relationship to body size. Herpetologica 41:194–205.

———, AND ———. 1987. Morphological constraints on egg size: a challenge to optimal egg size theory. Proc. Nat. Acad. Sci. 84:4145–4147.

GEORGES, A. 1984. Observations on the nesting and natural incubation of the long-necked tortoise *Chelodina expansa* in south-east Queensland. Herpetofauna 15:27–31.

———, C. J. LIMPUS, AND C. J. PARMENTER. 1993. Natural history of the Chelonia. *In* C. J. Glasby, G. J. B. Ross, and P. L. Beesley (eds.), Fauna of Australia, Vol. 2A. Amphibia and Reptilia, pp. 120–128. Australian Government Publishing Service. Canberra.

GIBBONS, J. W. 1982. Reproductive patterns in freshwater turtles. Herpetologica 38:222–227.

GOODE, J., AND J. RUSSELL. 1968. Incubation of eggs of three species of Chelid tortoises, and notes on their embryological development. Aust. J. Zool. 16:749–761.

GUTZKE, W. H., G. C. PARCKARD, M. J. PACKARD, AND T. J. BOARDMAN. 1987. Influence of the hydric and thermal environments on eggs and hatchlings of painted turtles (*Chrysemys picta*). Herpetologica 43: 393–404.

IVERSON, J. B., H. HIGGINS, A. SIRULIK, AND C. GRIFFITHS. 1997. Local and geographic variation in the reproductive biology of the snapping turtle (*Chelydra serpentina*). Herpetologica 53:96–117.

LEGLER, J. M. 1985. Australian Chelid turtles: reproductive patterns in wide-ranging taxa. *In* G. Grigg, G. R. Sine, and H. Ehmann (eds.), Biology of Australasian Frogs and Reptiles, pp. 117–123. Royal Zoological Society of New South Wales. Sydney.

LEGLER, J. M., AND A. GEORGES. 1993. Family Chelidae. *In* C. J. Glasby, G. J. B. Ross, and P. L. Beesley (eds.), Fauna of Australia, Vol. 2A. Amphibia and Reptilia, pp. 142–152. Australian Government Publishing Service. Canberra.

MCGINLEY, M. A., D. H. TEMME, AND M. A. GEBER. 1987. Parental investment in offspring in variable environments: theoretical and empirical considerations. Amer. Natur. 130:370–398.

MILLER, K. 1993. The improved performance of snapping turtles (*Chelydra serpentina*) hatched from eggs incubated on a wet substrate persists through the neonatal period. J. Herpetol. 27:228–233.

ROOSENBURG, W. M., AND A. E. DUNHAM. 1997. Allocation of reproductive output: egg- and clutch-size variation in the diamondback terrapin. Copeia 1997:290–297.

ROWE, J. W. 1994. Egg size and shape variation within and among Nebraskan painted turtle (*Chrysemys picta belli*) populations: relationships to clutch and maternal body size. Copeia 1994:1034–1040.

SMITH, C. C., AND S. D. FRETWELL. 1974. The optimal balance between size and number of offspring. Amer. Natur. 108:499–506.

SOTHERLAND, P. R., AND H. RAHN. 1987. On the composition of bird eggs. Condor 89:48–65.

YAMPOLSKY, L. Y., AND S. M. SCHEINER. 1996. Why larger offspring at lower temperatures? A demographic approach. Amer. Natur. 147:86–100.

Accepted: 14 July 1998.

Journal of Herpetology, Vol. 32, No. 4, pp. 596–598, 1998

Temperature Fails to Influence Hatchling Sex in another Genus and Species of Chelid Turtle, *Elusor macrurus*

ARTHUR GEORGES AND SALLY MCINNES, *Applied Ecology Research Group and CRC for Freshwater Ecology, University of Canberra, ACT 2601, Australia.*

Temperatures that prevail during the incubation of eggs in reptile nests can have a profound influence, not only on rate of development and duration of incubation (Yntema, 1978), but also on phenotypic outcomes, such as coloration, morphometrics, or sex (Ewert, 1979; Murray et al., 1990; Allsteadt and Lang, 1995). Reptiles typically exhibit one of two forms of sex determination—genotypic or environmental (Bull, 1980; Bull, 1983). The latter usually takes the form of temperature-dependent sex determination (TSD). For example, in most species of turtle with TSD, high temperatures produce 100% females and low temperatures produce 100% males (Bull and Vogt, 1979). A very narrow range of temperatures in between, called the threshold or pivotal temperature, may yield hatchlings of either sex.

Recent literature reviews by Ewert and Nelson (1991), Janzen and Paukstis (1991), Viets et al. (1994), and Lang and Andrews (1994) revealed that the distribution of TSD within the Reptilia is patchy at all taxonomic levels above species. All crocodilians studied to date have the trait, yet their sister taxon, Aves, does not. Within Squamata, many lizards have TSD, but it has not been demonstrated for any species of snake. At the family level, turtles in Carettochelyidae and Pelomedusidae have TSD (Alho et al., 1985; Webb et al., 1986; Ewert and Nelson, 1991), yet their sister taxa, Trionychidae and Chelidae respectively, apparently do not (Vogt and Bull, 1982; Bull et al., 1985). Within families, some agamid lizards have TSD and others do not, with similar examples known in the Gekkonidae and Emydidae. The trait is even variable among species of the same genus, as in *Clemmys* (Ewert and Nelson, 1991; Ewert et al., 1994).

Given such variation, one cannot argue convincingly for lack of TSD at any taxonomic level until having sampled a sufficiently wide range of its constituent taxa. The only chelid turtles studied thus far, the genera *Emydura* and *Chelodina*, lack TSD (Bull et al., 1985; Georges, 1988; Thompson, 1988). However, it cannot be said that Chelidae lack TSD in general because several genera remain unstudied. In this paper, we examine the effects of constant temperature incubation on hatchling sex ratios of *Elusor macrurus*, a recently described monotypic genus of Australian short-necked chelid turtle (Cann and Legler, 1994) with no clear affinities (Georges and Adams, 1992). We also provide some of the first data on the eggs, nests, and incubation of this elusive species.

Eggs of *Elusor macrurus* were collected from nests laid in sand bars adjacent to the Mary River, near Tiaro, Queensland. The site was searched thoroughly from 19–21 October 1991 for signs of nesting immediately following three days of rain. Nine freshly laid

nests yielded 120 eggs; three other nests were destroyed by foxes (*Vulpes vulpes*). Height above water, distance from water, depth to the top egg, and chamber depth were measured for each nest. Egg length, egg width, and egg weight were measured for each egg with vernier callipers and an electronic field balance. The eggs were buried in moist vermiculite (approximately 50% water by weight) and transported to Canberra by road.

Equal numbers of eggs that initiated development (as indicated by the white patch, Thompson, 1985) were systematically allocated to each of four incubators set at 27, 28, 30, and 32 C so that initially an equal number of eggs from each clutch was incubated at each temperature. Remaining eggs were allocated randomly across temperatures to bring each incubator's total to 15 eggs. The eggs were incubated on a fixed quantity of moist vermiculite (4 g water per 3 g vermiculite) in 500 ml circular plastic containers. The containers were weighed at weekly intervals and total moisture kept constant by the addition of water if necessary, but humidity in the containers was not measured. Temperatures (±0.1 C) in close proximity to the eggs were recorded twice daily with mercury thermometers calibrated against a NATA certified thermometer.

Hatchlings were weighed (±0.1 g) and killed by intracranial injection of sodium pentabarbitone. Weights included a small quantity of yolk that was internalised before or within 48 h of hatching. The right gonad, kidney, and associated ducts were removed, embedded in wax, sectioned, and dyed with haemotoxylin and eosin. The sex of each gonad was determined by examination under a light microscope according to criteria established in earlier studies (Georges, 1988).

Elusor macrurus lays 10–16 (mean 13.3, N = 9) white, hard-shelled, ovoid eggs averaging 34.13 ± 0.34 mm in length, 22.43 ± 0.19 mm in width, and 10.14 ± 0.21 g in weight (means given with standard errors based on N = 9 clutches, 120 eggs in total). Egg size ranged from 11.5 g (37.2 × 22.7 mm) to 8.6 g (31.9 × 21.6 mm). Eggs were deposited in a chamber constructed in sloping sand or sandy loam adjacent to water. Mean distance from water was 6.62 ± 0.76 m (1.4–9.5 m, N = 12) with a mean height above water of 2.50 ± 0.36 (0.70–4.04 m, N = 12). Nest chamber depths ranged from 16.5 to 20.0 cm (mean 18.4 ± 0.62 cm, N = 5) with depth to the top egg ranging from 7.5 to 13.2 cm (mean 10.7 ± 0.77 cm, N = 6).

All but four eggs initiated development of a white patch soon after collection or during transit. Approximate incubation periods are given in Table 1. Hatchlings had a mean carapace length of 32.5 ± 0.29, a mean carapace width of 30.3 ± 0.33, a mean shell depth of 13.9 ± 0.14, and a mean weight of 7.2 ± 0.10 g (N = 45).

There was no significant association between hatchling sex ratio and incubation temperature ($\chi^2 = 0.75$, df = 3, $P = 0.86$; Table 1). Pooling the data across temperatures yielded a sex ratio of male : female = 30:27 which was not significantly different from 1:1 ($\chi^2 = 0.07$, df = 1, $P = 0.79$).

This study has established that another distinct lineage of chelid turtle, *Elusor macrurus*, lacks TSD. Given the considerable variation in TSD among taxa at all levels of taxonomy and its relevance to the management of threatened species (Morreale et al., 1982; Vogt, 1994), studies of other chelid lineages are needed to determine if this pattern prevails throughout the family.

TABLE 1. Incubation period and outcomes of sexual differentiation for eggs of *Elusor macrurus* incubated at four different temperatures. Incubation periods should be regarded as approximate because they include a 5 day period during transit when temperatures could not be controlled.

Temperature (C)	N	Inc period (days)	Males	Females	Unsexed
27	15	62	6	8	1
28	15	52–55	8	6	1
30	15	46–48	8	6	1
32	15	41–46	8	7	0
Totals			30	27	3

Acknowledgments.—We would like to thank John Cann for sharing his extensive knowledge of Australian freshwater turtles without which this project would not have been possible. Mike Palmer-Allen for provided valuable technical assistance. This paper benefited greatly from the scrutiny it received by the Science Writers' Club at the University of Canberra and from comments made by Michael Ewert, Scott Thomson, Sean Doody, Matt Allanson, Patrick Driver and Wayne Robinson.

LITERATURE CITED

ALHO, C. J. R., T. M. S. DANNI, AND L. F. M. PADUA. 1985. Temperature-dependent sex determination in *Podocnemis expansa* (Testudinata: Pelomedusidae). Biotropica 17:75–78.

ALLSTEADT, J., AND J. W. LANG. 1995. Incubation temperature affects body size and energy reserves of hatchling American alligators (*Alligator mississippiensis*). Physiol. Zool. 68:76–97.

BULL, J. J. 1980. Sex determination in reptiles. Q. Rev. Biol. 55:3–21.

———. 1983. Evolution of sex determining mechanisms. Benjamin Cummings, California.

———, AND R. C. VOGT. 1979. Temperature-dependent sex determination in turtles. Science 206: 1186–1188.

———, J. M. LEGLER, AND R. C. VOGT. 1985. Non-temperature dependent sex determination in two suborders of turtles. Copeia 1985:784–786.

CANN, J., AND J. M. LEGLER. 1994. The Mary River Tortoise: a new genus and species of short-necked chelid from Queensland, Australia (Testudines: Pleurodira). Chel. Conserv. Biol. 1:81–96.

EWERT, M. A. 1979. The embryo and its egg: development and natural history. *In* M. Harless and H. Morlock (eds.), Turtles: Perspectives and Research, pp. 333–413. John Wiley, New York.

———, AND C. E. NELSON. 1991. Sex determination in turtles: diverse patterns and some possible adaptive values. Copeia 1991:50–69.

———, D. R. JACKSON, AND C. E. NELSON. 1994. Pat-

terns of temperature-dependent sex determination in turtles. J. Exp. Zool. 270:3–15.

GEORGES, A. 1988. Sex determination is independent of incubation temperature in another chelid turtle, *Chelodina longicollis*. Copeia 1988:248–254.

———, AND M. ADAMS. 1992. A phylogeny for Australian chelid turtles based on allozyme electrophoresis. Aust. J. Zool. 40:453–476.

JANZEN, F. J., AND G. L. PAUKSTIS. 1991. Environmental sex determination in reptiles: ecology, evolution and experimental design. Q. Rev. Biol. 66:149–179.

LANG, J. W., AND H. V. ANDREWS. 1994. Temperature-dependent sex determination in crocodilians. J. Exp. Zool. 270:28–44.

MORREALE, S. J., G. L. RUIZ, J. R. SPOTILA, AND E. A. STANDORA. 1982. Temperature-dependent sex determination: current practices threaten conservation of sea turtles. Science 216:1245–1247.

MURRAY, J. D., D. C. DEEMING, AND M. J. W. FERGUSON. 1990. Size-dependent pigmentation-pattern formation in embryos of *Alligator mississippiensis*: time of initiation of pattern generation mechanism. Proc. Roy. Soc. London (Ser. B) 239:279–293.

THOMPSON, M. B. 1985. Functional significance of the opaque white patch in eggs of *Emydura macquarii*. *In* G. Grigg, R. Shine, and H. Ehmann (eds.), Biology of Australian Frogs and Reptiles, pp. 387–395. Royal Zoological Society of New South Wales, Sydney.

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

VIETS, B. E., M. A. EWERT, L. G. TALENT, AND C. E. NELSON. 1994. Sex-determining mechanisms in squamate reptiles. J. Exp. Zool. 270:45–56.

VOGT, R. C. 1994. Temperature controlled sex determination as a tool for conservation. Chel. Conserv. Biol. 2:159–162.

———, AND J. J. BULL. 1982. Genotypic sex determination in the spiny softshell *Trionyx spiniferus* (Testudines: Trionychidae). Copeia 1982:699–700.

WEBB, G. J. W., D. CHOQUENOT, AND P. J. WHITEHEAD. 1986. Nests, eggs and embryonic development of *Carettochelys insculpta* (Chelonia: Carettochelidae) from northern Australia. J. Zool. (London) 1B:521–550.

YNTEMA, C. L. 1978. Incubation times for eggs of the turtle *Chelydra serpentina* (Testudines: Chelydridae) at various temperatures. Herpetologica 34:274–277.

Accepted: 14 July 1998.

Journal of Herpetology, Vol. 32, No. 4, pp. 598–601, 1998

Skin Glands of *Hyla japonica*

BENJAMIN A. SHEPHERD,[1] WILLIAM T. MCDOWELL,[2] AND JAN MARTAN,[1,3] [1]*Dept. of Zoology, Southern Illinois University at Carbondale, Carbondale, Illinois 62901, USA E-mail: bshepher@siu.edu* [2]*103 Town Country, Carbondale, Illinois 62901, USA.*

Hyla arborea var. *japonica* was first named by Günther (1858), and Stejneger (1907) recognized it as a subspecies *Hyla arborea japonica* (Günther). Subsequently, it was shown to be genetically distinct from *Hyla arborea* by Maeda and Matsui (1989). Kawamura et al. (1990) confirmed by hybridization crossing experiments that *Hyla japonica* is a distinct species. As far as we know, only Sokolov and Sakulina (1994) have compared the skins of these two species; however, mucous glands were not described. The establishment of *Hyla japonica* as a separate species from *Hyla arborea* and the paucity of information in the literature on the mucous glands of *Hyla japonica* encouraged us to conduct a histological study of the skin glands in this species.

Five *H. japonica* (non-breeding males) were obtained from N. Shinozaki, Japan Amphibian Laboratory, Nikko, Tochigi Prefecture, Japan, and transported to Southern Illinois University at Carbondale (SIUC). Frogs were killed with MS-222 and fixed in Baker's Formalin (10% formalin and 1% $CdCl_2$). Dorso-ventral strips of skin (3–4 mm wide) were excised from the body wall behind the front legs and in front of the hind legs, processed by standard paraffin methods, and serially sectioned at 6 μm or 15 μm. Following the collection of tissues for study, the animals were deposited in the SIUC Fluid Vertebrate Collection.

The following histological and histochemical methods were used: Harris hematoxylin-eosin (H-E), (Luna, 1968); Periodic acid-Schiff (PAS); Alcian blue 8GX (pH 2.5); Alcian blue 8GX (pH 2.5)/PAS; Mercury-bromphenol blue (HgBPB); Heidenhain's iron hematoxylin (HIH); and Mallory's phosphotungstic acid hematoxylin (MPAH) (Table 1).

All cutaneous glands were located in the stratum spongiosum of the dermis. In the dorsal skin, glands were surrounded dorsally and laterally by a sheath of melanophores (Fig. 1e). The glands were of a simple alveolar type, opening through their ducts on the external surface of the skin. All were encircled by a layer of myoepithelial cells (Fig. 1e) with more or less elongated nuclei. The long axes of 60 mucous and 60 granular glands were measured at 400× using an ocular micrometer. Granules in granular glands were measured at 1000×. Both types of glands were measured from dorsal and ventral skins, and the measurements were combined. Measurements of immature and mature glands of each type were grouped and the mean diameters calculated. Immature and mature stages of mucous and granular glands were present in skins, but mature granular glands containing granules predominated. The mean size of mucous glands was

[3] Deceased