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Effects of egg incubation condition on the post-hatching growth and performance of the snapping turtle, *Chelydra serpentina*

by

Kathleen M. Ryan

An Abstract of a Thesis
in
Biology

Submitted in Partial Fulfillment
of the requirements
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State University of New York
College at Buffalo
Department of Biology

MASTER

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ABSTRACT

The effect of egg incubation condition on the post-hatching growth and performance of the snapping turtle, *Chelydra serpentina*

The effect of incubation temperature on the post-hatching growth and performance capacities of the common snapping turtle, *Chelydra serpentina* was investigated in the laboratory. Turtle eggs were collected from four sites in New York State. The eggs were randomly assigned to four incubation temperature treatments to produce males (constant 26°C and downshifted 30-26-30°C) and females (constant 30°C and upshifted 26-30-26°C) under constant and altered temperature regimes. The incubation conditions resulted in 92% males from the constant 26°C group and 93% males from the downshifted (30-26-30°C) group. 100% females resulted from both the constant 30°C group and the upshifted (26-30-26°) group.

Turtles hatching from eggs incubated constantly at 26°C were significantly larger than hatchlings from eggs incubated at a constant 30°C or downshifted (30-26-30°C) (ANCOVA, df=3, F=5.56, p=0.0013).

Hatchlings were raised in individual aquaria at 25°C and fed earthworms and fish. After a 9-month growth period, turtles which had been incubated at a constant 30°C gained significantly more mass than did turtles from eggs which had

been downshifted or upshifted (ANOVA, df=3, F=4.71, p=0.007).

There was no extended effect of incubation condition on post-hatching performance and learning ability as measured by righting and feeding responses. Thus, the mass gain differences seen in this study suggest that physiological differences do result as the consequence of incubation condition. However, these physiological differences are not reflected in normal locomotive or feeding behavior.

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TABLE OF CONTENTS

	PAGE
LIST OF TABLES	vi
LIST OF FIGURES	viii
INTRODUCTION	1
EXPERIMENTAL DESIGN	7
MATERIALS AND METHODS	10
RESULTS	18
DISCUSSION	23
LITERATURE CITED	34
FIGURES	41
TABLES	53
APPENDIX	62

LIST OF TABLES

NUMBER	DESCRIPTION	PAGE
1.	One-way ANCOVA table showing the significant effect of incubation condition on the hatching mass of snapping turtles, *Chelydra serpentina*	51
2.	One-way nested ANOVA and tukey table showing the significant effect of incubation condition on the post-hatching mass gain of snapping turtles, *Chelydra serpentina*	52
3.	Model I 4 X 3 factorial ANOVA table showing the lack of significant effect of incubation condition on the feeding response time of snapping turtles, *Chelydra serpentina*	53
4.	Model I 4 X 3 factorial ANOVA table showing the effects of incubation condition and trial order on the latency period prior to the first righting response of turtles tested at 25°C	54
5.	Model I 4 X 3 factorial ANOVA table showing the effects of incubation condition and trial order on the frequency of righting response made by turtles tested at 25°C	55
6.	Model I 4 X 3 factorial ANOVA table showing the effects of incubation condition and trial order on the average response time of turtles tested at 25°C	56
7.	Model I 4 X 3 factorial ANOVA table showing the insignificant effect of incubation condition and ambient testing temperature on the latency period prior to the first response of turtles tested at 15°C, 25°C and 35°C	57

LIST OF TABLES
Continued

NUMBER	DESCRIPTION	PAGE
8.	Model I 4 X 3 factorial ANOVA table showing the effects of incubation condition and ambient testing temperature on the total number of righting responses made by turtles tested at 15°C, 25°C and 35°C	58
9.	Model I 4 X 3 factorial ANOVA table showing the effects of incubation condition and ambient testing temperature on the average response time of turtles tested at 15°C, 25°C and 35°C	59

LIST OF FIGURES

NUMBER	DESCRIPTION	PAGE
1.	Flow chart of general statistical procedures	39
2.	The significant scatterplot and regression of hatching mass and initial egg mass	40
3.	The experimental design for testing the effects of incubation condition and trial order or ambient testing temperature on the righting response variables and feeding responses of turtles	41
4.	Time course of incubation and days until hatching at four incubation conditions used in this experiment	42
5.	Feeding and testing schedule of the turtles followed during the righting response trials	43
6.	The significant effect of incubation condition on the hatching mass means of snapping turtles adjusted for initial egg mass	44
7.	The significant effect of incubation condition on the mass gain of snapping turtles over a 9-month period	45
8.	The significant effect of trial order on the latency period of juvenile snapping turtles prior to their first righting response when tested at 25°C	46
9.	The significant effect of trial order on the number of responses made by juvenile snapping turtles tested at 25°C	47

LIST OF FIGURES
Continued

NUMBER	DESCRIPTION	PAGE
10.	The significant effect of trial order on the average response time of juvenile snapping turtles tested at 25°C	48
11.	The significant effect of testing temperature on the frequency of righting responses made by juvenile turtles tested at 15°C, 25°C and 35°C	49
12.	The significant effect of testing temperature on the average response time of juvenile snapping turtles tested at 15°C, 25°C and 35°C	50

INTRODUCTION

Differential survival and reproduction are directly related to individual fitness (Pough, 1988). Therefore, understanding how environmental factors influence the growth and performance of individuals is of fundamental importance to ecologists. An understanding of the mechanisms by which vertebrate ectotherms are constrained by the interaction of their bioenergetic requirements and by the conditions of their physical environment may enable us to predict how they will respond to changes in their habitats caused by a combination of human and natural stresses (Spotila and Standora, 1987). It may be possible to use freshwater turtles as biological monitors of changing aquatic ecosystems, thereby providing a meaningful indicator of environmental quality (Bury, 1979). Research on the physiological ecology of turtles will help us to understand and predict how other vertebrate ectotherms would respond to climatic change and whether these animals, important components of many ecosystems, would survive (Spotila and Standora, 1987).

Miller et al. (1987) note than once turtles reach the juvenile stage, increased size and harder shells improve

their chance of survival. Large turtle hatchlings appear to be superior to smaller conspecifics in terms of escaping predators and obtaining food (Froese and Burghardt, 1974). A positive correlation between increased body size and reproductive success has been observed in many classes of animals (Zweifel, 1968; Mitchell, 1973; Nevo, 1973; Licht, 1976; Trivers, 1976; Tsubaki and Ono, 1978; Wilbur et al., 1978; Anderson and Fedak, 1985; Congdon and Gibbons, 1985; Roff, 1986; Bowen et al., 1987). Therefore, factors which influence the size of hatchlings and their subsequent growth rates are important components of the natural selection process in turtles.

Temperature is an important environmental factor with which all species must contend. Temperature influences biological processes as a rate function, as a signal to initiate processes, and as a threshold factor (Cossins and Bowler, 1987). Temperature effects on the growth and performance of a variety of organisms have been and will continue to be of particular interest to the many physiological ecologists who study ectotherms (Huey and Slatkin, 1976; Huey and Stevenson, 1979; Bennett, 1980; Huey and Stevenson, 1982; Van Berkum et al., 1983; Pough et al., 1983; Bull, 1987; Gutzke and Crews, 1988; Jensen, In Press; Pough, unpublished).

Incubation temperature of embryos is an important

facet of the biology of many reptiles because of its effect on sex determination. Although many reptiles exhibit genotypic sex determination (GSD) characterised by the presence of heteromorphic sex chromosomes (Bull, 1980), a large proportion of them exhibit environmental sex determination (ESD). Examples of ESD, where the sex of the offspring is determined by the conditions of the environment the eggs are placed in, include many species of nematodes (Christie, 1929; Anya, 1976) and some crustaceans (Ellenby, 1954). In these cases sex determination has been shown to be a function of parasitic density within the host organism where the eggs are laid. Temperature dependent sex determination (TSD) is an example of ESD (Bull, 1980). Some lizards, most or all crocodilians and most turtles lack heteromorphic sex chromosomes. Gonadal differentiation in these animals is determined by nest temperatures during a critical period of embryogenesis (Ynetma, 1968, 1976, 1978, 1979; Bull, 1980, 1987; Standora and Spotila, 1985; Gutzke and Crews, 1988). Shift experiments have defined temperature sensitive periods (TSPs) in a variety of animals exhibiting TSD (See Deeming and Ferguson, 1989, for review). Temperature during these sensitive periods effectively determines the sex of the embryo.

The mechanism resulting in sexual differentiation remains speculative at this point although Deeming and

Ferguson (1989) present an interesting hypothesis suggesting a possible mechanism for sexual determination in crocodiles. According to these researchers, TSPs define the end points of a period during which exposure of the embryo to a "dose of male determining factor" for an adequate amount of time results in a male hatchling. Inadequate dosage produces a female by default. Apparently, the male determining factor, of which there are a number of possible candidates (Mittwoch, 1971, 1973; Engel et al., 1981; Zaberski et al., 1982; Mittwoch, 1983; Standora and Spotila, 1985; Gutzke and Bull, 1986; Schlesinger, 1986; Bogart, 1987; Page et al., 1987; Bull et al., 1988), operates optimally at temperatures above 22°C and below 28°C. In Chelydra serpentina, 100% females result from low temperatures of 20-22°C and high temperatures of 28-30°C. 100% males result from incubation temperatures of 24-26°C (Yntema, 1968). It has been proposed that incubation conditions set the embryonic hypothalmus which governs the release of hormones which direct the development of other components of sexuality (Wilson et al., 1981; Gutzke and Crews, 1988). Thus, temperature as the primary sex determiner may act as a trigger that initiates a cascade of events culminating in adult sex differences. Optimal conditions during development could link the activity of the hypothalmus with the physiological requirements of sex

(Deeming and Ferguson, 1989). Reproductive behavior and endocrine physiology of adult leopard gekos has been shown to be influenced by the temperature experienced as embyos (Gutzke and Crews, 1988). Incubation temperature also has been shown to have an effect on the post-hatching growth of American alligators (Joanen et al., 1987).

The purpose of this study was to determine the effects of incubation temperature on the subsequent growth, performance capacity and behavior of snapping turtles, Chelydra serpentina, as examples of TSD reptiles. In the first part of the study turtle eggs were incubated under four different temperature treatments to produce males and females under constant and altered temperature regimes. The temperature switching procedure followed in this study was implemented as an attempt to separate the effects of sex from the effects of incubation temperature on the growth and performance variables measured. Hatchlings were then maintained at a constant temperature and photoperiod for several months. Growth was measured and experiments were performed to determine the performance capacity of the young turtles. Here I report that incubation condition had a significant effect on the hatching mass of the snapping turtles as well as on the post-hatching growth of the turtles.

There appears to be no long term effect of incubation

condition on performance as measured by the frequency of righting responses and average righting response time one year after hatching or on feeding reponse time 16 months after hatching.

EXPERIMENTAL DESIGN

The first question addressed by this study was: Is there a difference in the mass of hatchlings due to incubation condition? A general flow chart of the statistical procedures used in this study is presented in Figure 1. Following the suggestion of Packard and Boardman (1988) a scattergram was plotted. A linear regression was performed to determine if initial eggmass had a significant effect on the size of hatchlings. A significant relationship would indicate that an analysis of covariance using initial eggmass as the covariate would be worthwhile. Finding that that there was a relationship between initial eggmass and the size of turtles at hatching (Hatch mass = 1.19 +.65(Eggmass) ; r^2=0.6139, P=0.0240; Fig.2), an analysis of covariance (ANCOVA) was performed on hatchling mass data to determine the effects of incubation condition on hatchling size.

The next question addressed was: Does incubation conditon have an effect on the post-hatching mass gain of snapping turtles? Again, scattergrams and regression analysis were used to test for the effects of initial

eggmass and initial hatchling mass on the post-hatching mass gain of the turtles. Neither eggmass (r^2=0.0004, P=0.8858) nor hatching mass (r^2=0.00007, P=0.9541) was significant. To determine the effects of incubation condition on post-hatching mass gain, a 1-way analysis of variance (ANOVA) was performed on mass gain data. This was followed by a Tukey multiple range test for differences among incubation conditions.

The third question addressed was: Does incubation condition have an effect on the post-hatching performance of juvenile snapping turtles? The effects of animal size (mass, carapace and plastron lengths) on the performance variables of frequency of the righting response, latency period prior to the first righting response, average righting response time and feeding response time were tested using the scatterplot-regression analysis technique. The effects of eggmass and hatchling mass on the performance variables were tested in the same manner as for the first question. None of these factors was significant. Regression equations are given in Appendix 1.

The effect of incubation condition and testing order was analysed using data obtained from three trials all of which were conducted at the ambient testing temperature of 25°C. Frequency of righting response, latency period prior to the first righting response (hereafter referred to as

'latency period'), and average response time data were analysed using a Model I, 4 X 3 factorial ANOVA. Incubation condition and trial number were the fixed factors. The statistical design for all performance tests is presented in Figure 3. In order to simplify the interpretation of the statistical results, equal treatment cells were formed by indiscriminantly discarding all of the data from some turtles. All of the statistics for this study were run on SAS generated programs (SAS Institute Inc., Cary, N.C.; Version 6.6).

To test the effect of incubation condition on the righting response variables of the turtles tested at different ambient temperatures (15°C, 25°C and 35°C) a Model I, 4 X 3 factorial ANOVA was used. Incubation condition and testing temperature were the fixed factors.

Feeding response data were analysed using a Model I, 4 X 3 factorial ANOVA using incubation condition and trial number as the fixed factors.

METHODS AND MATERIALS

EXPERIMENTAL ANIMALS

In June of 1987, 180 Chelydra serpentina eggs from six clutches were obtained from four sites in New York State: three clutches from Prebble Swamp near Cortland, Cortland County, contained 18, 21 and 43 eggs, one clutch from Goodale Lake Cortland County, contained 28 eggs, one clutch from the Montezuma National Wildlife Refuge, Seneca County, contained 40 eggs and one clutch from Beaver Island State Park, Grand Island, Erie County, contained 30 eggs. None of the Beaver Island clutch hatched from the constant 26°C incubation group. Since turtles from some clutches and incubation conditions did not survive for the entire study, clutch effects could not be examined.

INCUBATION CONDITIONS

Eggs from each clutch were randomly assigned to four incubation conditions based on Yntema's (1968) staging experiments. Yntema's experiments documented a relationship between morphological characteristics of the embryo to

temperature and rate of development. At 30°C, the critical stage for sex determination begins at stage 14 which occurs 12 days after laying (stage 0). At 26°, the critical stage for sex determination begins at stage 16-17 which occurs 24 days after laying. Yntema also determined that development rate in the early embryo stages occurred 3.4 times faster at 30°C than at 20°C. A stage per day ratio was determined for both 26°C and 30°C and the eggs were temperature shifted prior to the onset of sexual determination. The switching procedure as well as the length of incubation time required for the eggs to hatch at each condition are presented in Figure 4. One group remained at 30°C for the entire incubation period. Another group remained at 26°C for the entire incubation period. The remaining two groups were switched back and forth between the two temperatures. Shifts were performed in the following manner: On day 6 of the experiment, one half of the eggs from the 30°C chamber (n=44) were transferred to the 26°C chamber. On day 9, one half of the original group of eggs placed in the 26°C (n=43) were transferred to the 30°C chamber. On day 31, the 30°C eggs which had been transferred to 26°C were returned to the 26°C chamber. On day 33, the 26°C eggs which had been transferred to 30°C were returned to the 26°C chamber (See Figure 4). The first temperature shift occurred prior to the predicted onset of embryonic sexual differentiation.

The embryos in group B would have been at stage 8.7 at the time of the upshift. The embryos in group D would have been at stage 10 at the time of the downshift. The return temperature shift occurred after the predicted completion of sexual differentiation (Ynetma, 1968). Eggs from the upshift group (B) spent a minimum of 35 days (mean of 40 days) at 26°C and a total of 25 days at 30°C. Eggs from the downshift group (D) spent a minimum of 41 days (mean of 47 days) at 30°C and a total of 24 days at 26°C.

The eggs (10 per bowl) were placed in covered 8-inch finger bowls containing moist vermiculite. Since the moisture content of the incubation medium has been shown to have an effect on the initial size of turtle hatchlings (Packard et al., 1987) the vermiculite was kept damp to the touch but with no standing water (Tracy et al., 1978). The eggs were buried in the vermiculite with the top half of the eggs exposed. The finger bowls were covered to prevent evaporation of the water and to keep the humidity more constant. On 5 June 1987 half of the eggs were placed in an environmental chamber set at 26°C(±1.0°C) and the remaining half in an environmental chamber set at 30°C (±0.5°C). On the seventh day of incubation I lowered the temperature in the 26°C chamber 1°C when I discovered that this chamber exhibited a higher temperature fluctuation when set at 26°C (within finger bowl-1.7°below to +1.3 above 26°C) than

when set at 25°C (within finger bowl -0.6° to +0.7° above 25°C). Finger bowls were rotated within each chamber every week. Temperatures inside the finger bowls were verified two to three times a day using a 24 gauge copper-constantan thermocouple and a digital Bailey Instruments Bat-12 thermocouple thermometer (Spotila et al., 1983).

At the onset of the experiment all eggs were weighed on a Mettler pl200 top loading balance (±0.005g). An artist's soft brush was used to clear the eggs of vermiculite. The eggs were weighed each week until the eggs pipped. On 17 July the eggs were dusted with Sulfadiazine to prevent mold spreading from infected to uninfected eggs (Ynetama, 1979). Pipping began 28 July 1987, 55 days after the experiment began. I continued to weigh unpipped eggs until 25 August 1987, 83 days after the experiment began.

GROWTH STUDY

Turtles were weighed (±0.005g) within 48 hours of emergence from their egg shells. Plastron and carapace lengths were measured (±0.05mm) with a dial caliper. Hatchlings from the four incubation conditions were randomly selected to be used for the growth and performance portion of this study. The turtles were individually housed in clear plastic aquaria (16cm X 32cm X 9cm) which had

holes in the lids to allow air exchange. The hatchlings were raised in a walk-in environmental chamber at a constant 25°C (±1.5°C) with a 12/12 photoperiod (Williamson et al., 1988). Temperatures within the aquaria were verified using the thermocouple thermometer. Aquaria were rotated daily within the chamber.

Three times a week, turtles ate a diet of earthworms and/or minnows dusted with Pervinal@, a vitamin supplement. All turtles were fed the same amount of food at each feeding. Food remained in the aquaria for 24 hours. The aquaria were cleaned every other day and chalk was placed in the aquaria to provide a calcium source. Masses, carapace and plastron lengths were recorded biweekly from 2 September 1987 to 4 June 1988.

PERFORMANCE EXPERIMENTS

Two separate experiments were conducted to determine the performance capabilities of the turtles after one year of growth. The turtles were kept at 25°C throughout the experiments and were not acclimated to the testing temperatures. The procedure for the first experiment was as follows: The turtle was placed carapace-down on a flat pine board surface. The turtle was allowed to right itself with no enticements. Each turtle was tested at three

ambient temperatures (15°C, 25°C and 35°C) in a lighted environmental chamber. Three questions were addressed with this experiment. First, did incubation condition have an effect on the number of righting responses performed by turtles at various ambient temperatures? The second question was: Did incubation condition have an effect on the latency time period prior to the first righting response of the turtles? Lastly, was the average response time per response of the turtles affected by incubation condition?

Each turtle, in its aquarium, was placed in the chamber 30 minutes prior to the performance test and removed from the chamber immediately after it was tested. The performance was timed with a stop watch, beginning with the placement of the turtle on its carapace and ending with the placement of all four feet on the surface. The turtle was immediately returned to its carapace upon righting itself. Each performance test lasted 30 minutes. Over the course of the study each turtle was tested five times. A tape recorder was used to record data during the tests. The latency period prior to the first righting response was noted as well as the number of responses which occurred. The length of time between responses was also recorded.

Performance tests were conducted 24 August 1988 through 10 October 1988. The turtles were fed 5 days prior

to each test. In order to avoid time of day and seasonal effects, turtles were randomly divided into four test groups. Each test group contained similiar number of turtles from each incubation condition. Figure 5 shows the feeding and test schedule of the test groups of turtles. All turtles were first tested at 25°C which was the temperature to which they had been acclimated. Next, they were tested at 15°C. Then, 25°C, followed by 35°C and again, at 25°C. The turtles were randomly selected from each test group in the following sequence of incubation conditions:

Test 1	(25°C)	A	B	C	D
Test 2	(15°C)	B	C	D	A
Test 3	(25°C)	C	D	B	A
Test 4	(35°C)	D	B	A	C
Test 5	(25°C)	A	B	C	D

Data from tests 1, 3 and 5 were analysed to test for performance differences which might be attributed to testing order. This answered the questions: Did learning take place over the course of the study and, if so, did incubation condition have an effect on the learning of the young turtles? Data from tests 2, 3 and 4 were analysed to test the effect of ambient testing temperature on the performance of turles from the different incubation conditions.

The second performance experiment addressed the question: Does incubation condition affect the feeding

response time of a young turtle when tested 16 months after hatching? Trials were conducted in each turtles's home aquarium at 25°C. A minnow was placed in the water 12 cm in front of the turtle. The stop watch was started and the lid replaced on the aquarium which was the normal feeding procedure. Each turtle was timed for a maximum of 5 minutes. Half of the turtles took longer than the allocated 5 minutes to ingest the offered food. In order to avoid a bias in favor of slow turtles, data was analysed ommitting data obtained from turtles that did not eat the offered food within the allocated 5 minutes.

RESULTS

Incubation conditions are referred to in this section as they appear in Figure 4. That is, the constant 26°C group will be referred to as 'A', the upshift 26-30-26°C group as 'B', the constant 30°C group as 'C' and the downshift 30-26-30°C group as 'D'.

The incubation conditions resulted in 100% females from the 30°C group, 92 % males from the 26°C group, 100% females from the upshift (26-30-26°C) group and 93 % males from the downshift (30-26-30°C) group. The sexes of the 55 turtles used in this study were confirmed by microscopic observation of the gonads in August, 1989. One turtle from the constant 26°C group was found to be female as was one turtle from the downshift (30-26-30°C) group. Data obtained from these two turtles were not used in the statistical anaylsis.

There was a significant effect of incubation condition on the adjusted mean hatching mass of snapping turtles (one way ANCOVA, n=104, df=3, F=5.65, P=0.0013; Table 1). Means of hatching masses, adjusted for variation presented by the initial egg mass of turtles, indicated that turtles hatched from eggs incubated at a constant 26°C were significantly

heavier ($\bar{X}$=8.53 g) than the hatchlings from eggs incubated at 30°C ($\bar{X}$=8.06 g) or downshifted from 30-26-30°C ($\bar{X}$=8.12 g Figure 6).

Neither egg mass (r^2=0.004, P=0.8858) nor hatching mass (r^2=0.0007, P=0.9541) had a significant effect on post-hatching mass gain. There was a continued effect of incubation condition on the growth of the hatchlings over a 9-month period (one-way ANOVA, n=44, df=3, F=5.35, P=0.0034; Table 2). Mean mass gain of turtles incubated at a constant 30°C was 68.19 grams. This was significantly greater than the mean mass gain of 53.85 grams accrued by the downshift turtles and the mean mass gain of 51.50 grams accrued by the upshift turtles. Mean mass gain of turtles incubated at a constant 26°C was 61.59 grams. This gain was not statistically different from the gain obtained by any of the other groups (Figure 7).

There was no significant effect of incubation condition or testing order on the feeding response time of turtles when they were tested 16 months after hatching (4 X 3 factorial ANOVA, P > 0.05; Table 3). Mean feeding response time in seconds for incubation groups were as follows: A=48, B=52, C=60 and D=60 (n=24). The mean feeding response time in seconds for order of testing was: first test = 63, second test = 49 and third test = 52 (n=24). Also, there was no significant effect of incubation condition on latency period

prior to the first righting response (4 X 3 factorial ANOVA, n=36, P > 0.05; Table 4; Means in seconds: A=211, B=222, C=147 and D=199) on the frequency of righting responses (4 X 3 factorial ANOVA, n=36 P > 0.05; Table 5; Mean number: A=52, B=52, C=47 and D=41), or average response time per response made by turtles (4 X 3 factorial ANOVA, n=36, P > 0.05; Table 6; Means in seconds: A=160, B=226, C=131, D=160. Incubation conditions did not have a significant effect on these variables during subsequent trials at 25 ^{o}C (Tables 4-6) or at trials at different ambient testing temperatures (4 X 3 factorial ANOVAs, n=40, P > 0.05; Tables 7-9).

The order of testing had a significant effect on all three righting response variables of turtles tested during subsequent trials at ambient testing temperature of 25 ^{o}C. Mean latency period prior to the first righting response was longest for turtles during the first trial ($\bar{X}$=390 seconds, 4 X 3 factorial ANOVA, n=36, df=2, F=7.44, P=0.0010: Table 4) and shortest during the third trial ($\bar{X}$=88 seconds) Some turtles failed to right themselves during the first trial. This is indicated by the 1800 seconds recorded for the test (Appendix 2). Since turtles from each of the four incubation conditions failed to right themselves during the first trial, but did right themselves during subsequent trials, I left these values in the analysis. Mean latency periods for trials two and three were not significantly different from

one another ($P > 0.05$). The total number of responses made by turtles was lowest during the first trial ($\bar{X}=29$; 4 X 3 factorial ANOVA, n=36, F=4.44, P=0.0144; Table 5). Again, the difference between mean number of responses was not significant for trials two ($\bar{X}=56$) and three ($\bar{X}=60$; Figure 9; $p > 0.05$). Average response time per response was also longest during the first trial (Model I 4 X 3 factorial ANOVA, n=36, df=2, F=6.64, p=0.0020; Table 6) and not significantly different for trials two ($\bar{X}=56$) and three ($\bar{X}=60$; Figure 10; $p > 0.05$). Comparing maximum range of latency period for trials two and three, with maximum range of average response time of turtles for trials two and three, it appeared that average response time was not solely a function of the amount of time turtles waited to make their first response (Appendix 2).

Neither ambient testing temperature nor incubation condition had a significant effect on the length of time a turtle remained on its carapace before making its first righting response (Table 7). Ambient testing temperature did have a significant effect on the number of responses made by turtles (Model I 4 X 3 factorial ANOVA, n=40, df=2, F=6.54, p=0.0021; Table 8). Mean number of responses was significantly lower at 15°C ($\bar{X}=37$) than at 25°C ($\bar{X}=57$) or at 35°C ($\bar{X}=78$). The mean number of responses made at 25°C was significantly greater than the number made at 15°C and

significantly lower than the number made at 35°C (Figure 11; p=0.05). Average response time was significantly slower at 15°C ($\bar{X}$=173 seconds) than at 35°C ($\bar{X}$=59 seconds), but the response time at 25°C ($\bar{X}$=76 seconds) was not significantly different than at 35°C or 15°C (4 X 3 factorial ANOVA, n=40, df=2, F=2.61, P=0.0784; Table 9). The maximum range indicated that some turtles failed to respond at 15°C (Appendix 2).

DISCUSSION

The results of this study suggest that incubation temperature is a factor which determines sex differentiation, the size of hatchlings and post-hatching growth of snapping turtles, Chelydra serpentina. This study failed to show an extended effect of incubation condition on post-hatching performance as measured by righting and feeding responses. Ambient testing temperature was found to have the expected effect for ectotherms (Cossins and Bowler, 1987), that is, faster responses at higher temperatures. Incubation condition did not affect the way animals responded at any of the test temperatures.

All of the turtles examined from the constant 30°C group and the upshift group (26-30-26°C) were found to be female. This was as expected and is well documented in the literature (Yntema, 1968,1978,1979,1981; Bull and Vogt, 1979; Bull, 1980; Gutzke and Paukstis, 1983,1984; Gutzke and Packard, 1985,1987; Packard et al., 1984,; Morreale et al., 1982; Standora and Spotila, 1985). One female was found in the constant 26°C and in the downshift (30-26-

30°C) group. This was a surprise. Since the hatching mass and growth behavior of these turtles were similiar to other same-clutch, same-incubation condition turtles, it is not likely that either the eggs or the turtles were mislabled. It is also unlikely, in the case of the 30-26-30°C female, that the egg was temperature shifted at an inappropriate time relative to other same-clutch eggs. All eggs were transferred on the same day. If this clutch was older than I believed when it was first obtained, it would be possible that the eggs were held too long at 30°C before they were shifted to 26°C. However, all other same-clutch downshifted turtles were male. Therefore, I rule out the possibility of procedural error in this finding. Other investigators have also obtained turtles of the opposite expected sex from controlled incubation experiments (Dr. G. Packard, personal comm.).

Bull and Vogt (1979, 1980) suggested that weak genetic factors which are usually over ridden by environmental influence may exist in TSD animals. Perhaps insufficient production of "male-factor" (Deeming and Ferguson, 1989) or inactivation of the male-factor receptor sites occurred due to some weak genetic effect. If snapping turtles follow the Deeming and Ferguson (1989) model for sex differentiation in crocodiles, then females result by default. In any event, the findings of this

study suggest the neccessity of continued investigations on sex-determination in snapping turtles and other TSD reptiles in order to clarify the molecular mechanism of sex determination in these animals.

Turtles in this study emerged from the shell sooner in eggs that were incubated for more days at 30°C than at 26°C. This was consistent with the findings obtained by other TSD researchers (Dodge et al., 1978; Gutzke, 1984; Ferguson and Joanen, 1982, 1983; Legler, 1960; Miller, 1985; Miller and Limpus, 1981; Mrosovsky and Yntema, 1980; Packard et al., 1987; Yntema, 1978; Joanen et al., 1987; Deeming and Ferguson, 1989). Increased temperature may be conducive to accelerated pre-hatching development rate thereby producing a mature embryo in a shorter amount of time (Gutzke, 1984; Miller, 1985; Packard et al.,1987). However, it appears that the increased metabolism also associated with higher temperatures (Packard et al., 1987) prevent the embryos from achieving maximum size at 30°C. Ferguson and Joanen (1982) also found that female alligators were larger from 30°C incubated eggs than were males from 34°C incubated eggs. The principle of allocation (Cody, 1966) states that energy used to power metabolism can not be converted into tissue. Therefore, it is reasonable to expect smaller hatchlings from eggs incubating at higher temperatures where metabolism is

increased. It would be interesting to know whether different incubation conditions result in continued mass differences in the animals at the end of a post-hatching growth period. Although turtles resulting from eggs incubated at 30°C were the smallest upon hatching in the current study, this group gained the most mass during the post-hatching growth portion of this study. Joanen and colleagues (1987) found that American alligators resulting from eggs incubated at 30.6°C and 31.7°C were larger at nine months post-hatching than were animals from eggs incubated at 29.4°C or 32.8°C. Interestingly, the alligators from 1987 study were the same size at hatching from all four incubation temperatures. Since there are differences in the way alligators and turtles respond to incubation temperature (for example, the reversal of the sex-producing temperatures), it is also possible that metabolic pathways may be affected differently by incubation temperature between the two species.

The mechanisms by which temperature regulates metabolism has not been as well studied in reptilian embryos as it has been in adult reptiles. There may be an optimal incubation temperature which produces maximal post-hatching growth in TSD animals. This aspect of their biology requires additional investigation for all TSD animals.

Snapping turtles incubated at a constant 30°C gained significantly more mass than did turtles from the down temperature shift (30-26-30°C) and up temperature shift (26-30-26°C) groups. This suggests that incubation factors, not neccessarily related to sexual differentiation, may have an effect on the post-hatching growth of juvenile snapping turtles. Theoretically, temperature changes during incubation may affect development in a variety of ways. For example, there may be an immediate increase or decrease in the rate of development (Cossins and Bowler, 1987). There may be an acclimation effect which is manifest in a change in development rate at the next temperature. There may also be a stress effect on the embryo. "Catch-up growth" as described by Sibley and Calow (1986) was observed in this study population of turtles. However, the mechanism by which temperature changes occurring during incubation may have effected this result in snapping turtles is not clear. Perhaps the mechanism is related to an acclimation effect. Perhaps the hypothalmus is indeed "set" during embryogensis as suggested by Deeming and Ferguson (1989). Metabolism in ectotherms is regulated by behavioral thermoregulation, which in turn is governed by the hypothalmus (Eckert et al., 1988). If we accept mass gain as a measure of the effeciency of various metabolic

pathways and associated enzyme production which in turn controls the rate of nutrient assimilation, then, clearly, post-hatching mass gain may be seen to be influenced by conditions incurred by the eggs during development. The observed relationship between incubation condition and post-hatching mass gain is intriquing and merits further investigation. Deeming and Ferguson (1989) found a relationship between incubation condition and the pigment patterns of American alligators. Variations of pigment were observed, but not quantified, in my study population of snapping turtles. A range of colors have been observed in wild populations of snapping turtles (Ernst and Barbour, 1972). Also, the coloration of ectotherms has been shown to be a factor in thermoregulatory behavior (Brodie, 1988). Therefore, an investigation testing the relationships between incubation condition and post-hatching thermoregulatory behavior, such as basking (in species that are known to do so) may provide valuable information pertaining to natural selection in TSD reptiles. Field experiments involving the mark-recapture of known incubation condition turtles would serve to evaluate the ecological significance of incubation condition on fitness and predation survival success in these animals.

Gutzke and Crews (1988) found a significant relationship between incubation condition and the adult

sex-related behaviors of courtship and egg laying in leopard geckos. Although locomotor performance should correlate with fitness (Arnold, 1983), incubation condition was not shown to affect the timing or frequency of the righting responses of snapping turtles. It appears that a snapping turtle's ability to return from a vulnerable position to one which offers greater protection from both predation and desiccation (Burger, 1976) is not influenced by sex or pre-hatching treatment (Tables 4-9). Miller and associates (1987) found a significant relationship between hydric incubation conditions and the locomotor capacities of snapping turtles tested shortly after hatching. Post-hatching acclimation during this experiment may have negated any long term effects of incubation on later performance. It has been shown that acclimation temperature not only influences ectotherm response but, in addition, ectotherms may be reacclimated with resultant differences in response (For review see White and Somero, 1982). Perhaps in the case of this study, incubation condition might also be considered an acclimation period. The post-hatching 25°C acclimation of these animals may have served to "reset" the hypothalmus and thereby offset any long term performance differences which may otherwise have been attributed to incubation condition. There was no difference in the way turtles from different

incubation conditions performed at various ambient testing temperatures. In general the results were typical of ectothermic animals: slower responses were made by the turtles at 15°C than at 25°C or at 35°C. Fewer responses were made by turtles at 15°C than at 25°C and at 25°C than at 35°C. There was no significant difference between means for incubation condition (Tables 7-9; Figures 11 and 12).

Testing the turtles three times at the same temperature indicates that mean performance of the turtles was influenced by previous testing. It might appear that these differences were due to learning or training. Some turtle species are "trainable". For example, Berry (1986) found that a captive population of juvenile desert tortises could be trained to extinguish certain aggressive behaviors in a few days by repeatedly touching the animals about the head. Spigel (1964) demonstrated that Chrysemys were able to learn a detour behavior over a course of five days when given two trials a day, four hours apart. However, other studies indicate that teaching behaviors to turtles is a slow process requiring repeated, often torturous deterrents (Granda et al., 1965). Although no known enticement was offered to encourage the turtles to respond or was any punishment incurred for a lack of response in this study, it appears that the turtles adjusted to the experimental conditions and improved their performance

after only one trial. This observed behavior may be due to the phenomenon of habit inhibition. A turtle's natural response to being placed on its carapace is to right itself. Perhaps the slower, less frequent righting responses seen during the first test were a reaction to the confusion which may have been created by placing the turtles in a new situation. There were no significant differences in the mean number of responses made by the turtles from the various incubation conditions. Therefore, it does not appear that incubation condition exerts any long term effect which may be reflected in a snapping turtle's ability to respond to unexpected changes in its environment. The results of this study suggest that long term incubation effects on behavior of snapping turtles may be more subtle that has been suggested by studies on other ectotherms (Bull,1897; Joanen et al., 1987; Deeming and Ferguson, 1989; Gutzke and Crews, 1988).

I did not find a significant association between feeding response time and incubation condition. The turtles in this study were accustomed to a steady food supply which remained in their aquaria for 24 hours, an adequate amount of time to be ingested. It is possible that differences in feeding response time was masked by previously learned feeding behavior. It is also possible that feeding response time, which may be looked upon as a

measure of sensory acuteness, is not associated with embryonic sex differentiation.

In general, my findings fail to support the hypothesis that incubation conditions affect the later post-hatching performance behavior of snapping turtles. This study was not designed to test specific sex-related behaviors such as courtship and mating as was the Gutzke and Crews (1988) study on leopard geckos. Snapping turtles mature at a much slower rate than lizards. Therefore a great deal more time would be required to test these parameters than could be allocated to the current study. However, this is an area of research that should be pursued for turtles as well. If, as in the case of leopard geckos, 20% of the adult female population is rendered sterile by the conditions encountered during development, it may be necessary to re-evaluate a number of current wildlife management practices relating to the restocking of endangered turtle species. Radioimmunoassays of blood concentrations of androgen and oestradiol were not performed on this study population of turtles. This type of information, particularly if combined with a release and recapture program, could provide useful information pertaining to the effect of incubation condition on adult fitness in turtles. The mass gain differences seen in this study suggest that physiological differences do result as

the consequence of incubation condition. However, these physiological differences are not reflected in normal locomotive or feeding behavior.

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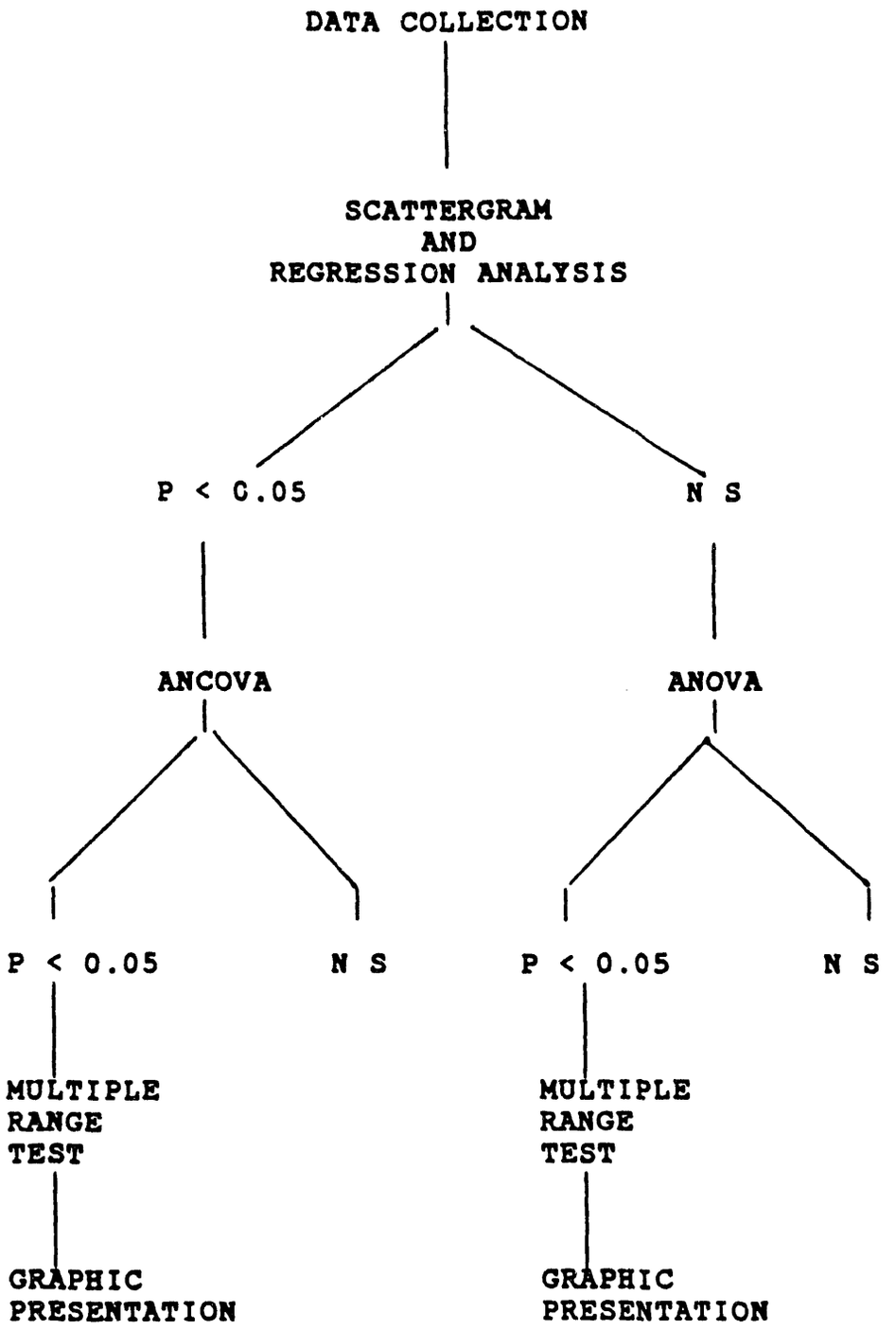

FIGURE 1. Flow chart of the general statistical procedures followed in this study.

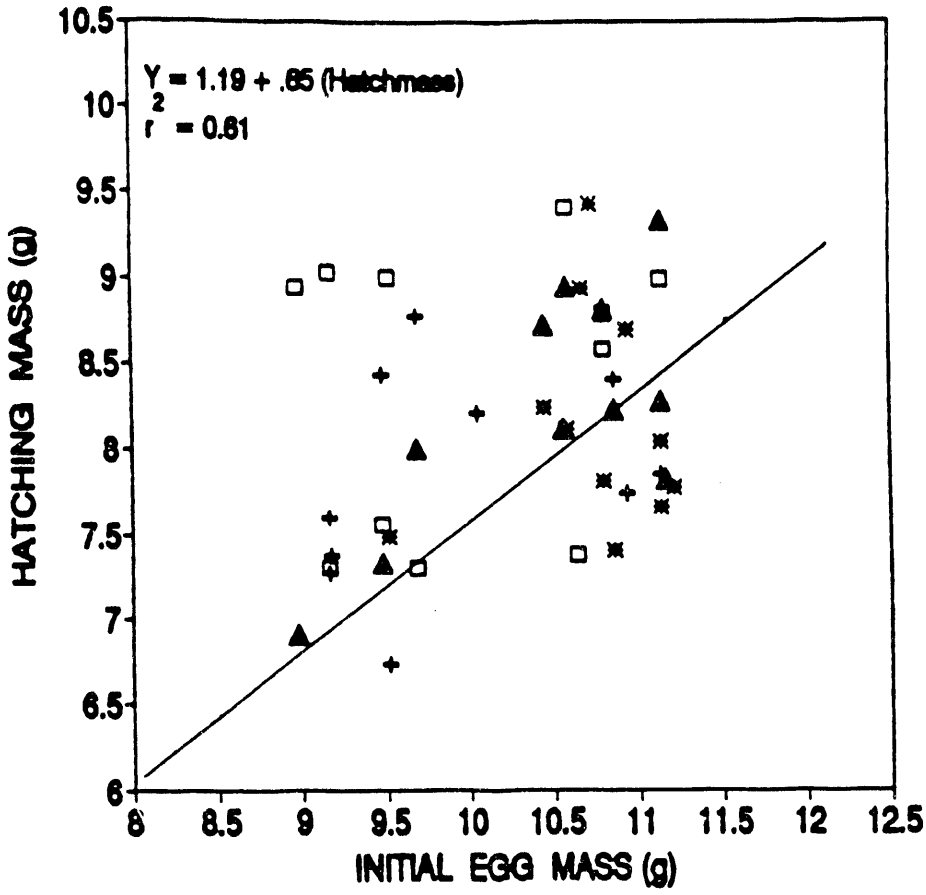

FIGURE 2. Scatterplot and regression analysis of hatching mass and initial egg mass. The symbols indicate incubation condition: Constant 26°C (□), upshift 26-30-26°C (+), constant 30°C (✱) and downshift 30-26-30°C (▲).

INCUBATION CONDITION

	A	B	C	D
1 (15°)	6 (9,10)	6 (9,10)	6 (9,10)	6 (9,10)
2 (25°)	6 (9,10)	6 (9,10)	6 (9,10)	6 (9,10)
3 (35°)	6 (9,10)	6 (9,10)	6 (9,10)	6 (9,10)

FIGURE 3. The experimental design used for testing righting response and feeding response data. The design is a Model I ANOVA with incubation condition and testing order (or testing temperature) as the fixed factors. Incubation conditions are designated by letters: constant 26°C (A), upshift 26-30-26°C (B), constant 30°C (C) and downshift 30-26-30°C (D). The numbers in the cells are the numbers of turtles tested. Six turtles from each incubation group were used for the feeding response trials. Nine turtles from each group were used for testing righting response at the ambient test temperature of 25°C. Ten turtles from each group were used for testing righting responses at three different ambient test temperatures. Testing order is shown by number. Testing temperature for the different ambient temperature test is given in parenthesis.

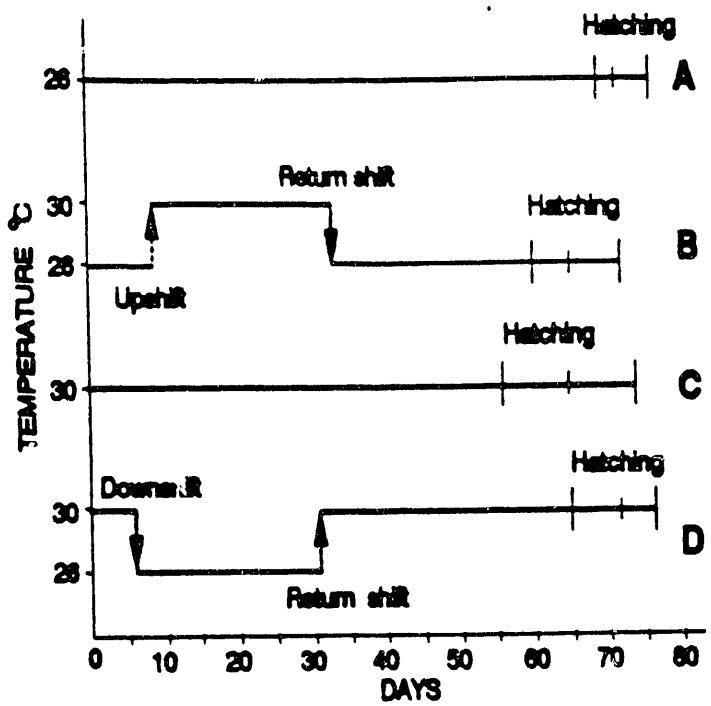

FIGURE 4. Time course of incubation at four incubation temperature conditions used in this experiment. The solid horizontal lines indicate the number of days each group of eggs spent at each temperature. The vertical arrows indicate when the eggs were temperature shifted. The vertical lines indicate first and last days of hatching for each group. Mean hatching day for each group is shown by the short vertical line. Incubation condition is shown by letter: constant 26°C (A), upshift 26-30-26°C (B), constant 30°C (C) and downshift 30-26-30°C (D).

	DAYS S	M	T	W	R	F	S
1					FEED ALL		
2				TR.1 G-1 FEED 1	TR.1 G-2 FEED 2 and 4	TR.1 G-3 FEED 3	
3	FEED 1	FEED 2	TR.1 G-4 FEED 4	FEED 3		TR.2 G-1 FEED 1	TR.2 G-2 FEED 2
4	FEED 4	TR.2 G-3 FEED 3		FEED 1	FEED 2	TR.2 G-4 FEED 4	FEED 3
5		TR.3 G-1 FEED 1	TR.2 G-2 FEED 2	FEED 4	TR.3 G-3 FEED 3		FEED 1
6	FEED 2	TR.3 G-4 FEED 4	FEED 3		TR.4 G-1 FEED 1	TR.4 G-2 FEED 2	FEED 4
7	TR.4 G-3 FEED 3		FEED 1		FEED 2	TR.4 G-4 FEED 4	FEED 3
8	TR.5 .5 G-1 FEED 1	TR.5 .5 G-4 FEED 4 and 1	FEED 2	TR.5 .5 G-3 FEED 3		TR.5 .5 G-3 FEED 1	TR.5 .5 G-1
9	TR.5 .5 G-4 FEED 4	TR.5 G-2 FEED 3					

FIGURE 5. This is the feeding and testing schedule which was followed during the righting response tests. Each testing group (G-) contains turtles from each incubation condition. Turtles were randomly placed in testing groups before testing began and were not switched between groups. Trials (TR.) 1, 3 and 5 were conducted at 25°C ambient temperature. Trial 2 was conducted at 15°C and trial 4 was conducted at 35°C. When a group is shown as being fed and tested on the same day, feeding occurred at the end of the day after testing was completed.

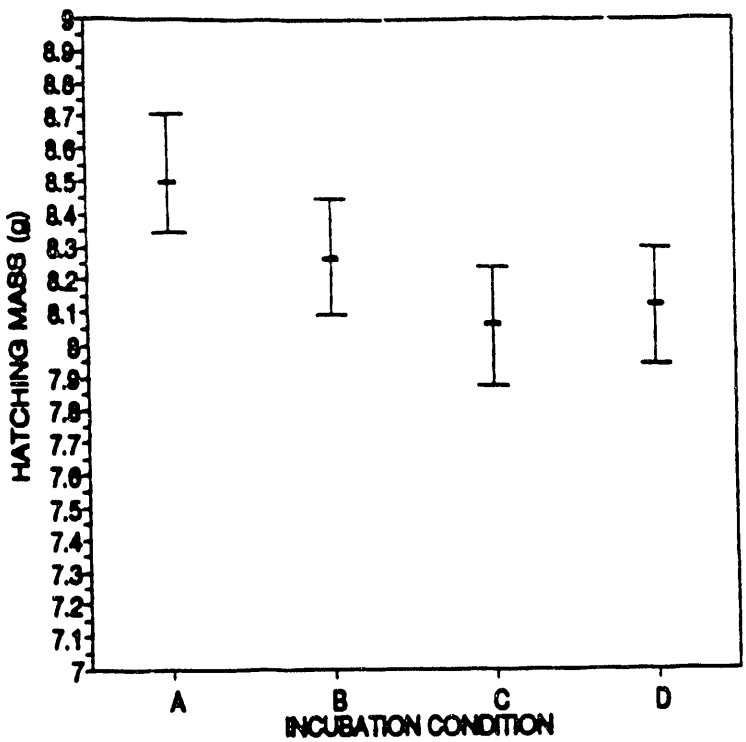

FIGURE 6. The effect of incubation condition on the mass of hatchling snapping turtles, *Chelydra serpentina*, adjusted for initial eggmass. Vertical lines indicated 2 standard errors of the mean. The center horizontal line on each vertical line indicates the mean hatching mass. Incubation condition is shown by letter: constant 26°C (A), upshift 26-30-26°C (B), constant 30°C (C) and downshift 30-26-30°C (D).

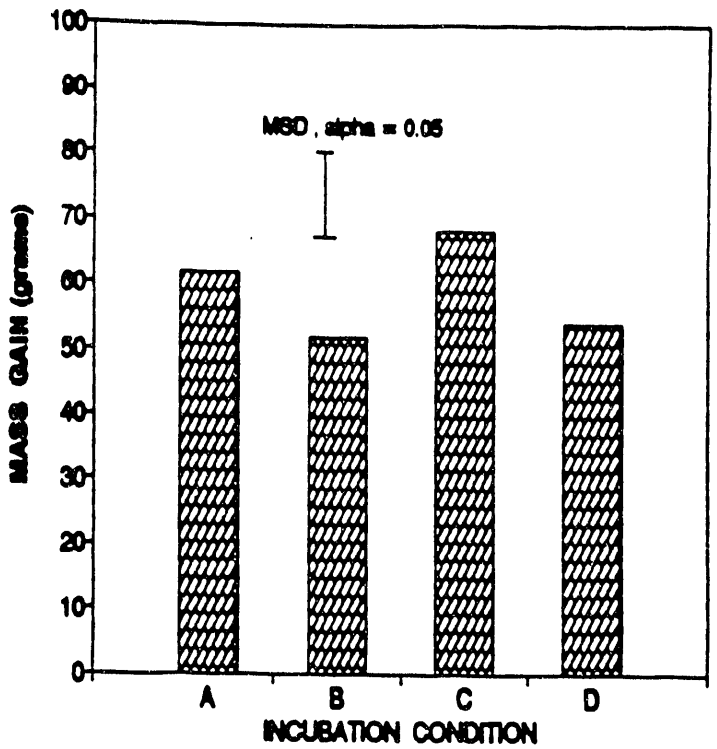

FIGURE 7. Bar graph of mean mass gain verses incubation condition for juvenile snapping turtles, Chelydra serpentina, (n=44) over a nine month growth period. Tukey's multiple range test clarified differences among means after data were analyzed using a one-way ANOVA. The minimum significant difference is 13.30 grams. Incubation condition is shown by letter: constant 26°C (A), upshift 26-30-26°C (B), constant 30°C (C) and downshift 30-26-30°C (D).

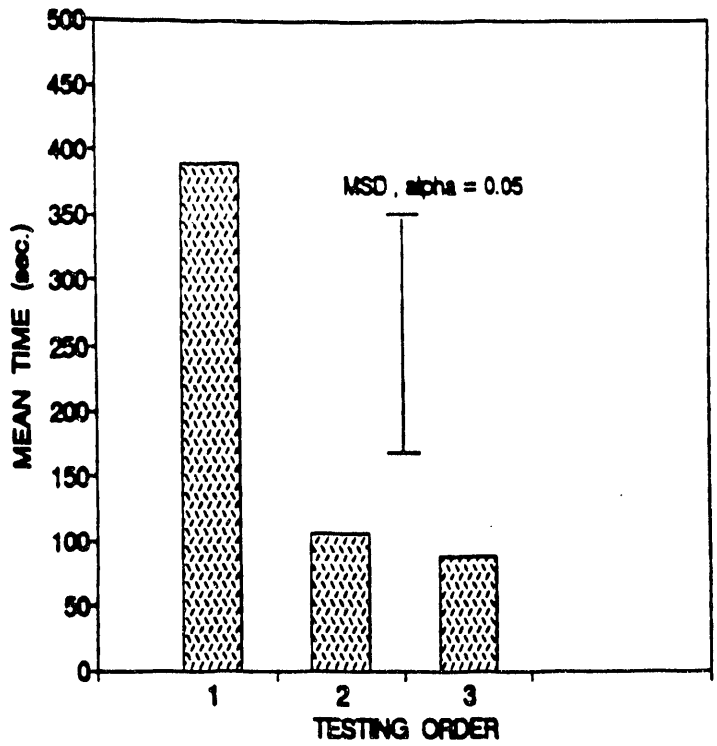

FIGURE 8. Bar graph of latency period of juvenile snapping turtles, Chelydra serpentina, (n=36), prior to their first righting response verses order of testing when tested at ambient 25°C. Tukey's multiple range test was used to clarify differences among means (minimum significant difference = 176.64 seconds) after data were analyzed with a model I 4 X 3 factorial ANOVA.

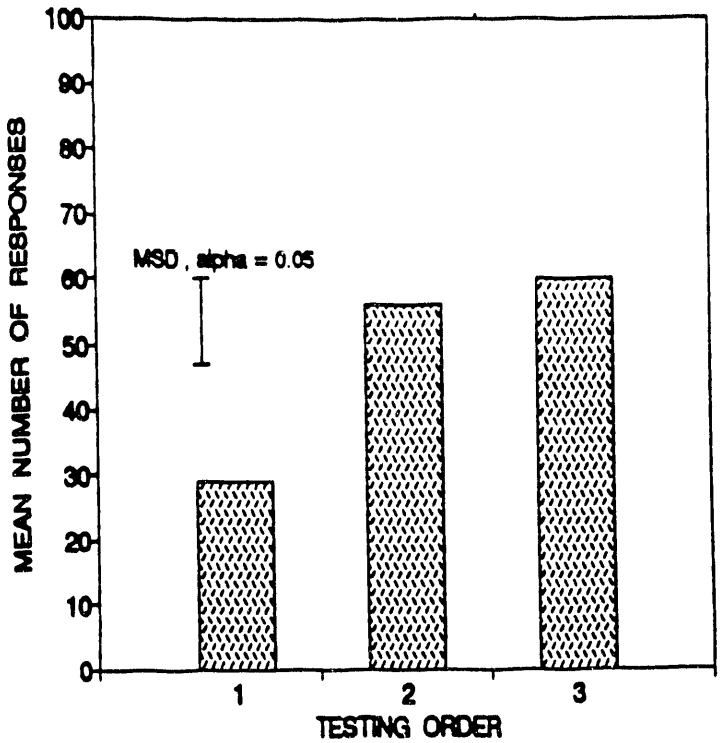

FIGURE 9. Bar graph of the number of righting responses made by juvenile snapping turtles, Chelydra serpentina, (n=36) verses order of testing when tested at ambient 25°C. Tukey's multiple range test was used to clarify differences among means (minimum significant difference = 12 responses) after data were analyszed with a model I 4 X 3 factorial ANOVA.

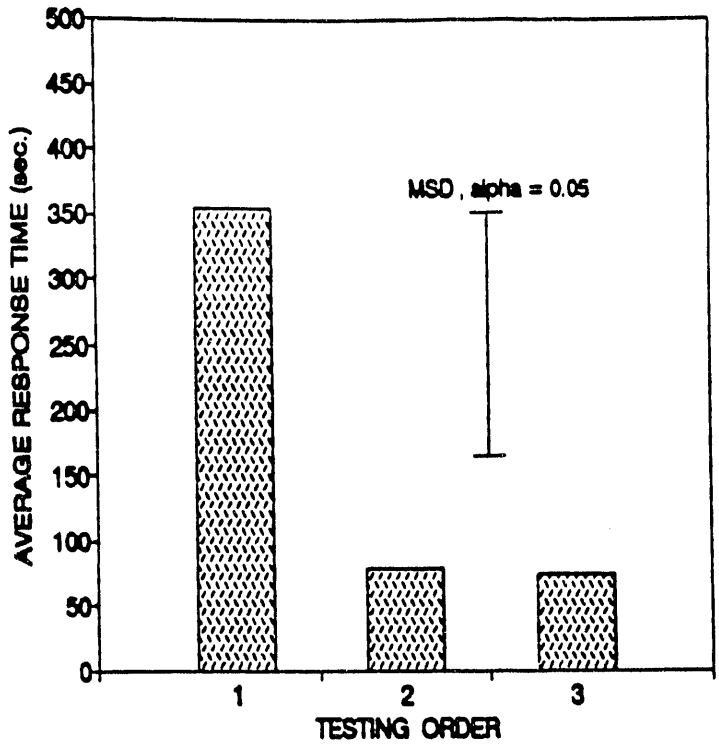

FIGURE 10. Bar graph of the number of average response time of juvenile snapping turtles, Chelydra serpentina, (n=36) verses order of testing when tested at ambient 25°C. Tukey's multiple range test was used to clarify differences among means (minimum significant difference = 181 seconds) after data were analyszed with a model I 4 X 3 factorial ANOVA.

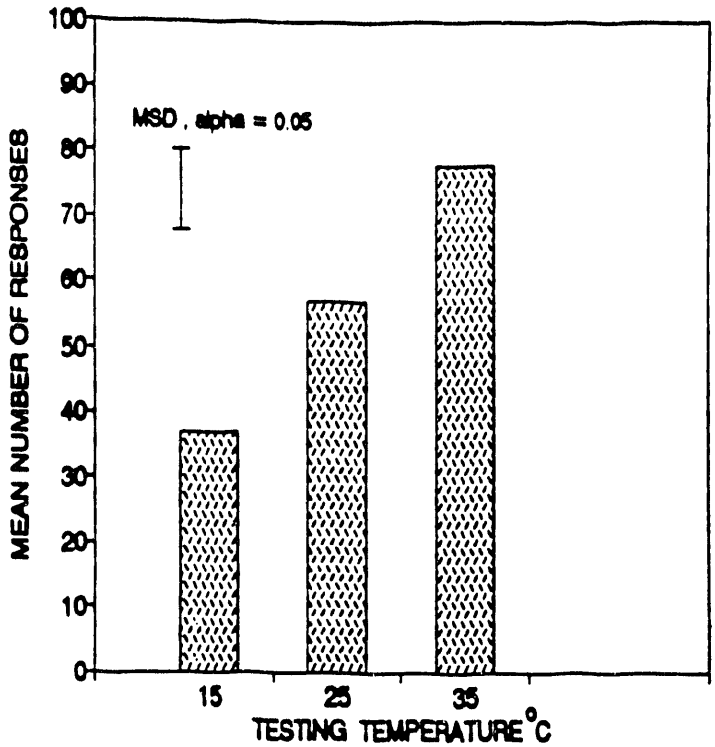

FIGURE 11. Bar graph of the number of righting responses made by juvenile snapping turtles, Chelydra serpentina, (n=40) verses testing temperature when tested at 15°C, 25°C, and 35°C ambient temperatures. Tukey's multiple range test was used to clarify differences among means (minimum significant difference = 12 responses) after data were analyszed with a model I 4 X 3 factorial ANOVA.

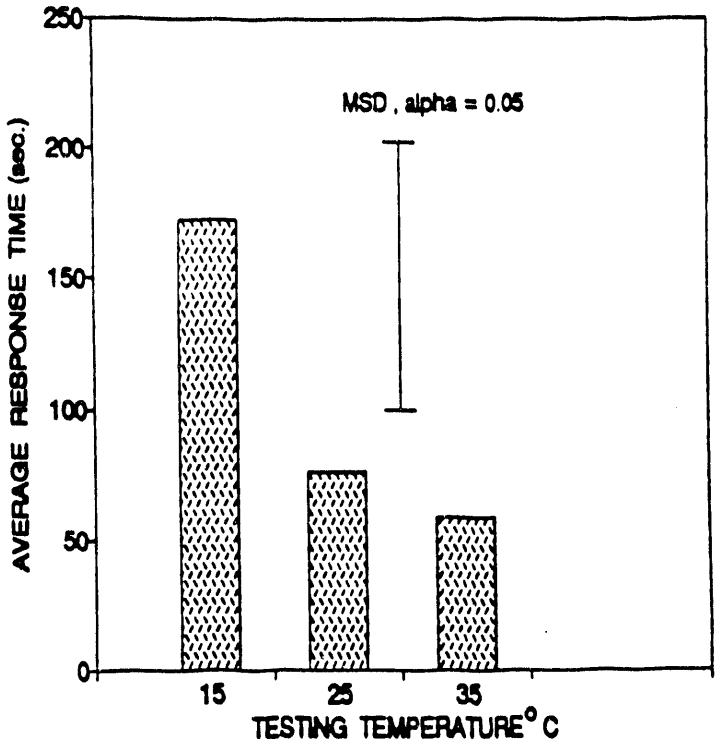

FIGURE 12. Bar graph of the average response time of juvenile snapping turtles, Chelydra serpentina (n=40), verses testing temperature when tested at 15°C, 25°C and 35°C ambient temperatures. Tukey's multiple range test was used to clarify differences among means (minimum significant difference = 104 seconds) after data were analyszed with a model I 4 X 3 factorial ANOVA.

TABLE 1. One-way ANCOVA table showing the effect of incubation condition on the mean mass of hatchling snapping turtles, *Chelydra serpentina* (n=104). The dependent variable is mass in grams. The covariate is the initial eggmass.

ANCOVA TABLE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P
Incubation Condition	3	3.4120	1.1373	5.65	0.0013
Eggmass	1	39.2044	39.2044	194.82	0.0001
Error	99	19.9223	0.2012	---	---

TABLE 2. One-way ANOVA showing the effects of incubation condition on the mass gain of hatchling snapping turtles, *Chelydra serpentina* (n=44) over a nine month growth period. The dependent variable is mass in grams.

ANOVA TABLE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P
Incubation Condition	3	1998.64	666.21	5.35	0.0034
Error	40	4981.59	124.54	---	---

TABLE 3. Model I 4 X 3 factorial ANOVA table showing the lack of stastical significance of the effects of incubation condition and testing order on the feeding response time of 16 month old snapping turtles, Chelydra serpentina (n=24). The dependent variable is time in seconds.

ANOVA TABLE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P
Incubation Condition	3	1878.82	626.27	0.18	0.9101
Testing Order	2	2437.69	1218.85	0.35	0.7071
Inc. Cond. * T. Order	6	11859.31	1976.55	0.57	0.7562
Error	60	209767.17	3496.12	---	----

TABLE 4. Model I 4 X 3 factorial ANOVA table showing the effects of incubation condition and testing order on the latency period prior to the first righting response of juvenile snapping turtles, *Chelydra serpentina*, (n=36) tested at the ambient temperature of 25 °C. The dependent variable is time in seconds.

ANOVA TABLE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P
Incubation Condition	3	88894.62	29631.54	0.21	0.8869
Testing Order	2	2066242.46	1033121.23	7.44	0.0010
Inc. Cond. * T. Order	6	194007.02	32334.50	0.23	0.9649
Error	96	13332037.79	138875.39	---	---

TABLE 5. Model I 4 X 3 factorial ANOVA table showing the effects of incubation condition and testing order on the total number of righting responses performed by young snapping turtles, Chelydra serpentina, (n=36) at the ambient testing temperature of 25°C. The dependent variable is the total number of completed righting responses.

ANOVA TABLE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P
Incubation Condition	3	2451.07	817.02	0.36	0.7805
Testing Order	2	20018.72	10009.36	4.44	0.0144
Inc. Cond. * T. Order	6	3331.87	555.31	0.25	0.9398
Error	96	216604.00	2256.29	---	---

TABLE 6. Model I 4 X 3 factorial ANOVA showing the effects of incubation condition and testing order on the average response time of young snapping turtles, *Chelydra serpentina*, (n=36) at the ambient testing temperature of 25°C. The dependent variable is average time in seconds.

ANOVA TABLE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P
Incubation Condition	3	130693.85	43564.62	0.31	0.8172
Testing Order	2	1858136.06	929068.03	6.64	0.0020
Inc. Cond. * T. Order	6	137945.87	22990.98	0.16	0.9856
Error	96	13437476.22	139973.71	---	---

TABLE 7. Model I 4 X 3 factorial ANOVA table showing the lack of statistical significance of the effects of incubation condition and ambient testing temperature on the latency period prior to the first righting response of juvenile snapping turtles (n=40) tested at three ambient temperatures (15°C, 25°C, 35°C). The dependent variable is time in seconds.

ANOVA TABLE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P
Incubation Condition	3	58693.83	19564.61	0.25	0.8590
Test Temperature	2	299903.72	149951.86	1.94	0.1488
Inc. Cond. * T. Temp.	6	431455.95	71909.33	0.93	0.4766
Error	108	8350537.10	77319.79	---	-----

TABLE 8. Model I 4 X 3 factorial ANOVA showing the effects of incubation condition and ambient testing temperature on the total number of righting responses made by juvenile snapping turtles (n=40) tested at three ambient temperatures (15°C, 25°C, 35°C). The dependent variable is the number of completed responses.

ANOVA TABLE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P
Incubation Condition	3	15052.16	5017.39	2.01	0.1166
Test Temperature	2	32643.47	16321.73	6.54	0.0021
Inc. Cond. * T. Temp.	6	4954.47	825.74	0.33	0.9193
Error	108	269388.50	2494.34	---	-----

TABLE 9. Model I 4 X 3 factorial ANOVA showing the effects of incubation condition and ambient testing temperature on average response time of juvenile snapping turtles (n=40) tested at three ambient temperatures (15°C, 25°C, 35°C). The dependent variable is average response time in seconds.

ANOVA TABLE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P
Incubation Condition	3	170804.43	56934.81	0.97	0.4077
Test Temperature	2	304710.05	152355.03	2.61	0.0784
Inc. Cond. * T. Temp.	6	213438.42	35573.07	0.61	0.7229
Error	108	6311276.40	58437.74	---	-----

APPENDIX 1. Table of regression equations and the significance of the relationship between the variables.

EQUATION	R^2	P
Hatching Mass = 1.19 + Eggmass (0.65)	0.6139	0.0240
Massgain = 59.49 + Hatchmass (-0.14)	0.0000	0.9541
Massgain = 61.54 + Eggmass (-0.31)	0.0000	0.8858
Y = Number of righting responses made at 25°C :		
Y = -21.79 + Plastron (1.64)	0.0184	0.3582
Y = 67.26 + Carapace (-0.13)	0.0003	0.9085
Y = 40.41 + Mass (0.22)	0.0070	0.5719
Y = 107.54 + Hatching Mass (-5.93)	0.0070	0.5677
Y = 68.07 + Eggmass (-0.90)	0.0002	0.9191
Y = Feeding response time:		
Y = 285.20 + Plastron (-2.71)	0.0108	0.4729
Y = 592.59 + Carapace (-6.73)	0.1002	0.0251
Y = 274.75 + Mass (-1.37)	0.0624	0.4729
Y = -53.50 + Hatching Mass (10.93)	0.0040	0.6623
Y = -191.52 + Eggmass (31.85)	0.0469	0.1309

APPENDIX 2. Table of righting response statistics by trial order of turtles tested at 25°C.

VARIABLE	TRIAL	RANGE	MEAN	STD. ERROR
LATENCY PERIOD (sec)	1	1 - 1800	389.94	97.47
	2	1 - 604	88.10	21.27
	3	1 - 931	105.81	29.21
AVERAGE RESPONSE TIME (sec)	1	7 - 1800	354.81	99.39
	2	5 - 482	79.33	17.12
	3	5 - 931	73.58	26.62
NUMBER OF RESPONSES	1	0 - 152	28.94	6.48
	2	2 - 237	55.58	8.65
	3	1 - 230	59.64	7.73

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