

Sex Term Dependence and Its Relation to Suitability in Neonates of *Peltocephalus Dumerilianus*

Carlos A. Almeida¹, Fernandez P. Diniz²

1. Department of Zoology, University of São Paulo, Brazil.,
2. Institute of Biosciences, Paulista State University, São Paulo, Brazil.

Received: March, 2023, Accepted and Published online: June, 2023

Abstract:

In the present paper the results of a fixed-temperature incubation trial with eggs from are analyzed and discussed Peltocephalus dumerilianus (Schweigger,1812) obtained by hormonal induction of eight females from which a total of 79 eggs was achieved; the existence of sex term dependence for the species is reaffirmed, with the birth of 45 males and 11 females; the existing relationships between the morphometry of females and the measurements of eggs and nestlings are also discussed, establishing a significant relationship between total volume of eggs per female with their morphometry. Morphometric measurements for the group of neonates obtained and maintained in a nine-month growth assay in the laboratory are compared, where it was found that only difference significantly exists for head width and weight in relation to sex, and it is validated that sex term dependence possesses suitability value for this species.

Keywords: Sex term dependence, allometry, proportion sexes, *Peltocephalus dumerilianus*, Brazil.

1. INTRODUCTION

Sexual determination is a fundamental biological process of profound importance for individual development and the formation of relationships between the sexes in natural populations (1). For some species of reptiles genetic sex determination (GERD) occurs and in others sex determination by temperature (STD) is given (sexual thermal determination) (2, 3, 4). In chelonians with STD, the usual pattern implies that at low incubation temperatures males are produced and at high incubation temperatures females are obtained (5, 6, 7). Nonetheless, for some species of chelonians the STD pattern manifests that at low and high incubation temperature females are produced already mean incubation temperatures are given as a result males (3,8). Additionally it is necessary to keep in mind that there is a first sensitivity period, which is that during which sex is irreversibly fixed and subsequent incubation temperatures are not relevant (7). The extent of the first sensitivity period will depend on the incubation temperature to be held (7). As a consequence of the cumulative effect of incubation temperature on sex determination, temperatures prior to the first sensitivity period influence sex determination, but not irreversibly, recognizing this stage as the second sensitivity period (7). There is a critical temperature (9) or pivotal temperature (10) or threshold temperature (11), which is just at which both sexes occur in equal proportions, Sexual determination in the embryo and that determined during the second third of the incubation period are related to the additive effects of the previously present temperature, since during the incubation period at least four daily hours of the selected: upper temperature are required or lower, having as reference the threshold temperature so that males or females are obtained preferentially (17). In chelonians due to multiple factors, among them: long periods of life and late maturity, do not allow to check easily whether some days of more or less during incubation would result in better advantages for the population, this makes STD, in this case, is considered an evolutionary enigma (18) and many aspects of their functioning and usefulness are still unclear, requiring therefore to delve into their research (19). In addition to the growth effect, incubation temperature affects performance in reptiles (20, 21). It is hypothetically proposed that the adaptive significance of STD, can be expressed for reptiles as an evolutionary advantage that would relate to aspects such as: phylogenetic inertia, which would indicate that it is an ancestral form of sexual determination without adaptive significance, acting on the group adaptation, being a strategy that facilitates internal dynamics, which may act by reducing the possibility

of noxious intercrossing, but does not clearly explain the case of those long-lived iteroparous organisms, such as chelonians that present annual interbreeding cohorts (3, 3). 18). It is considered that the differential options form the most robust proposal of all existing so far: they express that incubation temperature affects the suitability of males and females at birth, then STD can raise the suitability value to allow embryo development in the better incubation conditions (5, 6, 22, 23, 24, 25, 26). Equally, it can be interpreted that STD directly affects the phenotype and chances of optimal development of the individual (27). In the Podocnemididae of the Amazon the existence of STD on has been verified *Podonemis unifilis*, *P. erythrocephala*, *P. expansa*, *P. sextuberculata* and *Peltocephalus dumerilianus* (28, 29, 30, 31, 32). In the present study it was aimed to test an incubation temperature that produced more males than females under controlled management, while establishing the growth of neonates in the laboratory, their sexual dimorphism and the relationship of these factors to STD; the morphometric relationships of the induced females with respect to the eggs and nests obtained are likewise discussed.

2. PATIENTS AND METHODS

For obtaining the eggs destined for incubation in the laboratory, induction of gravid females, caught in natural environment between October 25 and November 5, 2004 in the Itú River, municipality of Barcelos, Amazonas, Brazil (00°24'10" S - 63°27'36" O y 00°24'39" S - 63°26'05" O). To establish the severity, inguinal palpation was performed (32). For each of the eight selected females, the following straight-line measurements were taken: carapace length (LC cm), carapace width (AC cm), plastron length (LP cm), animal height (A cm), and weight (P gr) (33, 34). Precision scales (Pesola) were used for weight records (1 Kg and 30 Kg ± 0.5 g). For the measurements pediatric ruler (1 - 100 cm ± 0.1 cm) and digital caliper 0 - 200 mm (Mitutoyo® IP 66 ± 0.01 mm). The obtaining of the eggs was performed in the laboratory of the Rio Negro Lodge Foundation (Barcelos, Amazonas). Each female was housed in a plastic box, with a water level that allowed them to rise to the surface to breathe, without being able to swim, maintaining a constant temperature of 28 °C throughout the process, for which a heater was installed in each box electric (Newattino®); continuous temperature record was taken through the use of digital thermometer (C/ MEM B3 - Thermomed® - 10 a + 60 °C ± 0.1°C). After a 12-hour acclimation period, 10 IU of synthetic oxytocin was injected subcutaneously® per kg of body weight (32).

The eggs obtained were marked individually; measured in their total length (X) and diameter (Y), using electronic gauge (Mitutoyo® IP 66 ± 0.01 mm); they were also weighed with portable electronic digital balance (Kenex 500 TS® ± 0.1gr) (32, 35) and the unit and total volume of each group or nest was calculated (33). The incubation was carried out in room with permanent air conditioning, as incubation chamber a plastic thermal box was used and as heat source and temperature distribution a thermostat (Climastac®) and an aquarium aeration pump (Aquaclear®) (32). The total incubation temperature was 30.16 oC (28.6 - 31.5) and the incubation temperature during the sensitivity period was 29.73 oC (28.93 - 30.83). The temperature records during incubation were taken with data loggers (Hobo®); the ambient humidity in the incubation chamber was maintained at saturation throughout the assay (36). The neonates were measured monthly throughout the growth experiment in the laboratory, taking: carapax length (LC cm), carapace width (AC cm), Plastron length (LP cm), animal height (A cm), width of the head (AK) and weight (P gr) (34, 35); they were marked on the marginal plates by means of cuts (37, 38), besides being identified by the number corresponding painting on the upper part of the carapax with non-toxic indelible ink. The neonates were kept in plastic trays 55 cm long, 35 cm wide and 19 cm deep (0.1925 m² of area and 0.382 m³ of volume). A diet based on commercial concentrated dog food was maintained three times a week, equivalent to 8% of weekly body mass, with water changes 6 times a month, maintaining an average ambient temperature of 26.9 oC. For sex verification the animals were sacrificed at ten months of age by sodium phenobarbital injection, opened by bridge to expose the abdominal cavity, fixed in 10% buffered formol, and preserved in alcohol 70%, they were then deposited in the collection of the Instituto Nacional de Pesquisas de Amazonas- INPA, to then observe gonadal differences under the microscope (39). The normality of the data was verified by the Kolmogorov- Smirnov fit test and visual inspection of the accumulated normal distribution figures; the statistical analyzes included X², multiple regression and discriminant factor analysis (40). The Analyzes of sexothermodependence were made by observing plots of daily temperature in relation to the number of days of incubation.

3. RESULTS

A total of 79 eggs were obtained from eight induced females, with a range of seven to 13 eggs/female ($X = 9.87$); on average presented: 56.65 mm (48.62 - 62.46. SD=2.401) in length, 34.52 mm (31.53-36.91. SD=1.585) in diameter and 35.25 cc (28 ,11- 40.78. SD=3.32) of volume (**Table 1**); in terms of egg weight, a mean of 38.72 g (32.5 - 43.7. SD = 2.815) was found (**Table 2**). When relating by a multiple regression test the morphometric measurements of the females (**Table 3**) with the number of eggs and with the total volume of each nest, it could be established that there was significant relationship between total volume of eggs per female with length of the carapace (LC), plastron length(LP) and A (Height) ($F(5,73)=32.112$ $p<0.05$ $R^2=0.6874$); no significant values were found for the number of eggs when compared with the same exposed variables. Regarding the total weight of eggs compared with the total weight of ovated and eggless females, significant values were determined in the multiple regression applied ($F(2,5)=332$, $p<0.005$ $R^2=0.9998$). The total incubation conducted with a mean of 30.16°C (28.6-31.5. SD=0.60042) had a duration of 97 days (96-98); taking as sensitivity period from day 31 to day 60, which corresponds to the second third of the total incubation time, the mean temperature in this lapse was 29.73 °C (28.93-30.83. SD=0.4891) (Figure 1). The neonates produced in this incubation process presented sexual determination by temperature (STD); of the 79 incubated eggs hatched 56 broods (70.88%), 45 males and 11 females, with a proportion of males equivalent to 80.37%, being highly significant the number of males ($X^2=20.64$. Gl1, $p<0.01$). The ratio between sexes was maintained throughout the experiment (75.77% of males) ($X^2 = 18.36$. Gl 1, $p<0.01$). Morphometrically the neonates presented in mm: LC = 51.22 (41.4-54.4), AC = 40.61 (32.1-45.3), LP = 42.08 (38.0-46.2).), AP = 30.32 (20.2-34.4), A = 24.38 (22-27.2), AK = 13.86 (12.57-15.86), and P = 31.32. 37g (26.4-34.8). When comparing by discriminant analysis the morphometric measurements of the growth experiment for the nine months, it was managed to establish that only there is difference significantly for head width (AK) and weight (P) in relation to sex ($F(9,408)=3,587$, $p< 0.05$).

Table 1. Volume calculation in cc for eggs and nestlings deP. Dumerilianus

Female	Eggs	Media	DS	Min.	Max.	Total Vol.
1	8	30.63	1,15	29.31	32.88	245.05
2	7	32.44	0.95	31.47	34.36	227.09
3	9	38.36	1.75	35.52	40.56	345.24
4	7	37.71	1,18	35.45	38.67	264.00
5	13	38.06	1.28	35.60	40.78	494.82
6	12	30.93	1.65	28,11	33.35	371.24
7	10	36.92	0.94	35.77	38.56	369.22
8	13	36.92	2,00	33.19	39.79	480.06

Table 2. Weight in grams of eggs and nestlings obtained forP. dumerilianus

female	1	2	3	4	5	6	7	8
Media	35.45	37.30	37.47	38.10	40.97	35.46	41.27	41.52
Min.	32.60	35.70	36.30	36.80	38.00	32.50	38.20	37.10
Max.	37.20	40.20	38.40	38.90	43.00	38.30	43.20	43.70
DS	1,329	1,431	0.632	0.772	1,294	1,726	1,631	1,665
total	283.60	261.10	337.23	266.7	532.48	425.52	412.7	539.63

Table 3. Morphometry of females ofP. dumerilianus induced (Carapax length (LC), carapax width (AC), plastron length (LP), height (A), and (P) weight).

Statistician	LC (cm)	AC (cm)	LP (cm)	A (cm)	P (g)
Media	32.88	23.23	26.02	12.97	4646.8
Min.	29.90	20.50	23,10	10.00	3200.0
Max.	34.50	25.20	27.20	14.20	5800.0
DS	1,354	1,433	1,382	1,173	856.3

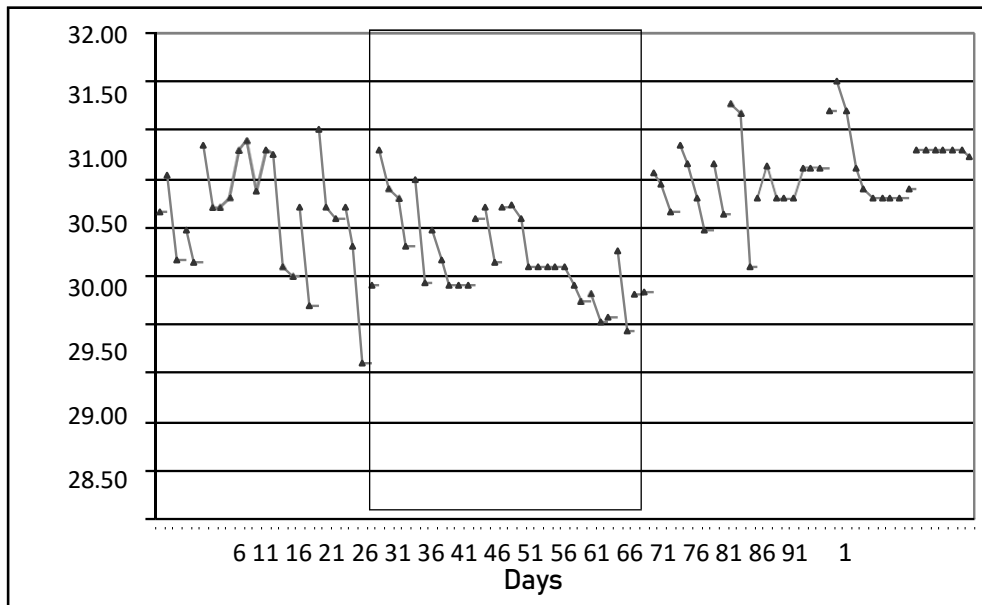


Figure 1. Incubation temperature in relation to the Process duration and box indicating the sensitivity period for *P. dumerilianus* Temperature oC

4. DISCUSSION

The nest size agrees in with existing data for the species (32, 41, 42). As far as the measurements of the eggs are concerned they are within the scope established for *P. dumerilianus* (39, 41, 42), but the recorded weight was lower. The duration of incubation in the laboratory coincides with that noted for the species (32, 41), but was 22% shorter than that established by Vogtet al (39) and 18.9% lower than that reported by Felix-Silva (42), in both cases reference is made to natural incubation. The smallest mature female encountered presented a carapax length (LC) and weight greater than that recorded by Vogt (32) for the Rio Negro, Brazil. In general female size presents significant relationship with total nest volume, equally there is a direct relationship between total nest mass and female body mass (42). The hatching rate is presented similar to that described by Félix-Silva (42) who found a value of 50% to 95%, but it is higher than that obtained by Vogt et al (39), in both cases for natural medium. It can be noted that sexual determination was presented by the incubation temperature (32), since during the sensitivity period a mean temperature of 29.73 oC (28.93-30.83) was maintained to produce significantly 80.37% of males, being the high temperatures that favored the results obtained. For neonates the measurements of carapax length (LC) and weight coincide with existing data for *P. dumerilianus* (32, 41). During the 270 days of the growth experiment, significant difference was found only for

head width (AK) and weight (P), with respect to sex, which is presented as a phenotypic expression of the species, starting at manifest in an early manner, differentiating males, which in this species are of larger body size than females (41). In this study no STD-linked adaptive responses were evidenced, the significant difference of sexes produced, being higher the proportion of males, is can be interpreted as an elevation of the suitability value at incubation (5, 6, 22, 23, 24, 25, 26). In comparison it has to be that the results found for males of *Chelydra serpentina* produced in the laboratory through STD management (24), show an increased growth; the males of *C. serpentina* apparently compete aggressively to enable reproduction which demonstrates that there is relationship with between sex and body size (43). This type of male reproductive behavior also characterizes a *P. dumerilianus*, finding an association between sex and allometry (44) which would allow us to conceptualize that if there is, in this sense, elevation of the suitability value. It can be asserted that the survival obtained in relation to sex was independent of STD, because during the whole of the study the sex ratio was significantly similar. It was evident that survival was not related to sex, incubation temperature, or manutering conditions; acquire, then, greater value the individual factors (44). In this case, according to the results it is interpreted that STD was selective in terms of the proportion of males obtained, but the absence of a certain measurable performance as a function of sex does not allow us to ascertain that there is a relationship with respect to incubation temperature. It can be established that there is favoring for one of the sexes by the incubation temperature, which allows to accept that STD, in this trial only presented suitability value (5, 11, 22, 23, 24, 25, 26; 27).

5. CONCLUSION

.....

Ethical Issues: All ethical issues were approved by the authors from the Iraqi Ministry of Health. Verbal and signed informed consents were obtained from all patients who included in the study during their first visit.

Conflict of interest: None

Source of funding: Authors declared no funding agency, or organization

6. Bibliography

1. West SA, Reece SE, Sheldon BC. Sex ratios. *Heredity* 2002 ; 88: 117-124.
2. Sites JWJ, Bickham JW, Haiduk MW. Derived X chromosome in the turtle genus *Staurotypus*. *Science* 1979; 206: 1410–1
3. Ewert MA, Nelson CE. Sex determination in turtles: patterns and some possible adaptive values. *Copeia* 1991; 1: 50-6
4. Sarre DS., Georges A, Quinn, A. The ends of a continuum: genetic and temperature dependent sex determination in reptiles. *BioEssays* 2004; 26: 639-6
5. Charnov EL, Bull JJ. When is sex environmentally determined?. *Nature* 1977; 266: 828–8
6. Bull JJ, Vogt RC. Temperature-dependent sex determination in turtles. *Science* 1979; 206: 1186–1188.
7. Bull JJ, Vogt RC. Temperature-sensitive periods of sex determination in Emydid turtles. *The Journal of Experimental Zoology* 1981; 218: 435–440.
8. Ewert MA, Jackson DR, Nelson CE. Patterns system of temperature sex determination in turtles. *The Journal of Experimental Zoology* 1994; 270: 3-1
9. Pieau C. Recent data on temperature-dependent sexual differentiation in embryos *Emys orbicularis* L. (Chelonian). *Bulletin of the Zoological Society of France* 1976; 4: 46-5
10. Yntema CL., Mrosovsky N. Critical periods and pivotal temperature for sex determination in loggerhead sea turtle. *Canadian Journal of Zoology* 1982; 60: 1012–1
11. Bull JJ. Sex determination in reptiles. *The Quarterly Review of Biology* 1980; 55: 3-2
12. Bull JJ, Vogt RC, McCoy CJ. Sex determination of temperatures in turtles: a geographic comparison . *Evolution* 1982; 36: 326-3
13. McCoy CJ, Vogt RC, Censky EJ. Temperature controlled sex determination in the sea turtle *Lepidochelys olivacea* . *Journal of Herpetology* 1983; 17: 404-406.
14. Limpus CJ, Reid P, Miller JD. Temperature dependent sex determination in Queensland sea turtles: intraspecific variation *Caretta caretta* . In: Grigg G, Shine R, Ehmann H. (Eds.). *Biology of Australian Frogs and Reptiles*. Royal Zoological Society. New South Wales, Australia; 1985: 343–351.
15. Mrosovsky N. Pivotal temperature for loggerhead turtles (*Caretta caretta*) from northern and southern beaches. *Canadian Journal of Zoology* 1988; 66: 61-6
16. Vogt RC., Flores-Villela O. Effects of incubation temperature on sex determination in a community of Neotropical freshwater turtles in southern Mexico. *Herpetology* 1992; 48: 265-2
17. Vogt RC. Temperature controlled sex determination as a tool for turtle conservation. *Chelonian Conservation and Biology* 1994; 1(2): 159-1
18. Shine R. Why is sex determined by nest temperature in many reptiles? *Tree* 1999; 14(5): 186-1

19. Bowden RM, Ewert MA, Nelson CE. Environmental sex determination in a reptile varies seasonally and with yolk hormones. *Proceedings of the Royal Society* 2000; B-267: 1745-1749.
20. Janzen FJ. An experimental analysis of natural selection on body size of hatchling turtles. *Ecology* 1993; 74: 332-3
21. Freedberg S, Ewert MA, Nelson CE. Environmental effects on fitness and consequences for sex allocation in a reptile with environmental sex determination. *Evolutionary Ecology Research* 2001; 3: 953-967.
22. Janzen FJ, Paukstis GL. Environmental sex determination in reptiles: ecology, evolution and experimental design. *The Quarterly Review of Biology* 1991; 66: 149-1
23. Burke RL. Adaptive value of sex determination mode and hatchling sex-ratio bias in reptiles. *Copeia* 1993; 1: 854-8
24. Rhen T, Lang JW. Phenotypic plasticity for growth in the common snapping turtle – effects of incubation-temperature, clutch, and their interaction. *The American Naturalist* 1995; 146: 726–747.
25. Shine R, Elphick MJ, Harlow PS. Sisters like it hot. *Nature* 1995; 378: 451–452.
26. St. Juliana JR, Bowden RM, Janzen FJ. The impact of behavioral and physiological maternal effects on offspring sex ratio in the common snapping turtle, *Chelydra serpentina*. *Behavioral Ecology and Sociobiology* 2004; 56: 270-2
27. Warner DA, Shine R. The adaptive significance of temperature-dependent sex determination: experimental tests with a short-lived lizard. *Evolution* 2005; 59(10):2209–2
28. Alho CJR. Conservation and management strategies for commonly exploited Amazonian turtles. *Biological Conservation* 1985; 32(2): 291-2
29. Remor de Souza R., Vogt RC. Influence of incubation temperature on sex and hatchling size in the Neotropical turtle *Podocnemis unifilis*. *Journal of Herpetology* 1994; 28(4): 453-4
30. Valenzuela N., Botero R, Martinez E. Field study of sex determination in *Podocnemis* expands from Colombian Amazon. *Herpetology* 1997; 53: 390-398.
31. Valenzuela N. Effects of constant, shift, and natural temperature on sex determination in *Podocnemis* expand turtles. *Ecology* 2001; 82(11):3010–3
32. Vogt RC. Turtles of Black River. In: Chao NL, Petry P, Prang G, Sonneschien L, Tlustý M. (Eds.). *Conservation and breeding of ornamental fish resources of the Rio Negro Basin, Amazonia. Brazil Project Piaba*. Ed. University of the Amazon. Manaus, Brazil; 2001: 245–262.
33. Vanzolini PE. A brief biometrical note on the reproductive biology of some South Americans *Podocnemis* (Testudines, Pelomedusidae). *Papeis Avulsos de Zoology* 1977; 31(5): 79-1

34. Seidel ME. Revision of the West Indian Emydid turtles (Testudines). American Museum Novitates 1988; 2918: 1-4
35. Medem F. Recommendations regarding how to count scaling and take dimensions of nests, eggs and specimens of the Crocodylia and Testudines. Lozania 1976; 20: 1-1
36. De La Ossa VJ. Scholarly guides to zoocry, with emphasis on reptiles. University of Sucre. Faculty of Agricultural Sciences. Depto. Zootechnics. Sincelejo, Sucre; 1999.
37. Zweifel RG. Long-term ecological studies of painted turtle populations, *Chrysemys pictura* on Long Island, NY American Museum Novitates 1989; 2952: 1-55.
38. Gibbons JW. Recommendations for future research on freshwater turtles: What are the questions?. In: Gibbons JW. (Ed.). Life history of the slider turtle. Smiths. Inst. Press. Washington, DC USA; 1990: 311–317.
39. Vogt RC, Cantarelli VC, De Carvalho GA. Reproduction of the Cabeçudu, in the Biological Reserve of Rio Trombetas, Para, Brazil. Chelonian Conservation and Biology 1994; 1(2): 145–1
40. Zar JH. Biostatistical analysis. Third edition. Prentice-Hall, Inc. Englewood Cliff; 1996.
41. Medem F. The Reproduction Of The “cabezón” Turtle *Peltocephalus tracaxa* (Spix). 1924 (Testudines. Pelomedusidae) in Colombia. Lozania 1983; 41: 1-1
42. Félix-Silva D. Reproductive Ecology of the “Head” (*Peltocephalus dumerilianus*) Testudines: Pelomedusidae, in Jaú National Park, Amazonas. Mastering Dissertation. UERJ/RJ. Rio de Janeiro, Brazil; 2004.
43. Janzen FJ., O'Steen S. An instance of male combat in the common snapping turtle (*Chelydra serpentina*). Bulletin of the Chicago Herpetological Society 1990; 25: 11-1
44. Fairbairn DJ. Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. Annual Review of Ecological Systems 1997; 28: 659-6
45. Mangel M., Stamps J. Trade-offs between growth and mortality and the maintenance of individual variation in growth. Evolutionary Ecology Research 2001; 3: 583-593.