



Incubation temperature and satiety influence general locomotor and exploratory behaviors in the common snapping turtle (*Chelydra serpentina*)

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ABSTRACT

Temperature during embryogenesis determines sex and has been shown to influence other physiological traits in reptiles. The common snapping turtle (*Chelydra serpentina*) is an ideal model for testing how temperature impacts behavior in species that display temperature-dependent sex determination. Behavioral assays are crucial to understanding how a changing climate may affect whole organism function in the snapping turtle. Currently, there are few behavioral assays for semi-aquatic vertebrates like turtles. In this study, we used digital cameras to record behavior of fed and fasted hatchling turtles from different incubation temperatures in an open field setting for 20 min in 2018 and repeated the experiment in 2019. Open fields were circular tanks filled with water to a depth of 3.5 cm. Each field was split into four quadrants and two zones (inner and outer). The amount of time turtles spent actively moving, total distance travelled, and several other measures were collected and summarized automatically from videos with open source image analysis software (ImageJ). Satiety and incubation temperature had significant effects on total distance moved, time spent moving, and time moving in the outer zone. These findings indicate that temperature during embryogenesis has a long-lasting effect on neural mechanisms underlying exploratory or general locomotor behavior in turtles.

1. Introduction

There are various modes of sex determination in the phylum Chordata, the most well-known being via sex chromosomes. However, many species of reptile and some species of fish display a phenomenon known as temperature-dependent sex determination (TSD). In these species, the sexual fate of the individual is triggered by environmental factors such as ambient temperature during embryonic development [1]. The common snapping turtle (*Chelydra serpentina*) is among those species. While temperature during embryogenesis determines sex in the common snapping turtle, temperature also has independent impacts on growth and thermoregulatory behavior [2–4]. It is likely that snapping turtles exposed to different temperatures during embryogenesis also display variation in other behaviors.

Ambient temperature during embryonic development has been shown to shape a wide variety of behaviors in other species. For example, it influences anti-predatory behavior in hatchling snakes [5], learning performance in sharks [6], foraging behavior in bearded dragon lizards [7], and locomotor behavior in bobwhite quail [8]. While size and speed of individuals could be indicative of fitness advantages, other factors like learning ability may also influence survival.

High correlations between size, speed, and learning ability have been found in lizards (*Bassiana duperreyi*) with incubation temperature causing these correlations [9]. Temperature has also been shown to have long-term effects on sociosexual behaviors in various reptiles. Social cognition in bearded dragons is affected by incubation temperature during development [10]. This study revealed that exposure to different temperatures during embryo development can cause variation in behavior long into adulthood, which could have fitness consequences. Similarly, incubation temperature during embryogenesis influences aggressive and sexual behavior in adult leopard geckos [11,12]. These behavioral effects are correlated with differences in metabolic capacity of brain nuclei that regulate sexual and aggressive behaviors in leopard geckos [13].

Another factor that has been shown to cause variation in behavior is the feeding status of the animal. In rodents, there is a well-characterized response to feeding called the behavioral satiety sequence in which animals eat, actively groom themselves, and then rest [14]. Snapping turtles have been observed to bury themselves in sediment in rivers and streams following feeding whereas unfed turtles do not bury themselves [15]. Some snakes have been shown to have decreased locomotor performance immediately following feeding on a test of predator

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avoidance [16]. In snapping turtles, feeding may similarly reduce spontaneous locomotor behavior.

However, there is a lack of information on the impact of incubation temperature and satiety on locomotor behavior in snapping turtles in a novel environment. Open field tests provide researchers with a means of assessing general locomotor ability, exploratory behavior, and anxiety [17]. One of the limitations of the open-field test is that a given aspect of an animal's performance may be attributable to multiple causes. For example, little movement could be indicative of either high anxiety or low locomotive ability. Because turtles are relatively non-social, it was assumed that variations in the turtles' behavior would primarily be related to general locomotor ability, foraging, and exploration.

Understanding how behavior is programmed by ambient temperature during embryogenesis could give us a valuable insight into the developmental basis for behavioral variation in snapping turtles and other reptiles while also providing a glimpse into how changing temperatures may affect this species in the future [18]. Species that display TSD are potentially vulnerable to extinction due to rapid climate change as warming temperatures skew population sex ratios [19]. An understanding of temperature effects on behavior in TSD species will inform future management decisions in a warming climate. Behavioral assays like the open field test will provide information regarding temperature effects on whole organism function in the common snapping turtle. The goal of this study was to use an open field approach to test whether incubation temperature during embryogenesis and satiety influence exploratory and general locomotor behaviors in *Chelydra serpentina* hatchlings.

2. Methods and materials

2.1. Animal husbandry

Eggs were collected in June of 2017 and 2018 from a captive breeding colony of snapping turtles at the University of North Dakota (UND). Adult females were palpated to assess their reproductive status in early June. Females with eggs were transferred to an outdoor aviary with natural substrate for nesting. The aviary was monitored at 2-h intervals from 10 pm to 10 am on a daily basis to directly observe females nesting. Females were identified by a unique combination of marks on their marginal scutes. Eggs were collected shortly after females finished nesting. Dam ID and nesting date were written on a post-it note placed with each clutch of eggs. Females were returned to their indoor stock tanks the day after nesting.

In 2017, we used three clutches from our breeding colony: Clutch 51 (n = 32), Clutch 57 (n = 28), Clutch 60 (n = 38). In 2018, we used six clutches from our breeding colony: Clutch 39 (n = 35), Clutch 41 (n = 19), Clutch 44 (n = 15), Clutch 48 (n = 17), Clutch 50 (n = 37), Clutch 51 (n = 28). Eggs were washed in tepid water within 24 h of oviposition. Eggs were individually labeled with unique clutch and egg numbers, placed in containers with moist vermiculite (mixed in 1:1 ratio of water to vermiculite by mass), and incubated at 22 °C (n = 54), 26 °C (n = 74), 27 °C (n = 55), or 28 °C (n = 66). These temperatures were chosen because 22 °C and 26 °C produce predominantly males, 27 °C produces a mixed sex ratio and 28 °C produces predominantly females in snapping turtles. Approximately even numbers of eggs from each clutch were assigned to each temperature treatment and further split between replicate incubators to avoid pseudoreplication.

We placed eggs in individual plastic cups labeled with their clutch ID, egg number, and incubation temperature a few days before turtles were expected to hatch. Turtles from the warmest temperature started hatching in late August, while turtles from the coldest temperature finished hatching in early October. Hatchling turtles in nature normally emerge from the nest and migrate to the nearest water body where they hibernate through the winter. Hatchlings in nature are not active until late spring when ice on lakes thaws and water temperature rises. To

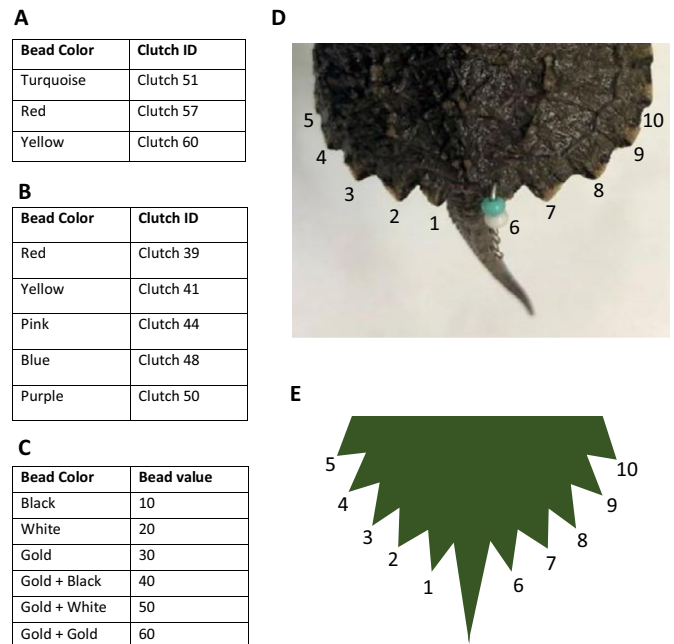


Fig. 1. Hatchling turtles were marked uniquely using colored beads attached to rear marginal scutes with a stainless-steel wire. One colored bead indicated clutch identity in (A) 2018 and (B) 2019. Additional beads were attached to indicate egg number using the following scheme. When looking at the turtle from above, (D and E) scutes to the left of the tail were numbered 1–5 and scutes to the right were numbered 6–10. (C) Black, white, and/or gold beads were added to indicate egg numbers greater than 10. Thus, egg number was the sum of the scute number (1–10) plus any black, white, and/or gold beads. For instance, a turtle from Clutch 51, Egg 6, in 2018 would have a single turquoise bead attached to scute number 6. A turtle from Clutch 51, Egg 26, in 2018 would have both a turquoise bead and a white bead attached to scute number 6, as shown in panel D.

mimic the general timeline of activity observed in nature, hatchlings were kept in their cups and fasted for 2–3 months during the winter. Turtles were sprayed daily with cold water to keep them well hydrated.

In early spring of 2018 (for eggs laid in 2017) and 2019 (for eggs laid in 2018), colored beads were attached to hatchlings' rear marginal scutes to permit individual subjects to be identified by their unique clutch and egg numbers (Fig. 1). Marked turtles were placed in cattle tanks filled with tap water to a depth of 2 to 5 cm. The tanks were set up with a slope to allow efficient draining and cleaning after turtles were fed. Turtles were given excess frozen smelt and allowed to feed ad lib for approximately 2 h every Monday, Wednesday, and Friday. Tanks had two platforms made of brick pavers to allow turtles to climb out of the water. One platform was placed under a heat lamp to allow turtles to behaviorally thermoregulate while the other platform was placed away from the heat source to allow basking in air at the ambient room temperature (~20 °C).

2.2. Open field, video acquisition, and processing

Six fiberglass aquaculture tanks with a diameter of 59 cm and a wall height of 55 cm were set up for open field tests in a separate room within the animal facilities at UND (Fig. 2). Water depth was approximately 3.5 cm. Water was changed between tests with different turtles. A miniature surveillance camera from Jet Security (Buena Park, CA) was fastened to a bracket 79 cm above water level. Hatchlings were recorded for twenty minutes either in a fasted state (n = 126) in the morning or shortly after being fed (n = 123) in the afternoon. Videos were saved as ASF files using a digital video recorder (DVR) from Advance Security (Bellevue, IL) programed to record at 30 frames per

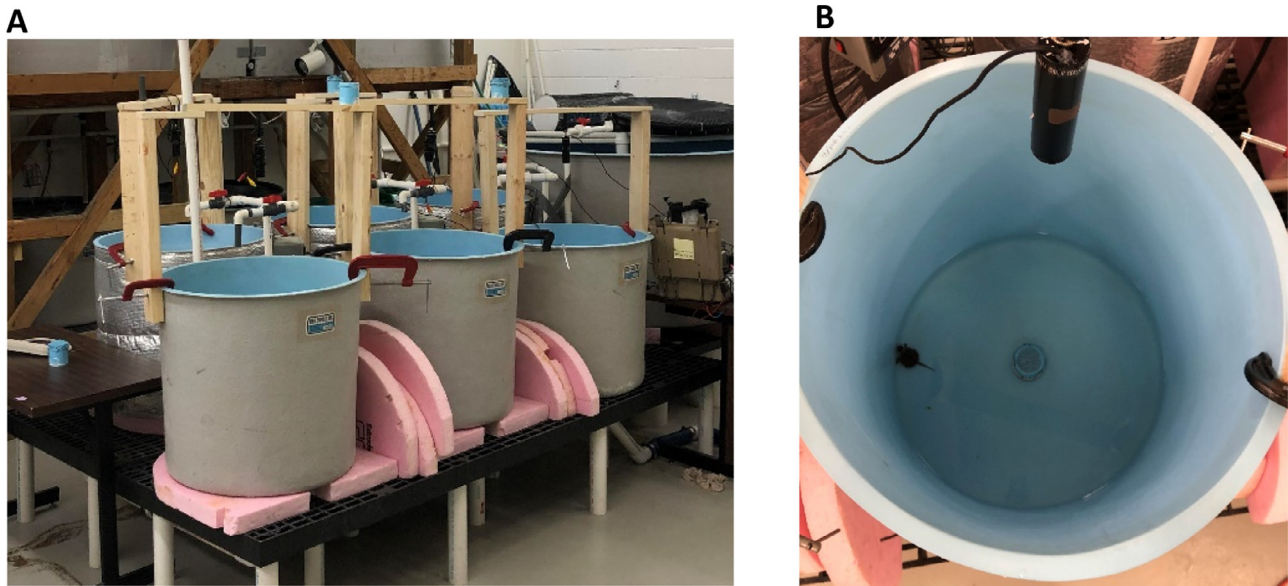


Fig. 2. Six fiberglass aquaculture tanks were set up for open field tests with hatchling snapping turtles. (A) Wooden brackets fastened to each tank were used for mounting digital cameras securely above the center of each tank. (B) View from above a tank showing the camera and a hatchling turtle on the left side of the open field arena.

second. Each video was split into individual jpegs with one frame per second for image processing and saved into respective directories using the Windows Command Prompt.

2.3. Fiji (ImageJ) processing

In order to collect data on the position of turtles in the open field from videos, we created a plugin for ImageJ. The videos were imported individually into Fiji as virtual stacks and converted into 8-bit greyscale with the macro. The origin of the image (center of the tank) along with inner and outer zones were then defined. The stack was duplicated and a blank image was created to cancel out the background. Blank frames were then deleted (i.e., images before turtles were placed in the open field). Finally, Cartesian coordinates of the hatchling were recorded in each frame. Coordinates of turtles were saved in csv files with their respective clutch and egg number. The plugin then used these coordinates with user-defined parameters (e.g., video resolution, frames per second, and moving threshold) to create measurements based on the location of the turtle in each frame at 1-second intervals during the 20-min test.

2.4. Statistical data processing

For seven clutches, it was possible to assign an equal number of subjects to each temperature and feeding status group. However, for the two smallest clutches (44 and 48), a nonequivalent number of subjects were tested in fed and fasted states. The results were analyzed using a mixed model, restricted maximum likelihood (REML) ANOVA, which is capable of handling unbalanced data. REML was also used to control for potential clutch-related differences in behavior (genetic differences between clutches could not be controlled, therefore they were treated as a random factor). Clutch, incubation temperature, and feeding status were independent variables in the model. Clutch identity and its interactions with other independent variables were analyzed as random effects. Incubation temperature, feeding status, and their interaction were analyzed as fixed effects. Year was also treated as a fixed effect, to test for differences between cohorts of hatchlings from 2018 and 2019. The significance of fixed effects was tested with F tests. The significance of random effects was tested with the Wald test. The null hypothesis for the Wald test is that variance components (due to random effects) are

equal to zero.

Multiple aspects of turtles' open-field performance were assessed so as to develop a robust profile of the ways in which incubation temperature and satiety affect behavior. Dependent variables included latency to begin moving, the total time spent moving, the average velocity (when the turtle was moving), the maximum velocity, the amount of time spent in the inner zone, the amount of time spent in the outer zone, and the total distance travelled. Latency was transformed with Log10 and the distance travelled was transformed with square root in order to meet the assumptions of ANOVA. JMP 13.0 software was used for all statistical analyses (SAS Institute, Cary, NC). All figures were created with Rstudio (version 1.2.5019).

3. Results

Incubation temperature ($F_{3,15} = 4.25$, $p = 0.02$) and feeding status ($F_{1,5} = 12.07$, $p = 0.02$) had significant effects on the amount of time hatchling turtles spent moving during open field tests, but the interaction between temperature and feeding status had no impact ($F_{3,203} = 1.33$, $p = 0.26$). Turtles incubated at a temperature of 22 °C moved for a longer period of time than turtles incubated at 26 °C, 27 °C, and 28 °C (Fig. 3A). Fasted turtles spent more time moving than satiated turtles (Fig. 3B). Time spent moving did not depend on the year in which the turtles hatched ($F_{1,6} = 0.51$, $p = 0.50$). None of the random effects influenced the time hatchlings spent moving in the open field: clutch ($p = 0.29$), clutch x temp ($p = 0.38$), clutch x satiety ($p = 0.74$), and clutch x temp x satiety ($p = 0.33$).

The total distance hatchling turtles travelled in open field tests was also affected by incubation temperature ($F_{3,232} = 4.63$, $p = 0.004$) and feeding status ($F_{1,5} = 11.70$, $p = 0.02$), while the temperature x feeding status interaction was nonsignificant ($F_{3,234} = 0.82$, $p = 0.49$). Turtles incubated at a temperature of 22 °C travelled farther than turtles incubated at 26 °C, 27 °C, and 28 °C (Fig. 3C). Fasted turtles travelled farther than satiated turtles (Fig. 3D). Year of testing did not influence the total distance travelled by hatchling turtles ($F_{1,6} = 1.45$, $p = 0.27$). None of the random effects influenced the distance turtles travelled in the open field: clutch ($p = 0.37$), clutch x temp ($p = 0.75$), clutch x satiety ($p = 0.71$), and clutch x temp x satiety ($p = 0.58$).

Incubation temperature ($F_{3,20} = 4.23$, $p = 0.02$) and feeding status ($F_{1,223} = 12.33$, $p = <0.001$) had significant effects on the amount of

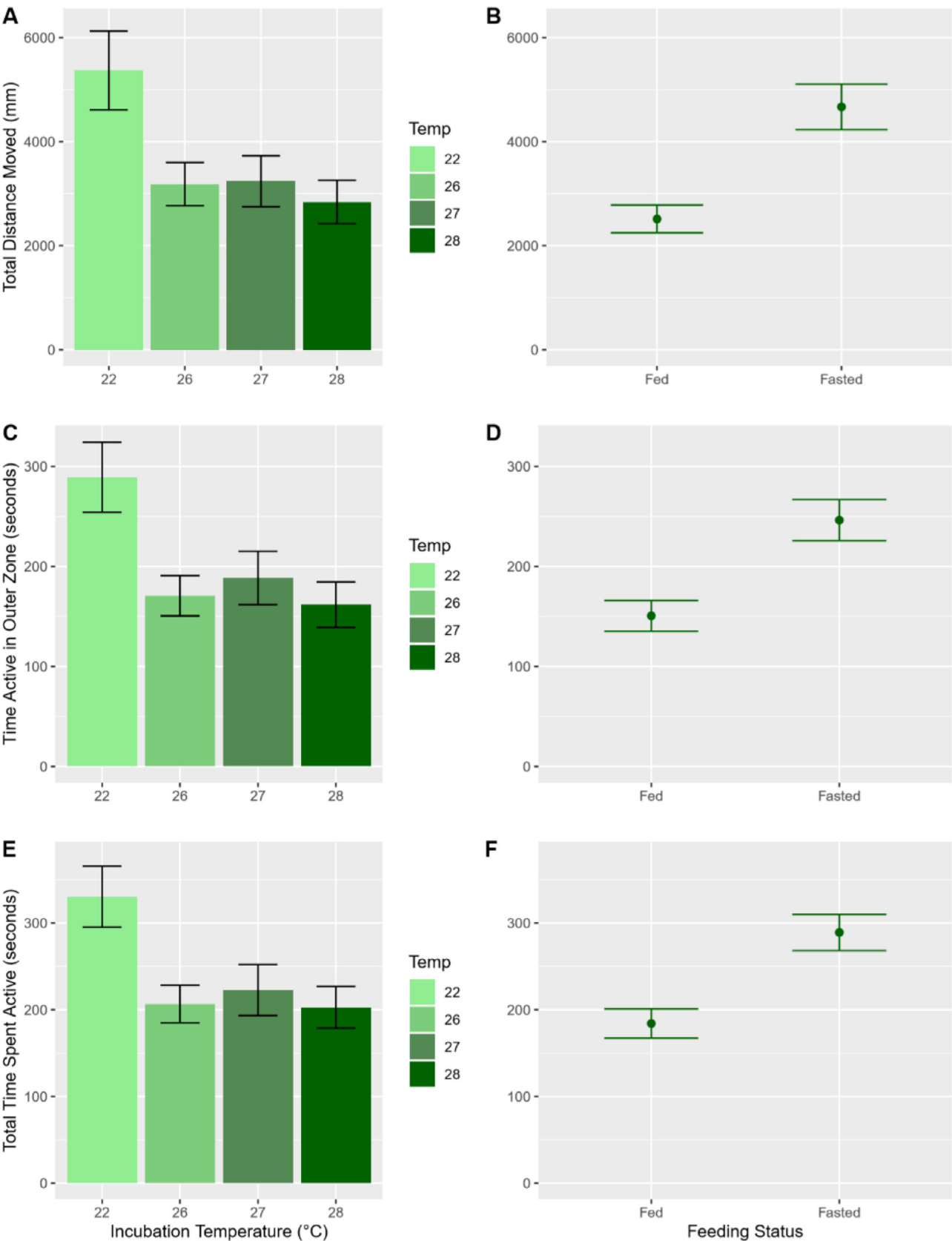


Fig. 3. Mean ($\pm$ SE) for Distance Moved (A and B), Time Active in Outer Zone (C and D), and Total Time Spent Active (E and F) as response variables with Incubation Temperature and Feeding Status as independent variables.

time turtles spent actively moving around the edge of the field. The temperature x feeding status interaction had no effect ($F_{3,220} = 1.39$, $p = 0.25$). Turtles incubated at the lowest temperature (22 °C) during embryogenesis spent more time actively swimming around the outer edge of the field than the hatchlings incubated at the other three temperatures (Fig. 3E). Fasted hatchlings spent more time moving around the outer ring than satiated hatchlings (see Fig. 3F). Year did not influence total distance travelled by hatchling turtles ($F_{1,6} = 0.31$, $p = 0.60$). None of the random effects influenced the distance turtles travelled in the open field: clutch ($p = 0.55$), clutch x temp ($p = 0.24$), clutch x satiety ($p = 0.79$), and clutch x temp x satiety ($p = 0.28$).

Incubation temperature had no effect on average velocity ($F_{3,232} = 1.71$, $p = 0.17$), latency to move ($F_{3,19} = 1.79$, $p = 0.18$), maximum velocity ($F_{3,231} = 1.33$, $p = 0.26$), or time spent in the inner zone ($F_{3,235} = 1.30$, $p = 0.27$). Feeding status had no effect on average velocity ($F_{1,13} = 0.49$, $p = 0.49$), latency ($F_{1,238} = 0.12$, $p = 0.72$), maximum velocity ($F_{1,5} = 1.98$, $p = 0.22$), or time spent in the inner zone ($F_{1,237} = 0.001$, $p = 0.97$). Year had no effect on average velocity ($F_{1,13} = 0.68$, $p = 0.43$), maximum velocity ($F_{1,4} = 0.67$, $p = 0.45$), or time spent in the inner zone ($F_{1,6} = 3.89$, $p = 0.09$). However, there was a significant difference in latency to move between turtles tested in 2018 versus turtles tested in 2019 ($F_{1,6} = 7.52$, $p = 0.03$).

None of the random effects influenced the average velocity of turtles in the open field: clutch ($p = 0.47$), clutch x temp ($p = 0.39$), clutch x satiety ($p = 0.24$), and clutch x temp x satiety ($p = 0.86$). Latency to move was not affected by clutch ($p = 0.14$), clutch x temp ($p = 0.26$), clutch x satiety ($p = 0.51$), or the clutch x temp x satiety interaction ($p = 1.0$). The maximum velocity of turtles was not influenced by clutch ($p = 0.60$), clutch x temp ($p = 0.65$), clutch x satiety ($p = 0.26$), or the clutch x temp x satiety interaction ($p = 0.80$). Variation among clutches for time spent in the inner zone of the open field approached significance ($p = 0.07$). However, no other random effects influenced the time turtles spent in the inner zone of the open field: clutch x temp ($p = 0.38$), clutch x satiety ($p = 0.19$), and clutch x temp x satiety ($p = 0.79$).

4. Discussion

Incubation temperature during embryogenesis can cause persistent morphological, physiological and behavioral changes in hatchling and juvenile reptiles and some of these changes even last into adulthood (reviewed in [18]). For instance, thermoregulatory behavior of hatchling snapping turtles and painted turtles is influenced by incubation temperature [2,3,20]. This led us to hypothesize there could also be differences in locomotor and exploratory behavior due to ambient temperature during embryogenesis. In this study, we examined the effects of incubation temperature and satiety on locomotor and exploratory behavior in the common snapping turtle.

We found that temperature during embryonic development altered exploratory and locomotor behavior of hatchling snapping turtles. Hatchlings incubated at 22 °C moved for a longer period of time and farther than turtles incubated at warmer temperatures when tested in a novel open field. These findings contrast with the relationship between incubation temperature and locomotor behavior obtained with other turtle species. Hatchling Chinese softshell turtles (*Pelodiscus sinensis*) incubated at 27 °C and 28 °C were found to travel longer distances than turtles incubated at 24 °C or 32 °C when tested in a terrestrial environment [21]. The same pattern emerges for swimming distance in olive ridley sea turtles (*Lepidochelys olivacea*) where hatchlings from intermediate temperatures swam farther than hatchlings incubated at either end of the temperature range [22]. In addition, hatchling loggerhead turtles (*Caretta caretta*) from lower incubation temperatures of 27 °C and 28 °C were significantly less active than hatchlings from temperatures between 28.5 °C and 31 °C [23]. Green sea turtle (*Chelonia mydas*) hatchlings incubated at cooler temperatures had decreased locomotor behavior compared to hatchlings from warmer temperatures [24]. Du

and Ji [21] hypothesized that embryos incubated at 33 °C and 24 °C had impaired locomotor behavior because they were incubated close to the upper and lower limit of temperatures that yield viable hatchlings in the Chinese softshell turtle.

Differences in incubation temperature effects on locomotor behavior between the snapping turtle and other turtle species could be due to shifts in thermal tolerance. For example, snapping turtle embryos incubated at 22 °C develop into viable hatchlings while *P. sinensis* embryos perish at incubation temperatures below 23 °C. Differences could also be due to variation in how animals from different parts of their geographic range are impacted by incubation temperature. Indeed, some species exhibit geographic variation in thermal tolerance based on latitude [25]. Further studies need to be conducted to find the upper and lower thermal limits for viable development of snapping turtle embryos as well as to characterize locomotor behavior across the entire range of viable incubation temperatures. These experiments would provide insight into optimal incubation temperatures and the shape of thermal reaction norms in the snapping turtle [18]. While incubation temperature affected the amount of time snapping turtle hatchlings spent moving and the total distance moved, it did not significantly affect the latency to move, average velocity while moving, or maximum velocity. By comparison, studies with other turtle species have shown that incubation temperature can alter hatchling velocity [21–23]. In various freshwater and sea turtle species, intermediate temperatures seem to produce hatchlings with better physiological performance than turtles incubated near the low or high end of the viable range of temperatures. We expect snapping turtles would follow suit in future experiments designed to measure maximal performance.

Incubation temperature has a direct organizing effect on the metabolic capacity and hormone sensitivity of the brain in leopard geckos [13,26–29]. Hormonal manipulation of leopard geckos caused changes in the volume and metabolic capacity of specific brain nuclei, which were correlated with behavioral differences [30]. Hormones control aggressive and sexual behaviors in the green anole lizard (*Anolis carolinensis*) [31], so variation in hormone sensitivity could influence certain behaviors in snapping turtles as well. Embryonic temperature has been shown to program thermoregulatory behavior in snapping turtles [2,3]. Both righting response and exploratory behaviors were found to be strongly repeatable (even after overwintering) and positively correlated in the red-eared slider turtle (*Trachemys scripta elegans*), suggesting that behavioral differences are consistent as hatchlings grow (behavioral types are programmed) [32]. This leads us to believe that incubation temperature effects on snapping turtle behavior observed in this study might persist beyond 6 months of age. Further studies would have to be performed to understand the neural mechanisms underlying temperature-induced variation in locomotor and thermoregulatory behavior for the common snapping turtle.

Satiety also has an impact on behavior in hatchlings. We detected significant differences in locomotor behavior based on whether hatchlings were fed or fasted before the open field test. Snapping turtle hatchlings that were fasted for 24 h moved more often and farther than turtles that were fed right before the test. There are two alternative explanations for this difference in exploratory behavior. Fed turtles could be resting more than fasted turtles to process the ingested meal (like the satiety sequence in rodents). On the other hand, fasted turtles could be displaying an increased level of foraging behavior. Differences in general locomotor activity after feeding may also be related to thermoregulation, which affects many physiological functions in reptiles [33]. Basking behaviors increase metabolic rate and digestion efficiency so it is not surprising that turtles would bask after being fed [34]. Prior research in the snapping turtle has shown that incubation temperature and satiety jointly affect thermoregulatory behavior in hatchlings [3]. Future research should focus on how incubation temperature during embryogenesis, ambient temperature of hatchlings, and satiety interact to influence resting and locomotor behaviors.

Little research has been done on the molecular, hormonal, or neural

basis of satiety in turtles. Cholecystokinin appears to be well conserved across mammalian species so it would be interesting to determine whether secretion of this hormone signals satiety in turtles like it does in mammals [35]. This is likely because the gastrointestinal tract of *Chrysemys picta* was shown to contain most of the regulatory peptides found in mammals [36]. Metabolism also changes during digestion. There was an increase in oxygen consumption in juvenile *Chrysemys picta* following feeding that dropped one day later to 68% of the value observed post-feeding [37]. In the green turtle, short-term fasting has also been shown to induce a catabolic state that may create a metabolic debt to be repaid when food becomes available [38]. The increase in metabolic demand following feeding could cause energy to be directed into digestion thereby decreasing exploratory behavior in snapping turtles.

We incubated eggs at constant temperatures throughout embryogenesis. In contrast, turtles from natural nests in the wild experience temperature fluctuations [39]. There is evidence that fluctuating incubation temperatures influence the development of some traits. However, the importance of these effects varies among species and populations [40–45]. Future research should examine how fluctuating temperatures in nature expose developing embryos to extreme temperatures, which could have a stronger negative impact on development than incubation at constant temperatures within the viable range. Turtle embryos are very sensitive to ambient temperature during development: raising incubation temperature by 1°C above the highest mean nest temperature recorded in the wild leads to reduced egg viability, hatchling growth rate, and decreased locomotor function in the Mary River turtle [39]. Further studies could investigate whether thermal fluctuations influence swimming performance and other locomotor behaviors of common snapping turtles.

Although the clutch effect for time spent in the inner zone of the open field approached significance, clutch identity and its interactions with the other independent variables did not influence any other aspects of exploratory and locomotor behavior in snapping turtles in this study. This contrasts with recent research reporting that clutch identity influences habitat choice, righting response, and latency to explore a terrestrial arena in red-eared slider turtles and overall swimming activity in loggerhead sea turtles [23,32]. Clutch effects may vary for different aspects of turtle behavior. While we found no variation among clutches for exploratory and locomotor behavior, other aspects of snapping turtle behavior could be significantly affected by clutch identity. To better understand clutch effects, future studies should examine clutch and incubation temperature effects with a greater variety of behaviors.

Another surprising finding from this study was that, while temperature and satiety both influenced exploratory and locomotor behavior, the interaction between these variables did not. By comparison, Rhen and Lang [3] found that incubation temperature during embryogenesis impacted how hatchling turtles thermoregulated after feeding. Turtles incubated at 24 °C preferred warmer ambient temperatures immediately following feeding, whereas turtles incubated at 26.5 °C or 29 °C did not change their thermal preference after feeding. This difference in thermoregulatory behavior after feeding (i.e., incubation temperature by satiety interaction) led us to hypothesize that incubation temperature and satiety might have an interactive effect on exploratory behavior. The lack of an interaction could be due to a variety of contextual factors. The most obvious difference is that the open field in this study was thermally homogenous (i.e., there was no heat source or thermal gradient). In addition, turtles in this study were tested alone in a novel environment, while the study of thermoregulatory behavior was conducted in the home tank with other turtles present. To the best of our knowledge, the current study is the first to examine how incubation temperature and satiety affect exploratory behaviors in turtles. More studies will have to be carried out to fully understand why temperature and satiety have independent effects in this environmental context.

This study has shown that exposure of different temperatures during embryogenesis impacts the behavior of *Chelydra serpentina* hatchlings in open field tests. Satiety also has a significant effect on hatchling behavior. We found that lower incubation temperatures produced more active hatchlings and that satiated hatchlings were less active than their fasted counterparts. While the open field test is a robust assay for variation in locomotor/exploratory behavior, one cannot be certain of the mechanisms underlying these differences in behavior. The neurological and physiological processes underlying variation in locomotor behavior are currently not well defined. In order to fully understand these mechanisms, additional molecular, histological, or physical assays should be considered [46].

Finally, incubation temperature induced differences in locomotor and exploratory behaviors could have fitness consequences (e.g., by affecting foraging success) for the common snapping turtle and other reptiles if these behaviors persist beyond the first few months of life. This study highlights the important role incubation temperature during development has on ectotherms by influencing spontaneous locomotor behaviors.

Declaration of Competing Interest

The authors declare no competing interests.

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References

- [1] D. Bachtrog, J.E. Mank, C.L. Peichel, M. Kirkpatrick, S.P. Otto, T.-L. Ashman, M.W. Hahn, J. Kitano, I. Mayrose, R. Ming, N. Perrin, L. Ross, N. Valenzuela, J.C. Vamori, The Tree of Sex Consortium, Sex determination: why so many ways of doing it? *PLoS Biol.* 12 (7) (2014), <https://doi.org/10.1371/journal.pbio.1001899>.
- [2] S. O'Steen, Embryonic temperature influence juvenile temperature choice and growth rate in snapping turtles *Chelydra serpentina*, *J. Exp. Zool.* 201 (1998) 439–449.
- [3] T. Rhen, J. Lang, Temperature during embryonic and juvenile development influences growth in hatchling snapping turtles, *Chelydra serpentina*, *J. Therm. Biol.* 24 (1) (1999) 33–41, [https://doi.org/10.1016/s0306-4565\(98\)00035-7](https://doi.org/10.1016/s0306-4565(98)00035-7).
- [4] T. Rhen, J.W. Lang, Phenotypic effects of incubation temperature in reptiles, *Temperature-Dependent Sex Determination in Vertebrates*, Smithsonian Institution, Washington, 2004, pp. 90–98.
- [5] J. Burger, Antipredator behaviour of hatchling snakes: effects of incubation temperature and simulated predators, *Anim. Behav.* 56 (3) (1998) 547–553, <https://doi.org/10.1006/anbe.1998.0809>.
- [6] C. Vila Pouca, C. Gervais, J. Reed, J. Michard, C. Brown, Quantity discrimination in Port Jackson sharks incubated under elevated temperatures, *Behav. Ecol. Sociobiol.* 73 (7) (2019), <https://doi.org/10.1007/s00265-019-2706-8>.
- [7] H. Siviter, D. Deeming, A. Wilkinson, Egg incubation temperature influences the growth and foraging behaviour of juvenile lizards, *Behav. Processes* 165 (2019) 9–13, <https://doi.org/10.1016/j.beproc.2019.06.003>.
- [8] S.C. Belnap, J.P. Curra, R. Lickliter, Prenatal incubation temperature affects neonatal precocial birds locomotor behavior, *Physiol. Behav.* 206 (2019) 51–58, <https://doi.org/10.1016/j.physbeh.2019.03.002>.
- [9] J. Amiel, T. Lindström, R. Shine, Egg incubation effects generate positive

- correlations between size, speed and learning ability in young lizards, *Anim. Cognit.* 17 (2) (2013) 337–347, <https://doi.org/10.1007/s10071-013-0665-4>.
- [10] H. Siviter, D. Deeming, M. van Giezen, A. Wilkinson, Incubation environment impacts the social cognition of adult lizards, *R. Soc. Open Sci.* 4 (11) (2017) 170742, <https://doi.org/10.1098/rsos.170742>.
- [11] D. Crews, J. Sakata, T. Rhen, Developmental effects on intersexual and intrasexual variation in growth and reproduction in a lizard with temperature-dependent sex determination, *Comp. Biochem. Physiol. Part C* 119 (3) (1998) 229–241, [https://doi.org/10.1016/S0742-8413\(98\)00012-7](https://doi.org/10.1016/S0742-8413(98)00012-7).
- [12] T. Rhen, D. Crews, Variation in reproductive behaviour within a sex: neural systems and endocrine activation, *J. Neuroendocrinol.* 14 (7) (2002) 517–531, <https://doi.org/10.1046/j.1365-2826.2002.00820.x>.
- [13] J.T. Sakata, D. Crews, Developmental sculpting of social phenotype and plasticity, *Neurosci. Biobehav. Rev.* 28 (2) (2004) 95–112, <https://doi.org/10.1016/j.neubiorev.2004.01.001>.
- [14] J.C. Halford, S.C. Wanninayake, J.E. Blundell, Behavioral satiety sequence (BSS) for the diagnosis of drug action on food intake, *Pharmacol. Biochem. Behav.* 61 (2) (1998) 159–168, [https://doi.org/10.1016/S0091-3057\(98\)00032-x](https://doi.org/10.1016/S0091-3057(98)00032-x).
- [15] G.P. Brown, R.J. Brooks, Thermal and behavioral responses to feeding in free-ranging turtles, *Chelydra serpentina*, *J. Herpetol.* 25 (3) (1991) 273, <https://doi.org/10.2307/1564584>.
- [16] T. Garland, S. Arnold, Effects of a full stomach on locomotor performance of juvenile garter snakes (*Thamnophis elegans*), *Copeia* 1983 (4) (1983) 1092–1096, <https://doi.org/10.2307/1445117>.
- [17] L. Prut, C. Belzung, The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review, *Eur. J. Pharmacol.* 463 (1–3) (2003) 3–33, [https://doi.org/10.1016/S0014-2999\(03\)01272-x](https://doi.org/10.1016/S0014-2999(03)01272-x).
- [18] S.K. Singh, D. Das, T. Rhen, Embryonic temperature programs phenotype in reptiles, *Front. Physiol.* 11 (2020) 35.
- [19] N. Valenzuela, R. Literman, J. Neuwald, B. Mizoguchi, J. Iverson, J. Riley, J. Litzgus, Extreme thermal fluctuations from climate change unexpectedly accelerate demographic collapse of vertebrates with temperature-dependent sex determination, *Sci. Rep.* 9 (1) (2019), <https://doi.org/10.1038/s41598-019-40597-4>.
- [20] J. Tamplin, A. Cyr, Effects of acclimation and egg-incubation temperature on selected temperature by hatchling western painted turtles (*Chrysemys picta bellii*), *J. Therm. Biol.* 36 (8) (2011) 507–514, <https://doi.org/10.1016/j.jtherbio.2011.09.001>.
- [21] W. Du, X. Ji, The effects of incubation thermal environments on size, locomotor performance and early growth of hatchling soft-shelled turtles, *Pelodiscus sinensis*, *J. Therm. Biol.* 28 (4) (2003) 279–286, [https://doi.org/10.1016/S0306-4565\(03\)00003-2](https://doi.org/10.1016/S0306-4565(03)00003-2).
- [22] M. Mueller, N. Ruiz-García, A. García-Gasca, F. Abreu-Grobois, Best swimmers hatch from intermediate temperatures: effect of incubation temperature on swimming performance of olive ridley sea turtle hatchlings, *J. Exp. Marine Biol. Ecol.* 519 (2019) 151186, <https://doi.org/10.1016/j.jembe.2019.151186>.
- [23] L. Fisher, M. Godfrey, D. Owens, Incubation temperature effects on hatchling performance in the loggerhead sea turtle (*Caretta caretta*), *Plos ONE* 9 (12) (2014) e114880, <https://doi.org/10.1371/journal.pone.0114880>.
- [24] E.A. Burgess, D.T. Booth, J.M. Lanyon, Swimming performance of hatchling green turtles is affected by incubation temperature, *Coral Reefs* 25 (3) (2006) 341–349, <https://doi.org/10.1007/s00338-006-0116-7>.
- [25] X. Ji, H. Huang, X. Hu, W. Du, Geographic variation in female reproductive characteristics and egg incubation of *Eumeces chinensis*, *Chin. J. Appl. Ecol.* 13 (6) (2002) 680–684.
- [26] D.L. Flores, D. Crews, Effect of hormonal manipulation on sociosexual behavior in adult female leopard geckos (*Eublepharis macularius*), a species with temperature-dependent sex determination, *Horm. Behav.* 29 (4) (1995) 458–473, <https://doi.org/10.1006/hbeh.1995.1277>.
- [27] T. Rhen, D. Crews, Embryonic temperature and gonadal sex organize male-typical sexual and aggressive behavior in a lizard with temperature-dependent sex determination, *Endocrinology* 140 (10) (1999) 4501–4508, <https://doi.org/10.1210/endo.140.10.7083>.
- [28] J.T. Sakata, P. Coomber, F. Gonzalez-Lima, D. Crews, Functional connectivity among limbic brain areas: differential effects of incubation temperature and gonadal sex in the leopard gecko, *Eublepharis macularius*, *Brain Behav. Evol.* 55 (3) (2000) 139–151, <https://doi.org/10.1159/00006648>.
- [29] J.T. Sakata, D. Crews, Embryonic temperature shapes behavioural change following social experience in male leopard geckos, *Eublepharis macularius*, *Anim. Behav.* 66 (5) (2003) 839–846, <https://doi.org/10.1006/anbe.2003.2294>.
- [30] D. Crews, P. Coomber, R. Baldwin, N. Azad, F. Gonzalez-Lima, Brain organization in a reptile lacking sex chromosomes: effects of gonadectomy and exogenous testosterone, *Horm. Behav.* 30 (4) (1996) 474–486, <https://doi.org/10.1006/hbeh.1996.0051>.
- [31] D. Crews, N. Greenberg, Social signals in lizards, *BioScience* 31 (1) (1981) 51–53, <https://doi.org/10.2307/1308180>.
- [32] A. Carter, R. Paitz, K. McGhee, R. Bowden, Turtle hatchlings show behavioral types that are robust to developmental manipulations, *Physiol. Behav.* 155 (2016) 46–55, <https://doi.org/10.1016/j.physbeh.2015.11.034>.
- [33] R.B. Huey, *Temperature, Physiology and the Ecology of Reptiles*, Academic Press, New York, 1982, pp. 25–91.
- [34] G. Bulté, G. Blouin-Demers, Estimating the energetic significance of basking behaviour in a temperate-zone turtle, *Ecoscience* 17 (2010) 387–393.
- [35] J. Crawley, R. Corwin, Biological actions of cholecystokinin, *Peptides* 15 (4) (1994) 731–755, [https://doi.org/10.1016/0196-9781\(94\)90104-x](https://doi.org/10.1016/0196-9781(94)90104-x).
- [36] D.A. Gapp, M.P. Kenny, J.M. Polak, The gastro-entero-pancreatic system of the turtle, *Chrysemys picta*, *Peptides* 6 (1985) 347–352, [https://doi.org/10.1016/0196-9781\(85\)90396-1](https://doi.org/10.1016/0196-9781(85)90396-1).
- [37] L. Sievert, G. Sievert, P. Cupp, Metabolic rate of feeding and fasting juvenile midland painted turtles, *Chrysemys picta*, *Comp. Biochem. Physiol. Part A* 90 (1) (1988) 157–159, [https://doi.org/10.1016/0300-9629\(88\)91022-5](https://doi.org/10.1016/0300-9629(88)91022-5).
- [38] D. March, E. Ariel, S. Munns, D. Rudd, D. Blyde, L. Christidis, B. Kelaher, Physiological changes in post-hatchling green turtles (*Chelonia mydas*) following short-term fasting: implications for release protocols, *Conserv. Physiol.* 7 (1) (2019), <https://doi.org/10.1093/conphys/coz016>.
- [39] M. Micheli-Campbell, H. Campbell, R. Cramp, D. Booth, C. Franklin, Staying cool, keeping strong: incubation temperature affects performance in a freshwater turtle, *J. Zool.* 285 (4) (2011) 266–273, <https://doi.org/10.1111/j.1469-7998.2011.00840.x>.
- [40] M. Micheli-Campbell, M. Gordos, H. Campbell, D. Booth, C. Franklin, The influence of daily temperature fluctuations during incubation upon the phenotype of a freshwater turtle, *J. Zool.* 288 (2) (2012) 143–150, <https://doi.org/10.1111/j.1469-7998.2012.00934.x>.
- [41] R. Andrews, T. Mathies, D. Warner, Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*, *Herpetol. Monogr.* 14 (2000) 420, <https://doi.org/10.2307/1467055>.
- [42] G. Ashmore, F. Janzen, Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures, *Oecologia* 134 (2) (2003) 182–188, <https://doi.org/10.1007/s00442-002-1109-z>.
- [43] X. Ji, J. Gao, J. Han, Phenotypic responses of hatchlings to constant versus fluctuating incubation temperatures in the multi-banded krait, *Bungarus multicinctus* (Elapidae), *Zool. Sci.* 24 (4) (2007) 384–390, <https://doi.org/10.2108/zsj.24.384>.
- [44] L. Lin, H. Li, H. An, X. Ji, Do temperature fluctuations during incubation always play an important role in shaping the phenotype of hatchling reptiles, *J. Therm. Biol.* 33 (3) (2008) 193–199, <https://doi.org/10.1016/j.jtherbio.2007.12.003>.
- [45] H. Li, Z. Zhou, T. Wu, Y. Wu, X. Ji, Do fluctuations in incubation temperature affect hatchling quality in the Chinese soft-shelled turtle *Pelodiscus sinensis*? *Aquaculture* 406/407 (2013) 91–96, <https://doi.org/10.1016/j.aquaculture.2013.05.011>.
- [46] K. Tatem, J. Quinn, A. Phadke, Q. Yu, H. Gordish-Dressman, K. Nagaraju, Behavioral and locomotor measurements using an open field activity monitoring system for skeletal muscle diseases, *J. Vis. Exp.* (91) (2014), <https://doi.org/10.3791/51785>.