mm, carapace widths (CW) between 162 and 175 mm, and body weights (BW) from 450 to 550 g.

Pronounced sexual dimorphism in size of *K. tecta* has been noted by other researchers who have reported that females are much larger than males. Moll (1987) measured two females and one male in India, recording CL of 183 and 153 mm for the females and 66 mm for the male. Minton (1966) measured two females and a male in Pakistan at respective CL of 173, 164, and 84 mm. Das (1991) also noted that females are much larger than males in India. The current study on *K. tecta* in Bangladesh recorded that females are only slightly larger than males, with the following means, standard deviations, and ranges: females ($n = 36$), 217 ± 1.7 (186–224) mm CL, 194 ± 4.03 (177–200) mm CW, 176 ± 2.2 (167–187) mm PL [plastron length], 84 ± 1.0 (76–85) mm CD [carapace depth], and 1050 ± 4.1 (830–1100) g BW; males ($n = 16$), 206 ± 4.0 (192–212) mm CL, 185 ± .5 (180–190) mm CW, 174 ± .5 (171–177) mm PL, 88 ± .5 (86–89) mm CD, and 935 ± 1.5 (842–950) g BW.

Usually, the two oviducts contained unequal numbers of eggs (mostly mature and shelled, but in some cases also immature ones). Out of 21 specimens dissected on 5 February 1993, only one specimen had equal numbers of eggs in the two oviducts. Females of 500 to 750 g BW lay a total of 14 to 26 eggs per season in two separate clutches. The first clutch consisted of 9 to 15 eggs, and the second clutch 7 to 11 eggs. Moll (1987) mentioned a clutch containing 8 eggs. Chaudhuri (1912) recorded that the roofed turtle laid 5 to 8 eggs at a time. Duda and Gupta (1982) reported a mean clutch size of 6.9, with a range of 4 to 10.

All eggs were ovoid with one end slightly pointed and the other blunter, whitish in color, and becoming slightly bluish at hatching time. The mean length of 44 eggs was 50.09 ± 0.25 mm (range 50–51 mm), mean width 20.45 ± 0.25 mm (range 20–21 mm), and mean weight 10.75 ± 0.04 g (range 10.4–10.9 g). Moll (1987) found eggs to average 37 x 21 mm and 10.74 g in India.

The 20 eggs placed in soil on 20 March developed visible blood spots after 18 days and embryos within 28 days, but failed to hatch as a result of red ant predation. The 12 eggs placed in 4 cm of sandy soil on 17 February developed blood spots within 15 days, embryos with eye spots within 25 days, moving embryos by the 40th day, and emergence of 4 hatchlings on the 71st day, for a hatching success of 33.3 %. The average hatchling weight was 16.2 g. Incubation temperatures varied between 27–30°C.

Literature Cited

Chaudhuri, B.L. 1912. Aquatic tortoises of the Middle Ganges and Brahmaputra. Rec. Indian Mus. (Calcutta) 7:212-214.

Daniel, J.C. 1983. The Book of Indian Reptiles. Bombay: Bombay Natural History Soc., 141 pp.

Das, I. 1991. Colour Guide to the Turtles and Tortoises of the Indian Subcontinent. Avon, England: R & A Publishing Ltd., 133 pp.

Duda, P.L., and Gupta, V.K. 1982. Transabdominal migration of ova in the freshwater turtles. Proc. Indian Acad. Sci. Anim. Sci. 91(2):189-197.

Fugler, C.M. 1984. The commercially exploited Chelonia of Bangladesh: taxonomy, ecology, reproductive biology and ontogeny. Bangladesh Fish. Inf. Bull. 2(1):1-52.

Khan, M.A.R. 1982. Chelonians of Bangladesh and their conservation. J. Bombay Nat. Hist. Soc. 79:110-116.

Minton, S.A., Jr. 1966. A contribution to the herpetology of West Pakistan. Bull. Amer. Mus. Nat. Hist. 134:27-184.

Moll, E.O. 1987. Survey of the freshwater turtles of India. Part II: The genus *Kachuga*. J. Bombay Nat. Hist. Soc. 84:7-25.

Smith, M.A. 1931. The Fauna of British India, including Ceylon and Burma. Reptilia and Amphibia. Vol. I. Loricata, Testudines. London: Taylor and Francis, 185 pp.

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Incubation Period and Sex Ratio of Hermann's Tortoise, *Testudo hermanni boettgeri*

Bert T. Eendebak[1]

[1]*Tortoise Study Centre Oosterbeek, van Limburg Stirumweg 22, 6861 WL Oosterbeek, The Netherlands*

The influence of temperature on the incubation period and sex of chelonians, especially aquatic species, has been studied by different authors. Extensive studies on *Emys orbicularis* have been published by Pieau (1971) and on various Emydinae species by Bull et al. (1982a). A recent review of temperature-dependent sex determination (TSD) in squamate reptiles is given by Spotila et al. (1994) and of TSD in turtles by Ewert et al. (1994). However, little detailed work has been published on measurements of the effect of incubation temperature on the incubation period and sex ratio of tortoises (Testudinidae). In this paper the author reports on a study of the incubation and sex determination of 741 eggs of *Testudo hermanni boettgeri* from which 312 tortoises hatched successfully.

Materials and Methods. — Eggs of *Testudo hermanni boettgeri* were laid from 1982–1994 by a group of 10 adult females kept in an outdoor terrarium in The Netherlands. The study was planned to investigate the effects of environmental parameters on the incubation period and sex ratio of *T. hermanni boettgeri*. Apart from the temperature, many other parameters were studied, e.g., characteristics of the parental female, nest location, time of nesting, order of laying within a clutch, egg weight, and humidity during the incubation period.

Nesting by the colony of captive *T. hermanni boettgeri* took place in the months of May, June, and July, usually between 1000 and 1200 hrs. All eggs were removed from the nests, marked with a pencil, weighed, inspected, and placed in incubators within one to three hrs after laying.

Incubation periods of the eggs were measured at constant incubation temperatures. Three different types of incubators were used. One incubator used bi-metal control,

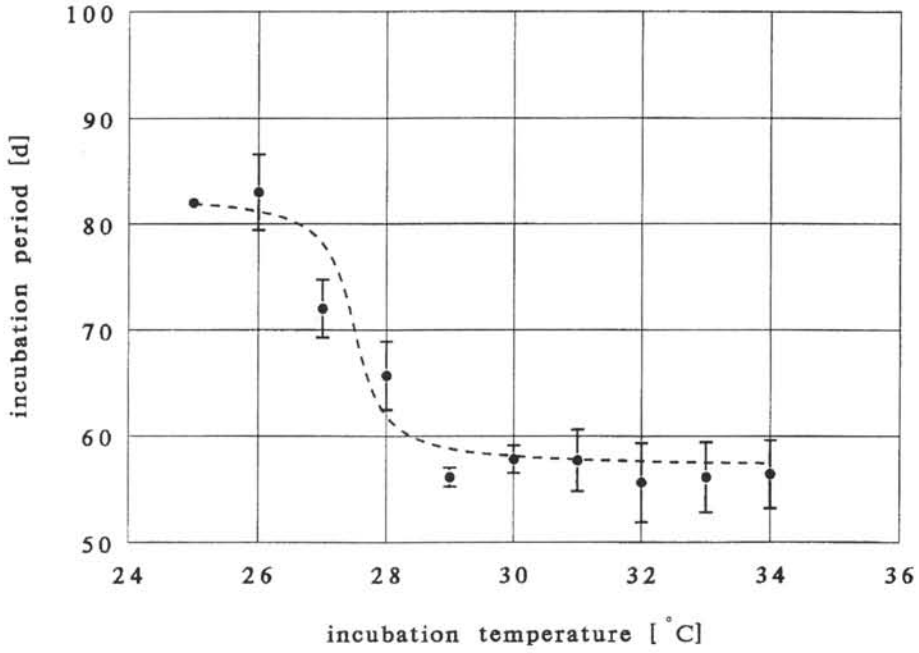

Figure 1. Incubation period of *Testudo hermanni boettgeri* as a function of the incubation temperature. Data are shown as mean and SD.

whereas the other two used NTC-sensed temperature elements and a temperature sensing bridge or TRIAC triggering circuit as control units. All these incubators usually regulated the intended temperature to within about 0.2°C. To achieve this result the bi-metal controller had to be adjusted almost daily.

Within an incubator the eggs were placed on a foam rubber subsoil and positioned in a Latin square design. Horizontal and vertical thermal gradients within the incubator were minimized by careful distribution of the heating cables. Variation from the intended temperature was limited to ca. 0.2°C, as monitored by movable thermocouple probes. In view of the control of temperature gradients the approach of the Latin square design as intended by Bull et al. (1982b) is not necessary. To reduce the influence of unknown genetic or environmental parameters the eggs of one clutch were equally distributed over the three incubators.

The incubation period as defined in this study is the time interval between oviposition and emergence of the full-grown hatchling. The length of time from pipping of the eggshell to the emergence of the hatchling is about one day. Immediately after laying, the eggs were inspected for possible development of blood-vessels which are normally only visible after 6–8 days of incubation. No sign of blood-vessels were detected, so a hypothetical developmental process within the female is unlikely and does not influence the results. In exceptional cases (ca. 2%) the hatchling did not hatch after the expected time. In these cases the egg was opened by hand two weeks after the end of the predetermined incubation period. These exceptional cases were not included in the data analyzed.

The hatchlings were marked individually by notching the marginal scutes according to the system used by the Charles Darwin Station on Santa Cruz Island (Thornton, 1971). Most of these hatchlings were distributed among members of the Dutch Tortoise Association. Only a relatively small number (ca. 25%, randomly selected) were kept until sex determination could take place. These juveniles were kept in the same outdoor terrarium as the colony of adult *T. hermanni boettgeri*.

The sex of the juveniles was determined by external characteristics such as the shape of the tail, carapace, plastron, or anal scutes (Stubbs et al., 1981). In most cases this determination can take place at a carapace length of ca. 10 cm at an age of 3–4 yrs. Juveniles were not defined as male or female until at least two different sex characteristics were present, this usually being the shape of the tail and anal scutes.

Results. — From the 741 eggs incubated at a constant temperature, 515 eggs were visibly fertilized, from which 312 tortoises hatched successfully. Table 1 shows the results of incubation temperatures of 25°C up to 34°C. Other environmental parameters such as characteristics of the parental

Table 1. Incubation period of *Testudo hermanni boettgeri.*

Incubation Temperature [°C]	Number of Eggs	Mean Incubation Period [days]	Standard Deviation
25	2	82.0	0.0
26	7	83.0	3.6
27	3	72.0	2.7
28	20	65.7	3.2
29	10	56.1	0.9
30	31	57.8	1.3
31	48	57.7	2.9
32	64	55.6	3.7
33	101	56.1	3.3
34	26	56.4	3.2

female, nest location, time of nesting, order of laying within a clutch, egg weight, and humidity during the incubation period were also studied, but no significant correlation of these parameters with the incubation period could be found. The numbers, however, were too small to justify definite conclusions. The eggs were weighed with an accuracy of ± 0.5 g. Weight-loss of the eggs during incubation averaged 30-40%. In 4 out of 120 clutches exceptional weight-loss (more than 60%) resulted in either dead embryos or short incubation periods. These exceptions are not included among the data presented in Table 1. The results, also presented in Fig. 1, show a relatively abrupt change from a low-temperature incubation period of ca. 83 days to a high-temperature incubation period of ca. 57 days. The transition period (in days) is defined as the average of the slow and fast incubation periods (here equal to 70 days), and the corresponding temperature is called the transition temperature (here equal to 27.5°C). This should not be confused with the threshold temperature, which is the incubation temperature that produces a sex ratio of 50%. A possible curve that fits the measured points was found to be the following mathematical approximation:

$$y = 70 - 26 arctan(3(x - 27.5))\pi^{-1} \qquad [1]$$

where: y = incubation period [days]
x = incubation temperature [°C]
27.5 = transition temperature [°C]
70 = transition period [days].

Mortality rates for incubation temperatures of 26 up to 32°C were in the range 20 to 30%. For incubation temperatures of 25, 33, and 34°C the mortality rate was about 50%, whereas at 24 and 35°C (ca. 20 eggs) the mortality rate was almost 100%. Hatchlings incubated at a constant temperature of 24 and 25°C are generally small, weak, and not able to hatch successfully, so the definition of the incubation period at these temperatures is questionable. For that reason the exact shape of the curve for incubation temperatures below 27°C in Fig. 1 cannot be determined.

Table 2. Sex ratios of *Testudo hermanni boettgeri*.

Incubation Temperature [°C]	Number of Males	Number of Females	Sex Ratio % Males
25	2	0	100
26	4	0	100
28	5	0	100
30	11	0	100
31	11	3	79
32	6	17	26
33	0	11	0
34	0	4	0

During the study it was found that in some cases *T. hermanni boettgeri* incubated at temperatures of 30–33°C developed their secondary sex characteristics only after a long time. In exceptional cases it took 8–10 years, which is long compared with the normal situation. This effect can lead to a male-oriented bias in the measured sex ratio if not all hatchlings of a specific year (or even better, a specific clutch) are sexed definitively. For that reason, only the numbers of males and females from clutches in which the sex of all hatchlings was determined were taken into account. A randomly selected group of ca. 25% of the hatchlings were kept until sex determination could take place. Table 2 and Fig. 2 give some results of the measured sex ratios (defined as the percentage of male hatchlings) of 74 *T. hermanni boettgeri*. Figure 2 shows a sharp transition between 30 and 33°C, with a sex ratio of 50% at a threshold temperature of 31.5°C.

Discussion. — Detailed information on the influence of incubation temperatures on incubation period and sex ratio

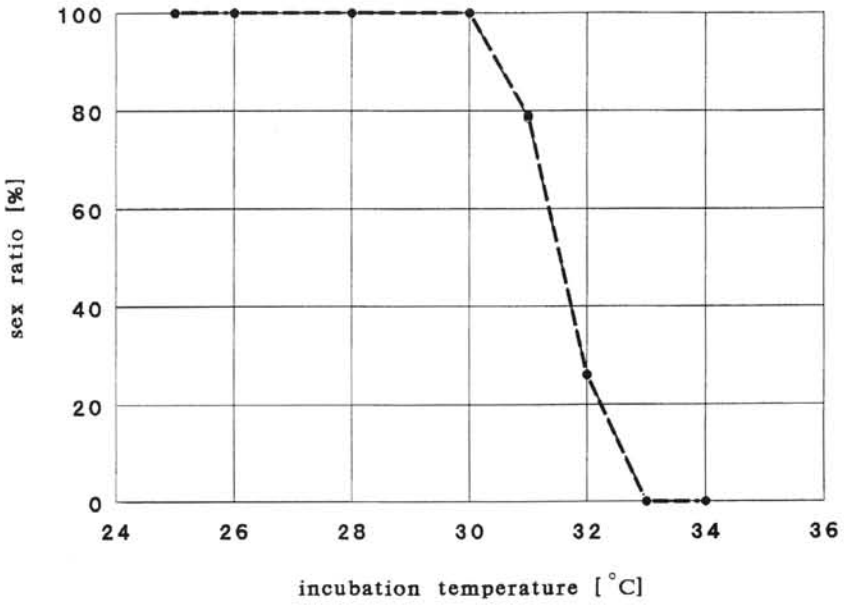

Figure 2. Sex ratio of *Testudo hermanni boettgeri* as a function of the incubation temperature. Data are shown as mean sex ratios of percentage males.

has been reported for *Emys orbicularis* by Pieau (1971) and Pieau and Dorizzi (1981), and for various Emydinae species by Bull et al. (1982a). Since then temperature-dependent sex determination (TSD) has been described in about eight families of turtles. A recent review of TSD is given by Ewert et al. (1994). For *Testudo hermanni* only sparse data are reported. Kirsche (1967) found an average incubation period of 63 days for an incubation temperature of 28–30°C, which is in accordance with the results of this study. Much longer incubation periods of more than 100 days are reported for wild populations by Swingland and Stubbs (1985). In the author's outdoor terrarium, some *T. hermanni boettgeri* hatched after 110 to 120 days, thanks to a relatively warm and dry Dutch summer.

In this study incubation temperatures of 24 to 34°C were used. Constant temperatures below 26 and above 32°C lead to increasing mortality rates. It appears that damaging incubation temperatures are similar to those that are avoided in wild populations by careful nesting site selection (Meek and Avery, 1988; Willemsen, 1991).

The incubation period was almost constant in the range 29 to 34°C, being ca. 57 days throughout. This result indicates that embryonic developmental rates are almost constant in that temperature range. A mortality rate of almost 100% at an incubation temperature of 24°C indicates that the minimum temperature for embryonic development for *T. hermanni boettgeri* is about 23 to 24°C. Using these results to test the model suggested by Georges (1989) to predict hatchling sex ratios when nest temperatures fluctuate will be difficult. In natural nests, temperatures will regularly drop below this minimum temperature of embryonic development.

Sex determination of hatchlings that were incubated at temperatures between 30 and 33°C was in some exceptional cases possible only after a long time. This phenomenon can be explained by the study of Pieau (1975), who has reported on the formation of intersexes of *Emys orbicularis* incubated at a threshold temperature of 28 to 29°C. Observations of the author, however, indicate that external sex characters will appear with time in almost all cases. Zaborski et al. (1982) suggested the possibility that some individuals which were intersexes as hatchling become phenotypic females afterwards. Whether sex ratios persist until the appearance of sexual dimorphism or whether sex reversal may occur cannot be determined, because sex determination in this study was based only upon external sex characteristics.

Comparison with sex determination results for the Emydinae shows a similar change from virtually all males to all females over a narrow temperature range. The threshold temperature for *T. hermanni*, however, was found in this study to be 31.5°C instead of 28 to 30°C for the Emydinae studied by Bull et al. (1982a). As suggested by Bull and Vogt (1979), different mortality rates of the embryos are not likely to occur because mortality rates were independent of the incubation temperatures within the region of 26 to 33°C.

Based on the effect of temperature on sex determination one could expect that relatively cold regions or periods may lead to temporarily lower threshold temperatures or favored male differentiation. This theory is not supported by field investigations as performed for Emydinae by Bull et al. (1982b). This lack of evidence could be explained by compensation by other environmental effects. However, in this study the incubation temperature was the only environmental parameter that had a detectable influence on the incubation period and sex ratio. Other parameters, such as genetically determined differences, nest location, time of nesting, order within a clutch, egg weight, and humidity during the incubation were investigated, but no correlations with the incubation period or sex ratio were found. Further investigations are needed to confirm these preliminary conclusions.

The most conspicuous differences from incubation in nature are the daily and seasonal variations in the incubation temperatures versus the constant incubation temperatures used in this study. In natural nests, the daytime soil temperature at the egg location can be up to 18°C higher than at night (Pieau, 1982), and this effect may easily overrule the influence of a small change in a constant average temperature. The results of this study show that fluctuations of the environmental temperature above 28°C does not influence the development rate, but can have a major effect on sex ratios. To what extent the number of hours of incubation or the embryonic development above the threshold temperature are decisive for sex determination is still an open question. For that reason this study is being continued by an investigation on the influence of variations of the incubation temperature on the sex ratio of *T. hermanni boettgeri*.

Literature Cited

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

BULL, J.J., MCCOY, C.J., AND VOGT, R.C. 1982a. Sex determining temperatures in turtles: a geographic comparison. Evolution 36(2):326-332.

BULL, J.J., VOGT, R.C., AND BULMER, M.G. 1982b. Heritability of sex ratio in turtles with environmental sex determination. Evolution 36(2):333-341.

EWERT, M.A., JACKSON, D.R., AND NELSON, C.E. 1994. Patterns of temperature-dependent sex determination in turtles. J. Exp. Zool. 270:3-15.

GEORGES, A. 1989. Female turtles from hot nests: is it duration of incubation or proportion of development at high temperatures that matters? Oecologia 81:323-328.

KIRSCHE, W. 1967. Zur Haltung, Zucht, und Ethologie der Griechische Landschildkröte. Salamandra 3:36-66.

MEEK, R., AND AVERY, R.A. 1988. Thermoregulation in chelonians. Herpetological Journal 1:253-259.

PIEAU, C. 1971. Sur la proportion sexuelle chez les embryons de deux cheloniens issus d'oeufs incubés artificiellement. C. R. Hebd. Séanc. Acad. Sci. Paris (D) 272:3071-3074.

PIEAU, C. 1975. Données recentes sur la differentiation en fonction de la temperature chez les embryons d'*Emys orbicularis*. Bull. Soc. Zool. France 101(Suppl. 4):46-53.

PIEAU, C. 1982. Modalities of the action of sexual differentiation in field developing embryos of the European pond turtle *Emys orbicularis*. J. Exper. Zool. 220:353-360.

PIEAU, C., AND DORIZZI, M. 1981. Determination of temperature

sensitive stages for sexual differentation of the gonads in embryos of the turtle *Emys orbicularis*. J. Morphol. 170:373-382.

SPOTILA, J.R., SPOTILA, L.D., AND KAUFER, N.F. 1994. Molecular mechanism of TSD in reptiles: a search for the magic bullet. J. Exp. Zool. 270:117-227.

STUBBS, D., HAILEY, A., TYLER, W., AND PULLFORD, E. 1981. Expedition to Greece 1980. University of London Natural Society.

SWINGLAND, I.R., AND STUBBS, D. 1985. The ecology of a Mediterranean tortoise (*Testudo hermanni*): reproduction. J. Zool. Lond. (A) 205:595-610.

THORNTON, I. 1971. Darwin's Islands. A natural history of the Galapagos. Garden City: AMNH Natural History Press, 322 pp.

WILLEMSEN, R.W. 1991. Differences in thermoregulation between *T. hermanni* and *T. marginata* and their ecological significance. Herpetological Journal 1:559-567.

ZABORSKI, P., DORIZZI, M., AND PIEAU, C. 1982. H-Y antigen expression in temperature sex-reversed turtles (*Emys orbicularis*). Differentiation 22:73-78.

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Growth of Head-Started Kemp's Ridley Sea Turtles (*Lepidochelys kempii*) Following Release

CHARLES W. CAILLOUET, JR.[1], CLARK T. FONTAINE[1], SHARON A. MANZELLA-TIRPAK[2], AND THEODORE D. WILLIAMS[1]

[1]*National Marine Fisheries Service, Southeast Fisheries Science Center, Galveston Laboratory, Galveston, Texas 77551 USA;* [2]*U.S. Army Corps of Engineers, Galveston District, Galveston, Texas 77550 USA*

The Kemp's ridley sea turtle (*Lepidochelys kempii*) captive-rearing, tagging, and release experiment called "head-start" began in 1978 as part of an endangered species recovery program (Woody, 1986, 1989; Phillips, 1989; Kemp's Ridley Recovery Team, 1992). Its goal was to establish a nesting colony at the Padre Island National Seashore near Corpus Christi, Texas through imprinting of hatchlings. Rearing the turtles in captivity at the National Marine Fisheries Service (NMFS) Laboratory in Galveston, Texas also gave them an early survival advantage over their wild counterparts (Caillouet et al., 1993). However, there have been no documented nestings of head-started Kemp's ridleys, although a number of recent nestings of the species in Florida and the Carolinas have been reported (Meylan et al., 1990; Anonymous, 1992, 1994; Palmatier, 1993; Bowen et al., 1994).

This paper fits growth curves to post-release straight carapace length (SCL, in cm) vs. age (t, in yr) of head-started Kemp's ridleys released along the Texas coast, based on tag returns from the Gulf of Mexico and adjacent bays. It also estimates t at sexual maturity, under the assumption that Kemp's ridleys mature sexually at least by the time they reach 60 cm in SCL (see Pritchard, 1989).

Materials and Methods. — From 1978 through 1992, 25,676 Kemp's ridley hatchlings (averaging 1712 per year and ranging from 250 to 3080 per year), were received for the experiment. Most (15,823) of the hatchlings were provided by the National Park Service's Padre Island National Seashore, but 9669 were obtained from the nesting beach near Rancho Nuevo, Tamaulipas, Mexico and 184 from a captive-breeding experiment at the Cayman Turtle Farm Ltd., Grand Cayman, B.W.I. Hatchlings from the Padre Island National Seashore were produced from eggs obtained from Rancho Nuevo, and the breeders at Cayman Turtle Farm Ltd. were originally obtained as hatchlings from Rancho Nuevo or as yearlings from the head-start experiment. Prior to release in the Gulf of Mexico or adjacent bays, 22,608 captive-reared Kemp's ridleys of the 1978–1992 year-classes were tagged with external, Hasco style 681, Monel or Inconel, foreflipper tags (Fontaine et al., 1993). Turtles released in the year following receipt as hatchlings were considered the standard in the head-start experiment. Older turtles were also released, but they were considered atypical due to the additional habituation to captive-rearing conditions they were exposed to during extended head-starting. Standard releases along the Texas coast totaled 18,790 turtles. Of these, 18,174 (96.7%) were released into the Gulf of Mexico and 616 (3.3%) into adjacent bays. The released turtles ranged in age from 0.80 to 1.27 yr, in geometric mean SCL from 13.9 to 31.0 cm, and in geometric mean weight from 0.463 to 4.839 kg.

Tag returns came from the NMFS Sea Turtle Stranding and Salvage Network (see Schroeder, 1989) and fishermen, both commercial and recreational. Fitting of growth curves was restricted to 117 tag returns from the Gulf of Mexico or adjacent bays for turtles that had been released along the Texas coast because:

(a) most head-started Kemp's ridleys were released along the Texas coast,

(b) except for a single tag return in 1994 (Richard Byles, U.S. Fish and Wildlife Service, Albuquerque, New Mexico, pers. comm., July 1994), there has been no direct evidence that Kemp's ridleys in the Atlantic return to the Gulf of Mexico (Schmid and Ogren, 1992),

(c) except for two recent nestings on the U.S. Atlantic coast (Anonymous, 1992; Palmatier, 1993; Bowen et al., 1994), the Kemp's ridley breeding population has been confined to the Gulf of Mexico (Pritchard, 1989), and

(d) growth of head-started Kemp's ridleys in the Atlantic appears to be slower than in the Gulf (Fontaine et al., 1989).

For individuals recaptured more than once, only the last of their tag returns was included in the data used to fit growth curves, so as not to give undue weight to such individuals.

Logistic, Gompertz, and von Bertalanffy growth curves were fitted to paired observations ($n = 117$) of SCL and t with the microcomputer program FISHPARM, version 3.0S (Prager et al., 1987). Because the data set contained only three points representing head-started turtles older than 5 yr