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Reproduction in four species of turtles at the National Zoological Park, Washington D.C., U.S.A.

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SINCE 1986, data have been recorded for reptiles and amphibians reproducing in the Reptile House at the National Zoological Park (NZP), Smithsonian Institution, Washington, DC, USA. These records represent an almost untapped wealth of information, and an opportunity to examine the reproductive biology of four species of turtles that have produced a sufficient number of clutches to provide significant data — the aquatic Australian Common Snake-necked Turtle, *Chelodina longicollis*, the aquatic South American Matamata, *Chelus fimbriatus*, the terrestrial African Pancake Tortoise, *Malacochersus tornieri*, and the terrestrial African Leopard Tortoise, *Geochelone pardalis*. Data concerning these clutches, eggs, and hatchlings are presented below.

METHODS AND MATERIALS

Husbandry conditions in the Reptile House for all specimens included a 12:12 hour artificial light regime, but overhead windows allow more or less light to enter depending on the season. The heating unit was set at 27°C (80°F); however, the air temperature was usually lowered to 25°C (78°F) because of frequent opening and closing of the outer doors by visitors. Water temperature in aquatic displays was also set at 27°C, but, like the air temperature, was actually about 25°C. The indoor enclosure housing *Geochelone pardalis* was opened, weather permitting, to an outside yard from mid-April through September so the tortoises could move there to obtain natural sunlight.

Geochelone pardalis and *Malacochersus tornieri* were fed a diet of mixed greens, carrots, and sweet potatoes once a day for six days each week. The aquatic turtles, *Chelodina longicollis*

and *Chelus fimbriatus*, were fed different diets: *C. longicollis* was presented chopped earthworms and crickets, ad libitum at least once, but sometimes twice, daily; *C. fimbriatus* had goldfish added to its tank at least once weekly for feeding ad libitum (if all the initial goldfish were consumed, more were added).

The masses of female *C. longicollis*, *C. fimbriatus*, and *M. tornieri*, and all eggs and hatchlings were measured with a triple-beam balance accurate to 0.1 g; those of the larger female *G. pardalis* were determined with a calibrated bathroom scale. In addition, the length and width of eggs, and hatchling carapace length were measured with dial calipers accurate to 0.1 mm. Straight-line carapace lengths of female turtles were measured with either the dial calipers, or by placing the larger individuals on a meter stick. When possible, other data recorded were oviposition and hatching dates, condition of individual eggs (fertile/infertile), female identification number, and male identification number. Eggs were collected, marked for identification, and arranged in ventilated plastic shoe boxes or Rubber Maid sweater boxes on a 1:1 vermiculate/water media, and were incubated at 27.5-28.5°C (82-84°F) in a Parcifol Incubator. Both temperature and length of incubation were recorded. An attempt was made to determine reproductive effort by calculation of the relative clutch mass (RCM), total clutch mass divided by the postparturient mass of the female turtle (Hirshfield & Tinkle, 1975; Vitt & Congdon, 1978; Shine, 1980; Cuellar, 1984; Seigel & Fitch, 1984; Barron, 1997).

Statistical procedures were all done using the SAS system (v. 8.12). Analyses done included standard t-tests, linear regression, spearman rank correlation, and rank regression (Birkes & Dodge, 1993).

RESULTS AND DISCUSSION

Geochelone pardalis

Thirty-three clutches containing 299 eggs were produced between 1984 and 1997; however, only females responsible for 17 clutches could accurately be determined--301248 (7), 302454 (5), 304001 (3), 303475 (1), and 301677 (1). Masses recorded for these females averaged 1.82 (1.13-2.40) kg. Clutch size averaged 9.6 (1-26) eggs. The single egg clutch is a new record, as this tortoise was previously known to lay 5-30 eggs per clutch, and 5-7 clutches per nesting season (Ernst & Barbour, 1989). No correlation between female mass and clutch size was evident. Two of the females that produced more than one clutch laid large clutches alternating with very small clutches: 301248--17 (1990), 24 (1991), 2 (February, 1992), 22 (August, 1992), 2 (November, 1992), 26 (August, 1997), and 20 (September, 1997) eggs in seven clutches, respectively; 302454--16 (1984), 2 (1989), 16 (1992), 11 (1993), and 17 (1993) eggs in five clutches, respectively. However, female 304001 did not follow this pattern, producing three clutches of 13 (1990), 9 (1991), and 9 (1992) eggs, respectively. Since several months to years passed between normal clutches and those containing few eggs, no cycle is apparent. Perhaps the eggs in clutches of two were infertile, and merely passed instead of being absorbed. Overall, six (18.8%) clutches contained 1-4 eggs, including the single clutch of four eggs attributed to the largest female (303475, 3.41 kg).

Masses of clutches with no damaged eggs averaged 471 (83.1-1,165.8) g. RCM for the 17 clutches identified with females for whom body masses at the time of oviposition were known averaged 0.285 (0.098-0.333); clutch masses averaged 37.3 (10.9-54.0)% of postpurient female body mass.

Data concerning the eggs are presented in Table 1. No significant differences were found between

the dimensions or masses of fertile versus infertile eggs. Regressing average egg and clutch masses on female mass also yielded no significant results.

Eggs were produced in all months, but June and July; 82% were laid from August-January, with most in August (18%) and November-January (45%). Clutch intervals for those laid by the three females mentioned above were: 302454 laid three clutches between 1st September 1992 and 29th October 1993 at a mean interval of 212 (80-344) days; 304001 produced three clutches between 6th August 1990 and 8th February 1992 at intervals of 180-359 (mean, 270) days; and 301248 laid five clutches between 4 November 1990 and 4 November 1992 at intervals averaging 175 (89-334) days, and also laid two clutches of 26 and 20 eggs, respectively, in 35 days in 1997 (16th August-20th September). Incubation averaged 141 (123-176) days.

The fertility rate was 58.1 (10-100)% for clutches with no damaged eggs. The hatching rate was relatively low--28.3 (0-100)% for all clutches; 53.8 (10-100)% in 100% fertile clutches. Seventy-five percent of neonates hatched in December and January (single clutches hatched in February, May and August). Thirteen hatchlings had 43.0-50.2 (mean, 46.0) mm carapace lengths, 39.1-41.4 (mean, 40.2) mm carapace widths, and masses of 22.4-33.7 (mean, 30.1) g. Four had 10-16 (mean, 13.8) mm x 7-17 (mean, 13.3) mm yolk sacs. The ratio of hatchling mass to fertile egg mass averaged 60.6%, and hatchling mass to female body mass averaged 1.6%.

Malacochersus tornieri

Twenty-five clutches containing 29 eggs were produced between 1986 and 1998; 13 were laid by three females--30272 (6), 304281 (4), and 304728 (3). Masses recorded for females which produced 15 clutches averaged 385.8 (310.6-558.0) g. Clutch size averaged 1.16 (1-3) eggs. Usually only a single egg is laid during an oviposition (Ernst & Barbour, 1989).

The largest female, 30272, laid a clutch of two eggs in 1995, and another of three eggs in 1998; each of her previous four clutches in 1992-1993

contained a single egg. She was heavier in 1995 (545 g) and 1998 (558 g) than in 1992 and 1993 (433-525 g), possibly indicating a direct relationship between clutch size and female body mass. Mass of the clutch containing two eggs was 50 g, compared to 17-26 g for her single egg clutches (the clutch of three eggs had one crushed and could not be compared). Female 304728 produced three single egg clutches at body masses of 366-394 g, and female 304281 laid four single egg clutches when 424-499 g.

RCM of nine clutches for which female masses were available and no eggs damaged were 0.030-0.102 (mean, 0.054). Clutch masses averaged 5.80 (3.05-11.54)% of postpurient female body mass. Individual egg masses averaged 59% of female body mass (fertile eggs, 5.5%; infertile eggs, 6.4%). Regression of both average egg mass and clutch mass on female mass yielded no significant results.

Data on the elongated eggs is presented in Table 1. No significant differences were found when comparing the dimensions and masses of fertile and infertile eggs. The fertility rate was 60.9% for clutches with no damaged eggs.

Oviposition occurred in every month, except August; 87% of the clutches were laid between September and April, with most (40%) in January and February. Female 30272 laid three clutches in 1992 (15th January, 15th September, 28th December) at intervals of 243, 104, and 88 days, respectively; female 304281 laid two clutches in 1994 (12 January, 4 May) 112 days apart, and female 304728 laid clutches on 27th November 1993 and 4th May 1994, 158 days apart. Incubation periods ranged from 154-175 days at 28.5°C to

Species	combined	fertile	infertile
<i>Geochelone pardalis</i>			
egg mass (gm)	47.4 (30.1-70.0)	50.2 (39.0-70.0)	46.0 (30.1-60.0)
egg length (mm)	45.4 (38.0-56.8)	45.6 (40.9-52.6)	45.3 (38.0-56.8)
egg width (mm)	41.9 (32.0-48.0)	42.7 (38.5-46.1)	41.6 (32.0-48.0)
<i>Malacochersus tornei</i>			
egg mass (gm)	22.3 (16.1-29.4)	20.8 (16.1-26.0)	23.9 (20.0-29.4)
egg length (mm)	43.0 (18.8-55.0)	43.4 (38.2-48.5)	42.7 (18.8-55.0)
egg width (mm)	19.2 (14.0-37.0)	28.0 (24.6-31.4)	27.5 (14.0-37.0)
<i>Chelodina longicollis</i>			
egg mass (gm)	6.0 (0.6-8.4)	6.5 (4.7-8.4)	5.2 (0.6-8.0)
egg length (mm)	29.6 (14.0-39.9)	30.2 (20.7-39.9)	28.5 (14.0-34.0)
egg width (mm)	18.5 (7.5-29.0)	18.9 (12.9-22.4)	17.8 (7.5-29.0)
<i>Chelus fimbriatus</i>			
egg mass (gm)	35.1 (15.3-46.2)	37.7 (31.3-46.2)	34.9 (15.3-41.0)
egg length (mm)	39.3 (36.3-47.0)	39.7 (36.3-42.7)	39.3 (37.0-47.0)
egg width (mm)	38.6 (36.5-40.4)	37.4 (36.5-38.5)	38.7 (37.0-40.4)

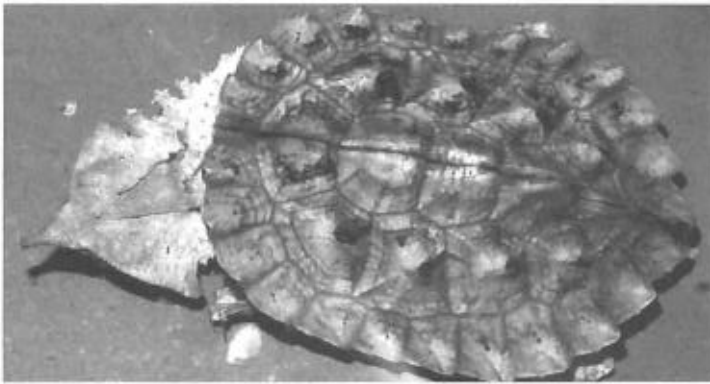
Table 1. Mean egg size measurements for all four species discussed. Figures in parentheses are ranges.

122-149 days at 29.5-31.0°C. Hatching dates were clustered in January-March (42.8%), but also occurred in June (28.6%) and September (28.6%).

Hatchlings had carapace lengths of 35.9-39.5 (mean, 37.5) mm and masses of 12.0-13.0 (mean, 12.4) g. The ratio of hatchling mass to fertile egg mass averaged 58.5%, and hatchling mass to female body mass averaged 3.2%.

Chelodina longicollis

Thirty-two clutches containing 255 eggs were produced in 1988-1998. Unfortunately, only two of the females that oviposited were identified. Most of the clutches were laid in the water-filled pool of the enclosure housing several females, making it impossible to determine which female was responsible for the clutch. Average mass and carapace length of the six females that probably produced clutches were 722.3 (578-951) g and 19.5 (17.5-24.0) cm, respectively. Female 305339 (650 g) produced a clutch of four eggs (clutch mass 23.6 g) on 23rd March 1992 (RCM, 0.035), and female 305145 (908 g) laid 10 eggs (six broken) in



Turtle species bred at the National Zoological Park, Washington. *Chelodina longicollis* (above) and *Chelus fimbriatus* (below). See also front cover.

a nest excavated to 10.0 cm deep in a flower planter on 8th March 1994.

Mean clutch size for the 32 clutches (255 eggs) was 8.0 (2-18) eggs; normally 6-24 eggs compose a clutch (Ernst & Barbour, 1989). Masses of 27 complete clutches (no eggs destroyed or damaged) averaged 41.9 (8.0-72.9) g. Measurement and weight data of the elongated eggs are presented in Table 1. Although suspect due to lack of independence, t-tests do indicate that egg length and egg mass differ between fertile and infertile eggs. This was confirmed visually by examination of box-and-whisker plots. Regressions of both average egg mass and clutch mass on female mass yielded no significant results. The mean fertility rate for the 255 eggs was 35.7 (0-100)%, and for clutches containing fertile eggs 54.7 (11.8-100)%. Eight clutches were infertile. One egg (24.9 mm X

19.4 mm x 6.7 g) in a clutch of 18 eggs laid 1st January 1995 contained two embryos.

Oviposition occurred from November to June, and in September. Most clutches (22, 68.8%) were laid between 1st January and 24th May, one clutch was laid on 30th June, another on 19th September, and five (15.6%) clutches were produced in November-December. Incubation periods ranged from 40-87 (mean, 59) days, depending on the incubation temperature (27.5-28.5°C). Hatching occurred between 30 January and 26 May, with peaks in March ($n=21$ days, 39.6%), May ($n=9$ days, 17.0%) and June ($n=13$ days, 24.5%). Eggs hatched on three days (5.7%) in January, four days (7.5%) in February, and three days (5.7%) in April.

Hatchlings had carapace lengths of 21.0-28.1 (mean, 25.5) mm and masses of 2.5-5.6 (mean, 4.5) g. The mean ratio of hatchling mass to fertile egg mass was 55.7%.

Chelus fimbriatus

Nineteen clutches were produced between 1990 and 1999. Only two females were identified as the parent of clutches (12)--30647 (six clutches between 14th August 1990 and 19th August 1996) and 304318 (five clutches between 9th December 1993 and 4 September 1997). These two females were also responsible for two other clutches laid on 18 January 1995 and 29 January 1999, but which female laid which clutch could not be determined. Female body mass at the time of ovipositing these clutches averaged 7.35 (5.40-11.62) kg. The larger female (30647) produced the following clutches: August 1990 (2 eggs), September 1990 (13 eggs), June 1991 (2 eggs), December-January 1992/1993 (12 eggs), August 1995 (7 eggs), and August 1996 (11 eggs). The

shorter female (304318) produced clutches in December 1993 (10 eggs), October 1995 (7 eggs), October 1996 (15 eggs), and September 1997 (8 eggs). The two clutches that could not be assigned to a specific female were laid 18th January 1995 (12 eggs) and 29th January 1999 (20 eggs). RCM of clutches produced by female 30647 averaged 0.050 (0.031-0.063), and the average percentage of clutch mass to female to female postparturient mass was 5.32 (3.25-6.78). Mean relative clutch mass for clutches produced by female 304318 was 0.065 (0.053-0.086), and the mean clutch mass to female postparturient mass was 6.93 (4.39-9.38). The combined average of these calculations for both females were 0.066 and 7.03%, respectively.

The 19 clutches of *Chelus fimbriatus* contained 149 eggs which averaged 7.84 (1-20) eggs. Ernst & Barbour (1989) reported a range of eggs per clutch of 12-28. Clutch masses averaged 299.35 (32.5-690.8) g.

Measurements and weights of 148 eggs (one was broken) are presented in Table 1. No significant differences in the dimensions and masses of fertile versus infertile eggs were found, and regressions of average egg mass and clutch mass on female mass also produced no significant results. Mean hatching percentage of fertile eggs was only 6.9%.

Spearman rank correlation indicated a possible significant association between hatching percentage and female mass ($p = .0002$). Further investigations using rank regression also showed significance ($P = .01923$) when hatching percentage was regressed on female mass. If true, it is interesting that the hatching percentage decreases with female mass; however, we suspect there may be some problems with data integrity. The other turtle species did not show any significant results when examining Spearman correlations or rank regressions involving hatching percentage and female mass.

Eighteen clutches (95%) totalling 147 eggs were laid between 5 August and 18 February, one clutch (5%) of two eggs was laid in July. Forty-two eggs (28.1%) (3 clutches) were laid in September, 23 eggs (15.4%) each in October (3 clutches) and December (4 clutches), and 22 eggs (14.8%) eggs were oviposited in August (4 clutches). Female

30647 laid two clutches in 1990 43 days apart. The mean interval between clutches for all clutches which could be accurately assigned to her was 437 (43-946) days; if the two unverified clutches are included, the average interval between clutches was 440.8 (43-893) days. The interval between the known clutches laid by female 304318 averaged 454.7 (315-678) days, if the two unverified clutches are included, the average interval between her clutches was 393.4 (315-512) days. The combined clutch interval average for the two females was 385.6 (43-747) days.

Incubation at 28.5-29.5°C averaged 303 (283-314) days. At hatchling, neonates had 42.9-47.8 mm carapaces (mean, 45.04 mm), and weighed 16.7-22.3 (mean, 18.8) g.

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