relatives of *Chelodina oblonga* are among the *Chelodina longicollis* group of species (including *C. novaeguineae*, *C. steindachneri*, *C. mccordi*, *C. reimanni*, and *C. pritchardi*) rather than the *C. expansa* group to which it bears the closest superficial similarity (including *C. parkeri*, *C. rugosa*, and *C. siebenrocki*). Electrophoretic comparisons yielded five synapomorphies uniting *C. oblonga* with the *C. longicollis* group (Georges and Adams, 1992), a result confirmed by recent comparisons of 12S mitochondrial gene sequences (J. Seddon, *pers. comm.*). A more distant relationship may explain the presence of well developed neurals in *C. oblonga* and the absence of exposed neurals in the *C. expansa* group of species.

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Temperature-Dependent Sex Determination and Hatching Success in the Gopher Tortoise (*Gopherus polyphemus*)

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The gopher tortoise (*Gopherus polyphemus*) is a large terrestrial turtle once common in the lower coastal plain of the southeastern United States. Gopher tortoise numbers have been dramatically reduced throughout their range, but the species now has local protection in each of the six states in which it occurs, as well as federal protection in Louisiana, Mississippi, and western Alabama. Conservation measures are diverse, and include suggestions that in some cases eggs may be collected and incubated, with hatchlings released in the wild.

Potential success of such conservation tactics depends on information about hatching success rates and sex determination mode for the species. If captive hatching rates are low, an artificial incubation program is unlikely to be very successful and other management actions may be more appropriate. Information on sex determination mode is important because application of naive incubation techniques may yield undesirable sex ratios in species with temperature-dependent sex determination (TSD) (Morreale et al., 1982). Alternatively, knowledgeable incubation techniques may facilitate favorable manipulations to fit management objectives of TSD species (Vogt, 1994). Although the congener *Gopherus agassizii* is known to have TSD (Spotila et al., 1994; Lewis-Winokur and Winokur, 1995), sex determination mode is not known for *G. polyphemus*. As variation in the mode of sex determination within a single genus has been reported in other turtles (e.g., *Clemmys*, Ewert and Nelson, 1991), as well as in lizards (Viets et al., 1994), it is appropriate to assess the sex determination mode of *G. polyphemus*.

Methods. — Eggs for this study were collected on 12 June 1992 by oxytocin injection (Ewert and Legler, 1978) of four gopher tortoises recently captured on the Tillman Sand Ridge in Jasper County, South Carolina. This population is

probably the northernmost population for the species (Wright, 1982). Tortoises in this area usually nest between the last week of May and the last week of June (Wright, 1982). Mean clutch size in our sample was 6.5 eggs (individual clutches of 3, 6, 7, and 10 eggs), which is considerably higher than the mean of 3.8 eggs per clutch (range = 2 to 6) for 23 nests reported by Wright (1982) for the same population, sampled in 1979. Our mean individual egg mass 36 hours after oviposition was 38.0 g (n = 26, range = 30.9–43.6 g, s.d. = 3.3), which is similar to Wright's (1982) determination of 38.8 g (n = 19, range = 35.4–44.7 g) for freshly laid eggs.

Clutches were split for incubation and eggs randomly assigned to constant temperature incubation at either 26, 29, or 32°C, starting thirty-six hours after oviposition. Eggs were placed in containers of moist vermiculite (1:1 water to vermiculite by mass). Containers were rotated within the incubators every two weeks, and at the same time containers were weighed and lost water was replaced. Variation in incubation temperature was evaluated regularly throughout incubation using minimum-maximum thermometers; temperatures never varied more than 1°C from desired temperatures. When the first egg pipped in each incubator, all individual eggs from that incubator were placed in separate containers of moist vermiculite to assure correct identification of hatchlings.

Results and Discussion. — Twenty eggs (77%) hatched (Table 1), and hatching success did not vary significantly by incubation temperature (2 x 3 Fisher's Exact Probability Test, P = 0.448). Hatching success also did not vary significantly among clutches from different mothers (2 x 4 Fisher's Exact Test, P = 0.085), although, given the small sample size utilized for this test, the small P value suggests that maternal origin may actually be important. Incubation duration (date of oviposition to date of emergence from egg) was not correlated with initial egg mass (r^2 = 0.002, P = 0.841). There were no significant differences in incubation duration due to maternal origin ($F_{3,16}$ = 0.689, P = 0.572), but incubation temperature did have a significant effect on incubation duration ($F_{2,17}$ = 30.067, P < 0.001). A combined ANOVA with both of these variables could not be performed because some of the resulting classes were too small.

The hatchlings (Fig. 1) were raised in captivity for 10 months, after which 17 of the 20 hatchlings died, apparently as a result of disease. While there is no significant relationship between survivorship beyond 10 months and incubation temperature (x^2 = 0.74, d.f. = 2, P > 0.10), it may be noteworthy that the three tortoises that survived beyond this period were all from 29°C incubations. Lewis-Winokur and Winokur (1995) found decreased post-hatching survivorship in *G. agassizii* that had been incubated at cool temperatures.

Fourteen of the 17 dead hatchlings were fixed in formalin upon death and later transferred to 70% ethanol for long term preservation. Sex was subsequently assessed by M.A. Ewert by macroscopic examination of gonads and Müllerian ducts. By the age of death of these tortoises (10 or more months) Müllerian ducts have fully regressed in males of most species of turtles (Ewert and Nelson, 1991; Ewert,
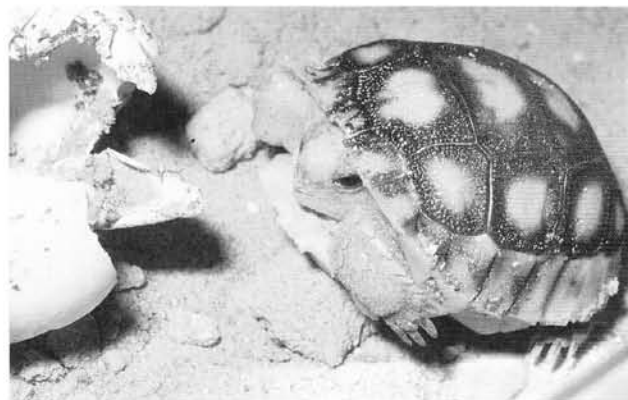

Figure 1. Hatchling *Gopherus polyphemus* from South Carolina.

pers. obs.) and have become either appreciably reduced or fully regressed in *G. agassizii* (Lewis-Winokur and Winokur, 1995). In our sample, Müllerian ducts were conspicuous in *G. polyphemus* individuals identified as females and absent or nearly absent in those identified as males. There were no intermediate conditions. Because it is more difficult to identify the sex of specimens after they have been transferred to alcohol (Ewert, *pers. obs.*), it is important to note that the identifications were made without foreknowledge of incubation temperature for any specific specimen, nor were identifications altered after the initial decision. The hatchling identification of one of the fourteen specimens was lost so it could not be assigned to an incubation temperature; it was eliminated from further analysis. Specimens were deposited in the research collection of the University of Michigan Museum of Zoology.

Sexually identified hatchlings from two different clutches were represented at each of the three incubation temperatures. Also, although all four of the adult females had at least some offspring among the 13 sexable specimens, only two had offspring from more than one incubation temperature. Sexable hatchlings from two clutches support
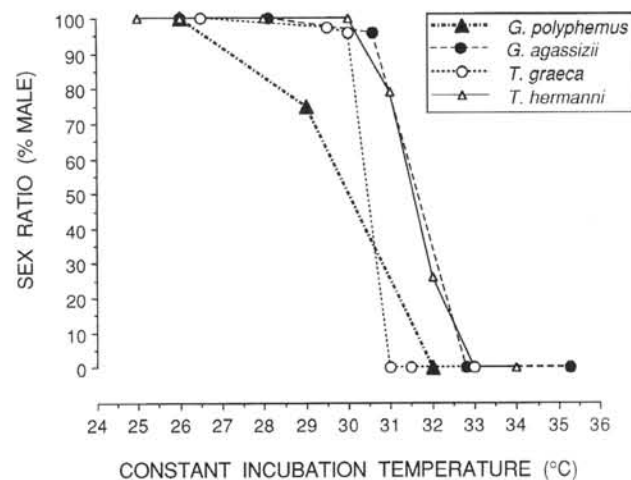

Figure 2. Sex ratios (% male offspring) produced at different incubation temperatures for the four tortoise species reported thus far. Sources of data: *Testudo graeca* (Pieau, 1972, 1975); *T. hermanni* (Eendebak, 1995); *Gopherus agassizii* (Spotila et al., 1994); *G. polyphemus* (this report).

Table 1. Incubation duration (days), percent hatching success, and gender of *Gopherus polyphemus* eggs and hatchlings.

Incubation Temperature (°C)	Eggs (n)	Incubation Duration Mean (s.d., range)	Hatchlings (n)	% Hatching Success	Males (n)	Females (n)	% Males
26	8	114.6 (9.1, 106–130)	5	63%	4	0	100%
29	9	97.0 (5.6, 88–102)	8	89%	3	1	75%
32	9	86.3 (4.1, 80–91)	7	78%	0	5	0%

the conclusion of males differentiating at cool temperatures (26 and 29°C) and females at the warmer temperature (32°C). Unfortunately, the single female hatchling from 29°C was the offspring of a mother from which there were no other sexable hatchlings. Therefore, further investigation of this anomaly is not possible.

Despite the small sample sizes, the differences in hatchling sex ratio by temperature are significant (Table 1, 2 x 3 Fisher's Exact Test, $P < 0.001$). Therefore, *Gopherus polyphemus* has TSD, as do all other tortoise species for which sex determination mode is known: *Testudo graeca* (Pieau, 1972, 1975), *T. hermanni* (Eendebak, 1995), *G. agassizii* (Spotila et al., 1994; Lewis-Winokur and Winokur, 1995).

Although we can only demonstrate that the pivotal temperature (incubation temperature that produces a 1:1 sex ratio) for *G. polyphemus* is between 29 and 32°C (Fig. 1), the fact that a single female hatchling resulted from incubation at 29°C suggests that the pivotal temperature may be in the lower end of this range. This supports the prediction by Spotila et al. (1994) that *G. polyphemus* would have a lower pivotal temperature than the 31.8°C they estimated for *G. agassizii*. Also, our findings for *G. polyphemus* are in concordance with the trend towards lower pivotal temperatures for turtles in the southeastern US relative to elsewhere in the US (Ewert et al., 1994).

Furthermore, we note that the available data suggest that members of the family Testudinidae (Fig. 2) have pivotal temperatures below those of Pelomedusidae, but above those of sea turtles and Chelydridae (Ewert et al., 1994; Mrosovsky, 1994; Souza and Vogt, 1994). Although few tortoise species have been evaluated, it is possible that *Gopherus polyphemus* may have an unusually low pivotal temperature relative to other tortoises. However, our sample sizes demonstrating TSD in this species are small, and further work is needed to assess pivotal temperature more precisely. Furthermore, our samples came from near the northern range limit for the species. *Gopherus polyphemus* has a fairly large latitudinal range, and variation within the range is possible. Therefore, our results should not be extrapolated to other parts of the range without local verification.

Management recommendations based on these results are not straightforward, because our data are limited and hatching success, sex ratios, and post-hatching vigor must all be taken into account when designing an artificial incubation program. Biased hatchling sex ratios, whether natural or not, may be desirable for management of some species (Vogt, 1994), but this choice must be assessed on a case-by-case basis (Mrosovsky and Godfrey, 1995). For management purposes, it is unclear whether it might be more important to maximize hatching success or to attempt to mimic natural incubation duration and/or sex ratios. Incuba-

tion temperature may also affect hatchling vigor as well as gender. For example, Spotila et al. (1994) showed that incubation temperature also affected post-hatching growth rates in *Gopherus agassizii*.

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