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# Sex Determination and Sex Ratios in *Crocodylus palustris*[1]

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SYNOPSIS. Incubation temperature determines sex in the mugger crocodile, *Crocodylus palustris*. Exclusively females are produced at constant temperatures of 28.0°C through 31°C. At 32.5°C, only males are produced. Both sexes are produced in varying proportions at 31.5, 32.0, and 33.0°C. Embryo survival is not affected within this range, but developmental rate and total incubation time are strongly temperature dependent. In natural nests laid in breeding enclosures, cool incubation temperatures produced only females whereas males were produced only in warm nests. Clutch sex ratios were female or male biased. Yearly sex ratios (=percent male) varied from 0.05 to 0.58; overall sex ratio during six nesting seasons was 0.24 (1 male : 3 females). Sex ratio and incubation time vary with nest location and temperature in a manner consistent with the constant temperature results. Incubation time decreases with increasing incubation temperature, and is an accurate predictor of sex ratio in the field and laboratory.

To date, temperature-dependent sex determination (TSD) has been reported in five species of *Crocodylus* and in three species of Alligatorinae; but the TSD patterns in these groups differ. The TSD pattern of *C. palustris* is similar to that of *C. porosus*. Nesting in *C. palustris* is synchronized with the seasonal availability of thermal regimes suitable for incubation. Resultant sex ratios are a consequence of when and where eggs are laid. Early nests are located in warm, sunny sites; in contrast, late season nests are located in the shade. An egg transplant experiment demonstrated that sex ratios could be altered by simple manipulations of nest temperatures in the field. The adaptive significance of TSD in crocodilians may relate to the influence of incubation temperature on various hatchling attributes, particularly growth.

## INTRODUCTION

Incubation temperature determines sex in alligators and crocodiles (Ferguson and Joanen, 1982, 1983; Webb and Smith, 1984; Hutton, 1987; Webb *et al.*, 1987). Temperature-dependent sex determination (TSD) has also been demonstrated in some, but not all, species of turtles and lizards (*e.g.*, Yntema, 1976; Bull, 1983, 1987; Raynaud and Pieau, 1985; Standora and Spotila, 1985; Webb *et al.*, 1986). Among crocodilians, the universal absence of heteromorphic sex chromosomes (Cohen and Gans, 1970; Singh and Ray-Chaudhuri, 1973; King *et al.*, 1986) points to the likelihood that all living Crocodilia exhibit TSD (Ferguson, 1985). However, the TSD patterns in the few studied representatives differ substantially, and this diversity argues that additional species will have to be studied before valid generalizations may emerge. From a management perspective, the relevant information for conservation and/or utilization programs must, of necessity, be species-specific.

In this paper, we report that incubation temperature determines sex in the mugger crocodile, *Crocodylus palustris*. Eggs were incubated at constant temperature to determine temperature effects on embryonic survival and development, incubation time, and sex. The sex ratios of natural nests in field enclosures were examined, and an egg transplant study was conducted to determine how nest location and time of nesting influence incubation temperatures and resultant sex ratios. In the discussion, we relate these results to other studies of TSD in crocodilians, examine the consequences of TSD on sex ratios and nesting, and comment briefly on the adaptive significance of TSD in crocodilians.

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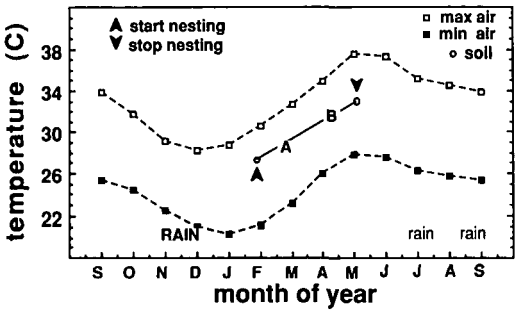


FIG. 1. Period of nesting for *Crocodylus palustris* at the Madras Crocodile Bank (start and stop denoted by arrows) in relation to ambient soil temperatures (at 30 cm depth; denoted by line between arrows), monthly mean air temperatures (mean daily maxima and minima; open and closed squares, respectively), and the annual pattern of precipitation (northeast monsoon: heavy rain from October thru December; southwest monsoon: intermittent rain from June through September). Weather data for Madras, Tamil Nadu, south India from Ramdas (1974).

## METHODS

### *Study species and locality*

The mugger crocodile (*Crocodylus palustris*) is a broad-snouted, heavily-armored, moderately-sized member of the speciose genus *Crocodylus*. It occurs throughout the Indian subcontinent south through Sri Lanka; major breeding populations exist in Sri Lanka and at scattered localities within India, principally in the northwest and in the south (Whitaker, 1987). The natural history of the species is outlined by Deraniyagala (1939), Groombridge (1982), and Whitaker and Whitaker (1979, 1984). A summary of the reproductive biology of the mugger (Whitaker and Whitaker, 1984) is based on extensive surveys of wild populations in India and Sri Lanka as well as on comprehensive studies of captives. Salient features of mugger nesting ecology in wild habitats and in breeding enclosures in south India are reviewed in Appendix 1.1.

We conducted this study at the Madras Crocodile Bank (=MCB; 12°50′N, 80°10′E) located on the Bay of Bengal coast, 35 km south of Madras in Tamil Nadu, southeast India. Adult crocodiles, primarily from localities in south India, are maintained in large breeding enclosures at the facility (Appendix 1.2). The annual mean temperature is 29.4°C and ranges from 20°C in January to 38°C in May. Annual rainfall is 1,200 mm and occurs primarily during the northeast monsoon (October–December) with sporadic precipitation from June to September. Nesting (February until early May) coincides with the period of minimal rainfall and increasing ambient temperatures; the annual cycle of temperature and precipitation (Ramdas, 1974) and the chronology of nesting at MCB are shown in Figure 1.

### *Nesting / incubation*

At MCB, we recorded the following data for each clutch laid from 1978 through 1983: identity of female, date of egg deposition, nest location, behavioral observations, and date of hatching. Eggs were removed from nests just prior to hatching. Hatchlings were marked individually and reared in cohort groups in outdoor pens. From 1984 through 1987, we observed egg deposition at each nest. Soil and ambient temperatures in and around nests were recorded during nest construction, egg laying, and egg removal. Eggs were collected the next morning; and each was weighed, measured, and marked. Viability of each egg was determined by candling for the presence of subembryonic fluid (Webb and Manolis, 1987).

Eggs for the constant temperature experiments were assigned randomly to 1–3 temperature treatments and placed in incubators within 12–16 hr of laying. Incubator design and incubation protocol are presented in Appendix 1.3. Incubation was initiated before the development of opaque banding (>95% instances). Throughout incubation, eggs were candled every 1–2 wk to detect dead embryos. Prior to hatching, we placed eggs in partitioned trays with lids to ensure that each hatchling could be related to its egg. At pipping, eggs were removed and placed in individual plastic containers. Hatching typically occurred within 12–48 hr of pipping. Hatchlings were weighed, measured, marked, and sexed. For determination of total incubation time, the day following nocturnal egg deposition was designated as "day 0"; the endpoint was the date the egg was pipped.

*Embryos / developmental rate*

Embryos were collected to determine developmental stage as a function of time and temperature. Procedures are outlined in Appendix 1.4. We preserved embryos in buffered 10% formalin and determined developmental stage by comparison with Ferguson (1985, 1987). Because developmental rate is not strictly linear throughout development and because stage at hatching varies with incubation temperature (for *Crocodylus johnstoni* and *C. porosus*; Webb *et al.*, 1987), total incubation time may be used only as an approximate indicator of how developmental rate varies with incubation temperature. In this paper, "approximate" developmental rate coefficients were computed by dividing total incubation time at 30°C by total incubation time at specified constant temperature.

*Egg transplant*

In 1986, we incubated eggs in natural nests and at transplant sites within the breeding enclosures. Eggs were removed from natural nests the morning following deposition and were processed as described above. Viable eggs in each clutch were divided into two equal groups. We returned one group to the original nest (30 cm depth), and the other was shifted to a transplant site (30 cm depth) selected to provide a thermal regime that differed from the original site. For example, if a natural nest was located in a warm, sunny site, the transplant site was located in a cool, shady location, and vice versa. In most instances, we located transplants in the shade and 1–3 m from natural nests. Moisture levels (% water by weight) were measured at natural *vs.* transplant nests, and did not differ significantly (Mann-Whitney *U*-test, $P > 0.05$; natural nests: $\bar{x} = 6.9\%$, $\pm 0.66$ SE, $n = 17$; transplant nests: $\bar{x} = 7.2\%$, $\pm 0.54$ SE, $n = 17$).

We measured soil temperature with a remote thermistor probe buried in the center of each egg group. Temperatures were monitored hourly throughout incubation, and daily maxima and minima were compiled for each site. We estimated hatching time by averaging the daily records from each site, and then calculating the total incubation time on the basis of the relationship shown in Figure 2. Eggs were removed from enclosure nests 1–5 days prior to hatching, placed in individual containers, and held in a shaded room at ambient temperature. Date of pipping was recorded, and then hatchlings were marked individually and subsequently sexed.

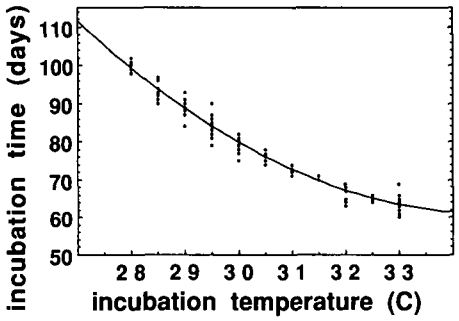


FIG. 2. Total incubation time (in days; from laying to pipping) for *Crocodylus palustris* eggs incubated at constant temperatures of 28.0–33.0°C. Data are tabulated in the Appendix: Table B.

*Temperature measurement*

We measured all temperatures with thermistor probes (YSI 400 series), digital thermometers and/or trace-recorders (Tegam, Rustrak). Probes, thermometers, and recorders were calibrated to an NBS-traceable certified thermometer, readable to 0.05°C. The accuracy of absolute temperature measurement was 0.1°C. Throughout incubation, the accuracy of temperature maintenance within individual incubators was $\pm 0.01$ to $\pm 0.02$°C (SE), and the extent of variation was $\pm 0.10$ to $\pm 0.20$°C (SD) (see Appendix 1.3).

*Sex determination / sex ratios*

We assigned sex by examining genitalia and, when possible, by inspecting gonads. Criteria and procedures for sexing are presented in Appendix 1.5. Sex ratios (=percent male) were determined for: (1) clutch groups of eggs incubated at constant temperatures in the laboratory, (2) clutches of eggs laid in natural nests in field enclosures during 1978 through 1983, and (3) groups of eggs incubated in natural and transplant nests in field enclosures in 1986. We sexed

juveniles from the 1978–1983 nests on the basis of cliteropenis size at ages of 1–6 yr in April 1984. Small samples (<4 juveniles/clutch) were excluded from analyses. Sex ratios calculated in this manner are approximate because each was based on juveniles surviving for 1–6 yr in rearing pens. Sex ratios calculated for the 1986 sample are exact because each included all late-stage embryos and dead young sexed by gonadal inspection as well as all living juveniles sexed at 1.5 yr of age.

*Analyses*

We used Statworks (Macintosh version, Cricket Software, 1985) for descriptive statistics, regression, paired comparisons with Wilcoxon signed rank test, and unpaired comparisons with the Mann-Whitney *U*-test. Significance was determined at the level of $P < 0.05$ (two-tailed).

## RESULTS

*Incubation at constant temperatures*

*Embryonic survival and development.*—Eggs from 36 clutches of 27 females were incubated at constant temperatures of 28–34°C at intervals of 0.5°C. Fertile eggs failed to complete development at 33.5 (n = 19) and at 34°C (n = 4). At 33.5°C, abnormal embryos were evident by 10–13 days; at 34°C, embryos died before day 20. Incubation below 28°C was not attempted. However, in two enclosure nests, eggs incubated at initial temperatures of 26.5–27.5°C for 5–10 days did develop normally; subsequent temperatures were >28°C. In addition, eggs in some nests experienced temperatures of 34–36°C during the last third of incubation. Thus, embryos are able to survive transient temperatures that range from 1 to 3°C above and below the viable range under constant conditions.

Embryonic survival was not affected by constant incubation temperatures between 28.0 and 33.0°C. However, embryonic viability did differ markedly among clutches irrespective of temperature treatment. Consequently, simple comparisons based on the percentage of eggs hatched at a given temperature relative to the number initially incubated are confounded by clutch effects. Direct comparison of eggs from the same clutch divided between two temperature treatments indicates that embryonic survival at 28–29°C and at 33°C did not differ from survival at mid-range temperatures. For example, for clutch 850805, 10 eggs were incubated at 28.0°C and 10 at 32.0°C; 10 hatchlings resulted from each treatment. For clutch 871002, 4 eggs were incubated at 29.5 and 4 at 33.0; 4 hatchlings resulted from each treatment. In similar fashion, no reduction in survivorship was noted in other two-way comparisons of paired temperatures, *e.g.*, 28.0 *vs.* 29.0, 28.5 *vs.* 29.5, 28.5 *vs.* 30.0, and 29.0 *vs.* 31.0°C (Appendix: Table A).

In *Crocodylus palustris,* development at 30°C takes 30 days to stage 20. This timetable is identical to that reported for *Alligator mississippiensis* and *C. johnstoni* (Ferguson 1987). For later stages at 30°C, the stage *vs.* age relationship of *C. palustris* is: stage 21 at 32 days, 22 at 38 days, 23 at 42 days, 24 at 46 days, 25 at 56 days, 27 at 68 days, and 28 at 80 days.

Embryonic development rate is temperature-dependent. The effect of temperature on development is indicated by comparing embryonic stages at intervals throughout incubation. At 12 days, embryos at 28°C were at stage 9.5 *vs.* stage 15.5 at 33°C. At 36 days, embryos at 28°C were at stage 19 *vs.* stage 24.5 at 33°C. At 60 days, embryos at 28°C were at stage 24 *vs.* stage 28 at 33°C. Approximate developmental rate coefficients for *Crocodylus palustris* are: 28.0°C = 0.7995; 28.5°C = 0.8571; 29.0°C = 0.8936; 29.5°C = 0.9545; 30.0°C = 1.000; 30.5°C = 1.046; 31.0°C = 1.130; 32.0°C = 1.193; 32.5°C = 1.220; 33.0°C = 1.259.

The relationship between total incubation time and incubation temperature for 297 eggs (34 clutches/25 females) is shown in Figure 2. Mean incubation time decreased from 99.8 days at 28°C to 63.4 days at 33°C. Incubation time may be predicted from incubation temperature (T = °C) by the regression formula:

$$\text{days} = 1{,}084.061 - 58.924T + 0.848T^2 \pm 0.052 \quad (R^2 = 0.969).$$

At increasing incubation temperatures,

development is accelerated and incubation time decreases. This effect is accentuated at lower temperatures. At 30°C, development was 1.25× faster than at 28°C, and 1.19× slower than at 32°C (Appendix: Table B).

*Sex determination.*—The relationship between sex and incubation temperature under constant conditions for 308 eggs (36 clutches/27 females) is summarized in Figure 3. Exclusively females were produced at and below 31°C. At higher temperatures, varying proportions of females were produced at all but one interval, 32.5°C. At 32.5°C, 100% males were produced. At 32°C, males comprised 59% of the total; and at 31.5 and 33°C, males were 22% and 31%, respectively, of the total. Thus, the production of males was restricted to a limited band of high temperatures, *i.e.*, 31.5–33°C. In contrast, females developed over the entire range of viable temperatures (28–33°C) with the exception of 32.5°C.

The available data indicate that sex ratio varies as a function of incubation temperature, but is independent of (a) female identity, (b) female age/reproductive history, and (c) first *vs.* second clutches of an individual female within a single season. At incubation temperatures of 31°C and below, 100% females resulted from "A" and "B" clutches of the same female (clutch 850801 at 30.5°C *vs.* 850808 at 29°C and 850808 at 28°C; Appendix: Table A). At and below 31°C, eggs from older, middle-aged females and eggs from younger females produced 100% females (age class 1–3; Appendix: Table A). Similarly, eggs from 3 to 6 different females produced identical sex ratios when incubated at the same temperature, *i.e.*, every interval between 28.0 and 31.0°C (Appendix: Table A).

At male-producing temperatures, differences in sex ratios were evident among clutches. At 32°C, percent male varied from 75% to 25%; and at 33°C, it varied from 75% to 21%. Sample size was small for such comparisons, and the significance, if any, of such differences is not apparent.

Sex ratio varies with total incubation time. Females were produced over the entire range of incubation times from 60–102 days; at incubation times of >72 days, exclusively females resulted. In contrast, males occurred over a restricted range of 60–71 days; at 64–66 days, 100% males were produced.

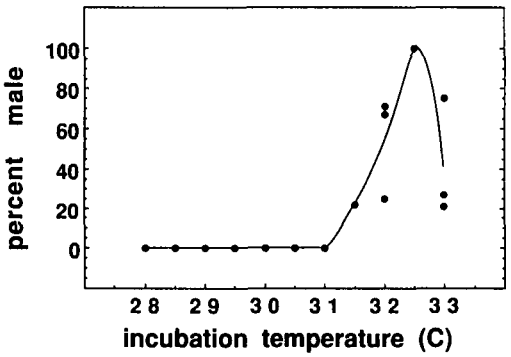


FIG. 3. Sex ratios (=percent male) of *Crocodylus palustris* eggs incubated at constant temperatures of 28.0–33.0°C at 0.5°C intervals. Line fitted by interpolation. Data are tabulated in the Appendix: Table A.

*Incubation in enclosure nests*

The sex ratios of groups of eggs incubated in field enclosures were examined to determine: (1) whether the sex ratios were biased, and (2) whether the ambient thermal regime experienced by an egg affected its sex. Two types of data were analyzed: (1) sex ratios for natural nests laid from 1978 through 1983, and (2) sex ratios in natural *vs.* transplant nests in 1986. During this field experiment, we monitored nest temperatures to characterize thermal environments during incubation and to relate these to nesting locations, times of nesting, incubation times, and the resultant sex ratios.

*Natural nests.*—We estimated the sex ratios of natural nests (1978–1983) by sexing juveniles (n = 734) in 1984. The results are shown in Table 1. These data indicate that (1) sex ratios on a per clutch basis were either unimodal (predominately all-female) or bimodal (all-female and all-male), (2) overall sex ratio was biased toward females, and (3) annual sex ratios varied by a factor of 10. For example, one half of the nests (26/52) were all-female. Only 40% (21/52) of the nests contained both males and females, and 10% (5/52) were all-male. In

TABLE 1. *Sex ratios in field nests of* Crocodylus palustris *at the Madras Crocodile Bank from 1978 to 1983 and in 1986.**

| Year | n | SR | Nests | % males per nest | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0 | 1–40 | 41–99 | 100 |
| 1978 | 43 | 0.21 | 5 | 3 | 1 | 1 | 0 |
| 1979 | 36 | 0.06 | 5 | 3 | 2 | 0 | 0 |
| 1980 | 33 | 0.58 | 5 | 0 | 1 | 3 | 1 |
| 1981 | 232 | 0.28 | 14 | 7 | 2 | 4 | 1 |
| 1982 | 184 | 0.37 | 13 | 5 | 2 | 4 | 2 |
| 1983 | 206 | 0.05 | 10 | 8 | 1 | 0 | 1 |
| 78-83 | 734 | 0.24 | 52 | 26 | 9 | 12 | 5 |
| 1986N | 181 | 0.44 | 20 | 7 | 4 | 4 | 5 |
| 1986T | 184 | 0.06 | 20 | 17 | 1 | 2 | 0 |

* N = number sexed; SR = annual sex ratio (=percent male); nests/year; distribution of nests into categories of "% males per nest." 78/83 indicates overall mean SR and summary totals. In 1986, 20 clutches were evenly divided into natural nests (=1986N) and transplant nests (=1986T); see text for explanation.

TABLE 2. *Comparison of total incubation times (days) for* Crocodylus palustris *eggs incubated at constant temperature, and in nests in field enclosures at the Madras Crocodile Bank during 1978–1983 and in 1986.**

| | 100% females | Mixed (m/f) | 100% males |
|---|---|---|---|
| Constant temperature incubation | 71–102 | 60–71 | 64–66 |
| Field incubation, 1978–1983 | 68–77, (60 and 65) | 65–77 | 63–70 |
| 1986N | 68–82 | 66–72 | 62–69 |
| 1986T | 72–94, (62 and 66) | 63–76 | — |

* (N = natural nests; T = transplant nests). Categories are for egg groups/nests producing 100% females, mixed sex ratios, and 100% males. Parentheses indicate outliers; see text for explanation.

1980, in contrast to other years, none of the nests were all-female; 80% (4/5) nests had sex ratios >40% male.

Overall sex ratio (percent male) averaged over six years was 0.24 or 1 male : 3 females. It varied from approximately 1 male : 19 females in 1979 (=0.05) and 1983 (=0.06) to 3 males : 2 females in 1980 (=0.58). The 1980 nesting season was clearly unusual because the total percentage of males produced was more than 2× the overall average. Conversely, in 1979 and in 1983, low percentages of males and relatively few all-male or mixed-sex nests were recorded (Table 1).

Sex ratio varied inversely with incubation time. The duration of incubation for nests producing 100% males ranged from 63–70 days. Mixed sex ratios were produced at incubation times of 65–77 days. Nests consisting of 100% females had incubation times of 68–77 days with outliers at 65 and 60 days (Table 2).

We analyzed the data from 1978–1983 to determine whether sex ratio varied with: (1) time of egg deposition, (2) nest location within enclosure, and (3) "A" *vs.* "B" clutches of individual females that nested twice in a season. Nests laid early (February to mid-March, n = 26) had a mean sex ratio of 0.186 (±0.058 SE) *vs.* 0.352 (±0.077 SE) for late nests (mid-March through April, n = 26). Nests deposited in open, sunny locations (n = 30) had a mean sex ratio of 0.247 (±0.063 SE) *vs.* 0.299 (±0.079 SE) for shaded nests (n = 22). The mean sex ratio of "A" nests (n = 34) was 0.227 (±0.058 SE) *vs.* 0.349 (±0.090 SE) for "B" nests (n = 18). None of these differences was significant (*U*-test, $P > 0.05$). In brief, there was a weak seasonal trend toward increasing numbers of males to be produced in late *vs.* early nests and in "B" *vs.* "A" clutches.

Stronger seasonal effects were expected based on the progressive increase in soil temperature measured during the nesting and incubation period (Figure 1). Temperatures at nest depth (30 cm) typically increased 0.5–1.0°C/week during this period. Temperatures rose from 27–28°C in early February to 33–34°C by early May. Soil temperatures also varied with location within an enclosure, and exposed sites had consistently elevated temperatures (±1–4°C at nest depth) relative to shaded ones.

The general pattern of nesting with respect to when and where eggs were deposited indicates that temperature may be an important component of nest site selection. Early in the season, nests were located predominantly in sunny, exposed sites (early: sun—19, shade—7). Late nests were located more often in shaded sites (late: sun—11, shade—15). The association of early-sun *vs.* late-shade was significant ($\chi^2 = 137$, $P < 0.0001$). A similar association was evident in the location of "A" *vs.* "B" clutches ($\chi^2 = 313$, $P < 0.0001$).

"A" nests were sunny (n = 25 *vs.* 9 in shade); in contrast, "B" nests were shaded (n = 13 *vs.* 5 in sun).

*Egg transplants.*—In 1986, each clutch was divided into two equal groups of eggs; and these were incubated in natural *vs.* transplant nests in the breeding enclosures. Females nested in 18 sunny locations and in two shaded sites. We positioned the corresponding transplant sites in 18 shaded sites and in two sunny locations.

The survival of embryos did not differ significantly in natural *vs.* transplant nests (Wilcoxon signed rank, T = 72.5, $P$ = 0.286). Overall, 80% (181/226) of the eggs in natural nests hatched *vs.* 81% (184/228) of the eggs in transplant nests. In paired comparisons of each clutch, survival was higher for eggs in natural *vs.* transplant nests in 10 instances, was equivalent in two instances, and was lower in 8 instances.

In the location selected by nesting females, twenty natural nests yielded an overall sex ratio of 0.44 or approximately 2 males : 3 females. The distribution of sex ratio per clutch was bimodal. Seven nests were all-female, and 5 were all-male. A higher than average proportion of males was produced in 1986, relative to the overall sex ratio of 1 male : 3 females for natural nests from 1978–1983. The 1986 result was similar to the male-biased annual sex ratios evident in 1980 and 1982 (natural nests "1986N," Table 1).

The effect of nest location on sex ratio is readily apparent. At transplant sites, sex ratios were markedly biased toward females. Twenty transplant nests yielded a combined sex ratio of 0.06 or approximately 1 male : 13 females. The distribution of sex ratio per clutch was unimodal; 17 (85%) of the transplanted egg groups were all-female (transplant nests "1986T," Table 1). Mean sex ratio was 0.06 (±0.042 SE) at transplant sites *vs.* 0.44 (±0.095 SE) in natural locations. The distributions of sex ratios resulting from the two treatments differed significantly (Wilcoxon signed rank, T = 12, $P < 0.01$).

Initial temperatures in natural nests were significantly warmer than at transplant sites (Wilcoxon signed rank, T = 9.0, $P < 0.0001$). In natural nests, values ranged from 28.1 to 32°C; the mean was 29.8°C (±0.26 SE, n = 20). In transplant sites, the mean was 28.9°C (±0.34 SE, n = 20); values ranged from 26.8 to 33°C.

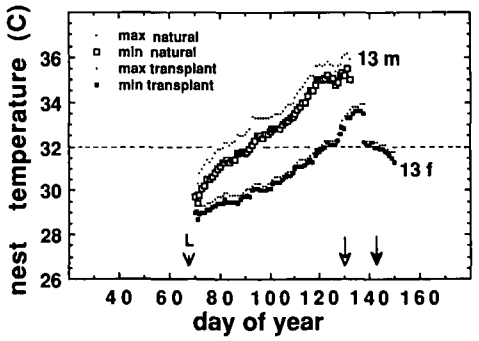


FIG. 4. Temperatures (°C) in a natural nest of *Crocodylus palustris* located in the sun and in a transplant nest in the shade. Daily maxima (small dots) and minima (open square = natural nest; solid square = transplant nest) at 30 cm depth are shown from date eggs were laid ("L" = arrow; day "0" = day 69 = 9 March) to hatching dates (open arrow = natural nest; solid arrow = transplant nest). Clutch (n = 26) was divided evenly between locations. Natural nest produced all males (n = 13); transplant nest produced all females (n = 13).

Representative temperatures in a natural nest with a sunny exposure and in the corresponding nest transplanted to a shady location are shown in Figure 4. A clutch of 26 viable eggs was evenly divided between the two sites; and each egg group was buried at nest depth (30 cm) along with a remote soil temperature probe. In the natural nest, daily maxima ranged from 29.9 to 36.2°C and averaged 33.6°C (±0.21 SE, n = 62). Daily minima ranged from 29.4 to 35.5°C and averaged 32.8°C (±0.22 SE, n = 62). The rate of temperature increase was approximately 0.1°C/day. At the transplant site, daily maxima ranged from 29.1 to 33.9°C and averaged 31.2°C (±0.15 SE, n = 77). Daily minima ranged from 28.7 to 33.6°C and averaged 30.9°C (±0.15 SE, n = 77). The rate of increase was approximately 0.05°C/day, or half the rate of increase in the natural nest.

Incubation time for eggs in the natural nest was 62 days *vs.* 77 days at the transplant site. The natural nest produced only males (13 males : no females); in contrast, only females resulted from incubation at the transplant site (no males: 13 females).

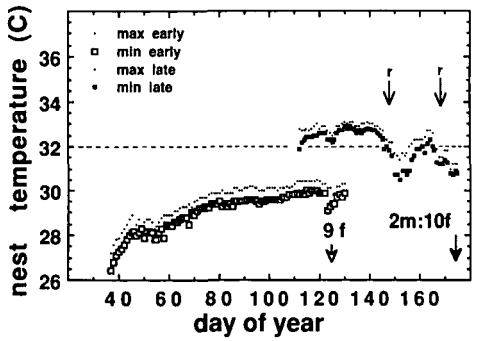


FIG. 5. Temperatures (°C) in early and late nests of *Crocodylus palustris*. Daily maxima (small dots) and minima (open square = early; solid square = late) at 30 cm depth are shown from date eggs were laid (start of each record) to hatching dates (open arrow = early; solid arrow = late). Intermittent days of rain indicated by "r" arrows. In the early nest, all females (n = 9) resulted; the late nest produced 2 males and 10 females.

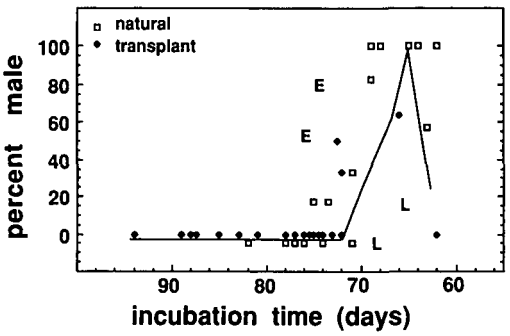


FIG. 6. Sex ratio (=percent male) as a function of total incubation time (in days, from laying to pipping) for clutches (n = 20; each clutch divided into 2 egg groups) of *Crocodylus palustris* incubated in natural (open symbol) *vs.* transplant (solid symbol) nests. Line indicates the results obtained from constant temperature incubation. Outlying values are indicated for two early (=E) and two late (=L) natural nests discussed in text.

The time of nesting also had major effects on incubation temperatures, on incubation times, and on resultant sex ratios. Incubation temperatures for an early *vs.* late nest are shown in Figure 5. In the early nest, temperatures were low initially, ranging from 26.4–27.2 to 28.1–28.9°C through day 10. At day 30, maximum and minimum were 29.3 and 28.8°C, respectively; by day 45, these were 30.0 and 29.3°C, respectively (Fig. 5). Incubation time was 94 days; 100% females were produced at this site.

In the late nest, temperatures were high initially, ranging from 31.9–32.9°C through day 10, and subsequently remained between 31.9 and 33°C through day 38. Rains on days 39, 56, and 62 resulted in rapidly falling soil temperatures to lows of 30.7–31.2°C on three occasions prior to hatching. At day 30, maximum and minimum were 33.0 and 32.7°C, respectively; by day 45, these were 32.0 and 30.9°C, respectively (Fig. 5). Incubation time was 65 days; the sex ratio in this nest was 0.17 (2 males : 10 females).

Incubation times for natural nests ranged from 62–82 days; mean incubation time was 71.2 days (±1.24 SE, n = 20). Mean incubation time for transplant nests was 77.8 days (±1.79 SE, n = 20); values ranged from 62–94 days. The distributions differed significantly (Wilcoxon signed rank, $T = 9.5$, $P < 0.001$). In natural nests, exclusively females were produced at incubation times of 68–82 days. Incubation times for all-male groups ranged from 62–69 days. In transplant nests, all-female groups had incubation times of 74–94 days with an outlier at 62 days; there were no all-male groups.

The relationship between incubation time and sex ratio for the 1986 data is shown in Figure 6; corresponding values for sex ratios and incubation times at constant temperature are also indicated. There is close agreement between laboratory and field results. In particular, long incubation times, >76 days, resulted in 100% female nests at constant incubation temperatures and in both field treatments. In the enclosures, incubation times for 100% males ranged over eight days; the median time was 65 days. This result was identical to the median incubation time that produced an all-male result at constant incubation temperature, *i.e.*, 65 days.

Outliers are evident for four natural nests and one transplant nest (Fig. 6). Two natural nests had long incubation times with higher than expected sex ratios (0.78 and 0.5 in 76 days), and two had brief incubation times with lower than expected sex ratios (0.0 in 68 days, and 0.17 in 65 days). The former were early nests in which ini-

tial temperatures were 28–29°C, but then rose rapidly to 32–33°C by mid-incubation. Low nest temperatures early in development resulted in an extended period of incubation. Temperatures in these nests were similar to those in the natural nest shown in Figure 4.

Sex ratios were lower than expected in two late nests (Fig. 6). Temperatures were 32–33°C during the first half of incubation; but, during the last half of incubation, temperatures repeatedly dropped to 30–31°C on days following rains. The initially high nest temperatures resulted in rapid development and a brief incubation period. In these nests, temperature profiles were similar to that shown for the late nest in Figure 5. Finally, in one late-season nest at a transplant site, the initial temperatures were 33.0–33.5°C, but declined to 30–31°C after rains during the middle third of incubation. Incubation time was brief, *i.e.,* 62 days; the sex ratio was 0.0.

## DISCUSSION

### *Survival and development*

Incubation temperature has demonstrable effects on embryo survival, development rate, and incubation time in *Crocodylus palustris.* Development to hatching proceeds normally at constant incubation temperatures of 28.0–33.0°C. This range coincides closely with the constant temperatures that result in high embryonic survival in the other species. These include: *Alligator mississippiensis,* 28–33°C (Ferguson and Joanen, 1983; Deeming and Ferguson, 1989); *Caiman crocodilus,* 28.0–33.0°C (Lang, unpublished data); *Crocodylus johnstoni,* 29.0–33.0°C (Webb *et al.,* 1983; Webb and Smith, 1984; Webb *et al.,* 1987); *Crocodylus niloticus,* 28.0–34.0°C (Hutton, 1987), *Crocodylus porosus,* 29.0–33.0°C (Webb *et al.,* 1987; Webb, 1989), and *Crocodylus siamensis,* 28–33°C (Lang, 1987*b*).

In *Crocodylus palustris,* the survival of embryos is not affected by incubation temperatures between 28.0 and 33.0°C, based on same-clutch comparisons of eggs incubated at mid-range *vs.* extreme temperatures. In contrast, incubation at constant temperatures of 33.5 and 34.0°C produces abnormal embryos that die at early stages. In other species, high temperatures (above specified ranges) result in the death and/or in the deformation of embryos/hatchlings. Low temperatures (below specified ranges) result in reduced viability of embryos/hatchlings and/or in eggs that fail to hatch (references cited above). In field nests of *C. palustris,* transient temperatures of 26–27°C early in development and of 34–36°C late in development did not have detrimental effects on developing embryos.

Embryonic development in *Crocodylus palustris,* as in most other species, is accelerated as incubation temperature increases within the viable range. Differences in stage as a function of incubation temperature are most apparent early in development, *i.e.,* stages 1–20. In general, incubation temperature affects the rate of differentiation and growth in *C. palustris* in a manner similar to the temperature effects on embryonic development reported for *C. johnstoni* and *C. porosus* (Webb *et al.,* 1987).

Temperature clearly is the major determinant of total incubation time in *Crocodylus palustris.* Incubation time decreases in a highly predictable manner at increasing constant temperatures. In the experiments reported here, incubation temperature explained 97% of the variation in incubation time. Incubation time is also temperature-dependent in *A. mississippiensis* (Ferguson, 1985), *Caiman crocodilus* (Lang, unpublished data), *Crocodylus niloticus* (Hutton, 1987), *C. johnstoni* and *C. porosus* (Webb *et al.,* 1987), and *C. siamensis* (Lang, 1987*b*).

Approximate developmental rate coefficients for *Crocodylus palustris* range from 0.89 at 29°C to 1.26 at 33°C. Comparable values reported for *C. johnstoni* are 0.84 and 1.30 respectively, and for *C. porosus* are 0.86 and 1.22 respectively (using Method B; Webb *et al.,* 1987). At 29°C, mean incubation time for *C. palustris* is 89.3 days, approximately 17 days faster than in either *C. johnstoni* or *C. porosus.* At 33°C, mean total incubation time for *C. palustris* is 63.4 days, approximately 4 days faster than in *C. johnstoni* and 12 days faster than in *C. porosus* (Webb *et al.,* 1987).

Overall, development in *Crocodylus palustris* is rapid relative to other crocodiles and to *Caiman crocodilus,* but slow relative to *Alligator mississippiensis.* The stage *vs.* age relationship at 30°C during days 1–30 (stages 1–20) is identical to that reported for *A. mississippiensis* and *Crocodylus johnstoni* (Ferguson, 1987). Development through later stages is retarded relative to *Alligator,* but advanced relative to *C. johnstoni.* At 30°C, total incubation time is slower than in *Alligator* by approximately 6 days (Joanen *et al.,* 1987), but faster than in *Caiman crocodilus* by 4 days (Lang, unpublished data), *Crocodylus johnstoni* by 9 days, *C. porosus* by 12 days (Webb *et al.,* 1987), and *C. niloticus* by 12 days (Hutton, 1987).

Development in *Crocodylus palustris* is presumably accelerated relative to other *Crocodylus* species late in development, at about stages 25–28. In this regard, it is interesting to note that the young of *C. palustris* and of *Alligator* typically hatch without a full set of erupted teeth, unlike *C. johnstoni* and *C. porosus* (Ferguson, 1987). At hatching, the external genitalia of *C. palustris* are also less well-differentiated relative to those of *C. johnstoni, C. porosus,* and *C. niloticus* (Webb *et al.,* 1984; Hutton, 1987).

*Sex determination*

Sex in *C. palustris* is determined by incubation temperature. In the laboratory, freshly-laid eggs incubated at constant temperatures of 28.0–33.0°C yielded 100% females at 31°C and below. Incubation at 31.5°C and above produced varying proportions of males and females at all but one temperature. At 32.5°C, 100% males resulted. The observed differences in primary sex ratios are not a consequence of differential mortality because embryo survival in these experiments was uniformly high (>90%) and did not differ appreciably among treatments. In the field, sex is determined by nest temperature during incubation, as evidenced by: (1) a large proportion of either all-female or all-male hatchlings from natural nests and overall yearly sex ratios that varied widely from 0.05 to 0.58 over six years, and (2) a field experiment in which the sex ratios of split-clutch egg groups varied as a function of nest location and incubation temperature. This comparison demonstrates conclusively that females are produced in cool nests whereas males are produced only in warm nests. This result confirms the general relationship between incubation temperature and hatchling sex which was based on constant conditions in the laboratory and extends it to eggs incubated in natural nests. Nesting in field enclosures corresponds closely to nesting by wild populations at various localities in south India (Appendix 1.1), so this relationship probably is valid generally for nests in nature.

In the crocodilian species studied to date, incubation temperature had direct and major effects on development rate which, in turn, determines incubation time. Webb *et al.* (1987) concluded that, for *Crocodylus johnstoni* and *C. porosus,* "development rate and total incubation time were as good, if not better, predictors of sex ratio than was temperature itself." The developmental rates associated with "maleness" are similar in three species of *Crocodylus.* In *C. palustris,* the highest proportion of males was produced at an approximate developmental rate coefficient of 1.22 (79.8 days at 30°C/65.4 days at 32.5°C). In *C. johnstoni* and *C. porosus,* developmental rates associated with maximum male production were 1.22 and 1.26, respectively (method B; Webb *et al.,* 1987). In *C. palustris,* maximum male production occurs at a mean incubation time which is 82% of the value at 30°C. Comparable values for *C. johnstoni* and *C. porosus* are 84% (74.0 days/89.0 days) and 87% (80.1 days/91.8 days).

In enclosure nests of *Crocodylus palustris,* the mean incubation time for all-male nests was 65 days or 81% of the mean incubation time at 30°C. This incubation time at constant temperature corresponds to 32.5°C, the temperature which produces 100% males (Figs. 2, 3). In *C. johnstoni,* field nests that yielded 100% males incubated in 72–74 days or 82% of the mean incubation time at 30°C (Webb and Smith, 1984). This incubation time at constant temperature

corresponds to 31.5°C, the temperature which produces the maximum number of males (method B; Webb *et al.*, 1987).

Because incubation temperature, developmental rate, and incubation time are functionally related, sex ratio varies predictably with incubation time. In *Crocodylus palustris*, lengthy incubation produces 100% females; on the other hand, incubation times that result in varying numbers of males are brief. Males were produced at incubation times of 60–71 days at constant temperatures (31.5–33.0°C). Corresponding incubation times in the field were 63–77 days in natural nests in 1978–1983, 62–72 days in 1986 natural nests, and 63–76 days in 1986 transplant nests. Sex ratios of 1.0 (100% males) were limited to incubation times of 64–66 days at constant temperature, and 63–70 days and 62–69 days in natural nests in 1978–1983 and 1986, respectively (Table 2). These data are remarkably coincident given that lab *vs.* field conditions differ, most notably with regard to constant *vs.* fluctuating thermal regimes. Thus, total incubation time is a useful predictor of sex ratio in *Crocodylus palustris* in the field as well as in the lab.

*TSD in crocodiles*

On the bases of incubation studies at constant temperature, temperature-dependent sex determination (TSD) has been demonstrated in five species of *Crocodylus* (subfamily Crocodilinae). These include *C. johnstoni* (Webb *et al.*, 1987), *C. niloticus* (Hutton, 1987), *C. palustris* (this study), *C. porosus* (Webb *et al.*, 1987), and *C. siamensis* (Lang, 1987*b*). The relationships between sex ratio and incubation temperature for these species are summarized in Figure 7. Major trends are: (1) only females are produced at low temperatures, <30–31°C; (2) varying proportions of females are produced at mid-range or high temperatures, >31–34°C; and (3) males are produced within a species-specific, restricted band of mid-range to high temperature, >31–34°C.

A notable feature of the TSD pattern seen in *Crocodylus palustris* is the clearly defined and narrow range of constant temperature that results in 100% males. A 0.5°C shift from 32.5°C yields 59% males at 32°C and 31% males at 33°C. In *Crocodylus johnstoni, C. porosus,* and *C. palustris,* the peak percentages of males occur at 31.5, 32.0, and 32.5°C, respectively. In *C. johnstoni,* males are produced only at 31–31.5°C and only in low proportions. In contrast, males are produced over a wider temperature range, at higher temperatures, and in high proportions in *C. porosus* and *C. palustris.*

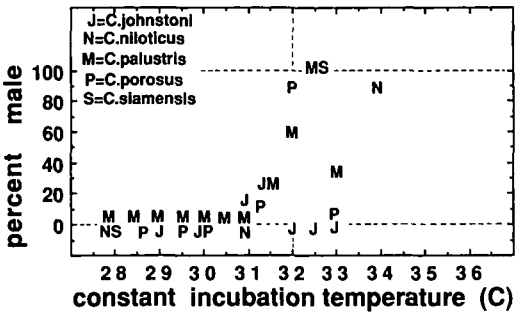


FIG. 7. Patterns of temperature-dependent sex determination (TSD) for five species of *Crocodylus,* subfamily Crocodilinae. Sex ratio (=percent male) varies with incubation at constant temperature (°C). Females are produced at low and high temperatures whereas males in varying proportions result at mid-range temperatures. References cited in text.

In all three species, females are produced at both low and high temperatures. In the other two species, high proportions of males occur at 32.5–33.0°C for *Crocodylus siamensis* and at 34.0°C for *C. niloticus,* but the data on these species are inadequate for further comparisons. For instance, it is not known whether higher temperatures, >33°C, would yield females in *C. siamensis.* Or if lower temperatures of 32–33°C might produce 100% males in *C. niloticus.* Hutton (1987) reports that for a single clutch of *C. niloticus,* 91% males (10:1) were produced at 32.5°C *vs.* 0% (0:11) at 31°C and 82% (9:2) at 34°C.

Overall, the pattern of TSD for *Crocodylus palustris* is similar to that for *C. porosus.* "Maleness" in these species does not require fluctuating and/or increasing temperatures as it apparently does in *C. johnstoni* (Webb *et al.*, 1987). However, increasing temperatures may augment "maleness"

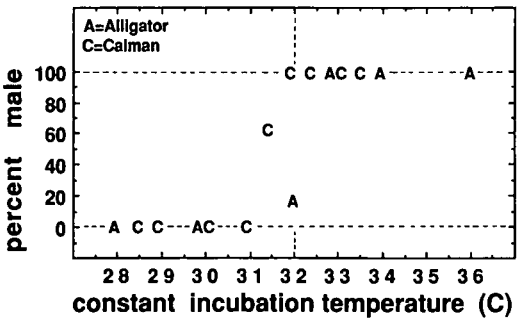


FIG. 8. Patterns of temperature-dependent sex determination (TSD) for *Alligator mississippiensis* and *Caiman crocodilus*, subfamily Alligatorinae. Sex ratio (=percent male) varies with incubation at constant temperature (°C). Females are produced at low temperatures and males are produced at high temperatures. References cited in text.

in *C. palustris* (Figure 4; Lang, unpublished data). In addition, female *C. palustris* that are produced at 33°C develop rapidly with short incubation times. Consequently, these resemble "high temperature females" in *C. johnstoni*, but may differ from female *C. porosus* in which at least some individuals incubated at 33°C had their early development retarded (Webb *et al.*, 1987).

Caution is required in making strict comparisons among species because nesting habits differ. The constant temperature utilized for laboratory studies may approximate field incubation conditions for certain species, *i.e.*, mound builders, but probably serves as a poor model for other species. The similarity of TSD patterns for *Crocodylus palustris* and *C. porosus* is especially noteworthy because these species differ substantially in nesting habits. On the other hand, the nesting ecologies of *C. palustris* and *C. johnstoni* are very similar, but their TSD patterns differ. These species and *C. niloticus* are hole nesters, whereas *C. porosus* and *C. siamensis* build mound nests. Thus, mode of nesting alone does not appear to explain the anomalous pattern exhibited by *C. johnstoni*.

### *Crocodiles* vs. *alligator / caiman*

The general pattern of TSD in *Crocodylus* species differs notably from the pattern exhibited by *Alligator mississippiensis* and *Caiman crocodilus* (Ferguson and Joanen, 1983; Lang, unpublished data). In these species, low incubation temperatures produce females; and high temperatures yield males (Fig. 8). Preliminary data on TSD in *Paleosuchus trigonatus* (Yamakoshi *et al.*, 1987) suggest a similar single-transition pattern in this additional member of the putative subfamily Alligatorinae. In *Alligator*, the transition from low-temperature females to males is about 32°C (13% males; Ferguson and Joanen, 1983), in *Caiman crocodilus* at 31.5°C (62% males; Lang, unpublished data), and in *Paleosuchus trigonatus* between 31 and 32°C (Magnusson, personal communication). In the *Crocodylus* species for which data are sufficient, the transition from low temperature females to males also occurs at 31–32°C. In summary, in *Crocodylus* species, high temperature females are produced. There are two transitions, from female to male and from male to high temperature female. In contrast, in *Alligator* and allies, there is a single transition; and no females are produced at high temperatures.

In *Crocodylus palustris*, preliminary data from switch-once experiments indicate that "femaleness" is irreversibly set by about stage 20. This stage corresponds to 30 days at 30°C incubation, or about 38% of total incubation time at 30°C. In contrast, "maleness" is irreversibly set later in development, by about stage 25. This corresponds to 45 days of incubation at 32°C or 67% of total incubation time at 32°C. These data indicate: (1) that the temperature sensitive period extends for up to two thirds of the developmental period in *C. palustris*, and (2) that "maleness" is committed later in development relative to "femaleness." Similar results have been reported for *C. porosus* and *C. johnstoni* (Webb *et al.*, 1987) and for *Alligator mississippiensis* (Deeming and Ferguson, 1989), but it should be noted that the temperature sensitive period appears to increase as incubation temperature increases (Webb *et al.*, 1987).

### *Sex ratios and nesting*

In this study, the overall sex ratio in field enclosures was 0.24, approximately 1 male : 3 females. Sex ratios varied from year to

year, and were clearly dependent on annual differences in seasonal weather patterns (Table 1; Lang, upublished data). Within a given season, sex ratio varied with the time of nesting as well as nest location. Unfortunately, no comparative data are available from wild populations of *Crocodylus palustris.* However, the similarities in the nesting ecology of the captive population at MCB and that of populations elsewhere in south India strongly suggest that the captive situation corresponds closely with nesting in nature (Appendix 1.1).

Female-biased sex ratios at hatching have been reported in wild populations of *Alligator mississippiensis* (mean sex ratio = 0.17; Ferguson and Joanen, 1983), *Crocodylus johnstoni* (mean sex ratio = 0.35; Webb and Smith, 1984), and *C. niloticus* (Hutton, 1987). In *C. johnstoni,* sex ratio varied with time of nesting and with geographic area; sex ratios tended to be high in nests that hatched early and to be low in late nests at specific localities (Webb and Smith, 1984). In *A. mississippiensis,* sex ratios were high in warm levee nests and low in cool marsh nests (Ferguson and Joanen, 1983).

For *Crocodylus palustris,* sex ratios in enclosure nests are a consequence of when and where the eggs are laid. Nesting occurs at the beginning of a warm, dry season characterized by increasing ambient temperatures and no precipitation. Nesting is initiated when soil temperatures approach 27–28°C, and is terminated before soil temperatures reach near-lethal limits for embryonic survival, and prior to the onset of intermittent rains. Soil temperatures increase gradually during the nesting period to 33–34°C (Figs. 1, 4, 5). Infrequent rains in late May and June result in rapid drops and marked fluctuations in nest temperatures. As a consequence, incubation time increases. Thus, nesting is timed to coincide with the period during which soil temperatures are high enough to sustain normal development and before soil temperatures rise to near-lethal limits and/or begin to fluctuate or drop with the onset of intermittent precipitation. In this region, water levels do not begin to rise until October, so flooding is not a significant source of egg mortality.

Within the window of thermal conditions conducive for successful incubation, there is a strong positive correlation between time of nesting and initial nest temperature. As initial nest temperature increases, total incubation time decreases, *i.e.,* development is accelerated. Sex ratio varies as a function of total incubation time as indicated in Figure 6. Thus, early and late nests have relatively long and short incubation times, respectively, and produce all females or a high proportion of females. Nests with intermediate incubation times result in varying proportions of males.

Nests situated in sunny locations have higher initial nest temperatures and shorter incubation times compared to nests located in the shade. In effect, nesting in sunny, exposed sites early in the season counteracts otherwise seasonally cool soil temperatures. Cool temperatures prolong incubation and, if too low, may compromise embryonic survival. Later in the season, ambient (shaded) soil temperatures have risen to 31–33°C; and soil temperatures in sunny locations approach the lethal upper limits for embryonic survival. Consequently, nesting in shaded, protected sites late in the season increases survivorship by reducing the risk that the eggs may overheat.

Nests that were laid early in the season were located in warm, sunny sites. In contrast, late season nests were located in the shade; and exposed sites were not used. These observations suggest that: (1) females select nest sites with particular thermal characteristics, and (2) that this behavior changes seasonally. If so, then such behavior may explain an earlier observation made regarding widely-spaced nest sites for "A" *vs.* "B" clutches (Whitaker and Whitaker, 1984). "A" clutches were typically located on an exposed, sunny bank on one side of the enclosure whereas "B" clutches were usually laid on an opposite bank that was heavily shaded.

The specific cues utilized by a nesting female in selecting a nest site are not well understood for any species of crocodilian, but likely include features directly related to the thermal properties of the nest (Lang,

1987*a*). In the wild and in field enclosures, female *Crocodylus palustris* typically excavate shallow depressions and construct trial nests in potential nesting areas. This activity starts weeks before egg deposition, but intensifies in the several days prior to egg laying. In field enclosures, we have observed that soil temperatures in trial nests are usually lower or higher than in the nests in which eggs are actually laid. Preliminary analyses indicate that there is a close match between the body temperature of a nesting female, the clutch temperature as the eggs are laid, and the soil temperature in the bottom of the nest. In several other species that construct soil nests, females dig "test" holes prior to egg laying (*e.g., C. johnstoni*: Webb, 1982; Smith, 1987; *C. niloticus*: Blake and Loveridge, 1987; *Gavialis gangeticus*: Bustard, 1980).

*Consequences of TSD*

On the basis of one model of TSD (Charnov and Bull, 1977; Bull, 1983, 1987), a species in which sex is environmentally determined is expected to exhibit other environmentally-induced effects that correlate with sex-specific fitness. In fact, incubation temperature has significant "nonsexual" effects in crocodilians (Webb, 1989) which may, in turn, have different fitness consequences for males *vs.* females. These include hatchling body size and weight (Hutton, 1987; Webb *et al.*, 1987), hatchling pigmentation patterns (Deeming and Ferguson, 1989; Lang, unpublished data), post-hatching growth rates (Hutton, 1987; Joanen *et al.*, 1987; Webb 1989), and post hatching thermal behavior (Lang, 1987*b*). Recent studies have indicated that male-producing incubation temperatures enhance post-hatching growth, and this effect of incubation temperature may constitute a selective advantage of TSD in crocodilians (Bull, 1987; Webb *et al.*, 1987; Webb, 1989). Studies at the Madras Crocodile Bank are currently underway to test this hypothesis by examining specific post-hatching attributes, *e.g.*, thermal selection and growth, of juvenile *C. palustris* as a function of incubation temperature.

The scenario outlined above emphasizes the important and far-reaching consequences of the nest environment on the biology of crocodilians. The potential for controlling sex and/or enhancing growth via incubation regimes has obvious significance for captive-breeding and rearing programs. In particular, the transplant experiment that we conducted with the eggs of *Crocodylus palustris* demonstrates the feasibility of successfully manipulating incubation conditions in the field. It is especially noteworthy that sex ratios were dramatically altered by relocating nests, but that embryonic survival was not compromised (Table 1). This experiment required only simple instruments for monitoring nest temperatures, information on handling eggs and sexing young, and a basic understanding of the nesting biology of the species.

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## APPENDIX

*Methods*

*Nesting ecology.*—Reproduction is seasonal, and coincides with warm, dry weather and with periods of low water. In south India, nesting occurs in the wild from February through April. Wild nests are usually located near permanent water (distance to water = 10 m) on the banks of ponds, tanks, and reservoirs or along the shorelines of rivers and streams. Wild nests are typically constructed in sandy soil (42 of 59 wild nests; 71%) at open, exposed sites (=sunny; 51 of 59 wild nests; 86%). Trial nests are a common feature of nesting at these localities. The females lay eggs (20–40 per clutch) in an L-shaped hole (25–35 cm in depth) dug by the female just prior to laying. Temperatures recorded in wild nests in south India early in incubation ranged from 29–33°C. In some of these nests, the presence of abnormal embryos and/or hatchlings suggests that incubation temperatures may at times be too high and/or too low. However, there is little evidence that desiccation and/or flooding are major sources of egg mortality in wild nests.

Details of nesting, particularly with regard to nest location and construction, in the breeding enclosures at MCB correspond closely with comparable data for wild nests elsewhere in south India (Whitaker and Whitaker, 1984). At MCB, nesting occurs during the warm, dry season (February through April; Figure 1). Most mature females lay two clutches (20–40 eggs per clutch) about 40 days apart during each breeding season. The regular production of two clutches per season is a phenomenon unique to this species at MCB, and has not yet been documented elsewhere. Our observations at MCB indicate that a second period of courtship and mating follows soon after the first clutch is laid, and that a second clutch, if conditions are favorable, is laid about 4–6 weeks later. Reliable records at Amaravathi Reservoir and at Chidambaram Waterworks in Tamil Nadu (for 1978–1983) indicate that nesting extends over three months (February through April). Intervals between nesting dates often exceed 4–5 wk. At these localities, some females may produce two clutches within a single season, but conclusive data on this point are lacking. Incubation times, which are indicative of ambient soil temperatures at nesting sites, average 67 days for wild nests *vs.* 68 days at MCB and correspond to mean incubation temperatures of 31.8–32.0°C.

*Breeding enclosures.*—Three breeding groups were comprised of 1–3 males and 6–40 mature females per group. Each group was housed in an enclosure (1,500 $m^2$) that contained an aquatic pool and surrounding terrain suitable for successful nesting (Whitaker, 1984; Whitaker and Whitaker, 1984; Lang *et al.,* 1986). Females producing eggs were middle-aged (approx. 12 yr old, >5 yr nesting), young (approx. 8 yr old, >2–5 yr nesting), and first/second season nesters (approx. 5–7 yrs old, <2 yr nesting). The younger females typically produced a high proportion of infertile eggs and/or clutches. Two clutches in one season ("A" and "B" nests) were produced primarily by older individuals.

*Incubator design / operation.*—Incubators were housed in an insulated room that was air-conditioned at 26 ± 1°C. A diesel generator provided auxiliary power. Local techniques were employed to stabilize daily thermal fluctuations in the building and included shading and insulating with thatch and straw.

Incubators were constructed of locally available, indigenous components. The basic unit consisted of a box of molded plastic foam insulation (3 cm thick, 50 × 30 × 40 cm high). An aquarium heater (90 watt) was cemented to a glass plate by positioning it directly above a length of flexible tubing connected to an aquarium air pump. The heater-hose unit was submerged in a shallow tray of water (6 cm depth) fitted into the bottom of the foam box. Operation of the heater/air pump was controlled by a contact thermoregulator (JUMO) and relay circuit. The mercury bulb of the thermoregulator was positioned in the bottom tray where it regulated water temperature to 0.1°C. This assembly provided a low-input source of

TABLE A. *Sex ratios of* Crocodylus palustris *eggs, grouped by clutch and incubated at constant temperatures of 28–33°C.**

| Temp | Clutch | Age | fm | n | M | F | SR |
|---|---|---|---|---|---|---|---|
| 28 | 850808 | 3B | met | 3 | 0 | 3 | 0.00 |
| | 872002 | 1A | 248 | 8 | 0 | 8 | 0.00 |
| | 872004 | 1A | 094 | 6 | 0 | 6 | 0.00 |
| | 872005 | 1A | 562 | 4 | 0 | 4 | 0.00 |
| | 872008 | 1A | 285 | 4 | 0 | 4 | 0.00 |
| | 872009 | 1A | 143 | 2 | 0 | 2 | 0.00 |
| | | | | 27 | 0 | 27 | 0.00 |
| 28.5 | 850805 | 3A | nov | 10 | 0 | 10 | 0.00 |
| | 850806 | 3A | vij | 10 | 0 | 10 | 0.00 |
| | 850807 | 3A | blk | 14 | 0 | 14 | 0.00 |
| | 851003 | 1A | 120 | 1 | 0 | 1 | — |
| | | | | 35 | 0 | 35 | 0.00 |
| 29.0 | 850804 | 3A | stp | 15 | 0 | 15 | 0.00 |
| | 850808 | 3B | met | 3 | 0 | 3 | 0.00 |
| | 851006 | 2A | 734 | 9 | 0 | 9 | 0.00 |
| | 851007 | 1A | 177 | 3 | 0 | 3 | 0.00 |
| | 851009 | 1A | 133 | 1 | 0 | 1 | — |
| | 872012 | 1A | 020 | 1 | 0 | 1 | — |
| | | | | 32 | 0 | 32 | 0.00 |
| 29.5 | 850807 | 3A | blk | 13 | 0 | 13 | 0.00 |
| | 870804 | 3A | vij | 1 | 0 | 1 | — |
| | 871002 | 2A | 987 | 4 | 0 | 4 | 0.00 |
| | 872008 | 1A | 285 | 4 | 0 | 4 | 0.00 |
| | | | | 22 | 0 | 22 | 0.00 |
| 30.0 | 850806 | 3A | vij | 17 | 0 | 17 | 0.00 |
| | 870801 | 2A | 981 | 8 | 0 | 8 | 0.00 |
| | 870802 | 2A | mis | 6 | 0 | 6 | 0.00 |
| | 870803 | 3A | stp | 6 | 0 | 6 | 0.00 |
| | 871004 | 2A | 073 | 3 | 0 | 3 | 0.00 |
| | 871007 | 2A | 091 | 3 | 0 | 3 | 0.00 |
| | 871008 | 2A | 237 | 1 | 0 | 1 | — |
| | 872014 | 1A | 326 | 2 | 0 | 2 | 0.00 |
| | | | | 46 | 0 | 46 | 0.00 |
| 30.5 | 850801 | 3A | met | 9 | 0 | 9 | 0.00 |
| | 872011 | 1A | ? | 5 | 0 | 5 | 0.00 |
| | 872013 | 1A | 011 | 3 | 0 | 3 | 0.00 |
| | | | | 17 | 0 | 17 | 0.00 |
| 31.0 | 850802 | 3A | mis | 9 | 0 | 9 | 0.00 |
| | 850804 | 3A | stp | 17 | 0 | 17 | 0.00 |
| | 851003 | 2A | 120 | 6 | 0 | 6 | 0.00 |
| | 851006 | 2A | 734 | 14 | 0 | 14 | 0.00 |
| | 851007 | 1A | 177 | 3 | 0 | 3 | 0.00 |
| | 851009 | 1A | 133 | 2 | 0 | 2 | 0.00 |
| | | | | 51 | 0 | 51 | 0.00 |
| 31.5 | 871016 | 2B | 984 | 9 | 2 | 7 | 0.22 |
| 32.0 | 850805 | 3A | nov | 10 | 7 | 3 | 0.70 |
| | 850809 | 3B | mis | 8 | 2 | 6 | 0.25 |
| | 850810 | 3B | blk | 13 | 9 | 4 | 0.69 |
| | 870805 | 3A | blk | 1 | 1 | 0 | — |
| | | | | 32 | 19 | 13 | 0.59 |
| 32.5 | 871012 | 2A | 624 | 8 | 8 | 0 | 1.00 |
| 33.0 | 850811 | 3B | nov | 14 | 3 | 11 | 0.21 |
| | 871002 | 2A | 978 | 4 | 3 | 1 | 0.75 |
| | 872010 | 1A | 259 | 11 | 3 | 8 | 0.27 |
| | | | | 29 | 9 | 20 | 0.31 |

* TEMP = incubation temperature (°C); CLUTCH = year, enclosure, nest; AGE: 1 = first/second season nester, 2 = young, 3 = middle-aged; A = first clutch/season. B = second clutch/season; FM = female identity; n = eggs in sample; M = male; F = female; SR = sex ratio (=percent male). Data are shown in Figure 3.

heat as well as a method for gently circulating water in the tray and air within the incubator.

Incubator temperature was set initially and thereafter monitored 2–6× daily using 1–2 thermistor probes which were sealed inside water-filled eggs. The "dummy egg" probe was placed in the center of each treatment tray, and the thermoregulator was adjusted to maintain egg temperature at the desired setting. Experimental eggs were placed in open plastic trays (38 × 27 × 6 cm high) surrounded by air. An incubator accommodated 2 trays (25 eggs/tray) stacked on top of 2 empty trays positioned above the bottom tray of heated water. Humidity within the incubator was high and stable (>99% relative humidity); fresh air was introduced into the incubator whenever the heater unit operated, normally for 2–5 min once every 1–3 hr. Eggs incubated in this manner did not show any evidence of desiccation, *e.g.*, weight loss was negligible and air spaces were not evident (as described by Whitehead, 1987).

Temperature gradients within a tray measured from a central point were 0.00 to 0.10°C, and between trays (top and below) were 0.00 to 0.20°C. Temperature

TABLE B. *Total incubation time for* Crocodylus palustris *eggs incubated at constant temperature.**

| | Incubation time (days) | | | | | |
|---|---|---|---|---|---|---|
| Temp | n | $\bar{x}$ | Median | Range | SD | SE |
| 28.0 | 22 | 99.8 | 99 | 98–102 | 1.28 | 0.27 |
| 28.5 | 35 | 93.1 | 92 | 90–97 | 2.28 | 0.39 |
| 29.0 | 32 | 89.3 | 89 | 84–93 | 2.24 | 0.40 |
| 29.5 | 22 | 83.6 | 83 | 79–90 | 3.08 | 0.66 |
| 30.0 | 42 | 79.8 | 80 | 75–82 | 1.58 | 0.24 |
| 30.5 | 17 | 76.3 | 77 | 74–78 | 1.21 | 0.29 |
| 31.0 | 51 | 72.8 | 73 | 71–74 | 0.97 | 0.14 |
| 31.5 | 9 | 70.6 | 71 | 70–71 | 0.53 | 0.18 |
| 32.0 | 32 | 66.9 | 67 | 63–69 | 1.72 | 0.30 |
| 32.5 | 8 | 65.4 | 65 | 64–66 | 0.73 | 0.24 |
| 33.0 | 27 | 63.4 | 63 | 60–69 | 3.07 | 0.59 |

* TEMP = incubation temperature (°C); n = sample size; $\bar{x}$ = mean; median; range; SD = standard deviation; SE = standard error; incubation time = number of days from laying to pipping. Data are shown in Figure 2.

in individual incubators was continuously recorded for 12–24 hr at 1 wk intervals. Tracings indicated no detectable temperature cycling (<0.10°C). In each incubator, eggs were shifted among and within trays to minimize gradient effects. Incubator temperature was adjusted downward periodically to compensate for metabolic heat production by embryos during the last half of incubation.

The incubation method employed in this study was similar to Method B, as described by Webb *et al.* (1987). An additional control on the method of incubation was provided by incubating eggs from a single clutch of *Crocodylus porosus* at 30°C and sampling eggs at specific times during development. This procedure provided a direct comparison of the data on age *vs.* stage at the standard temperature used in this study (30°C) with the results on *C. porosus* published by another laboratory (Webb *et al.*, 1987). Agreement was within ½ stage.

*Embryonic development.*—For a standard reference series, 2 clutches of eggs were incubated at 30°C and sampled at alternate days for the first 15 days of incubation, and at alternating 3–7 day intervals thereafter. To assess temperature effects on development, a third clutch was divided into treatments at 28, 31, 32, and 33°C, and sampled at 12, 24, 36, 48, and 60 days. The specific effects of constant incubation temperatures on embryonic development in *C. palustris* will be reported elsewhere, utilizing morphometric data to determine developmental rate coefficients as described by Webb *et al.* (1987).

*Sex determination.*—Gonads had been clearly differentiated into testes or ovaries in late stage embryos (stages 27–28), as well as in hatchlings and older animals. Sexing based on cliteropenis size (length, width) was definitive at 6 mo. At this age, errors were less than 2%; at 1 yr, error was negligible. Size criteria were determined by measuring the genitalia of growing hatchlings (1985 cohort) every 1–2 mo for 2 yr. Sex assignment based on cliteropenis size at 6 mo was confirmed by: (1) examining the gonads of a representative sample of each sex at 2 yr of age, and (2) examining cliteropenis size at 2 yr for the remainder.

For the 1985 and 1987 cohorts incubated at constant temperature, sexing was based on gonadal morphology for 33% of the animals (dead) and on sex organ size for those living at 2 yr and at 6 mo, respectively. The small samples incubated at 31.5°C (n = 9) and at 32.5°C (n = 8) were sexed by gonadal inspection. For all other animals, sexing was based on examination of genitalia at 1 yr or older and on gonadal morphology when possible.