattern. Furthermore, the enclosure areas were inspected each day to verify the presence of nests. When eggs were found they were transferred from the nests to a plastic recipient (37 x 23 x 7 cm) with a layer of 3 cm of vermiculite where they were artificially incubated. Eggs were accommodated on the substrate, simulating the same conditions they were found in the enclosure, and were sprinkled daily to maintain humidity. The recipients were placed in a closed room with a 250 W infrared lamp, positioned 40 cm above the plastic recipient, upholding the temperature close to 28°C. Temperature was controlled manually, by turning on or off the lamp according to the necessity. When external temperature was under 25°C during the night, the lamp was turned on until the next day.

In the last year of the present study eggs were numbered, weighted with an electronic balance Ohaus® L 200 to the nearest 0.1 g, and measured with a digital dial caliper Starrett® to the nearest 0.01 mm. They were identified through a black lead mark on the upper surface to avoid positional changes during transport and wrapping in an incubator Lyon®, model RL-1, specific for reptiles. Temperature and humidity were automatically controlled around 28-29°C and 80-90%, respectively. Eggs were examined every day and those that showed some abnormality, such as cracks or darkish color, were discarded.

An analysis of variance (ANOVA) was employed to test for sexual dimorphism, while descriptive statistics were performed to evaluate the variation showed by the analyzed parameters (Zar, 1999). Statistical tests were performed with a significance level of 0.05. Assumptions of normality and homoscedasticity were evaluated using Kolmogorov-Smirnov and Levene's test, respectively (Zar, 1999).

## RESULTS

### Morphological variation

Adult males and females showed no sexual dimorphism in carapace length ( $F_{1,14} = 2.05$ ;  $p < 0.17$ ;  $n = 16$ ), varying from 15.0-17.3 cm ( $\bar{X} = 16.5$ ;  $s = 0.8$ ;  $n = 6$ ) for males, and 15.2-19.5 cm ( $\bar{X} = 17.4$ ;  $s = 1.4$ ;  $n = 10$ ) for females. Other parameters of external morphology, pholidosis, and color pattern (such as cara-

pace shape, number of nuchal, marginals, vertebrals, and pleural scutes) agrees with data in the literature (Ernst and Barbour, 1989; Bonin *et al.*, 2006).

### Mating

Observations occurred from November to March, comprehending the rainy season. Four mating were analyzed (12 March 1996; 29 January 1997; 17 March 2000; and 22 November 2000). All mating occurred inside the water, during the afternoon between 16:00 and 17:30 hs, showing a regular pattern constituted by three conspicuous phases that are common to most Chelidae species: approximation, persecution, and copulation (Molina, 1998; Souza, 2004; Souza *et al.*, 2006; but see Discussion).

### Nests and clutches

We observed sixteen clutches (Table 1), although only the eggs from the last year were weighted and measured. Data on nesting behavior were not collected since eggs were laid during the night. Nests showed a slender subspherical concavity with 9 cm of mean diameter where eggs were laid. Nests were always found on leaf litter, near the roots of trees. Eggs were covered with leaves and a minor amount of sand. Clutch size varied from 2-4 subspherical eggs ( $\bar{X} = 3.3$ ;  $s = 0.7$ ;  $n = 16$ ); egg weight varied from 5.2-12.1 g ( $\bar{X} = 9.87$ ;  $s = 1.74$ ;  $n = 37$ ); length, considering the major axis, varied from 25.0-28.8 mm ( $\bar{X} = 26.83$ ;  $s = 1.15$ ;  $n = 37$ ). Adult specimens were not observed near the nests.

A clutch of *A. radiolata* in natural conditions was accidentally found by the senior author on 15 May 1999, near the Ossos lagoon, at the Municipality of

Búzios, State of Rio de Janeiro. The clutch had three eggs laid on a slender concavity dug on the soil, similar to those observed in captivity. Eggs were laid on the bottom of a bromeliad and the local presented a low incidence of light. All eggs weighted 10 g with length, considering the major axis, varying from 26.5-26.7 mm ( $\bar{X} = 26.6$ ;  $s = 0.1$ ,  $n = 3$ ). Nine eggs found on leaf litter along the margins of Geribá lagoon, Municipality of Búzios, were donated to RIOZOO on 8 September 1998. The donor was unable to report the quantity of nests in which the nine eggs were laid, but asserted that it was not a single clutch and that the nests were similar to those of captive females, although eggs were uncovered. They were in a sandy and primarily open area and weighted 6-11 g ( $\bar{X} = 9.4$ ;  $s = 1.7$ ;  $n = 9$ ) with length, considering the major axis, varying from 24.5-26.6 mm ( $\bar{X} = 25.6$ ;  $s = 0.8$ ;  $n = 9$ ). We found eggs in captivity to be significantly longer in their major axis ( $F_{1,47} = 7.70$ ;  $p < 0.01$ ;  $n = 49$ ), but not different in weight ( $F_{1,47} = 0.26$ ;  $p < 0.6$ ;  $n = 49$ ) than those found in natural conditions.

### Hatchlings and growth rate

During the period of study only four births occurred, all between the months of September and November. Three hatchlings arose from the clutch found on natural conditions at Geribá lagoon, whereas the fourth was born after being artificially incubated for 135 days.

Specimens showed a more prominent growth during the first month and between the third and sixth months of life (Fig. 1). The third specimen died on the second month and thus is not shown in the graphic. Growth was sharper during September and from January to March. Animals showed a more conspicuous increase in weight after the fourth month, with a slight decrease during the eighth and ninth months of life (Fig. 2), which correspond to the colder period of the year when specimens ingested lower quantities of food.

Specimens of *A. radiolata* showed ontogenetic variation of color pattern. Hatchlings have limbs, gular region, tail, and margins of carapace and plastron with conspicuous reddish color resting on a black background; the iris is black. The reddish color started to change to an orange tonality in the third month of life. After the fourth month, the animals showed no remains of reddish color, having a yellowish color lying on a black background. Concomitantly, the iris acquired a white color.

TABLE 1. Clutches of *Acanthochelys radiolata* observed during the period of study. “\*” denotes clutches found in natural conditions; “?” denotes eggs donated (unknown number of clutches).

Date	Number of clutches	Clutch size
30 April 1997	1	3
27 May 1997	1	3
28 January 1998	1	4
*8 September 1998	?	? (9 total)
*15 May 1999	1	3
25 Mach 2000	1	4
17 May 2000	3	3, 3, 3
7 September 2000	4	2, 3, 3, 4
21 November 2000	5	2, 3, 4, 4, 4

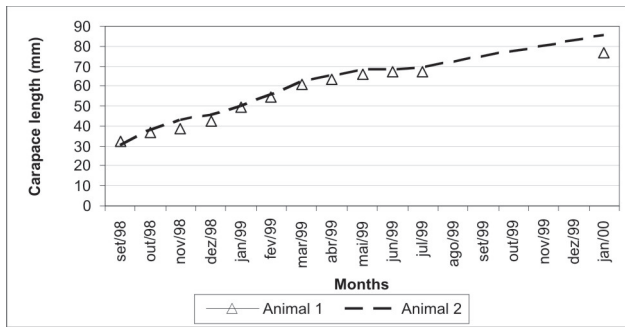


FIGURE 1. Carapace growth rate of two individuals of *A. radiolata* during the first ten months of life and after one year and four months.

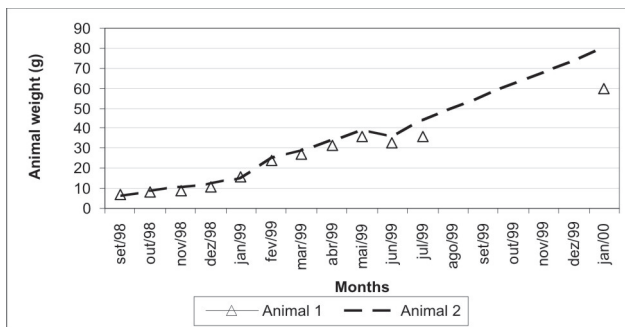


FIGURE 2. Weight of two individuals of *A. radiolata* during the first ten months of life and after one year and four months.

## DISCUSSION

### Mating

Mating behavior was described for many species of Chelidae (e.g. Medem, 1966; Rhodin and Mittermeier, 1976; Cann, 1978; Engberg, 1978; Murphy and Lamoreaux, 1978; Feldman, 1979; Medem, 1983; Molina, 1989; Souza, 2004; Souza *et al.*, 2006), although descriptions are generally brief. Molina (1998) registered mating of *Acanthochelys radiolata* in captivity between the months of September and February, which is in accordance with data presented herein (mating occurring between November and March). In the same work the author stated that mating of *A. spixii* (Duméril and Bibron, 1835) occurred between November and January. Horne (1993) points out that mating of *A. pallidipectoris* (Freiberg, 1945) in captivity occurred between September and November. Thus, mating in the genus *Acanthochelys* occurs in rainy and hot season.

Mating behavior of *A. radiolata* described by Molina (1998) is very similar to that we observed. Molina (1998) observed in a single occasion (in a total

of 10 observations) a phase denoted by this author as pre-copulation. This stage was not observed in the present study but comprehends the search by male for a suitable position that allows penetration. As mentioned by Molina (1998), the phases of pre-copulation and even persecution are not necessarily present during mating.

The phase of approximation during mating behavior is common among different chelonian species (Mahmoud, 1967; Murphy and Lamoreaux, 1978; Astort, 1984; Molina, 1989; 1998; Souza, 2004). The exam of cloacal region seems to be related to sex identification (Mahmoud, 1967; Carpenter, 1980) and the results of the present study corroborate this assertion since males apparently were capable of recognizing they were persecuting another male only after the exam of cloacal region of the persecuted male. Shealy (1976) and Souza (2004) argue that exam of cloacal region also facilitates the evaluation of female receptiveness. Considering *A. radiolata*, we observed that males sustained the persecution of females after the exam of cloacal region, even those that were not receptive. These results indicate that, at least for *A. radiolata*, exam of cloacal region is not sufficient to evaluate female receptiveness. Persecution is also present during mating behavior of other chelonian taxa, including Chelidae (Mahmoud, 1967; Astort, 1984; Molina, 1989, 1998), and according to Mahmoud (1967), this stage may have a stimulation function.

During copulation males of some chelonian taxa release their anterior limbs and keep themselves attached to females just by the posterior limbs (Lardie, 1975; Molina, 1989, 1998). Males of *A. radiolata* attach females using the four limbs during copulation. Extent of copulation is variable in chelonians (e.g. 10-30 minutes in *Phrynosoma geoffroanus*) (Molina, 1989), and in *A. radiolata* copulation lasted less than two minutes, probably due to the passive behavior of females in this phase.

### Nests and clutches

Eggs of *A. radiolata* were laid on concavities dug on the soil and covered with a minor amount of leaves and sand. This result contrasts with that found by Molina (1998) where eggs of *A. radiolata* were laid in nests 7.5 cm deep with an incubation chamber. This behavior may be a consequence of the lack of vegetation in the enclosures used by that author (Molina, pers. comm.), since eggs in the present study – both in captivity or natural conditions – were observed un-

der leaf litter near roots. Nests described herein are most similar to those described by Medem (1983) for *Platemys platycephala* (Schneider, 1792), with eggs laid on shallow grooves under leaf litter and, every so often, partially covered by sand.

Eggs of *A. radiolata* in the present study have subspherical shape, confirming Molina's (1998) findings, with length of major axis varying from 24.5-28.8 mm, and weighting 5.2-12.1 g. Horne (1993) described subspherical eggs for *A. pallidipectoris* varying from 25-28 mm considering the major axis, and weighting 8.8-9.4 g. D'Amato and Morato (1991) found subspherical eggs to *A. spixii* varying from 24.8-27.2 mm considering the major axis, and weighting 8.8-10.3 g. Cintra and Yamashita (1989) stated that *A. macrocephala* has spherical eggs, an exception to the genus, with diameter varying from 28-32.5 mm, and weighting 11-20 g. However, females of *A. macrocephala* reach larger carapace lengths than the other species of the genus, which could justify larger values of egg weighting and length presented by this taxon.

Clutches of *A. radiolata* were more often found in September, November, and May (Table 1). Molina (1998) found clutches of this taxon between June and October and of *A. spixii* between February and May. Cintra and Yamashita (1989) observed clutches of *A. macrocephala* between April and May, while clutches of *A. pallidipectoris* occurred between November and December, with a single clutch in March (Horne, 1993). According to this data clutches for the genus *Acanthochelys* may occur during most of the year, from September to June.

Clutch size varied from two to four eggs in the present study (three eggs in natural conditions), while Molina (1998) found clutches varying from one to six and one to four eggs in *A. radiolata* and *A. spixii*, respectively. D'Amato and Morato (1991) described a clutch size of four eggs to *A. spixii* in natural conditions. Horne (1993) found clutches varying from two to five eggs to *A. pallidipectoris* in captivity, and Cintra and Yamashita (1989) registered clutch size varying from four to eight eggs to *A. macrocephala* in natural conditions.

### Hatchlings

Hatchlings of *A. radiolata* have reddish blotches, contrasting with data from Ernst and Barbour (1989) who argue that neonates show yellowish blotches. The specimens examined in the present study showed this later pattern only after three months of life and,

based on these results, individuals analyzed by Ernst and Barbour (1989) probably were not hatchlings and had more than three months of life. There is no available information on ontogenetic variation of color pattern in *A. pallidipectoris*, although the remaining two species of the genus also show ontogenetic variation. Hatchlings of *A. spixii* have carapace, plastron, limbs, and tail with reddish blotches that are substituted by a uniform black color during ontogeny (D'Amato, 1992). Hatchlings of *A. macrocephala* show a reddish color (Cintra and Yamashita, 1989) and adults have a uniform dark grey color (Ernst and Barbour, 1989).

Hatchlings with striking color pattern are common in other species of Chelidae, such as *Chelus fimbriatus* and *Phrynops geoffroanus* (Ernst and Barbour, 1989; Molina, 1989). Probably this color pattern has a disruptive function and avoids predation (Edmunds, 1974; Owen, 1980). Adults have no marked disruptive pattern probably due to the most efficient protection offered by the carapace and by the different kind of predators.

### RESUMO

Dezesseis espécimes adultos (seis machos e dez fêmeas) de *Acanthochelys radiolata* de diferentes regiões do estado do Rio de Janeiro, Brasil, foram monitorados em cativeiro do ano de 1996 até o ano 2000 para se obter dados referentes à estação reprodutiva, oviposição, incubação e nascimento dos filhotes. Comportamento reprodutivo foi observado entre novembro e março e mostrou um padrão regular constituído por três fases conspícuas: aproximação, perseguição e cópula. Dezesseis desovas foram encontradas entre março e julho e quatorze ovos foram encontrados em condições naturais. Nascimento ocorreram entre setembro e novembro. Neonatos apresentavam a região gular, membros anteriores e posteriores, cauda e as bordas da carapaça e plastrão com manchas vermelhas sobre um fundo preto. Este padrão se modificou para uma tonalidade laranja no terceiro mês de vida.

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