

# Incubation Temperature Affects Body Size and Energy Reserves of Hatchling American Alligators (*Alligator mississippiensis*)

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## Abstract

*Incubation temperature determines sex in crocodylians and influences other features of hatchlings. We investigated the effects of incubation temperature, clutch, and sex on the dimensions, mass, and energy reserves of hatchling Alligator mississippiensis. Eggs from three clutches were incubated to pipping at constant temperatures of 29°, 31°, 32°, and 33° C; females were produced at 29° and 31° C, mixed sex ratios at 32° C, and males at 33° C. Incubation temperature affects hatchling size and yolk utilization in a consistent, but complex, pattern. Maximum yolk-free hatchling mass occurred at 32° C, and minimum mass at 31° C. Similarly, dimensions were maximized at 32° C and minimized at 29° C. Residual yolk mass was inversely related to yolk-free hatchling mass. Higher yolk mass occurred at 31° and 33° C; at 29° and 32° C, values were lower. However, fat mass remained constant in all clutches. Strong clutch effects were present in nearly all traits. At 32° C, males were heavier and had less yolk than females. These differential effects of incubation temperature, and consequently sex, affect important hatchling characteristics and may influence hatchling survival and fitness, in accord with recent theory.*

## Introduction

Incubation temperature determines sex in all crocodylians examined to date (Ferguson and Joanen 1983; Hutton 1987; Lang 1987; Webb et al. 1987; Lang, Andrews, and Whitaker 1989; Lang and Andrews 1994). In alligators,

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constant incubation temperatures ( $T$ )  $\leq 31.5^{\circ}\text{C}$  produce only females, only males are produced at  $32.5^{\circ}\text{C} \leq T \leq 33.0^{\circ}\text{C}$ , and  $T \geq 35.0^{\circ}\text{C}$  produce only females. Transitional temperatures at  $31.5^{\circ}\text{C} < T < 32.5^{\circ}\text{C}$  and  $33.0^{\circ}\text{C} < T < 35.0^{\circ}\text{C}$  result in mixed sex ratios (Lang and Andrews 1994). Incubation temperature also affects hatchling size, body mass, and yolk mass (Ferguson and Joanen 1983; Deeming and Ferguson 1989a). Recent studies also suggest that incubation temperature influences the posthatching growth of juvenile crocodilians (Joanen, McNease, and Ferguson 1987; Webb and Cooper-Preston 1989). Survival, and consequently fitness, may differ between males and females if there are nonsexual effects of incubation temperature correlated with sex (Charnov and Bull 1977; Bull and Charnov 1989). Our study focuses on various hatchling attributes that are affected by incubation temperature and may be correlated with sex.

Previous studies may have failed to distinguish similar patterns for two reasons. First, consistent definitions of hatchling mass and yolk mass are lacking. A critical distinction must be made between total hatchling mass, which does not account for differences in yolk mass, and yolk-free hatchling mass, which emphasizes actual body mass. Ferguson and Joanen (1983) used the term "absorbed abdominal yolk" to describe the amount of yolk available to the hatchling. But some hatchlings emerge with a considerably unabsorbed yolk. Schulte (1989) defined two types of yolk, that is, yolk within the abdominal cavity and yolk external to the abdominal cavity. These terms are unnecessary because the yolk is contained within only one compartment, the yolk sac, and all yolk will eventually be internalized and used for growth regardless of its initial location. Webb et al. (1987) defined "residual yolk" as the amount of yolk at pipping; this is in accord with "spare yolk," a term used in avian ecology (Romanoff 1967; Ar et al. 1987). Webb's definition will be used here.

Second, the reported effects of incubation temperature on alligator hatchling mass and yolk mass are contradictory (Ferguson and Joanen 1983; Joanen et al. 1987; Deeming and Ferguson 1989a; Schulte 1989). Small sample sizes and limited ranges of incubation temperatures have complicated these studies. Ecotype variation is an unlikely explanation for the different results found in these studies because most studies were made in southwestern Louisiana. In contrast, the effects of incubation temperature on the Australian freshwater crocodile, *Crocodylus johnstoni*, have been studied over a large range of temperatures (Webb et al. 1987; Whitehead, Webb, and Seymour 1990). However, these studies did not account for clutch effects and did not provide detailed locality data on egg collection. Hatchlings from different localities may show differences not exhibited by hatchlings from a single population.

In this study, we examine the effects of incubation temperature on hatching characteristics to answer the following questions: (1) Does incubation temperature affect the dimensions and mass of hatchling alligators? (2) Does incubation temperature affect hatchling energy reserves? (3) Does clutch affect hatchling features? (4) Do differences exist between males and females incubated at 32°C? We discuss our findings with respect to previous studies and relate them to prevailing theory on the adaptive significance of temperature-dependent sex determination (TSD) in reptiles.

## Material and Methods

### *Experimental Design*

We collected fresh eggs of *Alligator mississippiensis* within several days of laying in June 1991, at Rockefeller Wildlife Refuge in southwestern Louisiana. Fertile eggs ( $n = 126$ ) from three clutches were incubated to pipping at constant temperatures  $\pm 0.1^\circ\text{C}$  of 29° ( $n = 30$ ), 31° ( $n = 29$ ), 32° ( $n = 38$ ), and 33°C ( $n = 29$ ) in foam box incubators (Lang et al. 1989).

Upon pipping, each egg was frozen in an airtight plastic container and later thawed for data collection. We measured 13 dimensions and the total mass of each hatchling. Hatchlings were dissected by cutting the ventral midline from the throat to 2 mm anterior to the cloaca. The yolk sac was cut at the junction with the small intestine, removed, and weighed. We removed loosely associated fat bodies lining the ventral and lateral walls of the abdomen. A discrete fat body attached to the small intestine on the right side of the abdomen was also removed. This "intestinal" fat body was compact, globular, and darker than the smaller whitish fat bodies lining the abdominal wall. The intestinal and abdominal fat bodies were grouped into one category, abdominal fat bodies, because of the small mass and sample sizes. We removed all fat bodies in the tail from the posterior edge of the cloaca to the second single tail crest.

We determined gonadal sex macroscopically by shape, texture, and color of the gonads and by the presence or absence of oviducts (Forbes 1940a, 1940b; Ferguson and Joanen 1983; Hutton 1987). Representative samples of gonads were sectioned and stained for histological examination; these procedures and the criteria for diagnosing sex are outlined in Lance and Bogart (1992). In all cases, the gonadal sex determination was confirmed by the histological diagnosis. Hatchlings were preserved in 10% formalin and reexamined later to confirm sex. Constant incubation at 29° and 31°C produced 100% females and at 33°C produced 100% males. Mixed sex ratios (males:females) resulted from constant incubation at 32°C (total = 24:14,

clutch 1 = 10:3, clutch 2 = 8:4, clutch 3 = 6:7). Mean incubation time (days) varied: 84 d at 29°C, 71 d at 31°C, 66 d at 32°C, and 63 d at 33°C (Lang and Andrews 1994).

### *Measurements*

We measured initial egg, total hatchling, yolk-free hatchling, residual yolk, total fat, abdominal fat, and tail fat wet masses to  $\pm 0.01$  g using an Ohaus E300D analytic balance. We assumed that differences in yolk wet mass reflect proportional differences in energy content. Two hatchling dimensions were measured ventrally with a metric ruler to  $\pm 1$  mm: snout-vent length (measured to the posterior edge of the cloaca), and total length (measured to the tip of the tail). We measured 11 dimensions dorsally with calipers to  $\pm 0.1$  mm according to Deeming and Ferguson (1990): trunk length, inter-limb length, front-limb length, hind-limb length, head length, head width, head height, snout length, midsnout width, snout width at nares, and eye length.

### *Standardizing to Initial Egg Mass*

Initial egg mass explained significant variation ( $P < 0.05$ ) in total hatchling, yolk-free hatchling, and residual yolk masses by regression. These variables were standardized to initial egg mass for graphical purposes only; actual statistical tests that accounted for initial egg mass were performed by ANCOVA (see below). Lines were drawn on graphs to illustrate patterns and show significant interactions.

As an example, yolk-free hatchling mass was standardized to initial egg mass by using the regression equation of yolk-free hatchling mass on initial egg mass to calculate a predicted yolk-free hatchling mass for each observed value. Standardized yolk-free hatchling mass was obtained by adding the residual yolk-free hatchling mass (actual yolk-free hatchling mass - predicted yolk-free hatchling mass) to the mean yolk-free hatchling mass. The resulting standardized masses were then plotted for each temperature-by-clutch group. This separated the effect of initial egg mass from the effects of temperature and clutch. The following regression equations were used:

$$\text{Total Hatchling Mass} = 8.51 + 0.57 (\text{initial egg mass})$$

$$(r^2 = 0.66, P < 0.001),$$

$$\text{Yolk-Free Hatchling Mass} = 18.81 + 0.37 (\text{initial egg mass})$$

TABLE 1  
*Sample means and descriptive statistics of dimensions and mass parameters of hatching alligators from three clutches incubated at four constant temperatures*

| Variable                                | $\bar{X}$ | Minimum | Maximum | SD   | Variance | SEM | <i>n</i> |
|-----------------------------------------|-----------|---------|---------|------|----------|-----|----------|
| Egg measurements:                       |           |         |         |      |          |     |          |
| Egg length (mm) . . . . .               | 71.8      | 66.3    | 78.5    | 2.11 | 4.46     | .19 | 126      |
| Egg width (mm) . . . . .                | 42.7      | 40.5    | 44.2    | .67  | .45      | .06 | 126      |
| Initial egg mass (EMS) (g) . . . . .    | 75.85     | 66.88   | 84.20   | 3.24 | 10.47    | .29 | 126      |
| Hatchling mass (g):                     |           |         |         |      |          |     |          |
| Total hatchling mass (TOTHMS) . . . . . | 51.82     | 44.92   | 57.18   | 2.27 | 5.15     | .20 | 126      |
| Yolk-free hatchling mass . . . . .      | 46.49     | 41.44   | 51.30   | 2.01 | 4.03     | .18 | 126      |
| Hatchling dimensions (mm):              |           |         |         |      |          |     |          |
| Snout-vent length . . . . .             | 123       | 112     | 131     | 3.80 | 14.43    | .34 | 126      |
| Total length . . . . .                  | 245       | 224     | 263     | 7.77 | 60.36    | .69 | 126      |
| Trunk length . . . . .                  | 75.7      | 67.3    | 82.3    | 3.27 | 10.69    | .31 | 110      |
| Interlimb length . . . . .              | 57.2      | 51.7    | 69.7    | 2.37 | 5.63     | .23 | 110      |
| Front limb length . . . . .             | 46.8      | 41.4    | 51.3    | 1.95 | 3.79     | .19 | 110      |

|                                |      |      |       |       |        |      |     |
|--------------------------------|------|------|-------|-------|--------|------|-----|
| Hind limb length               | 57.6 | 52.7 | 61.8  | 2.09  | 4.36   | .20  | 110 |
| Head length                    | 35.0 | 32.7 | 36.8  | .81   | .66    | .07  | 126 |
| Head width                     | 20.4 | 19.5 | 21.4  | .41   | .17    | .04  | 110 |
| Head height                    | 16.9 | 15.4 | 18.4  | .56   | .31    | .05  | 110 |
| Snout length                   | 14.6 | 11.6 | 16.9  | 1.00  | .99    | .10  | 110 |
| Midsnout length                | 13.6 | 11.6 | 15.9  | .93   | .87    | .09  | 110 |
| Snout length at nares          | 8.9  | 7.4  | 10.3  | .63   | .40    | .06  | 110 |
| Eye length                     | 11.6 | 10.2 | 12.7  | .51   | .26    | .05  | 110 |
| Hatchling energy reserves (g): |      |      |       |       |        |      |     |
| Residual yolk mass (YMS)       | 5.33 | 2.20 | 8.86  | 1.34  | 1.81   | .120 | 126 |
| Total fat mass (TOTFAT)        | 2.21 | 1.79 | 2.70  | .178  | .032   | .016 | 126 |
| Abdominal fat mass             | .58  | .35  | .78   | .096  | .009   | .009 | 126 |
| Tail fat mass                  | 1.63 | 1.26 | 2.00  | .147  | .022   | .013 | 126 |
| Mass ratios (%):               |      |      |       |       |        |      |     |
| TOTHMS/EMS                     | 68.3 | 62.4 | 71.5  | 1.77  | 3.12   | .16  | 126 |
| YMS/TOTHMS                     | 10.2 | 4.9  | 17.3  | 2.43  | 5.91   | .22  | 126 |
| TOTFAT/TOTHMS                  | 4.3  | 3.5  | 5.1   | .30   | .092   | .027 | 126 |
| TOTFAT/YMS                     | 44.2 | 23.4 | 100.0 | 12.42 | 154.25 | 1.11 | 126 |

Note. Initial egg mass is represented by EMS, total hatchling mass by TOTHMS, residual yolk mass by YMS, and total fat mass by TOTFAT.

$$(r^2 = 0.35, P < 0.001),$$

$$\text{Residual Yolk Mass} = -10.30 + 0.21 (\text{initial egg mass})$$

$$(r^2 = 0.25, P < 0.001).$$

Other parameters were not standardized because their regressions did not explain significant variation or because the  $r^2$  values were low. However, after accounting for main treatment effects in ANCOVA, egg mass was a significant covariate with snout-vent length, total length, and snout width at nares.

### *Statistical Analysis*

We used SAS (SAS Institute 1987) and SYSTAT 5.2 (SYSTAT 1992) to test the effect of incubation temperature on various hatchling parameters by ANCOVA using a mixed model design. Initial egg mass was the covariate with two factors, temperature and clutch. For the purpose of analysis, the clutch factor represents any genetic and/or maternal effects beyond initial egg mass, which had already been removed. Differences between males and females at 32°C were tested with another ANCOVA consisting of the covariate (initial egg mass) and two factors, sex and clutch. Significance levels for main effects were acceptable at  $P < 0.05$  (two-tailed). Certain tests produced significant interactions with initial egg mass. In these cases, the conditions of ANCOVA were violated, so the main effects could not be tested. If a temperature-by-clutch interaction was not significant ( $P < 0.1$ ), it was removed from the final model.

## **Results**

Sample means and descriptive statistics of hatchling parameters are shown in table 1. Clutch means and descriptive statistics of initial egg mass of hatchling alligators appear in table 2. Additional results are shown in Allsteadt (1993).

### *Hatchling Mass*

Total hatchling mass did not vary significantly among incubation temperatures (fig. 1; ANCOVA,  $F = 4.14$ ,  $P = 0.07$ ). However, the effect of clutch explained significant variation in total hatchling mass ( $F = 28.13$ ,  $P < 0.0001$ ). The temperature-by-clutch interaction was significant when sexes were pooled ( $F = 8.90$ ,  $P < 0.0001$ ) but was only significant for females when

TABLE 2

*Clutch means and descriptive statistics of initial egg mass of hatchling alligators from three clutches*

| Initial Egg Mass (g) | $\bar{X}$ | Minimum | Maximum | SD   | Variance | SEM | <i>n</i> | CS |
|----------------------|-----------|---------|---------|------|----------|-----|----------|----|
| Clutch 1 . . . . .   | 74.65     | 66.88   | 81.21   | 3.53 | 12.43    | .54 | 43       | 51 |
| Clutch 2 . . . . .   | 74.98     | 69.48   | 81.91   | 2.58 | 6.67     | .40 | 42       | 45 |
| Clutch 3 . . . . .   | 78.00     | 72.40   | 84.20   | 2.40 | 5.75     | .37 | 41       | 45 |

Note. Clutch size is designated by CS.

sexes were analyzed separately ( $F = 3.63$ ,  $P = 0.01$ ). At 32°C, total hatchling mass did not vary significantly between males and females ( $F = 1.20$ ,  $P = 0.28$ ).

Yolk-free hatchling mass did not vary significantly with temperature when sexes were pooled (fig. 1;  $F = 4.25$ ,  $P = 0.06$ ). However, when sexes were analyzed separately, the effect of temperature was significant for males ( $F = 31.99$ ,  $P < 0.0001$ ) but not for females ( $F = 3.16$ ,  $P = 0.15$ ). Clutch significantly affected yolk-free hatchling mass when sexes were pooled ( $F = 24.32$ ,  $P < 0.0001$ ). When the sexes were analyzed separately, clutch was significant for females ( $F = 14.30$ ,  $P < 0.0001$ ) but not for males ( $F = 0.61$ ,  $P = 0.55$ ). The temperature-by-clutch interaction was significant when sexes were pooled ( $F = 6.51$ ,  $P < 0.0001$ ) and for females ( $F = 3.60$ ,  $P = 0.01$ ). At 32°C, males weighed significantly more than females ( $F = 12.55$ ,  $P = 0.001$ ).

### *Body Dimensions*

Incubation temperature significantly affected snout-vent length (ANCOVA,  $F = 9.69$ ,  $P = 0.01$ ), total length ( $F = 18.48$ ,  $P = 0.002$ ), trunk length ( $F = 9.16$ ,  $P = 0.01$ ), interlimb length ( $F = 4.56$ ,  $P = 0.05$ ), and front-limb length ( $F = 15.73$ ,  $P = 0.003$ ) in a similar pattern (fig. 2). Maximum dimensions were generally produced at 32°C, whereas minimum values occurred at 29°C. Body dimensions generally showed a similar pattern of the effect of clutch for females: clutch 1 > clutch 2 > clutch 3. Clutch significantly affected snout-vent length ( $F = 22.56$ ,  $P < 0.0001$ ), total length ( $F = 28.03$ ,  $P < 0.0001$ ), trunk length ( $F = 10.75$ ,  $P < 0.0001$ ), and front-limb length ( $F = 5.24$ ,  $P = 0.007$ ), but not interlimb length ( $F = 2.03$ ,  $P = 0.14$ ).



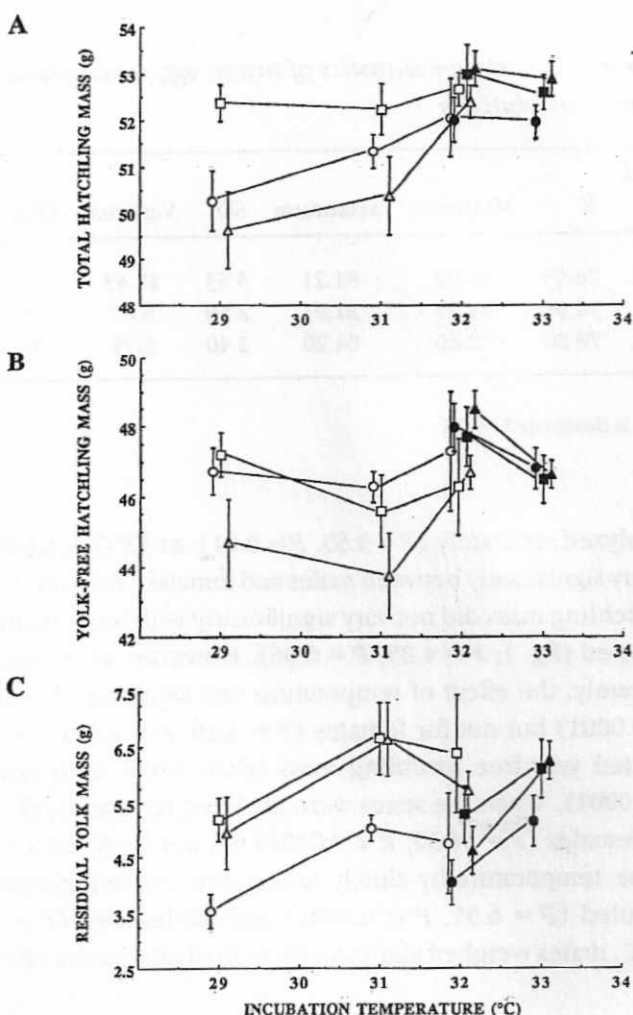


Fig. 1. Relationship between incubation temperature and (A) total hatchling mass, (B) yolk-free hatchling mass, and (C) residual yolk mass of hatchling alligators. Masses were standardized to initial egg mass by simple regression. Clutch 1 = circles; clutch 2 = squares; clutch 3 = triangles; females = open symbols; males = closed symbols. Means were offset around each constant temperature to prevent overlapping error bars ( $\pm 2$  SEM). Lines were drawn to show interaction effects.

Temperature-by-clutch interactions were significant for snout-vent length ( $F = 4.32$ ,  $P = 0.0006$ ), total length ( $F = 4.24$ ,  $P = 0.0007$ ), trunk length ( $F = 2.83$ ,  $P = 0.01$ ), interlimb length ( $F = 3.29$ ,  $P = 0.005$ ), and front-limb length ( $F = 2.45$ ,  $P = 0.03$ ). At  $32^{\circ}\text{C}$ , differences in body dimensions between the sexes were not significant (ANCOVA,  $P > 0.40$ ).

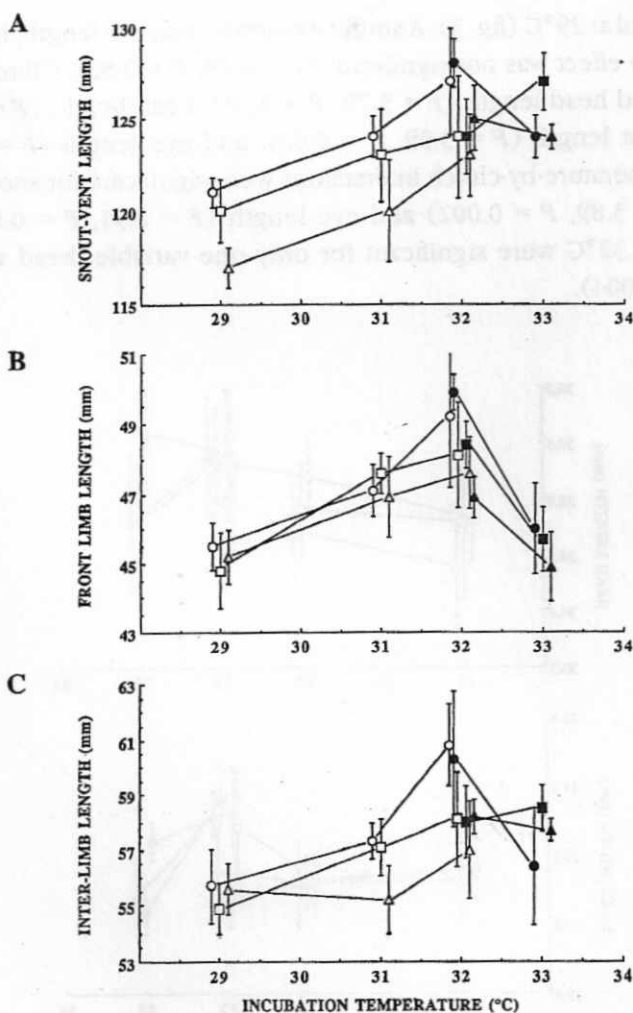


Fig. 2. Relationship between incubation temperature and (A) snout-vent length, (B) front-limb length, and (C) interlimb length of hatching alligators. Clutch 1 = circles; clutch 2 = squares; clutch 3 = triangles; females = open symbols; males = closed symbols. Means are displayed as in fig. 1.

#### Head Dimensions

Incubation temperature had significant effects on head length (ANCOVA,  $F = 3.24$ ,  $P = 0.03$ ), head width ( $F = 6.84$ ,  $P = 0.0003$ ), head height ( $F = 18.56$ ,  $P < 0.0001$ ), snout length ( $F = 28.11$ ,  $P < 0.0001$ ), midsnout width ( $F = 14.31$ ,  $P = 0.004$ ), and snout width at nares ( $F = 7.79$ ,  $P = 0.02$ ). Maxima for most head dimensions occurred at 32° or 33°C, whereas minima gen-

erally occurred at 29°C (fig. 3). A single exception was eye length, for which a temperature effect was not significant ( $F = 0.29$ ,  $P = 0.83$ ). Clutch significantly affected head length ( $F = 3.79$ ,  $P = 0.03$ ), head height ( $F = 3.58$ ,  $P = 0.03$ ), snout length ( $F = 3.99$ ,  $P = 0.02$ ), and eye length ( $F = 3.64$ ,  $P = 0.03$ ). Temperature-by-clutch interactions were significant for snout width at nares ( $F = 3.89$ ,  $P = 0.002$ ) and eye length ( $F = 2.31$ ,  $P = 0.04$ ). Sex differences at 32°C were significant for only one variable, head width ( $F = 9.80$ ,  $P = 0.004$ ).

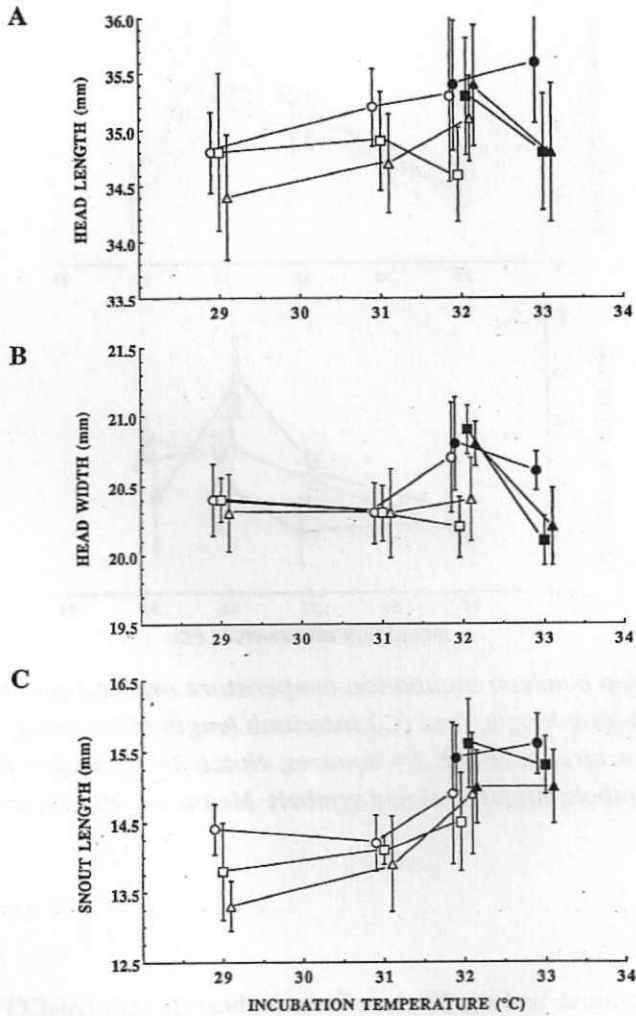


Fig. 3. Relationship between incubation temperature and (A) head length, (B) head width, and (C) snout length of hatchling alligators. Clutch 1 = circles; clutch 2 = squares; clutch 3 = triangles; females = open symbols; males = closed symbols. Means are displayed as in fig. 1.

### Energy Reserves

Incubation temperature significantly affected residual yolk mass (ANCOVA,  $F = 33.64$ ,  $P < 0.0001$ ). Residual yolk mass was inversely related to yolk-free hatchling mass and was higher at 31° and 33°C, whereas lower values occurred at 29° and 32°C (fig. 1). Clutch also explained significant variation in residual yolk mass ( $F = 56.30$ ,  $P < 0.0001$ ). However, the temperature-by-clutch interaction was not significant. At 32°C, the mean yolk mass of females weighed significantly more than that of males ( $F = 12.40$ ,  $P = 0.001$ ).

Total fat mass was relatively constant, only varying  $\pm 0.25$  g, and was not significantly affected by temperature ( $F = 0.36$ ,  $P = 0.78$ ). The effect of clutch on total fat mass was significant for males only ( $F = 8.48$ ,  $P = 0.0007$ ). The temperature-by-clutch interaction was only significant when sexes were pooled ( $F = 25.55$ ,  $P < 0.0001$ ). At 32°C, there was no significant difference between sexes ( $F = 0.24$ ,  $P = 0.63$ ).

The effects of incubation temperature ( $F = 4.40$ ,  $P = 0.09$ ) and clutch ( $F = 1.58$ ,  $P = 0.21$ ) on abdominal fat mass were not significant for females. Similarly, the temperature-by-clutch interaction was not significant among females ( $F = 2.24$ ,  $P = 0.07$ ). At 32°C, there were no sex differences in abdominal fat mass ( $F = 0.70$ ,  $P = 0.41$ ).

Temperature did not significantly affect tail fat mass when sexes were pooled ( $F = 1.47$ ,  $P = 0.31$ ), but was significant for females ( $F = 3.27$ ,  $P = 0.04$ ). Clutch explained significant variation in tail fat mass ( $F = 5.20$ ,  $P = 0.007$ ). The temperature-by-clutch interaction for tail fat mass was significant ( $F = 3.71$ ,  $P = 0.002$ ). At 32°C, sex differences in tail fat mass were not significant ( $F = 0.00$ ,  $P = 0.95$ ).

## Discussion

### Hatchling Mass

Total hatchling mass and yolk-free hatchling mass did not vary significantly with incubation temperature. However, the effect of temperature on hatchling mass ( $P < 0.10$ ) may be biologically significant. All three clutches showed the same response to temperature. Also, yolk-free hatchling mass of males did vary significantly; a maximum occurred at 32°C and a minimum at 33°C. Increasing the sample size of clutches may reveal a temperature effect that is statistically significant.

Previous studies of *Alligator mississippiensis* found significant differences among temperatures for both hatchling mass parameters, but these results were highly variable. Maximum total hatchling mass was reported at 30°C (Ferguson and Joanen 1983), 29.4° and 32.8°C (Joanen et al. 1987), and

34°C (Schulte 1989). Ferguson and Joanen (1983) found maximum yolk-free hatchling mass at 34°C, whereas 30°C was reported in a later study (Deeming and Ferguson 1989a).

The highly significant temperature-by-clutch interactions found in our study may explain these inconsistencies. However, previous studies (references cited above) had small sample sizes, small ranges of temperature, failed to distinguish between total and yolk-free hatchling mass, and/or did not account for clutch effects. In Schulte's study (1989), the clutch effect was controlled as a blocking factor; however, hatchling mass was not standardized to initial egg mass. In addition, incubation at 34°C produces mixed sex ratios that are female biased (Lang and Andrews 1994), not 100% male as had been reported in previous studies (reviewed in Deeming and Ferguson [1991]).

In *Crocodylus johnstoni*, yolk-free hatchling mass was inversely related to yolk mass and decreased consistently over the range of viable incubation temperatures from 28° to 34°C (Manolis, Webb, and Dempsey 1987; Webb et al. 1987; Whitehead et al. 1990). According to these reports, increased embryonic growth was explained by long incubation periods at low temperatures allowing embryos to metabolize more yolk (Manolis et al. 1987; Deeming and Ferguson 1989a). However, convincing data have not been shown for *Crocodylus porosus* (Webb and Cooper-Preston 1989). Moreover, clutch effects have not been evaluated in these studies.

The maximum yolk-free hatchling mass of alligators in this study (32°C) is not consistent with that of *C. johnstoni*, which occurred at the lowest incubation temperature (28°C). In the turtle *Chelydra serpentina*, intermediate incubation temperatures produced maximum total hatchling mass (Packard et al. 1988; Brooks et al. 1991). Intermediate temperatures also maximized the hatchling mass of some non-TSD reptiles (Gutzke and Packard 1987; Van Damme et al. 1992) and some birds (Romanoff, Smith, and Sullivan 1938; Romanoff 1944; Deeming and Ferguson 1991; Vleck and Hoyt 1991).

#### *Hatchling Dimensions*

Hatchling dimensions varied significantly with incubation temperature; maximum dimensions occurred at 32°C, whereas minimum values occurred at 29°C. Significant temperature effects on hatchling body dimensions, in addition to mass, indicate that the differences in body mass are due to actual variations in growth as opposed to other factors, that is, changes in water content or fat storage (Hutton 1987; Whitehead et al. 1990). Intermediate incubation temperatures also maximized hatchling size in *Crocodylus nil-*

*oticus*; hatchlings from 31.0°C were longer than those from 28.0° or 34.0°C (Hutton 1987). However, Schulte (1989) found that the total length of alligator hatchlings was greater at 30°C than at 32° or 34°C. In *Caiman crocodilus*, total length of hatchlings may increase with incubation temperatures between 30° and 34°C (Campos 1993). Other studies have not reported significant temperature effects on hatchling linear dimensions in alligators (Ferguson and Joanen 1983; Deeming and Ferguson 1989a, 1990) or in crocodiles (Webb and Cooper-Preston 1989).

In the turtle *Chrysemys picta*, low and intermediate incubation temperatures produced long carapace lengths, whereas high temperatures produced shorter lengths (Gutzke et al. 1987; Packard, Packard, and Birchard 1989). In non-TSD species, intermediate temperatures also maximized snout-vent length of the lizard *Podarcis muralis* (Van Damme et al. 1992) and of three snakes, *Pituophis melanoleucus*, *Coluber constrictor*, and *Lampropeltis getulus* (Gutzke and Packard 1987; Burger 1990).

#### *Energy Reserves*

Incubation temperature strongly affected the pattern of yolk utilization in embryonic alligators and resulted in large yolk differences at hatching. Residual yolk mass was inversely related to yolk-free hatchling mass; larger yolk masses occurred at 31° and 33°C, whereas smaller yolk masses occurred at 29° and 32°C. The fluctuating values of yolk mass suggest that the pattern of yolk utilization is more complex than previously hypothesized.

Previous studies have reported inconsistent values for hatchling yolk mass in alligators. Maximum yolk mass occurred at 30°C (Ferguson and Joanen 1983; Schulte 1989) and at 33°C (Deeming and Ferguson 1989a). The results of these previous studies are subject to error from small sample sizes, large clutch effects, and significant temperature-by-clutch interactions. In one of these early studies (Ferguson and Joanen 1983), low-temperature embryos were sacrificed early in development; thus, the report of large yolk mass at low temperatures was misleading (Webb et al. 1987). Also, in previous studies, yolk mass was not standardized to initial egg mass.

In *C. johnstoni* hatchlings, residual yolk mass increased consistently over the range of viable incubation temperatures from 28° to 34°C and was negatively correlated to yolk-free body mass (Webb et al. 1987; Whitehead et al. 1990; Whitehead, Seymour, and Webb 1992). This pattern of yolk utilization was due to the long incubation periods at low temperatures allowing embryos to metabolize more yolk with consequent increases in growth (Manolis et al. 1987; Deeming and Ferguson 1989a). In the turtle *C. serpentina*, hatchling yolk mass increased with incubation temperature as in

*C. johnstoni*; however, low viable temperatures ( $\leq 26^{\circ}\text{C}$ ) were not examined (Packard et al. 1988).

Incubation temperature did not affect hatchling fat mass in this study and has not been reported in other reptiles. The difference of the intestinal fat body from the other abdominal fat bodies may suggest a biochemical difference in lipid content. Some mammals use a second fat, brown fat, for thermogenesis and acclimating to cold ambient temperatures (Eckert and Randall 1983). The intestinal fat body of alligators may serve as an alternate energy source when yolk and/or food are limited.

Fat bodies provide substantial energy reserves to the hatchling in addition to yolk lipids. The consistent fat mass among hatchlings from different incubation temperatures indicates that fat bodies are conserved more than yolk mass. Snell and Tracy (1985) found that abdominal fat bodies were metabolized slower than the yolk mass and functioned as long-term energy reserves in the iguana *Conolophus subcristatus*. Fat bodies also compliment the energy reserves of hatchling crocodiles (Whitehead et al. 1990). In green iguanas, the largest hatchlings converted more yolk to fat bodies, presumably because of optimal hydric conditions (Werner 1988). These trends suggest that fat bodies may be important during environmental stress if yolk reserves are quickly depleted. In adult reptiles, fat bodies are utilized during hibernation, severe and unfavorable environmental conditions, and seasonal reproductive cycles (Fox 1977; Duvall, Guillette, and Jones 1982; Gregory 1982; Seigel and Ford 1987).

### *Adaptive Significance*

How dimensions, body mass, and yolk mass of hatchlings actually influence the survival of alligators is not clear. This study revealed two patterns of growth of hatchling alligators: (1) large hatchlings with a small yolk mass and (2) small hatchlings with a large yolk mass. Variable incubation temperatures in nature may maintain variation in hatchling size, hatchling mass, and yolk mass. The adaptive value of morphological variation may be that hatchlings from different nest temperatures are preadapted to various environmental conditions (Lang 1987; Deeming and Ferguson 1991).

The advantages of large body size produced at  $32^{\circ}\text{C}$  probably outweigh those of large yolk mass at  $33^{\circ}\text{C}$ . Large body size may confer a head start in growth to hatchlings incubated at  $32^{\circ}\text{C}$ . Whereas large size alone may reduce the number of potential predators, large hatchlings with small yolks have greater mobility and agility to forage and avoid predation. Longer limbs and trunks may translate into advantages in locomotion even among individuals with similar body masses. Small hatchlings with large yolk masses

appear to move awkwardly and consequently are probably slower and more susceptible to predation. Maximum posthatching growth rates of other reptiles occur at intermediate incubation temperatures (Joanen et al. 1987; Webb and Cooper-Preston 1989; Brooks et al. 1991; McKnight and Gutzke 1993; Rhen 1993).

Fischer et al. (1991) contend that hatchling alligators do not effectively capture small prey, and they imply that a large yolk mass is more adaptive. However, hatchling alligators are adept predators of insects (Crouch 1977). Furthermore, hatching normally occurs in late summer when food resources, for example, insects, fish, amphibians, shrimps, and crustaceans, are locally abundant. Naive predators at first, hatchlings maintained at high temperatures have a lower mortality, metabolize residual yolk rapidly, and begin to feed 3–4 d posthatching (Joanen and McNease 1976, 1977, 1991; Crouch 1977; J. W. Lang, unpublished observations). Wild hatchlings may thermoregulate at high temperatures, as do captives immediately after hatching (Lang 1987), and probably begin feeding before the yolk is completely absorbed. Feeding actually increased the metabolism of the residual yolk in birds (Romanoff 1944). In this study, hatchlings from all temperatures received a substantial investment of yolk, sufficient to fuel a hatchling until it begins feeding.

The residual yolk of hatchling reptiles is an important energy resource (Ewert 1985; Congdon 1989; Congdon and Gibbons 1990). Large yolk mass may increase hatchling survival when food is scarce and/or environmental conditions are severe (Whitehead and Seymour 1990; Ewert 1991). In alligator eggs incubated at 32°C ( $\pm 1^\circ\text{C}$ ), only 26% of the original energy contained in the initial yolk was used for embryogenesis, whereas 74% was transferred to the hatchling body and residual yolk (Congdon and Gibbons 1989; Fischer et al. 1991). The energy-dense yolk apparently supports the hatchling alligator for several weeks or months after hatching (Fischer et al. 1991). However, although yolk is still found in 2–3-mo-old hatchlings maintained in captivity at high temperatures and fed regularly, the importance of residual yolk is probably overstated by Fischer et al. (1991), for reasons explained above. Under normal conditions, yolk reserves function as an immediate source of energy at hatching and represent only a secondary resource once feeding commences (1–2 wk posthatching).

#### *Clutch Effects and Temperature-by-Clutch Interactions*

This study revealed strong clutch effects on most hatchling parameters. In addition, there were highly significant temperature-by-clutch interactions; the clutches responded differently to temperature. These factors probably account for the wide variation in hatchling features reported in previous



studies. The significant clutch effects and significant temperature-by-clutch interactions in this study imply that there are genetic and/or maternal effects on hatchling parameters in addition to the strong temperature effect.

Clutch effects on hatchling mass have been reported previously in alligators (Schulte 1989), crocodiles (Hutton 1987), turtles (Brooks et al. 1991; McKnight and Gutzke 1993), and iguanas (Troyer 1983; Werner 1988). Temperature-by-clutch interactions on body length were observed in *C. niloticus* hatchlings (Hutton 1987). Ricklefs and Cullen (1973) reported that yolk mass was affected by clutch in *Iguana iguana*. Clutch and temperature-by-clutch interactions have strong effects on the survivorship and growth of hatchling snapping turtles, *C. serpentina* (Rhen 1993). Clutch also influences temperature-dependent sex ratios in crocodylians (Lang and Andrews 1994), and in turtles these clutch effects are significantly heritable (Bull, Vogt, and Bulmer 1982; Janzen 1992).

Temperature-by-clutch interactions may represent a mechanism for maintaining genetic variation in hatchling parameters (Via and Lande 1987; Gillespie and Turelli 1989). This variation could be adaptive to organisms inhabiting spatially or temporally unpredictable environments (Kaplan and Cooper 1984; Gabriel and Lynch 1992). Different genotypes would produce different phenotypes, each adapted to specific conditions. Specialized genotypes may be favored over generalized ones because they have a higher geometric mean fitness in heterogeneous environments (Kaplan and Cooper 1984). The temperature-by-clutch interactions would augment this variation by allowing one genotype to produce a variety of phenotypes and by allowing different genotypes to produce the same phenotype (Sultan and Bazzaz 1993). Thus, the temperature-by-clutch interactions could be selected if the relative fitnesses of different genotypes change in different environments.

### Sex Differences

At 32°C, sex differences were highly significant for yolk-free hatchling mass, yolk mass, and head width. The residual yolk of females weighed more than that of males, but yolk-free hatchling mass and head width were greater in males than in females. A previous study found that the total hatchling mass of males was slightly greater than that of females at 32°C (Joanen et al. 1987). In contrast, other studies have not reported sex differences at 32°C in hatchling alligators (Ferguson and Joanen 1983; Schulte 1989). However, incubation temperature significantly affected the size of hatchling genitalia in a pattern similar to the effect of temperature on snout-vent length in this study (Allsteadt and Lang, in press).

The sex differences in this study support the hypothesis of temperature-dependent fitness to explain the maintenance of TSD in reptiles (Charnov and Bull 1977; Bull and Bulmer 1989; Bull and Charnov 1989). This hypothesis predicts that TSD would be favored over genetic sex determination when fitness is affected by environmental conditions and when one sex experiences a differential fitness over the other under specific conditions. Differences in some traits (e.g., hatchling size, energy reserves, and post-hatching growth rates) may lend a selective advantage to one sex over the other (Lang et al. 1989; Deeming and Ferguson 1989*b*) and may ultimately be expressed as the sexual dimorphism in adults; for example, male alligators grow to a larger adult size than females.

### *Conclusions*

The lack of ecological studies makes inferences on the adaptive significance of hatchling features highly speculative. Experimental studies are difficult to use as predictive tools because temperature-by-clutch interactions introduce wide variation in hatchling traits; these interactions may account for the large discrepancies among previous studies. During this study, Lang and Andrews (1994) determined that high temperatures ( $\geq 33.5^{\circ}\text{C}$ ) also produce female alligators. High temperatures should result in small hatchlings with large yolk reserves; these hatchlings will probably experience lower survival than larger hatchlings from intermediate temperatures. Hatchling traits are affected by incubation temperature, clutch, and temperature-by-clutch interactions. Temperature-by-clutch interactions may increase genetic variation in hatchling parameters as an adaptive response to a heterogeneous environment. This study raises more questions about TSD in alligators: How are hatchling traits related to adult sexual fitness? How do these traits affect the long-term survival of alligators?

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