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Effect of Egg-Incubation Temperature on Growth and Selected Temperature of *Apalone Spinifera*, the Spiny Softshell Turtle

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EFFECT OF EGG-INCUBATION TEMPERATURE ON GROWTH AND SELECTED TEMPERATURE OF *APALONE SPINIFERA*, THE SPINY SOFTSHELL TURTLE

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ABSTRACT

In order to survive, turtle hatchlings must be able to choose optimal thermal locations that promote growth and activity through thermoregulation. In some softshell turtle species, embryonic incubation conditions may influence post-hatching phenotypic characters, such as growth rates and temperature preference. I tested the effect of egg-incubation temperature on growth and thermal preferences of *Apalone spinifera* hatchlings, from Butler County, Iowa. Egg-incubation temperature did not affect growth of 10 juvenile *Apalone spinifera* (Reptilia: Testudines: Trionychidae). After 5 months, hatchlings incubated at 30°C did not differ in size from the turtles incubated at 25°C. In an aquatic thermal gradient from 14–34°C, egg-incubation temperature affected temperature selection among 10 *Apalone spinifera* hatchlings. Egg-incubation temperature was directly related to selected temperature in 3–5 month-old hatchling *Apalone spinifera* acclimated to 22°C. Hatchlings incubated at 25°C chose the coldest temperature available (14°C) more frequently than any other temperature available; turtles incubated at 30°C selected the warmest temperature available (34°C) more often than any other temperature. In the gradient tank, turtles from both egg-incubation temperature treatments visited more chambers (4.00 ± 0.2) and relocated (7.56 ± 0.7) more frequently between chambers during single-temperature control tests than during gradient runs. These results indicate that juvenile *A. spinifera* from Iowa can identify temperature differences and select preferred temperatures within at least a 4°C range. This ability to select temperature within a narrow range is a feature that may affect fitness in *Apalone spinifera*.

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INTRODUCTION

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Ectothermic Reptiles

Reptiles are ectotherms. As such, their body temperature is dependent on the warmth of their surroundings (Carroll 1969). This dependence on the environment plays a vital role in their health, physical performance, growth rate, reproduction, and defense mechanisms (Carroll 1969; Smith et al. 1981; Williamson et al. 1989). As a result, ambient temperature may profoundly affect reptile behavior and physiology (Spotila and Standora 1985; Williamson et al. 1989). While many studies on thermal regulatory responses have been conducted on reptilian species, there is little known about thermal regulation in the few turtle species that display genetic sex determination (GSD) (Tamplin 2006, 2009; Feltz and Tamplin 2007).

Softshell turtles are a GSD species and display cryptic behavior that may influence habitat selection as well as their ability to thermoregulate (Graham and Hutchison 1979; Spotila et al. 1984; Nebeker and Bury 2001). North American softshell turtles (*Apalone* spp.) regulate their temperature through aquatic and aerial basking. To insure survival, the hatchlings of softshell turtles must be able to maintain proper body temperature in addition to finding ample food supply, avoiding predators, and socializing (Smith et al. 1981; Graham and Graham 1997; Feltz and Tamplin 2007). In order to promote increased fitness among turtle hatchlings, it is important that optimal environmental and developmental conditions in turtles be understood.

The Influences of Ambient Temperature

Turtles must keep their body at an optimal temperature range to facilitate efficient metabolic function, nutrient digestion, and immune responses (Huey 1982; Hammond et al.

1988; Knight et al. 1990). Thus, it is imperative that hatchlings are capable of sensing temperature differences and locating favorable thermal niches for proper thermoregulation (Tamplin 2006, 2009). Environmental temperatures during embryonic incubation periods influence sex in turtle species that display temperature-dependent sex determination (TSD) (reviewed by Valenzuela 2004; Valenzuela and Lance 2004). Softshell species do not possess TSD, but embryonic temperature may affect their development and post-hatching phenotype (Nebeker and Bury 2001; Feltz and Tamplin 2007; Tamplin 2009).

Temperature Affects Physiology

Egg-incubation temperature affects the potential fitness of turtles and impacts their physiology on several levels. Primarily, the features that are influenced include the following: righting time (inverting after being placed on its backside), ability to move on land and in water, defense mechanisms, growth rate, oxygen consumption, and post-hatching survival (Rhen and Lang 1995; Doody 1999; Nebeker and Bury 2001; Ashmore and Janzen 2003; Feltz and Tamplin 2007; Tamplin 2009). In addition to these factors, incubation temperature may also influence post-hatching phenotypes (e.g., coloration, markings, and shape) as well as temperature selection in at least some TSD species (O'Steen 1998; Rhen and Lang 1995, Tamplin and Cyr in press).

Natural History of Spiny Softshell Turtles

Habitat Preferences and Distribution

The spiny softshell turtle (*Apalone spinifera*) lives in freshwater habitats. Although it is primarily a riverine turtle, *A. spinifera* may also be found in lakes, streams, and ponds. Within these aquatic environments, softshells prefer soft bottoms that are typically composed of sand,

gravel, or mixed sediments (Ernst and Lovich 2009). These substrates allow softshells to burrow easily, a cryptic response used to hide from predators. Shelter is also found under aquatic forms such as fallen trees and branches that are submerged underwater. In Iowa, female softshells prefer open water more than males (Williamson and Christiansen 1981).

Apalone spinifera is endemic to North America. The species ranges west of the Mississippi River, from Minnesota west to Montana and south to Arkansas (Ernst and Lovich 2009). The subspecies native to Iowa is the Western spiny softshell, *Apalone spinifera hartwegi.* It may be found as far south as Louisiana and Oklahoma and north into parts of Minnesota and South Dakota.

Spiny Softshell Anatomy and Physiology

The shell of a spiny softshell turtle is leathery, flat, round, and may or may not possess a low central keel. A pronounced feature of *Apalone spinifera* is their tube-like snout with nostrils that contain a septal ridge (Ernst and Lovich 2009). *Apalone spinifera* also possess small dots on their carapace as hatchlings. Males will retain these spots while females become mottled with blotches. In addition to these traits, all four feet are webbed to aid their locomotion in water (Ernst and Lovich 2009). *Apalone spinifera* are agile swimmers that primarily use their forelimbs to move through the water and bury in the substrate. As a result, spiny softshells typically have twice the amount of forelimb surface area than turtles that possess hard shells (Pace et al. 2001; Ernst and Lovich 2009).

Spiny softshells are a unique group of reptiles because, like fish, they possess the ability to extract oxygen from water. When fully submerged, gas exchange may occur through the skin of *A. spinifera* (Stone et al. 1992; Stone and Iverson 1999). Up to 85% carbon dioxide and 38%

oxygen gas may be exchanged through their skin and allow them to stay underwater for long periods of time. If the water is highly oxygenated, spiny softshells have the ability to stay under water for several days (Reese et al. 2003). Because of their leathery shell, *A. spinifera* has an excess amount of permeable skin that must be kept moist. If a spiny softshell does not receive water within three days, it may dehydrate and die (Ernst and Lovich 2009).

Spiny softshell turtles are able to thermoregulate remarkably well in adverse situations. This is primarily due to the fact that softshells heat faster than they cool (Smith et al. 1981). When placed in the same temperature, the warming heart rates of adult *A. spinifera* are much faster than cooling heart rates. As a hatchling, *A. spinifera* can heat its body up two times faster than it cools down (Smith et al. 1981). These thermal properties allow *A. spinifera* to maintain optimal temperature ranges for longer time periods than aquatic ectotherms of similar size (Smith et al. 1981).

Reproductive Behavior

In spiny softshell turtles, mating typically occurs in the spring months (April–May), and eggs are laid in the summer (May–August). Eggs are preferably laid in sand and close to a water source to insure the eggs are kept moist throughout development (Ernst and Lovich 2009). During the incubation period, temperature does not determine the sex of the softshell. Their sex is determined genetically (GSD), but which, or if, specific chromosomes determine their sexual dimorphic features have yet to be determined in this species (Bull and Vogt 1979; Doody 1999; Greenbaum and Carr 200; Janzen and Paukstis 1991). During embryonic development, differentiation of the sexes appears to happen at the same time as in TSD turtle species, but softshells possess a much lower concentration of testosterone and estradiol-17β. This further

indicates that softshells utilize a different strategy in sex determination than TSD turtles (Janzen and Paukstis 1991; Janzen et al. 1998).

When spiny softshells reach adulthood, females are typically much larger than males. Adult spiny softshell females are roughly 1.6 times larger than males (Ernst and Lovich 2009). The average plastron length in males is 8–10 cm whereas female plastron length is approximately 18–20 cm. Adult males are identified by their thick, long tails and, as adults, their shells possess dark ocelli, spots, and lines (Ernst and Lovich 2009). Sexually mature females lose much of their dark spotting and display a blotchy, mottled appearance. In addition to their coloration, females possess short, small tails. Sexual dimorphism can also be seen in spiny softshell hatchlings. The coloring of females tends to be brownish olive while males often display hues of gray (Graham and Cobb 1998; Ernst and Lovich 2009).

General Behavior and Feeding Habits

North American Softshell turtles are active during the day and are highly aquatic. During periods of activity, they spend most of their time searching for food or basking in shallow water (Williams and Christiansen 1981; Graham and Graham 1997). At night they tend to sleep in submerged vegetation or are buried under lake or river sediments. In Iowa, *A. spinifera* is typically active from April through October and hibernates during winter. Adults are more likely to be active during colder months than juveniles (Ernst and Lovich 2009).

Apalone spinifera are carnivorous; they are known to eat the following items: crayfish, snails, insects, fish, small amphibians and snakes (Ernst and Lovich 2009). In Iowa, Williams and Christiansen (1981) reported that the diet of spiny softshell turtles consists of approximately 55% crayfish, 42% fish, and 25% insects. To find such food items, *A. spinifera* will force their

snouts into dense vegetation or probe under pebbles and vigorously bite their prey (Ernst and Lovich 2009).

Socialization between spiny softshell turtles is often aggressive. In captivity, larger *A. spinifera* tend to dominate smaller individuals, primarily in competition for resources (Ernst and Lovich 2009). These aggressions occur particularly between males in the form of biting. In contrast, spiny softshells do not show these aggressive tendencies when interacting with other hard shelled turtle species (Ernst and Lovich 2009).

The primary defense mechanism of spiny softshell turtles is violent biting and scratching (Ernst and Lovich 2009). *Apalone spinifera* possess long, thick necks that allow them to whip their heads around and bite a predator with much vigor. A secondary defense mechanism is illustrated by its cryptic coloration and burrowing behavior. According to Ernst and Lovich (2009), *A. spinifera* produce specific levels of melanin to match the bottom substrate that they inhabit.

Behavior of Turtles in Thermal Gradients

In laboratory settings, the substrate provided may influence the behavior of softshell turtles. Utilizing an aquatic thermal gradient, Nebeker and Bury (2001) reported that smooth softshells (*Apalone mutica*) selected a higher temperature (27°C) with a sand substrate and did not show a temperature preference without a substrate. In addition to this study, Feltz and Tamplin (2007) determined that spiny softshell turtles (*Apalone spinifera hartwegi*) from Iowa chose the warmest temperature (30°C) available across various substrate treatments (i.e., sand, gravel, and no substrate). Further testing examined the effect of sand removal from the two

warmest chambers. Under these conditions, *A. spinifera* chose the warmest temperature (24°C) with a sand substrate demonstrating that substrate may affect temperature selection.

Egg-incubation and acclimation temperature may also play an important role in how turtles respond to a thermal gradient. In a laboratory setting, Tamplin and Cyr (in press) found that temperature selection in Western painted turtles (*Chrysemys picta bellii*), a TSD species, is inversely related to incubation temperature. Painted turtles incubated at 30°C chose the coldest temperature (14°C) available, and those incubated at 27.5°C chose the warmest temperature (34°C) (Tamplin and Cyr, in press). Tamplin (2006, 2009) demonstrated that juvenile wood turtles (*Glyptemys insculpta*), a GSD species, acclimated to 20°C chose the warmest temperature available (27°C) and avoided the coldest temperature (12°C) in a thermal gradient of 12–27°C.

Selection of high temperatures has been previously demonstrated in a laboratory setting by common snapping turtles (*Chelydra serpentina*), painted turtles (*Chrysemys picta*), wood turtles (*Glyptemys insculpta*), Florida red-bellied turtles (*Pseudemys nelsoni*), and softshells (*Apalone mutica* and *Apalone spinifera*) (Schuett and Gatten 1980; Rhen and Lang 1995; O'Steen 1998; Bury et al. 2000; Nebeker and Bury 2000, 2001; Feltz and Tamplin 2007; Tamplin 2006, 2009; Tamplin and Cyr, in press). Within aquatic thermal gradients of temperatures in their normal active range, turtle hatchlings and yearlings selected warmer temperatures (27–33°C) within a narrow range. These studies suggest that juvenile turtles are able to detect differences in temperature and may select warmer temperatures to promote thermoregulation.

Statement of Purpose

This study was designed to test the effect of egg-incubation temperature on future growth rates and the thermal preferences of *Apalone spinifera* hatchlings, a turtle species that exhibits genetic sex determination (GSD). Data produced by the present study on *A. spinifera* was compared to previously generated data from GSD species (wood turtles, *Glyptemys insculpta*) and TSD species (common snapping turtles, *Chelydra serpentina*, and painted turtles, *Chrysemys picta*).

HYPOTHESES TESTED

This study tested the effect of egg-incubation temperature on growth and selected temperature of 10 *Apalone spinifera* hatchlings from Butler County, IA. I tested the following hypotheses: 1) that high incubation temperature correlates with larger body size at hatching and juvenile size at 3–6 months of age when maintained at the same ambient temperature (22°C); and 2) that both egg incubation treatment groups will select warmer temperatures in an aquatic thermal gradient when given temperature choices within their normal active range.

MATERIALS AND METHODS

Egg Collection and Incubation

Fourteen *Apalone spinifera* eggs from the same nest were collected in Butler County, Iowa on 29 June 2009 from a sand bank adjacent to the West Fork Cedar River. The eggs were numbered, weighed (± 0.01g), measured (± 0.01mm), and incubated at one of two different temperatures (25 and 30°C). The total clutch wet mass was 134.5 g. Mean (± SD) egg mass (11.21 ± 0.92 g); range = 9.69–12.60 g and mean (± SD) diameter (27.21 ± 0.70 mm); range = 26.20 –28.44 mm was also calculated for the nest. During incubation, the eggs were placed in a moist sand substrate (see Feltz and Tamplin 2007) and incubated at one of the two treatment temperatures. Hatching occurred between 60 and 99 days post-nesting date. The warmer (30°C) incubation group hatched approximately one month earlier (28–29 August 2009) than the colder (25°C) incubation group (27 September – 6 October 2009). Twelve of the 14 eggs were fertile, and 10 hatchlings (5 from each treatment group) were selected for further analysis.

Hatchling Care

Hatchlings were identified by their egg number. Non-toxic white paint was applied to the carapace in order to identify individual turtles (Galbraith and Brooks 1984; Priest and Franklin 2002). Hatchlings were housed in separate 1.4 L chambers that measured 35 (l) x 17.5 (w) x 12 (h) cm. The turtles were maintained at 22 ± 0.5°C and the maintenance chambers possessed the following features: pea gravel substrate with wet (water depth = 6 cm) and dry areas, artificial vegetation, and fluorescent and UVA and UVB lamps (12h light: 12h dark). The tubs were cleaned on a weekly basis with hot water and anti-bacterial soap and rinsed thoroughly with reverse osmosis filtered water. In addition to a weekly cleaning, fresh water and waste removal

was provided every other day. Turtles were fed pelleted turtle food, crushed fish food flakes, and dried miniature shrimp 3–4 days per week. Additionally, turtles were fed approximately 24 hours before monthly growth measurements and experimental test runs in the gradient tank.

By three months of age, the turtles were large enough to easily withstand conditions of the gradient tank. Hatchlings were tested in two groups of five turtles based on their incubation temperature. Gradient and control tests were run in a sequential order from January to April 2010 in a way that permitted each turtle from each treatment group to be tested an equal number of times.

Measuring Growth

Growth was recorded monthly during the experimental period. Hatchling growth was based on eight morphological parameters: mass (M), carapace length (CL), plastron length (PL), head width (HW), shell width (SW), shell height (SH), plastron to vent length (PV), vent to tail length (VT), and total tail length (TTL). Mean (±SD) measurement values for M, CL, and PL were averaged to determine the body size of the turtles during the testing period.

Temperature Gradients

The gradient tank (Figure 1) measured 176 (l) x 84 (w) x 15 (h) cm. Within the tank, there were 6 chambers of equal size [69 (l) x 27 (w) x 15 (h) cm] and a common area [15 (l) X 176 (w) x 15 (h) cm]. The common area served as a passageway for turtles to move in and out of each chamber. The tank and chamber walls were composed of molded polyethylene. Insulating board, 5–7cm, thick was also used to cover the outer tank and interior of each chamber wall. Reverse osmosis water used in the gradient tank was thermally controlled, filtered, and aerated in

one of two 1500 L insulated source tanks and pumped through an insulated manifold into the gradient tank. Chamber temperatures were regulated by dispensing varying amounts of warm water (36°C) and cold water (10°C) to one end of each chamber. This regulation was done by mixing water at the head end, aerating it, and then sending it to an outlet valve at the opposite end of each chamber. I controlled water flow rate with needle valves and adjusted them throughout each test period in order to maintain the desired temperature in each chamber. The flow rate varied between 1–1.8 L per minute per chamber. Each chamber held the following items: two aeration stones (a 15 cm bar at the start of the chamber and a 45 cm bar that ran the length of the chamber), a 1 cm sand substrate, and artificial vegetation. This vegetation served as a resting area and provided cover for the turtles.

Water temperature (± 0.1°C) was measured during each experimental run with two remote input temperature probes in each chamber; one was inserted near the head of the chamber and another was placed in the middle of the chamber. I used high flow rates (1–1.8 L per minute) and through aeration to prevent the formation of thermal pockets, to eliminate thermal stratification, and to minimize any potential temperature fluctuation within chambers. Within each chamber, temperature variation was generally ≤ 1.5°C, and all chambers were maintained at temperatures within 2.3°C of the target temperature. Occasionally, aeration was briefly interrupted to assist in locating a cryptic turtle; these interruptions produced an occasional momentary temperature spike that in each case was immediately restored back to the target temperature.

In gradient tests, the turtles were provided a choice of six temperatures ranging between 14 and 34°C, at 4°C intervals. For control runs, all chambers were maintained at 22°C with 0°C

intervals. All runs were performed under lighting similar to the holding area of the turtles and occurred between 12:00 and 19:30 hours.

Chambers were assigned a temperature for gradient runs (n = 5, total gradient observations = 720 for each treatment group) and control chambers were numbered (n = 5, total control observations = 720 for each treatment group). In gradient testing, chamber temperatures were arranged in one of two ways: sequential temperatures from high to low, and randomly assigned temperatures among the six chambers. The sequential arrangement was reversed on alternating test days, so that the high and low extremes were in opposite chambers of the previous run. The mixed arrangement was run the same number of times as that of the sequential arrangement. Control and gradient tests were also alternated. For gradient tests, the turtles were placed in the common area at a location that was closest to their acclimation temperature (22°C). For control tests, all chambers were maintained at 22°C, so the turtles were put in the same location as the previous gradient run. Once in the gradient tank, the turtles were given 1 hour to habituate to their surroundings. After 1 hour, observations of individual location began.

For each test, I recorded the location of each turtle every 10 minutes for 3 hours (18 total observations per individual). If a turtle was located in the common area, they were assigned to the chamber with the closest proximity. Turtles that switched chambers between 10 minute observations were noted as relocated.

Data Analysis

Growth between the two treatment groups was compared through unpaired t-tests (Abacus Concepts, 1994). Egg size, mean M, CL, and PL were analyzed at hatching size, testing

size, and final size (i.e., at the end of testing) to determine if significant differences (alpha = 0.05) existed between egg-incubation treatments.

The following calculations were computed for both control and gradient tests: mean (± SE) number of chamber relocations per test, percentage of observations involving relocation, and mean (± SE) number of chambers visited. The first observation could not be considered a potential relocation; as a result, the maximum number of chamber relocations per individual per test was 17. In addition, the mean number of observations at each temperature was calculated for gradient runs, and for each chamber in control runs, and compared by egg-incubation temperature. One-way ANOVA (SPSS Inc., Chicago, IL) and unpaired t-tests (SPSS Inc., Chicago, IL) were used to determine if egg-incubation temperature produced significant effects ($p < 0.05$) on movement and activity. If a significant difference existed, multiple comparisons tests (Tukey B) were run to determine if egg-incubation temperature affected temperature (or chamber) selection, the mean number of chambers used, and the mean number of relocations. These data were then compared to data previously generated from other GSD and TSD species (wood turtles, common snapping turtles and painted turtles).

RESULTS

Egg Size

Unpaired t-test analysis indicated that mean (±SD) egg mass and mean (±SD) egg diameter were not significantly different (Egg Mass: d.f. = 8, t-value = 0.969, p = 0.3609; Egg Diameter: d.f. = 8, t-value = 0.418, p = 0.6866) between egg-incubation treatment groups. Mean (±SD) egg mass of the 30°C incubation treatment group was 10.90 ± 0.82 g, and mean (±SD) egg diameter was 27.18 ± 0.75 mm. Mean (±SD) egg mass of the 25°C treatment mean (±SD) egg mass was 11.57 ± 1.16 g and mean (±SD) egg diameter was 27.38 ± 0.76 mm.

Post-Hatching Growth

The 30°C incubation treatment mean (± SD) hatching mass was 8.90 ± 0.64 g; range = 7.94–9.73 g, mean (± SD) CL was (42.47 ± 2.31 mm); range = 39.04–45.54 mm, and mean (± SD) PL was 29.95 ± 0.60 mm; range = 28.99–30.34 mm. Mean (± SD) hatching mass of 25°C incubation treatment was 8.32 ± 0.92 g; range 6.82–9.00 g, mean (± SD) CL was 40.27 ± 2.79 mm; range 35.63–42.67 mm, and mean (± SD) PL was 29.48 ± 1.05 mm; range 28.09–30.77 mm. Unpaired t-tests indicated that mean hatching mass, CL, and PL were not significantly different (M: d.f. = 8, t-value = 1.163, p = 0.2784; CL: d.f. = 8, t-value = 1.358, p = 0.2114; PL: d.f. = 8, t-value = 0.846, p = 0.4129) between the treatment groups.

Body mass during testing (3–6 months) was also not significantly different (M: d.f. = 28, t-value = 1.047, p = 0.0989) between temperature treatment groups. The mean (±SD) mass for 30°C incubation group was 9.58 ± 1.05 g; range 8.12–11.62 g. The 25°C incubation group mean (±SD) mass was 9.00 ± 0.82 g; range 7.55–10.30 g. In contrast, the testing size of 30°C incubation treatment mean (±SD) CL was 44.48 ± 1.07 mm; range 41.69–45.72 mm and mean

(±SD) PL was 32.86 ± 1.14 mm; range 31.20–35.16 mm. The 25°C incubation treatment mean (±SD) CL was 42.87 ± 2.07 mm; range = 38.28–45.08 mm and mean (±SD) PL was 31.67 ± 1.22 mm; range 29.24–34.10 mm. Unpaired t-tests indicated that the testing CL and PL lengths were significantly different (CL: d.f. = 28, t-value = 2.677, $p = 0.0123$; PL: d.f. = 28, t-value = 2.756, $p = 0.0102$) between treatment groups.

After 5 months, body size did not differ between treatment groups (M: d.f. = 8, T-value = 1.672, $p = 0.1332$; CL: d.f. = 8, t-value = 1.674, $p = 0.1326$; PL: d.f. = 8, t-value = 1.640, $p = 0.1395$). The 30°C incubation treatment final mean (± SD) mass (10.22 ± 1.30 g); range = 8.12–11.62 g, mean (± SD) CL (44.95 ± 0.62 mm); range 44.33–45.70 mm, and mean (± SD) PL (33.53 ± 1.35 mm); range = 31.45–35.16 mm were similar to the 25°C incubation treatment final mean (± SD) mass (9.05 ± 0.87 g); range = 7.98–10.30 g, mean (± SD) CL (44.95 ± 0.62 mm); range 39.65–44.90 mm, and mean (± SD) PL (32.23 ± 1.36 mm); range 30.94–34.10 mm (Table 9 and 10).

Temperature Selection

Across all gradient tests, egg-incubation temperature of *A. spinifera* 3–5 month old hatchlings affected temperature selection (Figure 2 and 3). The warmer (30°C) egg-incubation treatment group chose the warmest temperature available (34°C = 34.0% of observations) more often than any other temperature (Table 1). The colder (25°C) egg-incubation treatment group selected the coldest temperature available (14°C = 42.2% of observations) more frequently than any other temperature (Table 2).

In gradient tests, one-way ANOVA indicated that chamber temperature significantly (30°C Incubation Treatment: d.f. = 5, F-value = 4.563, $p = 0.002$; 25°C Incubation Treatment:

d.f. = 5, F-value = 4.972, $p = 0.001$) influenced chamber selection of both treatment groups. Tukey B multiple comparisons tests indicated that turtles incubated at 30°C chose 34°C significantly (alpha = 0.05) more often than other temperatures across all gradient tests. In hatchlings incubated at 25°C, turtles chose 14°C significantly (alpha = 0.05) more often across all gradient tests.

Movement and Activity

Egg-incubation temperature affected movement and activity patterns in both aquatic thermal gradient and control tests. Turtles from both incubation groups relocated between chambers more often in single-temperature control tests (mean ± SE) than during gradient tests. Unpaired t-tests indicated that more chamber relocations occurred during control tests than gradient runs between both incubation groups (d.f. = 14, t-value = -7.747, $p < 0.0005$). Chamber relocations between treatment groups were not significantly different during gradient tests (d.f. = 14, t-value = -1.186, $p = 0.255$). Hatchlings incubated at 25°C generated 4.73 (± 1.0) relocations for gradient tests and 10.10 (± 0.4) for control tests. For hatchlings incubated at 30°C, the number of chamber relocations for gradient tests was 3.33 (± 0.6) and 5.03 (± 0.5) for control tests.

In addition to relocations, both incubation groups visited significantly more chambers during control tests (d.f. = 14, t-value = -5.051, $p < 0.0005$) than gradient tests (d.f. = 14, t-value = -1.654, $p = 0.120$). Hatchlings incubated at 25°C visited a mean (±SE) chamber number of 3.38 (± 0.4) in gradient tests and 4.63 (± 0.1) in control tests. Turtles incubated at 30 °C visited 2.68 (± 0.2) chambers in gradient tests and 3.38 (± 0.2) chambers in control tests. In control tests for the 25°C incubation group, 75.3% (± 1.7%) of the hatchlings changed chambers at least once;

in gradient tests, 56.3% (± 6.4%) of the turtles changed temperatures at least once. 56.3% (± 3.5%) of hatchlings incubated at 30°C changed chambers at least once during control tests; in gradient tests, 44.6 (± 2.9%) hatchlings changed temperatures at least once (Tables 5–8).

During control tests run at their acclimation temperature (22°C), both treatment groups showed a slight preference for the end chambers of the tank, yet most runs produced no sequential patterns (Table 3 and 4). The turtles' tendency to choose the end chambers during control tests may be attributed to the rectangular setup of the tank, rather than a preference for a specific chamber over another. In a control setting, the hatchlings would often swim in the common areas of the tank, stop when they reached the end of the tank, and subsequently enter the end chambers. This trend was found particularly in turtles incubated at 25°C during control runs. Multiple comparisons tests (Tukey B) indicated that turtles incubated at 25°C relocated significantly (alpha = 0.05) more frequently than 30°C incubation treatment. Additionally, hatchlings incubated at 25°C visited significantly more chambers in control tests than 30°C incubated turtles. To avoid a bias of preference for the ends of the tank, the sequential temperature patterns in the gradient were reversed, and an equal number of runs with the temperatures mixed (i.e., the extreme temperatures located interiorly) were performed during gradient tests.

DISCUSSION

Compared to adults of the same species, turtle hatchlings are more susceptible to predation and have higher mortality rates. Juvenile turtles possess smaller body mass than adult turtles making them more vulnerable to changes in environmental factors. For survival, hatchlings must be able to maintain proper body temperature in addition to finding ample food supply and avoiding predators (Graham and Graham 1997). To increase fitness, turtle hatchlings must be able to choose optimal thermal locations that promote growth and activity through thermoregulation. Selection of warmer temperature niches are correlated with increased growth rates for turtles, because it facilitates proper metabolic function, nutrient digestion, and immune responses (Huey 1982; Hammond et al. 1988; Knight et al. 1990).

Egg-Incubation Influences Growth

Egg-incubation temperature did not affect total growth of juvenile *Apalone spinifera hartwegi*, from Butler County, Iowa. Hatchlings incubated at 25°C and 30°C did not significantly differ at the beginning or end of the 5–6 month measuring period. My hypothesis (1), that high incubation temperature correlates with a larger hatching size and juvenile (3–6 months) size maintained at the same ambient temperature, was rejected. Results did indicate that testing size of 30°C incubation group was significantly larger in carapace and plastron length, but mass was not significantly different between the two treatment groups. This difference in size during testing may be due to the 1 month difference in hatch dates between treatment groups, or it may be an artifact of the small sample size. Because they were larger, the 30°C incubation group possessed more surface area, thus they could potentially lose heat faster than the 25°C incubation group. This heat loss may further explain the preference for warm temperatures demonstrated by

the 30°C incubation treatment. By the time the experiment concluded, mean body size of turtles incubated at 25°C was equivalent to the mean body size of the 30°C incubation group.

Egg-Incubation Influences Temperature Selection

Different embryonic incubation temperatures (25, 30°C) affected temperature selection in 3–5 month old *Apalone spinifera hartwegi* acclimated to 22°C. Turtles incubated at 25°C chose colder temperatures in the gradient; hatchlings incubated at 30°C chose warmer temperatures in the gradient. My hypothesis (2), that both incubation treatment groups will select warmer temperatures when given temperature choices within their normal active range, was partially rejected. Turtles incubated at 30°C chose warmer temperatures, whereas the 25°C incubation group chose colder temperatures. Mean comparisons of temperature selected between incubation groups indicated that 34°C was chosen significantly more often by hatchlings incubated at 30°C, but 14°C was chosen significantly more by turtles incubated at 25°C. This indicates that juvenile *A. spinifera* may choose temperatures directly related to their egg-incubation temperature.

These results do not correspond with the data from common snapping turtles, *Chelydra serpentina*, and painted turtles, *Chrysemys picta,* both of which are TSD species. These two TSD species displayed temperature preferences that were inversely related to incubation temperature in an aquatic thermal gradient (O'Steen, 1998; Rhen and Lang, 1999; Tamplin and Cyr, in press). Data from the present study suggest that *Apalone spinifera* from Butler County, Iowa may be directly influenced by egg-incubation temperature.

In both control and gradient tests, the turtles typically remained cryptic and were buried in the sand substrate. Occasionally, turtles would bask in the artificial vegetation or move within the chamber. Movement within a chamber was primarily caused by interactions with other

turtles. Generally, interactions were neutral and passive, yet some aggressive (chasing, head butting, and biting) and submissive behavior (head withdrawal) was observed. In gradient runs, turtles from both incubation groups would sometimes enter the common area, switch to a new chamber for a few minutes, and then go back to the previous chamber visited. In control runs, turtles from both incubation groups were more active and changed chambers frequently; in these runs, turtles were found swimming in the common areas more often as well.

Conclusion

This evidence indicates that juvenile North American *Apalone spinifera hartwegi* from Iowa can identify temperature increments and select preferred temperatures within at least a 4°C range. Data from the present study suggest that embryonic thermal experience may have a profound effect on future temperature selection but not on body size of juvenile *A. spinifera* after 5 months of age. In an aquatic thermal gradient of 14–34°C, *A. spinifera* hatchlings selected temperatures that corresponded directly to their egg-incubation temperature (i.e., 25°C incubation group chose 14°C; 30°C incubation group chose 34°C).

These results may provide insight to the variability of previous studies thermoregulatory behavior of turtles. Causes of thermoregulatory behavior in turtles are complex and possibly differ between species and populations (e.g., *Chelydra serpentina, Chrysemys picta, Glyptemys insculpta, Apalone mutica* and *Apalone spinifera*) (Rhen and Lang 1995; O'Steen 1998; Nebeker and Bury 2001; Tamplin 2006, 2009; Tamplin and Cyr, in press). As a result, thermal preferences from more turtle species, and the environmental factors that influence them, need to be further identified. With more evidence, a better understanding of the developmental factors that influence environmental selection patterns in turtles may be revealed. The present study may

be improved by using a longer testing period for morphometric size data, increasing the sample size, incorporating eggs from different nests, and utilizing a circular tank to avoid the observed end chamber preferences during control runs. It is imperative that optimal environmental and developmental conditions in turtles be understood, so that conservation and management programs can better promote increased fitness among turtle offspring.

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TABLES

Table 1. Number of observations of 5 spiny softshell turtle (*Apalone spinifera*) hatchlings (4–6 months old), incubated at 30°C and acclimated to 22°C, at 10 minute intervals for 3 hours, at each temperature in an aquatic thermal gradient tests of 14–34°C.

Gradient: 30°C Incubation Treatment

Test	14°C	18°C	22°C	26°C	30°C	34°C	Total # of Observations
1	22 (24.4%)	20 (22.2%)	20 (22.2%)	2 (2.2%)	6 (6.7%)	20 (22.2%)	90 (99.9%)
2	42 (46.7%)	18 (20.0%)	1 (1.1%)	14 (15.6%)	9 (10.0%)	6 (6.7%)	90 (100.1%)
3	1 (1.1%)	0 (0.0%)	15 (16.7%)	23 (25.6%)	2 (2.2%)	49 (54.4%)	90 (100.0%)
4	0 (0.0%)	33 (36.7%)	7 (7.8%)	4 (4.4%)	14 (15.6%)	32 (35.5%)	90 (100.0%)
5	26 (28.9%)	19 (21.1%)	5 (5.6%)	1 (1.1%)	7 (7.8%)	32 (35.6%)	90 (100.1%)
6	17 (18.9%)	7 (7.8%)	19 (21.1%)	3 (3.3%)	1 (1.1%)	43 (47.8%)	90 (100.0%)
7	1 (1.1%)	11 (12.2%)	19 (21.1%)	12 (13.3%)	20 (22.2%)	27 (30.0%)	90 (99.9%)
8	1 (1.1%)	9 (10.0%)	27 (30.0%)	9 (10.0%)	8 (8.9%)	36 (40.0%)	90 (100.0%)
Sum (%)	110 (15.3%)	117 (16.3%)	113 (15.7%)	68 (9.4%)	67 (9.3%)	245 (34.0%)	720 (100.0%)

Number of Observations

Table 2. Number of observations of 5 spiny softshell turtle (*Apalone spinifera hartwegi*) hatchlings (3–5 months old), incubated at 25°C and acclimated to 22°C, at 10 minute intervals for 3 hours, at each temperature in an aquatic thermal gradient tests of 14–34°C.

Gradient: 25°C Incubation Treatment

Test	14°C	18°C	22°C	26°C	30°C	34°C	Total # of Observations
1	78 (86.7%)	3 (3.3%)	0 (0.0%)	1 (1.1%)	5 (5.6%)	3 (3.3%)	90 (100.0%)
2	82 (91.1%)	7 (7.8%)	1 (1.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	90 (100.0%)
3	53 (58.9%)	2 (2.2%)	13 (14.4%)	8 (8.9%)	3 (3.3%)	11 (12.2%)	90 (99.9%)
4	32 (35.6%)	14 (15.6%)	11 (12.2%)	13 (14.4%)	12 (13.3%)	8 (8.9%)	90 (100.0%)
5	12 (13.3%)	11 (12.2%)	7 (7.8%)	8 (8.9%)	20 (22.2%)	32 (35.6%)	90 (100.0%)
6	30 (33.3%)	9 (10.0%)	2 (2.2%)	12 (13.3%	9 (10.0%)	28 (31.1%)	90 (99.9%)
7	8 (8.9%)	23 (25.7%)	4 (4.4%)	30 (33.3%)	14 (15.6%)	11 (12.2%)	90 (100.1%)
8	9 (10.0%)	21 (23.3%)	22 (24.4%)	7 (7.8%)	10 (11.1%)	21 (23.3%)	90 (99.9%)
Sum (%)	304 (42.2%)	90 (12.5%)	60 (8.3%)	79 (11.0%)	73 (10.1%)	114 (15.8%)	720 (99.9%)

Number of Observations

Table 3. Number of observations of 5 spiny softshell turtle (*Apalone spinifera hartwegi*) hatchlings (4–6 months old), incubated at 30°C and acclimated to 22°C, at 10 minute intervals for 3 hours, at each chamber in control tests (n = 720). Control chambers were recorded for each chamber as if gradient was in effect, but temperature of all chambers was 22°C.

Control: 30°C Incubation Treatment

Number of Observations

Test	1	2	3	4	5	6	Total # of Observations
1	14 (15.6%)	15 (16.7%)	19 (21.1%)	2 (2.2%)	5 (5.6%)	35 (38.9%)	90 (100.1%)
2	26 (28.9%)	9 (10.0%)	21 (23.3%)	6 (6.7%)	6 (6.7%)	22 (24.4%)	90 (100.0%)
3	31 (34.4%)	15 (16.7%)	20 (22.2%)	1 (1.1%)	2 (2.2%)	21 (23.3%)	90 (99.9%)
4	22 (24.4%)	5 (5.6%)	0 (0.0%)	3 (3.3%)	43 (47.8%)	17 (18.9%)	90 (100.0%)
5	9 (10.0%)	9 (10.0%)	9 (10.0%)	2 (2.2%)	15 (16.7%)	46 (51.1%)	90 (100.0%)
6	26 (28.9%)	9 (10.0%)	6 (6.7%)	3 (3.3%)	3 (3.3%)	43 (47.8%)	90 (100.0%)
7	30 (33.3%)	3 (3.3%)	10 (11.1%)	3 (3.3%)	13 (14.4%)	31 (34.4%)	90 (99.9%)
8	36 (40.0%)	5 (5.6%)	4 (4.4%)	1 (1.1%)	9 (10.0%)	35 (38.9%)	90 (100.0%)
Sum (%)	194 (26.9%)	70 (9.7%)	89 (12.4%)	21 (2.9%)	96 (13.3%)	250 (34.7%)	720 (99.9%)

Table 4. Number of observations of 5 spiny softshell turtle (*Apalone spinifera hartwegi*) hatchlings (3–5 months old), incubated at 25°C and acclimated to 22°C, at 10 minute intervals for 3 hours, at each chamber in control tests (n = 720). Control chambers were recorded for each chamber as if gradient was in effect, but temperature of all chambers was 22°C.

Control: 25°C Incubation Treatment

Test	1	2	3	4	5	6	Total # of Observations
1	30 (33.3%)	10 (11.1%)	4 (4.4%)	13 (14.4%)	6 (6.7%)	27 (30.0%)	90 (99.9%)
2	29 (32.2%)	7 (7.8%)	17 (18.9%)	1 (1.1%)	8 (8.9%)	28 (31.1%)	90 (100.0%)
3	32 (35.6%)	7 (7.8%)	8 (8.9%)	5 (5.6%)	6 (6.7%)	32 (35.6%)	90 (100.1%)
4	35 (38.9%)	4 (4.4%)	11 (12.2%)	4 (4.4%)	6 (6.7%)	30 (33.3%)	90 (99.9%)
5	37 (41.1%)	18 (20.0%)	9 (10.0%)	6 (6.7%)	3 (3.3%)	17 (18.9%)	90 (100.0%)
6	17 (18.9%)	9 (10.0%)	6 (6.7%)	5 (5.6%)	12 (13.3%)	41 (45.6%)	90 (100.1%)
7	32 (35.6%)	3 (3.3%)	3 (3.3%)	3 (3.3%)	13 (14.4%)	36 (40.0%)	90 (99.9%)
8	44 (48.9%)	7 (7.8%)	1 (1.1%)	4 (4.4%)	5 (5.6%)	29 (32.2%)	90 (100.0%)
Sum (%)	256 (35.6%)	65 (9.0%)	59 (8.2%)	41 (5.7%)	59 (8.2%)	240 (33.2%)	720 (99.9%)

Number of Observations

Table 5. Mean number of chamber relocations, percentage of observations that involved a relocation, percentage of turtles with at least one relocation, and mean number and percentage of chambers visited for aquatic thermal gradient tests (14–34°C) with 5 spiny softshell turtle (*Apalone spinifera hartwegi*) hatchlings (4–6 months old) incubated at 30°C and acclimated to 22°C. Observations were made in 10 minute intervals for 3 hours.

Gradient: 30°C Incubation Treatment

Test #:	1	2	3	4	5	6	7	8	$\bar{X} \pm SE$
Mean # Relocations:	2.00	2.80	3.80	3.40	3.00	1.60	3.00	7.00	3.33 ± 0.6
% Obs Relocated:	11.1	15.6	21.1	18.9	16.7	8.9	16.7	38.9	18.5 ± 3.2
% Turtles Relocated:	40.0	40.0	60.0	80.0	80.0	60.0	60.0	80.0	62.5 ± 5.9
Mean # Chambers:	2.20	2.00	2.80	2.80	2.80	2.40	2.80	3.60	2.68 ± 0.2
% Chambers:	36.7	33.3	46.7	46.7	46.7	40.0	46.7	60.0	44.6 ± 2.9

Table 6. Mean number of chamber relocations, percentage of observations that involved a relocation, percentage of turtles with at least one relocation, and mean number and percentage of chambers visited for aquatic thermal gradient tests (14–34°C) with 5 spiny softshell turtle (*Apalone spinifera hartwegi*) hatchlings (3–5 months old) incubated at 25°C and acclimated to 22°C. Observations were made in 10 minute intervals for 3 hours.

Gradient: 25°C Incubation Treatment

Test #	1	2	3	4	5	6	7	8	$\bar{X} \pm SE$
Mean # Relocations:	1.20	0.60	4.20	8.00	3.80	4.80	8.40	6.80	4.73 ± 1.0
% Obs Relocated:	6.7	3.3	23.3	44.4	21.1	26.7	46.7	32.7	25.6 ± 5.6
% Turtles Relocated:	60.0	40.0	60.0	80.0	100.0	100.0	100.0	100.0	80.0 ± 8.5
Mean # Chambers:	2.00	1.60	3.20	4.20	3.40	3.60	4.60	4.40	3.38 ± 0.4
% Chambers:	33.3	26.7	53.3	70.0	56.7	60.0	76.7	73.3	56.3 ± 6.4

Table 7. Mean number of chamber relocations, percentage of observations that involved a relocation, percentage of turtles with at least one relocation, and mean number and percentage of chambers visited for control tests (22°C) with 5 spiny softshell turtle (*Apalone spinifera hartwegi*) hatchlings (4–6 months old) incubated at 30°C and acclimated to 22°C. Observations were made in 10 minute intervals for 3 hours. Control test observations were recorded in each chamber as though the gradient was in effect.

Control: 30°C Incubation Treatment

Test #:	1	2	3	4	5	6	7	8	$\bar{X} \pm SE$
Mean # Relocations:	2.60	5.60	3.60	6.20	3.80	5.00	6.40	7.00	5.03 ± 0.5
% Obs Relocated:	14.4	31.1	20.0	34.3	21.1	27.8	35.6	38.9	27.9 ± 3.1
% Turtles Relocated:	60.0	80.0	80.0	60.0	80.0	100.0	80.0	100.0	80.0 ± 5.3
Mean # Chambers:	2.20	4.00	3.20	3.20	3.40	3.40	3.40	4.20	3.38 ± 0.2
% Chambers:	36.7	66.7	53.3	53.3	56.7	56.7	56.7	70.0	56.3 ± 3.5

Table 8. Mean number of chamber relocations, percentage of observations that involved a relocation, percentage of turtles with at least one relocation, and mean number and percentage of chambers visited for control tests (22°C) with 5 spiny softshell turtle (*Apalone spinifera hartwegi*) hatchlings (3–5 months old) incubated at 30°C and acclimated to 22°C. Observations were made in 10 minute intervals for 3 hours. Control test observations were recorded in each chamber as though the gradient was in effect.

Control: 25°C Incubation Treatment

Test #:	1	2	3	4	5	6	7	8	$\bar{X} \pm SE$
Mean # Relocations:	10.20	11.40	10.20	10.80	8.00	10.60	9.60	10.00	10.10 ± 0.4
% Obs Relocated:	56.7	63.3	56.6	71.1	44.4	58.9	53.3	55.6	57.5 ± 2.7
% Turtles Relocated:	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0 ± 0.0
Mean # Chambers:	5.20	4.60	4.40	4.80	4.20	5.00	4.60	4.20	4.63 ± 0.1
% Chambers:	72.2	76.6	73.3	80.0	70.0	83.3	76.7	70.0	75.3 ± 1.7

Table 9. Individual growth data for 5 *Apalone spinifera hartwegi* (with assigned number) incubated at 30°C over 6 months. The symbol key indicates the start and stop of the testing period.

Individual Growth: 30°C Incubation Treatment

		Turtle 1			Turtle 2			Turtle 3		
	Date Measured	M(g)	CL(mm)	PL(mm)	M(g)	CL(mm)	PL(mm)	M(g)	CL(mm)	PL(mm)
Hatch Date	9/4/2009	9.01	42.59	30.2	8.84	42.78	30.34	7.94	39.04	28.89
Month 1	10/16/2009	8.63	43.51	30.61	8.94	43.71	32.28	7.45	40.66	30.58
Month 2	11/4/2009	8.83	43.69	31	9.47	44.2	32.32	8.18	41.2	32.13
Month 3	12/4/2009	8.28	43.74	31.2	9.18	44.46	32.4	7.13	41.29	32.13
*Month 4	1/4/2010	8.34	43.8	31.2	9.77	44.9	33.04	8.32	41.69	32.24
Month 5	2/4/2010	8.12	44.39	31.2	10.12	45.07	33.11	9.03	43.56	32.3
**Month 6	3/4/2010	8.12	44.62	31.45	11.62	45.52	33.82	10.09	44.56	33.93

Turtle 4			Turtle 5		
M(g)	CL(mm)	PL(mm)	M(g)	CL(mm)	PL(mm)
9	42.38	30.22	9.73	45.54	30.09
9.19	43.65	32.29	9.32	45.54	31.86
9.42	43.65	32.29	10.09	45.54	31.86
9.15	43.74	32.63	8.89	45.56	32.04
9.21	43.77	32.65	9.7	45.66	32.41
9.87	43.88	34.34	10.2	45.72	32.72
10.83	44.33	35.16	10.43	45.7	33.31

Symbol Key
Start Testing = *
End Testing = **

Table 10. Individual growth data for 5 *Apalone spinifera hartwegi* (with assigned number) incubated at 25°C over 5 months. The symbol key indicates the start and stop of the testing period.

Individual Growth: 25°C Incubation Treatment

		Turtle 6			Turtle 7			Turtle 8		
	Date Measured	**M(g)**	**CL(mm)**	**PL(mm)**	**M(g)**	**CL(mm)**	**PL(mm)**	**M(g)**	**CL(mm)**	**PL(mm)**
Hatch Date	10/2/2009	8.08	40.83	28.8	8.73	42.12	30.77	8.98	42.67	29.98
Month 1	11/4/2009	8.74	42.87	30.8	8.96	43.03	31.05	9.84	42.74	30.47
Month 2	12/4/2009	9.25	43.39	31.5	8.52	43.2	31.33	9.5	43.79	30.73
***Month 3**	1/4/2010	9.29	43.33	32.06	8.53	43.24	31.72	9.62	44.01	30.96
Month 4	2/4/2010	9.4	43.43	32.06	8.38	43.24	32.01	9.62	45.08	30.96
****Month 5**	3/4/2010	9.16	43.73	32.1	8.56	43.31	32.27	9.24	44.9	31.72

Turtle 9			Turtle 10		
M(g)	**CL(mm)**	**PL(mm)**	**M(g)**	**CL(mm)**	**PL(mm)**
6.82	35.63	28.09	9	40.09	22.77
7.4	36.84	28.48	9.37	42.82	31.94
7.55	37.53	28.95	9.11	42.82	32.18
7.55	38.28	29.24	9.44	43.09	32.33
7.86	39.35	29.56	10.06	43.55	32.98
7.98	39.65	30.94	10.3	44.8	34.1

Color Key
Start Testing = *
End Testing = **

FIGURES

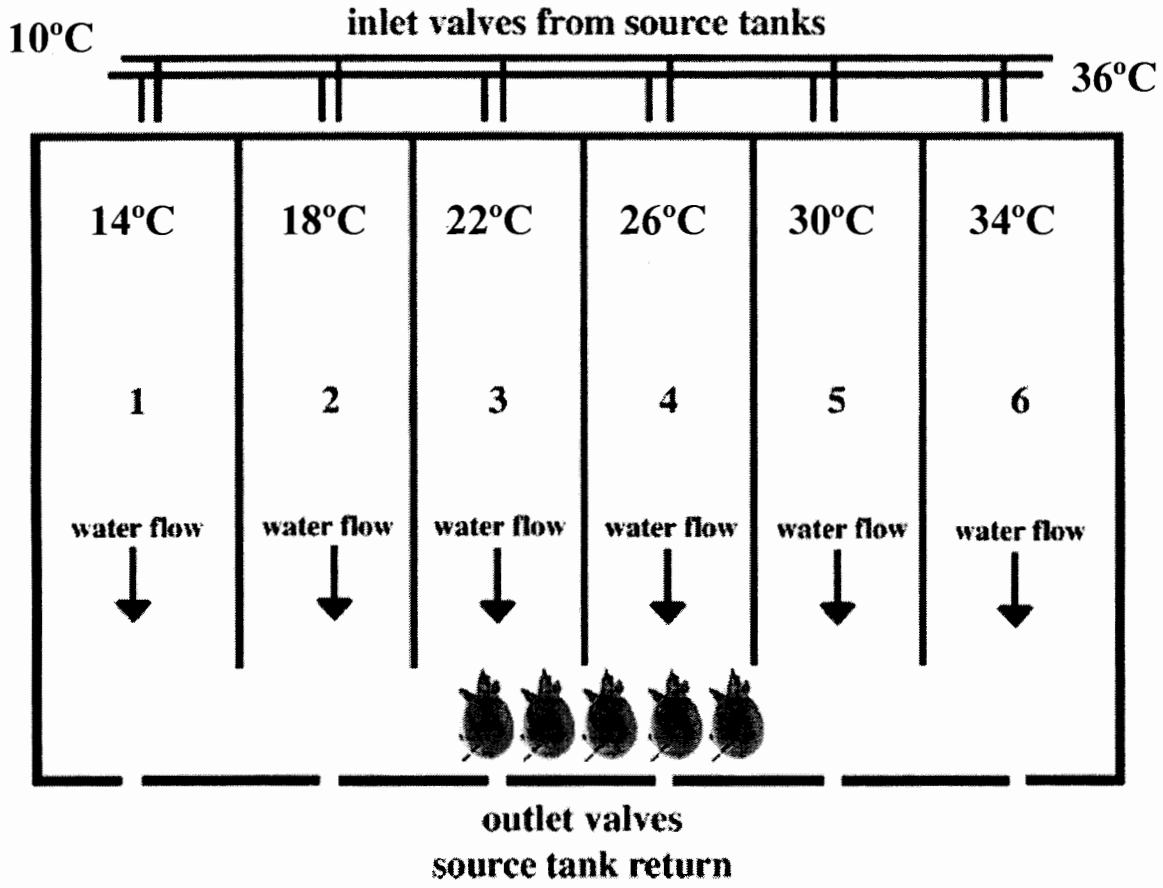

Figure 1. Diagram of the test chamber. Chamber numbers correspond to single-temperature control tests; the temperature of each chamber is indicated for gradient tests only. Sequential gradients were reversed on alternating test runs and an equal number of mixed-temperature runs were performed.

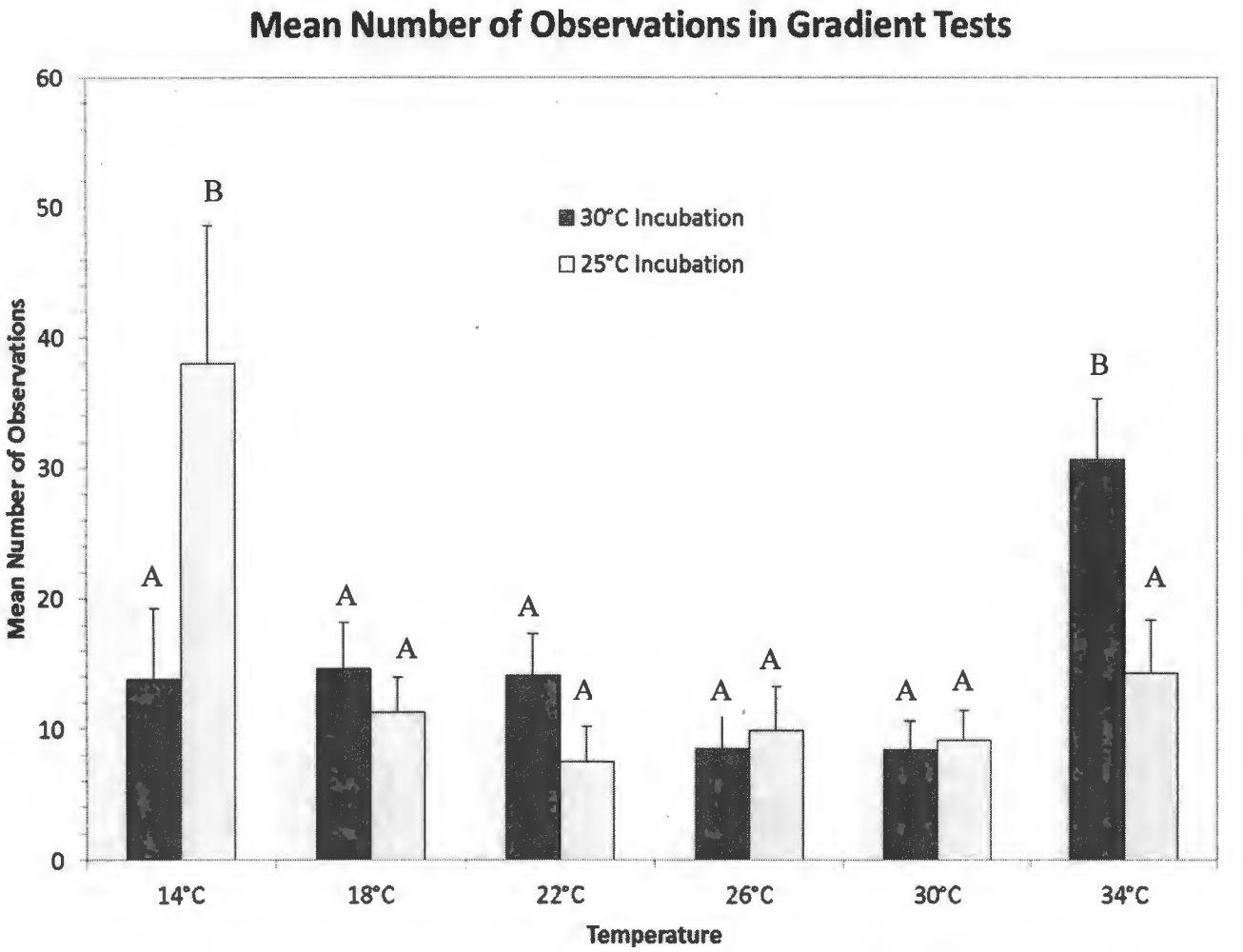

Figure 2. The mean (+ SE) number of observations for 5 hatchling *Apalone spinifera hartwegi* from egg-incubation treatments (25°C and 30°C) at each temperature in a 14–34°C aquatic thermal gradient. Observations were made every 10 minutes for 3 hours (18 observations per turtle per test run; total observations per treatment group = 720). Letters indicate homogenous subsets of chamber selection as determined by Tukey B multiple comparisons tests.

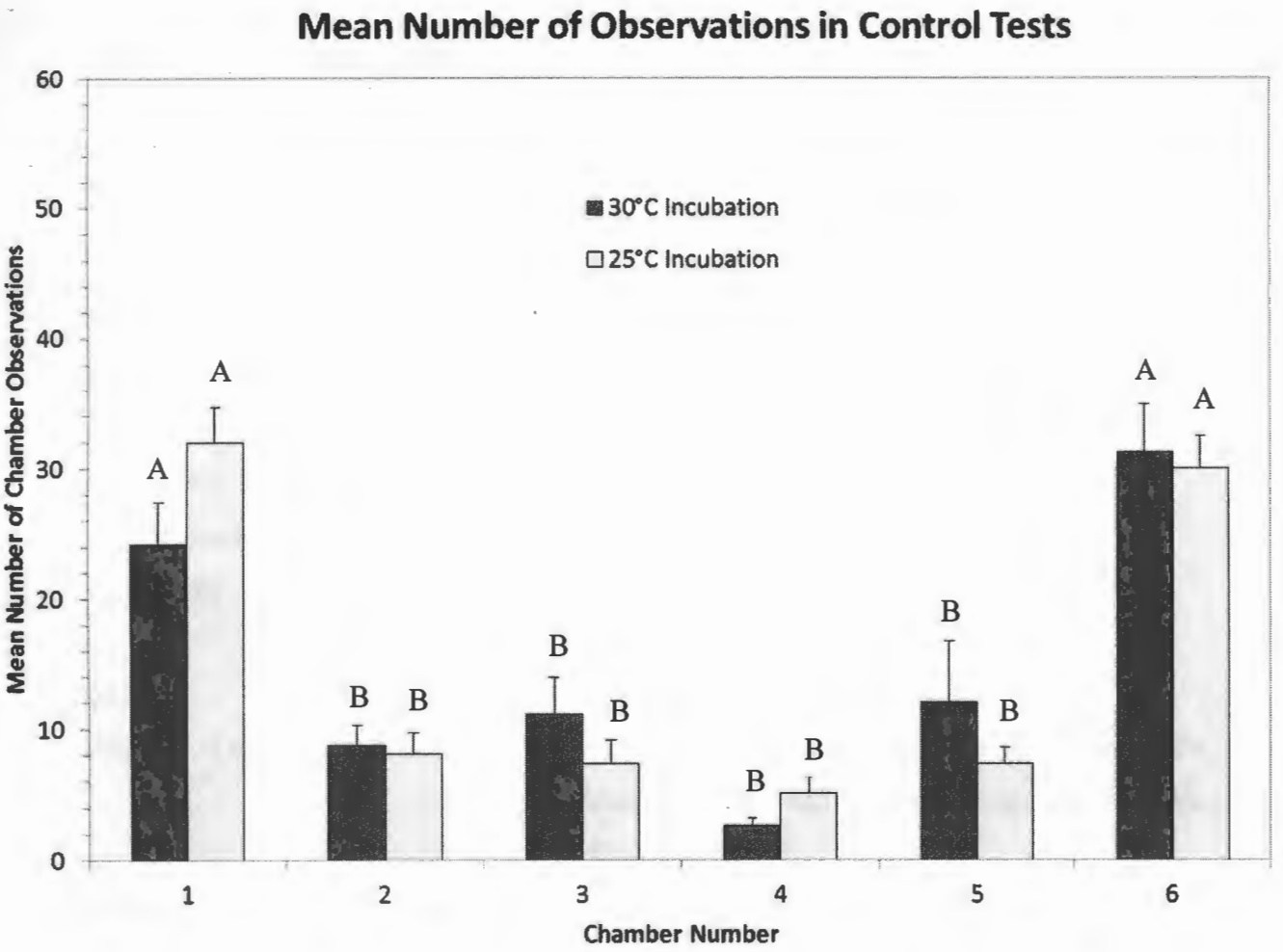

Figure 3. The mean (+ SE) number of observations for 5 hatchling *Apalone spinifera hartwegi* from egg-incubation treatments (25°C and 30°C) in each chamber in single-temperature (22°C) control tests. Observations were made every 10 minutes for 3 hours (18 observations per turtle per test run; total observations per treatment group = 720). Letters indicate homogenous subsets of chamber selection as determined by Tukey B multiple comparisons tests.

APPENDICES

Appendix A. Scientific Terms.

Term	Definition
Aquatic thermal gradient	System of chambers filled with varying water temperatures
Carapace	The back (or shell) of a turtle
Cryptic	An organism's ability to camouflage itself
Ectotherms	Body temperature is control by environment
Genetic sex-determination	Genes determine sex of organism
Homogenous	Relating to something of the same
Incubation	Environmental temperature during embryonic development
Keel	Protruding bony spine on turtle
ANOVA (One-way)	Analysis of variance; a statistical procedure used to compare means of two or more samples (SPSS Inc., Chicago, IL)
Plastron	Underside (or belly) of a turtle's shell
Reverse osmosis	Purification process of water
Substrate	Substance (sand) used to cover bottom of gradient tank
Selected temperature	Aquatic temperature chosen by turtle in gradient
Septal ridge	Division of nostril cavity dividing it into two
Temperature sex-determination	Environmental temperature determines sex of organism
T-test	determines whether the means of two groups are statistically different from each other (Abacus Concepts, 1994)
Tukey B	statistical procedure that compares the means of treatment groups in pairs (SPSS Inc., Chicago, IL)
Vent	Anal opening

Appendix B. Abbreviations used.

Abbreviation	Definition
CL	Carapace length
cm	Centimeters
g	Grams
GSD	Genetic sex-determination
h	height
HW	Head width
l	Length
L	Liters
M	Mass
mm	Millimeters
p	Alpha
PL	Plastron length
PV	Plastron to vent length
SD	Standard deviation
SH	Shell height
SW	Shell width
TSD	Temperature sex-determination
TTL	Total tail length
UVA	Ultraviolet A wavelengths
UVB	Ultraviolet B wavelengths
VT	Vent to tail
w	Width

Appendix C. Symbols used.

Symbol	Definition
%	Percent
>	Greater than
<	Less than
≤	Less than or equal to
≥	Greater than or equal to
±	Plus or minus
°C	Degrees Celsius