

Incubation temperature in the wild influences hatchling phenotype of two freshwater turtle species

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ABSTRACT

Background: The nest environment influences phenotypic traits of hatchling turtles. Female turtles select nest sites that promote hatchling survival, and alter nesting behaviour in response to changing environments. Differences in phenotype generated by incubation environment could provide variation in traits that natural selection can act upon. The relationship between incubation temperature in the laboratory and post-hatching phenotype is well documented, but whether incubation in nature generates biologically meaningful levels of phenotypic variation is less well studied.

Questions: (1) What are the effects of canopy cover, laying date, and nest depth on incubation temperature? (2) What are the relationships between incubation temperature, egg mass, and hatchling phenotype? (3) What are the sex-specific effects of incubation temperature on phenotypic variation in two turtles with temperature-dependent sex determination?

Organisms: Painted turtle (*Chrysemys picta*) and snapping turtle (*Chelydra serpentina*) nests and hatchlings from Algonquin Park, Ontario, Canada.

Methods: In 2010 and 2011, we measured canopy cover at nests and hourly temperatures within nests throughout incubation. Post-parturition, we measured egg mass of each clutch. After emergence, we measured hatchling righting response (time taken to flip from carapace to plastron), carapace length, and mass.

Conclusions: Canopy cover and oviposition date did not affect nest temperature, but nest depth influenced daily temperature variance in snapping turtle nests. However, limited variation in environmental characteristics suggests that a female's ability to select microhabitats that adaptively affect offspring survivorship or phenotype is limited. Female painted turtles with heavier eggs selected nest sites that were warmer. Nest incubation temperature was related to multiple hatchling characteristics. Painted turtle hatchling carapace length was positively related to mean incubation temperature, but snapping turtle hatchling size was not related to incubation temperature. Painted turtle hatchling righting response was not related to incubation temperature, but snapping turtle hatchlings from warmer nests righted themselves more quickly and hatchlings from nests with greater temperature variance righted more slowly. Our predicted nest sex ratios suggested that warmer nests with heavier eggs would produce female hatchlings. Also, in both species, carapace length was greater for hatchlings from nests

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predicted to produce females than from nests predicted to produce males. These findings from a natural setting help to inform future research on the adaptive significance of temperature-dependent sex determination.

Keywords: righting response, oviposition date, temperature-dependent sex determination, constant temperature equivalent, temperature-dependent differential fitness hypothesis, Charnov-Bull hypothesis, maternal condition-dependent choice hypothesis.

INTRODUCTION

In reptiles, incubation temperature influences hatchling size, body condition, frequency of deformities, and offspring sex (Bull, 1980; McKnight and Gutzke, 1993; Diaz-Paniagua *et al.*, 1997; Hewavitharath and Parmenter, 2001; Booth *et al.*, 2004; Refsnider, 2013; Riley and Litzgus, 2013). In many turtle, lizard, and snake species, incubation temperature also affects post-hatching performance (e.g. righting ability, swimming speed), and performance differences can be long-lasting and reflect future survival (Burger, 1989; Janzen, 1993a, 1995; Shine *et al.*, 1997; Freedberg *et al.*, 2001, 2004). More broadly, incubation temperature is related to hatchling survival, growth rates, metabolism, behaviours, and habitat selection (Brooks *et al.*, 1991; Van Damme *et al.*, 1992; O'Steen, 1998; Rhen and Lang, 1998; Du and Ji, 2003). Thus, incubation temperature determines a substantial portion of an individual reptile's characteristics.

Female turtles may use the environmental characteristics surrounding nests, such as canopy and vegetation cover, to select nest location; canopy cover may, in turn, affect nest temperature, and consequently hatchling phenotypes (Weisrock and Janzen, 1999; Cotter and Sheil, 2014). Additionally, female painted turtles (*Chrysemys picta*) show plasticity in oviposition date and nest depth in response to environmental cues, which can affect nest temperature and thus hatchling phenotypes (Wilson, 1998; Morjan, 2003a; Shawanz and Janzen, 2008). Female nest selection has important implications for maximizing hatchling fitness in light of environmental change [e.g. nest sex ratio in a changing climate (Wilson, 1998; Schwanz and Janzen, 2008)].

Studies examining the effects of incubation conditions on post-hatching fitness in reptiles have largely been based on incubation at constant temperatures in the laboratory (Paitz *et al.*, 2010; Neuwald and Valenzuela, 2011). Nest temperatures fluctuate naturally throughout incubation, and laboratory studies that incubated eggs at programmed fluctuating temperatures found differences in hatchling developmental time, sex ratio, mass, and locomotor performance compared with eggs incubated under constant conditions (Schwarzkopf and Brooks, 1985; Doody, 1999; Ashmore and Janzen, 2003; Du and Ji, 2003; Booth, 2006; Mullins and Janzen, 2006; Les *et al.*, 2007; Paitz *et al.*, 2010). Although temperature variation has a marked effect on the development of reptiles, potentially other less well examined, naturally varying environmental variables may play important roles in determining post-hatching phenotype and fitness.

In many reptiles, offspring sex is also determined by the embryonic developmental environment, a system known as 'environmental sex determination' [ESD (Bull, 1980; Schwarzkopf and Brooks, 1985; Hanson *et al.*, 1998; Pieau *et al.*, 1999; Valenzuela, 2004)]. Temperature-dependent sex determination (TSD), a form of ESD, is particularly common in long-lived reptiles (turtles, crocodilians, tuatara). Many researchers have hypothesized about the adaptive nature of TSD because of this commonality. Perhaps the most widely studied hypothesis is the 'temperature-dependent differential fitness' or 'Charnov-Bull' hypothesis, which asserts that TSD is adaptive if developmental environment differentially impacts sex-specific fitness

(Charnov and Bull, 1977; Valenzuela, 2004). It predicts that TSD maximizes offspring fitness by matching offspring sex with the incubation environment that optimizes the lifetime fitness of that sex. Warner and Shine (2008) showed support for this hypothesis by demonstrating that reproductive success for each sex in a short-lived lizard (*Amphibolurus muricatus*) is maximized at the incubation temperature producing that sex. A less widely examined hypothesis to explain the adaptive nature of TSD is the 'maternal condition-dependent choice' hypothesis, which proposes that mothers select developmental environments to produce the sex that would benefit most from their maternal effects, favouring TSD (Roosenburg, 1996). This hypothesis predicts that mothers carrying larger eggs will preferentially lay nests in environments that produce the sex that benefits most from greater egg mass. This model is predicated on the assumption that larger eggs produce larger hatchlings, and one sex may benefit more from larger hatchling size. Although larger egg (and hatchling) mass may be disproportionately favourable to female lifetime fitness in species where females grow larger (Roosenburg and Kelley, 1996), the evidence does not consistently show that females lay larger eggs in female-producing environments (Roosenburg, 1996; Morjan, 2003b).

To determine if natural incubation environments generate biologically meaningful phenotypic variation in reptiles, the effects of incubation environment on hatchling phenotypes must be studied under natural conditions (Shine, 1999). First, we examined whether nest incubation temperatures were affected by nest depth, canopy cover, and nest lay date for snapping turtles (*Chelydra serpentina*) and painted turtles (*Chrysemys picta marginata*) at their northern range limits. Second, we examined whether natural incubation temperature had an effect on egg mass and hatchling phenotype (righting response and body size) in these two turtle species. Third, we predicted clutch sex ratios and interpreted whether sex-specific phenotypic differences exist in nature that could differentially impact fitness of the sexes (in accordance with Charnov and Bull, 1977).

METHODS

Study area

The study was conducted at two sites in western Algonquin Park, Ontario, Canada. The first study site is located at the Wildlife Research Station (45°35'N, 78°30'W) within the North Madawaska watershed. Nesting sites around the Wildlife Research Station vary from natural sand dunes beside lakes to gravel embankments along access roads and Highway 60. The second study site, the Arowhon area (west of the Wildlife Research Station), is bisected by a gravel railway embankment built in 1895, now decommissioned as a rail-line but used as a public hiking trail (Mizzy Lake Trail); the sparsely vegetated gravel embankment is the main oviposition site for both turtle species (Schwarzkopf and Brooks, 1985; Hughes, 2003).

Our study area is contained within the Algonquin-Lake Nipissing ecoregion, and is a rugged landscape underlain by Precambrian Shield outcrops (Ontario Ministry of Natural Resources, 1998). Elevations on the west side of Algonquin Park (370–570 m above sea level) are higher than the surrounding landscape, and this creates a colder and wetter climate (Ontario Ministry of Natural Resources, 1998). This climate is representative of the northern range limits of both turtle species.

Field methodology

In 2010, nest site monitoring began on 20 May and ended on 20 June. In 2011, nest site monitoring occurred between 5 June and 4 July. Nest monitoring began when females started to congregate in aquatic habitats adjacent to nest sites, and/or terrestrial nest searching behaviour was observed. Researchers monitored nest sites visually on foot. Monitoring occurred during peak nesting for both species (Ernst and Lovich, 2009): in the morning from dawn (c. 5 am) to about 10 am, and in the afternoon from just prior to dusk (c. 5 pm) until after dark, so long as there was nesting activity. Monitoring of nesting areas ceased when no nesting activity was seen for three days in succession.

Nests were excavated after females had completed oviposition. Eggs were removed and placed in plastic bins lined with moistened vermiculite (1 : 1 ratio of vermiculite to water by weight). As eggs were removed, they were numbered using a pencil to ensure they were returned to the nest in the same order and orientation. The depth to the top and bottom of the nest cavity was measured to the nearest 0.1 cm using a ruler. After the eggs were removed, nests were filled with excavated soil to reduce desiccation. Nest locations were marked with metal stakes and flagging tape. Eggs were transported to the laboratory at the Wildlife Research Station, where they were weighed with a digital scale to the nearest 0.1 g (SP202, Scout Pro, Ohaus Corporation, Pine Brook, NJ). Processing and return of eggs to the nest occurred within 24 hours of oviposition, prior to adherence of the vitelline membrane to the inner shell surface when a 'white spot' forms on the top of the egg (Yntema, 1968; Rafferty and Reina, 2012); this ensured no trauma to developing embryos (Samson *et al.*, 2007).

Most nests were fitted with predator-exclusion cages to prevent depredation of eggs and hatchlings. A variety of nest cage types were used to test the effects of nest caging on hatchling fitness for another study (Riley and Litzgus, 2013). Each nest was randomly assigned to a nest caging treatment (above-ground or below-ground hardware cloth, uncaged control) and cages were deployed during egg reburial. These cage types did not affect incubation temperature in either species (Riley and Litzgus, 2013). The eggs were reburied in the original nest cavity, at the original depths and in the original order, with a temperature data logger in the centre of the clutch. The temperature data logger used was either a waterproofed iButton® (accuracy of $\pm 1^\circ\text{C}$ or $\pm 0.5^\circ\text{C}$; Thermochron DS1921G, Dallas Semiconductor, Sunnyvale, CA) or a HOBO StowAway® (accuracy of $\pm 0.2^\circ\text{C}$; TidbiT TBI32-05 + 37, Onset Computer Corp., Bourne, CA). Temperature readings from data loggers of different types, and with and without waterproofing, did not differ [our data: $F_{3,2480} = 2.01$, $P = 0.94$; Roznik and Alford (2012)]. Data loggers recorded temperature hourly. In late August 2010 and 2011, close to the date when emergence of hatchlings was anticipated, above-ground cages were fitted to the uncaged nests to trap emerging hatchlings.

Canopy cover (%) was measured using a densiometer positioned 30 cm above the nest (Lemmon, 1957). In 2010, once per month from oviposition to October, and on the day of emergence, canopy cover at nests was measured. In 2011, canopy cover was measured once a fortnight from oviposition to October, and on the day of emergence.

Daily monitoring for hatchling emergence began a few days before the predicted emergence period: 63 days post-oviposition for snapping turtles, and 89 days post-oviposition for painted turtles (Ernst and Lovich, 2009). The first snapping and painted turtles both emerged on 25 August in 2010, and on 27 August and 1 September for painted turtles and snapping turtles respectively in 2011. When hatchlings emerged from their nests, all

the hatchlings and unhatched eggs were collected and transported to the laboratory at the Wildlife Research Station for processing. Hatchlings ceased emerging on 30 September 2010 and 28 September 2011.

Midline carapace length of each hatchling was measured to the nearest 0.01 mm using digital calipers (3148, Traceable Digital Calipers, Control Company, Friendswood, TX). Hatchling mass was measured to the nearest 0.1 g using a digital scale (SP202, Scout Pro, Ohaus Corporation, Pine Brook, NJ).

After exiting the nest, hatchlings often disperse overland to their overwintering habitat (Paterson *et al.*, 2014; Riley *et al.*, 2014). Efficient locomotion allows hatchlings to escape predation, desiccation, and drowning. A turtle that cannot quickly right itself is more likely to succumb to these mortality risks (Finkler and Claussen, 1997). Thus, hatchling performance in righting response trials indirectly relates to future survival (Delmas *et al.*, 2007). Our righting response test consisted of placing each hatchling on its carapace on a flat cloth-covered board (30 × 15 cm) and waiting for the turtle to flip onto its plastron. Two variables were timed to the nearest 0.01 s using a digital stopwatch: (1) latency period, which is the time from placement on the turtle's carapace until the first righting attempt; and (2) righting period, which is the time from first righting attempt until successful righting (Freedberg *et al.*, 2004; Delmas *et al.*, 2007; Rasmussen and Litzgus, 2010; Riley and Litzgus, 2013; Riley *et al.*, 2014). To account for any differences in laboratory temperature during righting trials, the ambient temperature of the room was recorded for each righting trial. Trials were recorded with a digital camera (Photosmart R742, HewPackard Development Company, Mississauga, ON), and latency period and righting period were scored from recordings. If a turtle did not right itself within 15 minutes, it was removed from analysis. Measurements and righting trials occurred within 24 hours of emergence, after which hatchlings were released back at their natal nests.

Analyses

Hourly incubation temperatures were extracted from data loggers and summarized by calculating both average daily nest temperature and average daily temperature variance from 48 hours post-oviposition until 24 hours pre-emergence for each nest for both species. Daily temperature variance describes the degree to which temperatures fluctuate around the mean within 24 hours (Paitz *et al.*, 2010; Neuwald and Valenzuela, 2011). Temperature variance has been shown to influence hatchling development (Schwarzkopf and Brooks, 1985; Doody, 1999; Ashmore and Janzen, 2003; Mullins and Janzen, 2006; Les *et al.*, 2007; Paitz *et al.*, 2010).

Percentage canopy cover was averaged across incubation for each nest. Data were then transformed using $\log_{10}(y + 1)$ to ensure normality. Nest lay date was coded in annual numeric sequence (Wilimovsky, 1990). Hatchling righting response variables (latency period and righting period) were averaged for each clutch, and were transformed using $\log_{10}(y + 1)$ to ensure normality before statistical analysis. Finally, hatchling carapace length and mass, and egg mass were averaged for each clutch.

Multiple linear regressions were constructed to determine whether nest depth, canopy cover or lay date affected incubation temperature (daily mean and daily variance). In addition to the main variables, these models also included the additional factor of year. Multiple linear regressions were also used to determine if nest temperature variables influenced egg mass, and hatchling righting response (latency period and righting period, tested separately), mass, and carapace length. Linear regressions used to examine whether daily nest temperature was related to egg mass also included the factor of year. We also

examined whether hatchling size (carapace length and mass) was related to egg mass, using a linear regression that also included year. Linear regressions used to examine the relationship between incubation temperature (daily mean and daily variance, tested separately) and righting period also included the factors of laboratory temperature during each righting trial [which is known to affect ectotherm performance (Hutchison *et al.*, 1966)] and year. Linear regressions used to examine the relationships between incubation temperature variables and hatchling carapace length and mass also included year.

We were also interested in examining the potential relationship between sex-determining temperatures and post-hatching phenotype, so we calculated the constant temperature equivalent [CTE (Georges, 1989)] for each nest. For each nest, mean daily nest temperature and daily range of temperatures over the middle third of incubation were calculated, coinciding with the temperature-sensitive period of sex determination. For *C. serpentina*, we used a minimal developmental temperature (T_0) of 16°C (Freedberg *et al.*, 2011), whereas for *Ch. picta* T_0 was set at 14°C (Les *et al.*, 2007). The aforementioned values were used to parameterize equation (4) from Georges (1989), which was solved via iterative methods in Excel. Calculated CTEs were used to designate individual nests as female- or male-producing. Specifically, nests with CTEs above the estimated species-specific pivotal temperature for sex determination for this geographic region [*Ch. picta* = 27.5°C (Schwarzkopf and Brooks, 1985); *C. serpentina* = 27.1°C (Ewert *et al.*, 1994)] were considered ‘female-producing’, whereas nests with CTEs below the pivotal temperature were considered ‘male-producing’. Although female *C. serpentina* are also produced below a lower sex-determining threshold of 22°C (Ewert *et al.*, 1994), no nests in our study had a CTE below this threshold.

We could not identify the sex of hatchlings from their gonads because our study was conducted at a long-term study site for painted and snapping turtles, and sacrificing cohorts of hatchlings for gender identification was not logistically viable. The CTE accounts for the impact of variation in development rate associated with fluctuating daily nest temperature, and has been shown to be an accurate predictor of nest sex ratios in turtles with environmental sex determination (Georges *et al.*, 1994, reviewed in Telemeco *et al.*, 2013). While individual nests near the pivotal temperature may theoretically produce mixed sex ratios, mixed-sex clutches in the wild are rare in *Chrysemys* and *Chelydra* (Janzen, 1994; Kolbe and Janzen, 2002) and very few nests fell in the predicted transitional range in our study (see Results). Because mixed-sex nest sex ratios can only be determined from temperature data when precise data on the sex-determining response curve are available for that geographic region, we did not feel that we could reasonably predict specific nest sex ratios for turtles in our population. We thus simply presumed nests to be male- or female-producing. Student’s unequal variance *t*-tests were used to determine whether the sexes differed in egg mass and hatchling phenotypic variables [carapace length, mass, and righting response transformed using $\log_{10}(x + 1)$].

In all statistical tests, assumptions of normality and homogeneity of variance were verified. Data were transformed to ensure normality as needed (see above for specifics). No significant interactions were found in the models with multiple factors, so only main effects were tested and reported. All summary data are reported as the mean $\pm$ 1 standard error. The significance level of $\alpha = 0.05$ was used for all statistical tests. Linear models were constructed in R (R Development Core Team, 2012).

RESULTS

Mean painted turtle daily nest temperature was $22.81 \pm 0.29^\circ\text{C}$ (min: 19.55°C ; max: 24.70°C). Mean painted turtle nest temperature variance within a 24 hour period was $11.67 \pm 0.53^\circ\text{C}$ (min: 6.02°C ; max: 16.37°C). Mean snapping turtle daily nest temperature was $23.21 \pm 0.16^\circ\text{C}$ (min: 20.83°C ; max: 24.94°C). Mean snapping turtle daily nest temperature variance was $10.19 \pm 0.33^\circ\text{C}$ (min: 6.16°C ; max: 15.30°C).

Environmental effects on nest temperature

Canopy cover and lay date were not correlated with nest temperatures for either species. In painted turtles, canopy cover was not related to mean daily nest temperature ($F_{1,17} = 0.14$, $P = 0.71$) or mean nest temperature variance ($F_{1,17} = 1.58$, $P = 0.23$). In snapping turtles, canopy cover was not related to mean daily nest temperature ($F_{1,43} = 1.11$, $P = 0.30$) or mean nest temperature variance ($F_{1,43} = 0.20$, $P = 0.65$). In painted turtles, nest lay date was not related to mean daily temperature ($F_{1,17} = 0.17$, $P = 0.69$) or mean daily temperature variance ($F_{1,17} = 0.19$, $P = 0.67$). In snapping turtles, lay date was not related to mean daily temperature ($F_{1,43} = 0.37$, $P = 0.55$) or mean daily temperature variance ($F_{1,43} = 0.12$, $P = 0.73$).

Nest depth was negatively related to nest temperature variance in snapping turtle nests ($F_{1,40} = 9.76$, $P = 0.01$), in that deeper nests had less daily variation in temperature (Fig. 1), but nest depth was not related to mean nest temperatures ($F_{1,40} = 2.22$, $P = 0.14$). Painted turtle nest depth was not related to mean nest temperature ($F_{1,17} = 0.36$, $P = 0.56$) or nest temperature variance ($F_{1,17} = 0.09$, $P = 0.77$).

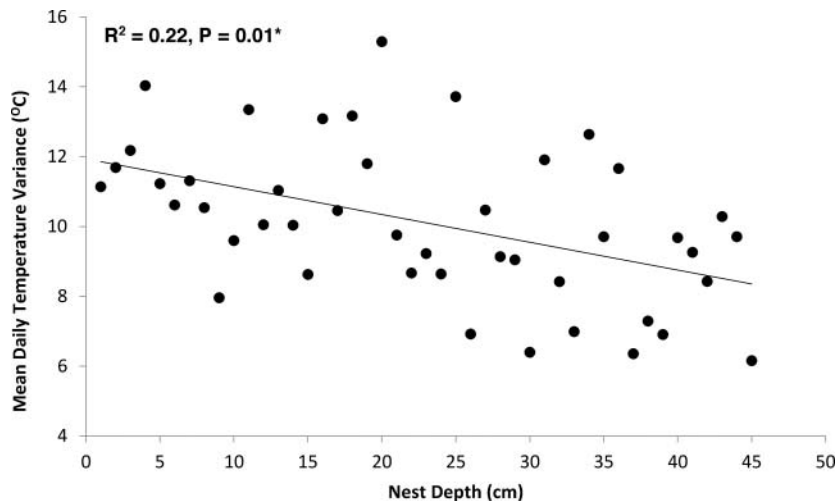


Fig. 1. Nest depth and mean daily nest temperature variance were significantly, negatively related in snapping turtles (*Chelydra serpentina*); deeper nests exhibited less temperature variance.

Relationships between nest temperature, egg mass, and hatchling phenotype

Painted turtles

In painted turtles, average daily nest temperature was marginally, positively related to egg mass ($F_{1,17} = 4.41$, $P = 0.05$; Fig. 2). Hatchling carapace length was not related to egg mass ($F_{1,17} = 1.58$, $P = 0.22$), but hatchling mass was significantly, positively related to egg mass ($F_{1,17} = 17.33$, $P < 0.01$).

On average, painted turtle latency period was 82.0 ± 20.4 s, and righting period was 4.7 ± 1.4 s. Neither latency (LP) nor righting period (RP) was related to mean daily nest temperature (LP: $F_{1,17} = 0.09$, $P = 0.77$; RP: $F_{1,17} = 0.79$, $P = 0.42$). The righting response variables were also not significantly related to daily nest temperature variance (LP: $F_{1,17} = 1.92$, $P = 0.18$; RP: $F_{1,17} = 0.19$, $P = 0.67$). Latency period exhibited a non-significant, negative trend with daily temperature variance, whereas righting period exhibited a non-significant, positive trend.

Painted turtle carapace length was positively related to mean daily nest temperature ($F_{1,17} = 4.81$, $P = 0.04$; Fig. 3), but no relationship was detected between carapace length and daily nest temperature variance ($F_{1,17} = 0.08$, $P = 0.78$). Hatchling mass was not related to mean daily nest temperature ($F_{1,17} = 0.06$, $P = 0.81$) or daily nest temperature variance ($F_{1,17} = 0.19$, $P = 0.67$).

Snapping turtles

Average daily nest temperature over the course of incubation was not related to egg mass ($F_{1,43} = 1.16$, $P = 0.29$). However, both hatchling carapace length ($F_{1,43} = 12.79$, $P < 0.01$) and hatchling mass ($F_{1,43} = 17.34$, $P < 0.01$) were positively related to egg mass.

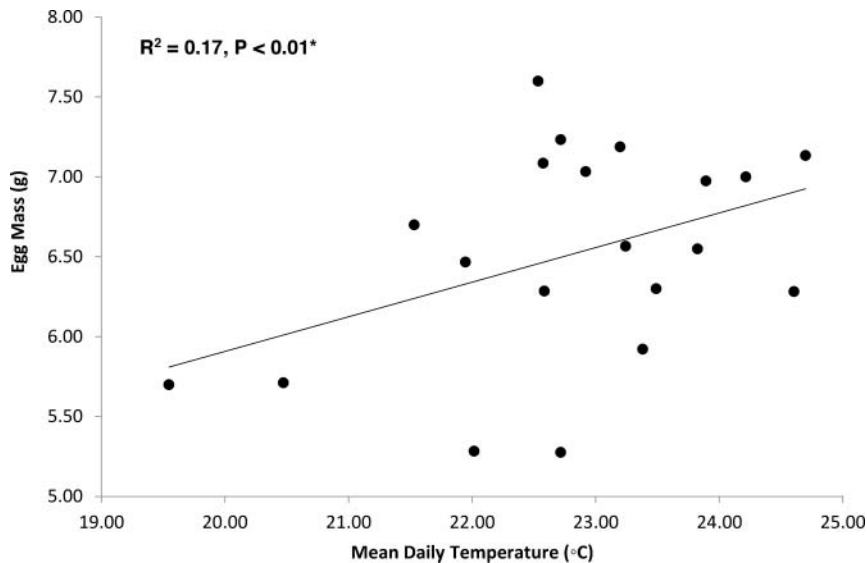


Fig. 2. Egg mass and mean daily nest temperature were significantly, positively related in painted turtles (*Chrysemys picta*); nests in which females laid larger eggs experienced warmer nest temperatures.

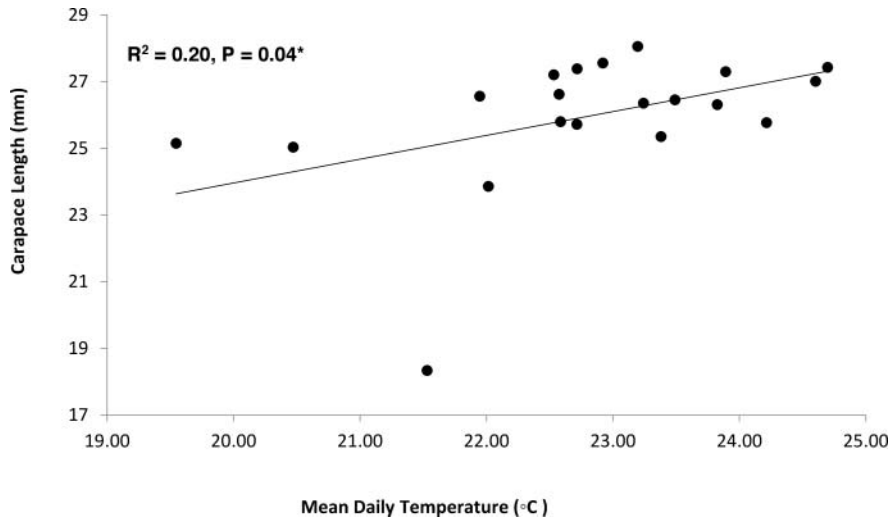


Fig. 3. Mean daily nest temperature and painted turtle (*Chrysemys picta*) hatchling carapace length were positively related; colder nests produced hatchlings with smaller carapace lengths.

Snapping turtle latency period averaged 83.1 ± 8.5 s, and righting period averaged 8.0 ± 1.9 s. Latency period was not related to nest temperature variables (mean: $F_{1,43} = 0.75$, $P = 0.39$, Fig. 4A; variance: $F_{1,43} = 1.01$, $P = 0.32$, Fig. 3B). Latency period exhibited a non-significant, negative trend with mean nest temperature and a non-significant, positive trend with nest temperature variance. Righting period was negatively related to daily mean nest temperature ($F_{1,43} = 5.47$, $P = 0.02$, Fig. 3A) and was positively related to daily temperature variance ($F_{1,43} = 7.75$, $P = 0.008$, Fig. 4B).

Snapping turtle carapace length was not related to mean daily nest temperature ($F_{1,43} = 2.82$, $P = 0.10$) or daily nest temperature variance ($F_{1,43} = 0.21$, $P = 0.65$). Similarly, hatchling mass was not related to mean daily nest temperature ($F_{1,43} = 1.16$, $P = 0.69$) or daily nest temperature variance ($F_{1,43} = 0.12$, $P = 0.73$).

Sex determination of nests

Based on the predicted constant temperature equivalents, 32% ($n = 15/47$) of snapping turtle clutches were predicted to be predominately male (evolutionary-ecology.com/data/2863Appendix.pdf, Table A1). Similarly, 30% ($n = 6/20$) of painted turtle clutches were predicted to be predominately male (2863Appendix.pdf, Table A2). In painted turtles, neither latency period ($t_{11} = -1.67$, $P = 0.12$) nor righting period ($t_{17} = -0.47$, $P = 0.65$) was significantly different between individuals from predominately male- and female-producing nests (Table 1). In painted turtles, female-producing nests were characterized by heavier eggs with an average mass of 6.76 ± 0.15 g, and male-producing nests were characterized by lighter eggs with an average mass of 5.93 ± 0.20 g ($t_{11} = 3.25$, $P < 0.01$; Table 1). Painted turtle hatchling carapace length was significantly larger for individuals from female-producing nests than male-producing nests ($t_6 = 5.26$, $P = 0.05$; Table 1). Painted turtle hatchling mass was not significantly different between nests predicted to be female- and male-producing ($t_6 = 0.56$, $P = 0.60$; Table 1).

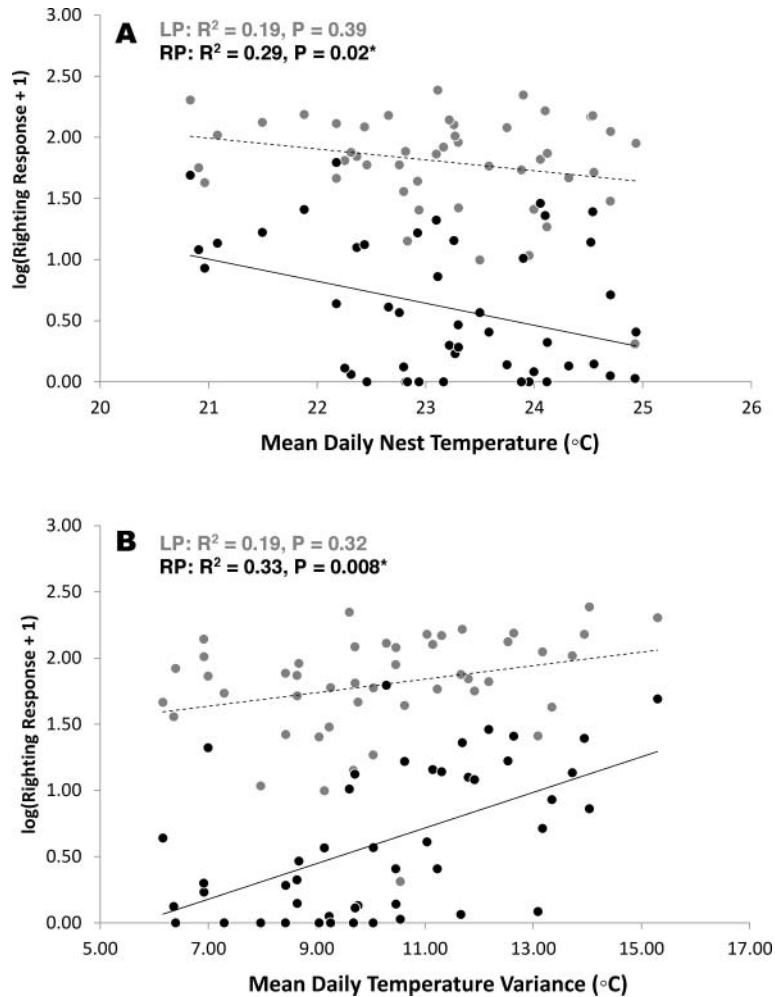


Fig. 4. The relationship between mean daily nest temperature (A) and daily temperature variance (B) and righting response, which is divided into latency period (grey circles and dashed trend line) and righting period (black circles and solid trend line) for snapping turtle (*Chelydra serpentina*) clutches.

In snapping turtles, neither latency period ($t_{41} = -0.10$, $P = 0.92$) nor righting period ($t_{24} = 0.39$, $P = 0.70$) was different between individuals from predominately male- and female-producing nests. Egg mass was not related to whether a nest was predicted to be male- or female-producing ($t_{27} = 0.69$, $P = 0.50$). Snapping turtle hatchling carapace length was significantly larger in female-producing nests relative to male-producing nests ($t_{34} = 3.75$, $P < 0.05$; Table 1). Hatchling mass did not differ between female- and male-producing nests ($t_{30} = 1.86$, $P = 0.07$).

Table 1. Mean nest temperature, righting response variables, carapace length, mass, and egg mass for male- and female-producing nests as predicted using constant temperature equivalents (CTEs) for painted turtle (*Chrysemys picta*) and snapping turtle (*Chelydra serpentina*) hatchlings from Algonquin Park, Ontario, Canada

Predominant sex ratio of nests	Daily mean temperature (°C)	Daily temperature variance (°C)	Latency period (s)	Righting period (s)	Hatchling carapace length (mm)	Hatchling mass (g)	Egg mass (g)
Painted turtle							
Male (<i>n</i> = 6)	21.59 ± 0.57	11.39 ± 1.52	149.7 ± 56.3	3.5 ± 0.6	23.92 ± 1.15	4.65 ± 0.43	5.93 ± 0.20
Female (<i>n</i> = 14)	23.33 ± 0.82	11.82 ± 0.46	53.0 ± 36.9	5.2 ± 2.0	26.84 ± 0.18	4.89 ± 0.12	6.76 ± 0.15
Snapping turtle							
Male (<i>n</i> = 15)	22.57 ± 0.18	9.03 ± 0.53	74.1 ± 10.9	9.0 ± 4.3	27.84 ± 0.30	9.03 ± 0.28	11.77 ± 0.42
Female (<i>n</i> = 31)	23.45 ± 0.20	10.80 ± 0.40	88.9 ± 11.1	7.7 ± 1.9	29.47 ± 0.33	9.68 ± 0.21	12.12 ± 0.28

Note: Data are displayed as mean ± standard error.

DISCUSSION

Environmental effects on nest temperature

No significant relationships were found between canopy cover and nest temperature variables for either species. In contrast to our results, other studies with painted turtles have found that canopy cover was negatively related to mean nest incubation temperature (Janzen, 1995; Weisrock and Janzen, 1999; Morjan, 2003a; St. Juliana *et al.*, 2004; Cotter and Sheil, 2014). Also, we observed no relationships between nest lay date and temperature variables for either species. In contrast, Cotter and Sheil (2014) anecdotally observed a trend of increasing nest temperature from the first to last nest laid at their study site in Ohio. Interestingly, snapping turtle nest depth was negatively related to daily temperature variance. In New Mexico, cooler painted turtle nests were also deeper and located closer to standing water than warm nests (Morjan, 2003a). Thus, nest depth appears to be responsible for some of the variation we observed in nest temperature in snapping turtles. Our study site in Algonquin Park is at the northern range limit of the two study species, thus our sites may be cooler than those at more southerly latitudes (Bobyne and Brooks, 1994). At northern latitudes, the active season for ectotherms is often shorter than it is at southern latitudes (Christiansen and Moll, 1973). In Illinois, the relationship between canopy cover and nest temperature was absent during two cooler summers (Janzen, 1994), which reflects our result that in the cooler, northern limit of *Ch. picta*'s range, there is no correlation between these two variables. At our study site, mean nest temperature was similar to that in nests studied by Cotter and Sheil (2014) but the range of nest canopy cover found at our study site was much more restricted than at theirs [nest canopy cover ranged from 0 to 54% for painted turtle nests and from 0 to 37% for snapping turtle nests in our study, and from 5 to 90% for painted turtle nests in that of Cotter and Sheil (2014)]. Restricted variation in canopy cover, and the restricted active season at our study site, may limit a female's ability to select microhabitats to affect offspring survivorship or size, but snapping turtles may be able to alter nest depth in order to positively influence hatchling morphometrics.

Overall, our findings indicate that there is no substantial variation in environmental characteristics (other than snapping turtle nest depth) at our study site, and that lay date, canopy cover, and painted turtle nest depth may not be main drivers of nest temperature variation. This is not to say that females select nests at random. Maternal nest-site choice is well documented in snapping and painted turtles (Kolbe and Janzen, 2002; Mitchell *et al.*, 2013). Nest-site choice is a behavioural maternal effect that at other study sites has been found to alter clutch phenotype [e.g. sex ratio (St. Juliana *et al.*, 2004; Mitchell *et al.*, 2013)]. It may simply be that lay date and canopy cover at our study site do not vary enough to impact females' ability to select nest environment in order to significantly affect offspring survivorship, size or sex ratio. Other factors that we did not measure (e.g. soil hydration) may have stronger influences on hatchling phenotype in our population.

Relationships between nest temperature, egg mass, and hatchling phenotype

The temperature–size rule (TSR), thought to be a general biological law for ectotherms, states that individuals reared in warmer temperatures mature at a smaller body size than those reared in cooler temperatures (Atkinson, 1994). In support of the TSR, smaller painted and snapping turtle hatchlings were produced at higher temperatures when incubated in the laboratory and under constant temperature regimes (Gutzke *et al.*, 1987; Brooks *et al.*, 1991). In

our study, in which we examined naturally varying nest temperatures, carapace length of hatchling painted turtles was positively related to mean nest temperature; this is opposite to the expectations of the TSR. Similarly, Mitchell *et al.* (2015) found a positive relationship between hatchling size and natural nest temperatures in *Ch. picta* nests in Iowa. Also at odds with the predictions of the TSR, we found that neither snapping turtle carapace length nor mass was related to nest temperature characteristics. The unexpected nature of our results and those of Mitchell *et al.* (2015) exemplifies the importance of examining ecological phenomena in natural settings. In our study, we focused on the impact that natural nest temperatures have on hatchling characteristics, but other environmental variables may also affect hatchling phenotype. Moisture levels in nests affect *Ch. picta* and *C. serpentina* hatchling phenotype: wetter nests produce heavier and larger hatchlings (Brooks *et al.*, 1991; Finkler, 1999; Packard and Packard, 2001). From studies on *Ch. picta* in the laboratory, size and mass of hatchlings are more strongly impacted by water potential than temperature within nests (Packard *et al.*, 1989; Cagle *et al.*, 1993). Thus, to truly understand how natural nests impact hatchling characteristics, multiple environmental factors must be considered. Incubation temperature exhibits a different relationship with hatchling size in the laboratory versus in the field, and this difference points to a potentially important role of thermal variance on developmental physiology.

Temperature characteristics of painted turtle nests were not significantly related to hatchling locomotor performance. In another field study, Cotter and Sheil (2014) similarly found no relationship between incubation temperature and locomotor performance in *Ch. picta*. In contrast, Refsnider (2013) found that in natural *Ch. picta* nests, increased temperature variance resulted in increased righting response and swimming speed. Nest temperature variance was substantially higher in our study than in that of Refsnider (2013): more than half of the *Ch. picta* nests in our study experienced a temperature variance of greater than 10°C, while none of the nests in Refsnider's study did. It is likely that very high temperature variance is associated with negative impacts on physiology, as it increases time spent at thermal extremes that are suboptimal developmental environments (Freedberg and Wade, 2004). Interestingly, if we examine only the nests with lower variance (<12°C) in our study, a negative association appears between temperature variance and righting time, although reduced sample size precludes statistical confirmation of this trend. More research is required to understand the relationship between temperature variance and hatchling phenotype throughout the range of *Ch. picta*.

Snapping turtle latency period was not significantly related to nest temperature characteristics. Conversely, righting period, the time it takes for the turtle to actively right itself, was related to nest temperature characteristics. The righting period portion of the righting response directly relates to motor function, and could have implications for juvenile survival (Delmas *et al.*, 2007). Hatchlings incubated in nests with lower mean daily temperatures had faster righting periods. Laboratory studies that incubated eggs under constant temperatures have also found a relationship between mean incubation temperature and locomotor performance (Janzen, 1995; Freedberg *et al.*, 2001, 2004; Booth *et al.*, 2004). Janzen (1994) found that *C. serpentina* hatchlings from cooler incubation temperatures swam faster than those from warmer treatments, and *C. serpentina* hatchlings from nests incubated in median temperatures ran faster on land than those incubated in extreme temperatures (Janzen, 1994); however, because the results from different types of performance measures may be affected by different levels of motivation to complete each task, it is difficult to directly compare results among behavioural trials.

In our study, higher nest temperature variance resulted in a slower righting period in *C. serpentina* hatchlings. In contrast, laboratory studies have found that fluctuating temperatures enhance locomotor performance (Ashmore and Janzen, 2003; Booth, 2006), and that increased temperature variance increased mass, survival, and immune response (Du and Ji, 2003; Les *et al.*, 2007, 2009). These laboratory studies suggest that temperature fluctuations enhance hatchling fitness, but the level of variance tested in these studies [$\pm 4^{\circ}\text{C}$ in Ashmore and Janzen (2003), $\pm 3^{\circ}\text{C}$ in Les *et al.* (2007, 2009), and $\pm 2\text{--}3^{\circ}\text{C}$ in Du and Ji (2003)] was far lower than that we observed in most natural nests (mean of $\pm 10\text{--}11^{\circ}\text{C}$) and presumably was not sufficient to reach thermal extremes that are harmful to development. The high level of variance characterizing nests of both species in our study may have caused development to proceed at temperatures that had negative impacts on physiology.

Phenotype at hatching is affected by nest temperature, but once the hatchlings emerge from the nest, does this phenotype remain with the individual over the long term, and does it have fitness consequences? Survivorship of hatchling *C. serpentina* appears to be size-dependent, with natural selection favouring larger hatchling size [*aka* the ‘bigger is better’ hypothesis (Janzen, 1993b)]. Similarly, larger *Trachemys scripta elegans* hatchlings released in an area with avian predators had higher survivorship (Janzen *et al.*, 2000; Myers *et al.*, 2007). Support for the ‘bigger is better’ hypothesis may be associated with the specific predator assemblage within a geographic area, as support for this hypothesis is contested (Congdon *et al.*, 1999; Paterson *et al.*, 2014). Hatchling size may benefit survival immediately after hatching during terrestrial movements overland to overwintering sites, and selection is most likely strongest at this life-stage due to high mortality (Ernst and Lovich, 2009; Paterson *et al.*, 2012, 2014). In the laboratory, incubation temperature has long-lasting effects on righting response in *Graptemys ouachitensis* and *T. scripta elegans*: turtles incubated at warmer temperatures righted themselves more quickly immediately post-hatching as well as after a year in captivity (Freedberg *et al.*, 2004). If hatching phenotype persists, then the differences in hatchling size and performance ability that we observed may affect individual fitness later in life. A potential model system to test this hypothesis would be a shorter-lived turtle (e.g. the chicken turtle, *Deirochelys reticularia*).

Predicted sex ratio of nests

In snapping turtles, most viable constant incubation temperatures produce exclusively or almost entirely (>90%) one sex, with a narrow range of approximately 1°C encompassing the pivotal temperatures that produce a more even sex ratio (Ewert *et al.*, 2005). Similarly, narrow ranges for mixed-sex clutches are found in painted turtles (Bull, 1985). In our study, only 4 of 20 clutch CTEs fell in this 1°C range surrounding the pivotal temperature in *Ch. picta*, and 4 of 47 clutch CTEs fell in the 1°C range surrounding the upper pivotal temperature in *C. serpentina* with no CTEs residing near the lower pivotal temperature. While within-nest heterogeneity in temperature may create additional temperature variation, the large proportion of nest CTEs that fall considerably out of the mixed-sex range (Table 1) suggests that mixed-sex clutches were relatively rare at our site.

Based on the calculated CTEs, the sex ratio for both species was probably female-biased (2863Appendix.pdf, Tables A1 and A2). The adult snapping turtle sex ratio is unknown at our study site, while the sex ratio in the adult painted turtle population is 3.44 females per male (Samson, 2003). This highly skewed sex ratio has been consistent within the adult population for over 20 years (Samson, 2003 and unpublished data). Because females are under strong

selection to invest approximately equally in the sexes under temperature-dependent sex determination (Bull, 1980), this skew suggests that painted turtles at our site do not select their nests to influence the sex ratios as theorized by Janzen (1994). Natural nest temperatures that were estimated to produce mainly males were colder and less variable for both species; however, these sites may have been undesirable owing to factors associated with nest construction. Although Schwanz and Janzen (2008) found that females adjust their nest microhabitat selection to influence sex ratio of hatchlings, perhaps environmental conditions limit the opportunity for the production of viable male-producing nests at our study site. Morjan (2003b) modelled that there is likely low potential to adjust sex ratio through nest-site selection by female painted turtles despite the presence of a significant relationship between vegetation cover and nest sex ratio at her study site. Our failure to detect any relationship between canopy cover and nest temperature suggests that the potential for sex ratio adjustment may be even further limited at our site.

In both snapping and painted turtles, hatchling carapace lengths were larger for female-producing nests than male-producing ones. The 'temperature-dependent differential fitness' or 'Charnov-Bull' hypothesis for the adaptive significance of temperature-dependent sex determination (TSD) suggests that phenotypic differences associated with incubation at male versus female temperatures may facilitate sex-specific differences in fitness (Rhen and Lang, 1998). Although it is logistically challenging to determine the impacts of hatchling size on lifetime fitness in long-lived species, our findings suggest that the developmental environments producing each sex are associated with significant differences in the phenotypic traits of offspring, which could potentially be acted upon by sex-specific selective pressures. Spencer and Janzen (2014) suggested that sex-specific selection pressures may not only occur later in life, but may act upon hatchlings during their first winter; overwintering temperatures were found to affect *Ch. picta* male and female hatchling metabolic rates and energy reserves differently. This suggests that sex differences in hatchling size caused by nest temperature may immediately impact fitness of turtles. Yet, differences in size associated with incubation temperature and egg mass were seen in 3-year-old diamondback terrapins (*Malaclemys terrapin*), suggesting that mechanisms underlying size differences in turtles can persist for several years (Roosenburg and Kelley, 1996). In snapping turtles, variation in hatchling size is not correlated with post-hatching growth in the laboratory (Brooks *et al.*, 1991; Bobyn and Brooks, 1994); however, larger hatchlings are favoured in competitive interactions (Froese and Burghardt, 1974), which suggests a positive feedback system may exist for hatchling size in the field. Hatchling size is associated with several other functionally important traits in turtles, including terrestrial movement (Janzen, 1993b; Tucker, 2000), competitive ability (Froese and Burghardt, 1974), swimming speed (Myers *et al.*, 2007), and post-hatching survival (Janzen *et al.*, 2000), providing additional opportunities for sex-specific selection to act.

Painted turtle nests that were warmer and predicted to be female-producing were characterized by a larger egg mass than those that were cooler and predicted to be male-producing. If females select nest sites depending on the characteristics of their eggs, then this nest-site choice may have an impact on hatchling phenotype. Similarly, Roosenburg (1996) found that *Malaclemys terrapin* females selected warmer nest sites for larger eggs (but see Morjan, 2003b). However, the mechanism a female turtle uses to assess her pre-laid egg size and how she might use this knowledge during nesting selection is unknown, and thus more research is required to fully interpret our findings. In *M. terrapin*, egg mass is correlated with juvenile body mass in females, but not in males; this relationship is predicted to speed female maturation by 2–3 years while having no effect on male maturation (Roosenburg and Kelley, 1996).

If painted turtles similarly experience sex-specific benefits of egg size, larger eggs laid in female-producing environments could produce sex-specific fitness consequences and could provide support for the adaptive significance of TSD (Roosenburg, 1996). Alternatively, more metabolic heat may be produced from larger developing embryos, and this may increase the temperature within the nest cavity and affect TSD (Carr and Hirth, 1961; Standora and Spotila, 1985), although this potential for heating has been regarded as too little or too late in incubation to be significant (Maloney *et al.*, 1990; Booth and Astill, 2001). Nevertheless, it is a potential avenue for future research. In snapping turtles, there was no relationship between egg mass and nest temperature. Two pivotal temperatures characterize TSD in this species, thus the relationship between female nest-site choice and egg size may be more complex. Furthermore, dimorphism in adult size shows considerable intraspecific variation in snapping turtles [from larger males to no dimorphism (Galbraith *et al.*, 1988)], so a clear relationship between egg mass and nest incubation condition may not be expected.

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REFERENCES

- Ashmore, G.M. and Janzen, F.J. 2003. Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia*, **134**: 182–188.
- Atkinson, D. 1994. Temperature and organism size: a biological law for ectotherms. *Adv. Ecol. Res.*, **25**: 1–58.
- Boby, M.L. and Brooks, R.J. 1994. Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling turtles (*Chelydra serpentina*). *J. Zool.*, **233**: 233–257.
- Booth, D.T. 2006. Influence of incubation temperature on hatchling phenotype in reptiles. *Physiol. Biochem. Zool.*, **79**: 274–281.
- Booth, D.T. and Astill, K. 2001. Temperature variation within and between nests of the green sea turtle, *Chelonia mydas* (Chelonia: Cheloniidae) on Heron Island, Great Barrier Reef. *Austral. J. Zool.*, **49**: 71–84.
- Booth, D.T., Burgess, E., McCosker, J. and Lanyon, J.M. 2004. The influence of incubation temperature on post-hatching fitness characteristics of turtles. *Int. Congr. Ser.*, **1275**: 226–233.
- Brooks, R.J., Boby, M.L., Galbraith, D.A., Layfield, J.A. and Nancekivell, E.G. 1991. Maternal and environmental influences on growth and survival of embryonic and hatchling snapping turtles (*Chelydra serpentina*). *Can. J. Zool.*, **69**: 2667–2676.
- Bull, J.J. 1980. Sex determination in reptiles. *Quart. Rev. Biol.*, **55**: 3–21.
- Bull, J.J. 1985. Sex ratio and nest temperature in turtles: comparing field and laboratory data. *Ecology*, **66**: 1115–1122.
- Burger, J. 1989. Incubation-temperature has long-term effects on behavior of young pine snakes (*Pituophis melanoleucus*). *Behav. Ecol. Sociobiol.*, **24**: 201–207.

- Cagle, K.D., Packard, G.C., Miller, K. and Packard, J. 1993. Effects of the microclimate in natural nests on development of embryonic painted turtles, *Chrysemys picta*. *Funct. Ecol.*, **7**: 653–660.
- Carr, A. and Hirth, H. 1961. Social facilitation in green turtle siblings. *Anim. Behav.*, **9**: 1–2.
- Charnov, E.L. and Bull, J. 1977. When is sex environmentally determined? *Nature*, **266**: 829–830.
- Christiansen, J.L. and Moll, E.O. 1973. Latitudinal reproductive variation within a single subspecies of painted turtle, *Chrysemys picta bellii*. *Herpetologica*, **1973**: 152–163.
- Congdon, J.D., Nagle, R.D., Dunham, A.E., Beck, C.W., Kinney, O.M. and Yeomans, S.R. 1999. The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentina*): an evaluation of the 'bigger is better' hypothesis. *Oecologia*, **121**: 224–235.
- Cotter, J.T. and Sheil, C.A. 2014. Hatchling sex ratios and locomotor performance of midland painted turtles (*Chrysemys picta marginata*). *J. N. Am. Herpetol.*, **2**: 3–6.
- Delmas, V., Baudry, E., Girondot, M. and Prevot-Julliard, A. 2007. The righting response as a fitness index in freshwater turtles. *Biol. J. Linn. Soc.*, **91**: 99–109.
- Díaz-Paniagua, C., Keller, C. and Andreu, A.C. 1997. Hatching success, delay of emergence and hatchling biometry of the spur-thighed tortoise, *Testudo graeca*, in south-western Spain. *J. Zool.*, **243**: 543–553.
- Doody, S. 1999. A test of comparative influences of constant and fluctuating incubation temperatures on phenotypes of hatchling turtles. *Chelonian Conserv. Biol.*, **3**: 529–531.
- Du, W. and Ji, X. 2003. The effects of incubation thermal environments on size, locomotor performance, and early growth of hatchling soft-shell turtles, *Pelodiscus sinensis*. *J. Thermal Biol.*, **28**: 279–286.
- Ernst, C.H. and Lovich, J.E. 2009. *Turtles of the United States and Canada* (2nd edn.). Baltimore, MD: Hopkins Fullfillment Service.
- Ewert, M.A., Jackson, D. and Nelson, C.E. 1994. Patterns of temperature-dependent sex determination in turtles. *J. Exp. Zool.*, **270**: 3–15.
- Ewert, M.A., Lang, J.W. and Nelson, C.E. 2005. Geographic variation in the pattern of temperature-dependent sex determination in the American snapping turtle (*Chelydra serpentina*). *J. Zool.*, **265**: 81–95.
- Finkler, M.S. 1999. Influence of water availability during incubation on hatchling size, body composition, desiccation tolerance, and terrestrial locomotor performance in the snapping turtle *Chelydra serpentina*. *Physiol. Biochem. Zool.*, **72**: 714–722.
- Finkler, M.S. and Claussen, D.L. 1997. Use of the tail in terrestrial locomotor activities of juvenile *Chelydra serpentina*. *Copeia*, **1997**: 884–887.
- Freedberg, S. and Wade, M.J. 2004. Male combat favours female-biased sex ratios under environmental sex determination. *Anim. Behav.*, **67**: 177–181.
- Freedberg, S., Ewert, M.A. and Nelson, C.E. 2001. Environmental effects on fitness and consequences for sex allocation in a reptile with environmental sex determination. *Evol. Ecol. Res.*, **3**: 953–967.
- Freedberg, S., Stumpf, A.L., Ewert, M.A. and Nelson, C.E. 2004. Developmental environment has long-lasting effects on behavioural performance in two turtles with environmental sex determination. *Evol. Ecol. Res.*, **6**: 739–747.
- Freedberg, S., Lee, C. and Pappas, M. 2011. Agricultural practices alter sex ratios in a reptile with environmental sex determination. *Biol. Conserv.*, **144**: 1159–1166.
- Froese, A.D. and Burghardt, G.M. 1974. Food competition in captive juvenile snapping turtles, *Chelydra serpentina*. *Anim. Behav.*, **22**: 735–740.
- Galbraith, D.A., Bishop, C.A., Brooks, R.J., Simser, W.L. and Lampman, K.P. 1988. Factors affecting the density of populations of common snapping turtles (*Chelydra serpentina serpentina*). *Can. J. Zool.*, **66**: 1233–1240.
- Georges, A. 1989. Female turtles from hot nests: is it duration of incubation or proportion of development at high temperatures that matters? *Oecologia*, **81**: 323–328.

- Georges, A., Limpus, C. and Stoutjesdijk, R. 1994. Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *J. Exp. Zool.*, **270**: 432–444.
- Gutzke, W.H.N., Packard, G.C., Packard, M.J. and Boardman, T.J. 1987. Influence of the hydric and thermal environments on eggs and hatchlings of painted turtles (*Chrysemys picta*). *Herpetologica*, **43**: 393–404.
- Hanson, J., Wibbels, T. and Martin, R.E. 1998. Predicted female bias in sex ratios of hatchling loggerhead sea turtles from a Florida nesting beach. *Can. J. Zool.*, **76**: 1850–1861.
- Hewavisenthi, S. and Parmenter, C.J. 2001. Influence of incubation environment on the development of the flatback turtle (*Natator depressus*). *Copeia*, **2001**: 668–682.
- Hughes, E.J. 2003. Nest-site selection, hatching success and hatchling over-winter success in painted turtles (*Chrysemys picta*). Unpublished MSc thesis, University of Guelph, Guelph, Ontario.
- Hutchison, V.H., Vinegar, A. and Kosk, R.J. 1966. Critical thermal maxima in turtles. *Herpetologica*, **22**: 31–41.
- Janzen, F.J. 1993a. The influence of incubation temperature and family on eggs, embryos, and hatchlings of the smooth softshell turtle (*Apalone mutica*). *Physiol. Zool.*, **66**: 349–373.
- Janzen, F.J. 1993b. An experimental analysis of natural selection on body size of hatchling turtles. *Ecology*, **74**: 332–341.
- Janzen, F.J. 1994. Vegetational cover predicts the sex ratio of hatchling turtles in natural nests. *Ecology*, **75**: 1593–1599.
- Janzen, F.J. 1995. Experimental evidence for the evolutionary significance of temperature-dependent sex-determination. *Evolution*, **45**: 864–873.
- Janzen, F.J., Tucker, J.K. and Paukstis, G.L. 2000. Experimental analysis of an early life-history stage: selection on size of hatchling turtles. *Ecology*, **81**: 2290–2304.
- Kolbe, J.J. and Janzen, F.J. 2002. Impact of nest-site selection on nest success and nest temperature in natural and disturbed habitats. *Ecology*, **83**: 269–281.
- Lemmon, P.E. 1957. A new instrument for measuring forest overstory density. *J. Forestry*, **55**: 667–668.
- Les, H.L., Paitz, R.T. and Bowden, R.M. 2007. Experimental test of the effects of fluctuating incubation temperatures on hatchling phenotype. *J. Exp. Zool.*, **307A**: 274–280.
- Les, H.L., Paitz, R.T. and Bowden, R.M. 2009. Living at extremes: development at the edges of viable temperature under constant and fluctuating conditions. *Physiol. Biochem. Zool.*, **82**: 105–112.
- Maloney, J.E., Darian-Smith, C., Takahashi, Y. and Limpus, C.J. 1990. The environment for development of the embryonic loggerhead turtle (*Caretta caretta*) in Queensland. *Copeia*, **1990**: 378–387.
- McKnight, C.M. and Gutzke, W.H.N. 1993. Effects of the embryonic environment and of hatchling housing conditions on growth of young snapping turtles (*Chelydra serpentina*). *Copeia*, **1993**: 475–482.
- Mitchell, T.S., Maciel, J.A. and Janzen, F.J. 2013. Does sex-ratio selection influence nest-site choice in a reptile with temperature-dependent sex determination? *Proc. R. Soc. Lond. B*, **280**: 20132460.
- Mitchell, T.S., Maciel, J.A. and Janzen, F.J. 2015. Maternal effects influence phenotypes and survival during early life stages in an aquatic turtle. *Funct. Ecol.*, **29**: 268–276.
- Morjan, C.L. 2003a. Variation in nesting patterns affecting nest temperatures in two populations of painted turtles (*Chrysemys picta*) with temperature-dependent sex determination. *Behav. Ecol. Sociobiol.*, **53**: 254–261.
- Morjan, C.L. 2003b. How rapidly can maternal behavior affecting primary sex ratio evolve in a reptile with environmental sex determination? *Am. Nat.*, **162**: 205–219.
- Mullins, M.A. and Janzen, F.J. 2006. Phenotypic effects of thermal means and variances on smooth softshell turtle (*Apalone mutica*) embryos and hatchlings. *Herpetologica*, **62**: 21–36.

- Myers, E.M., Tucker, J.K. and Chandler, C.H. 2007. Experimental analysis of body size and shape during critical life-history events of hatchling slider turtles, *Trachemys scripta elegans*. *Funct. Ecol.*, **21**: 1106–1114.
- Neuwald, J.L. and Valenzuela, N. 2011. The lesser known challenge of climate change: the variance and sex-reversal in vertebrates with temperature-dependent sex determination. *PLoS One*, **6**: e18117.
- Ontario Ministry of Natural Resources. 1998. *Algonquin Provincial Park Management Plan*. Ontario, Canada: Queen's Printer for Ontario.
- O'Steen, S. 1998. Embryonic temperature influences juvenile temperature and growth rate in snapping turtles *Chelydra serpentina*. *J. Exp. Biol.*, **201**: 439–449.
- Packard, G.C. and Packard, M.J. 2001. Environmentally induced variation in size, energy reserves and hydration of hatchling painted turtles, *Chrysemys picta*. *Funct. Ecol.*, **15**: 481–489.
- Packard, G.C., Packard, M.J. and Birchard, G.F. 1989. Sexual differentiation and hatching success by painted turtles incubating in different thermal and hydric environments. *Herpetologica*, **45**: 385–392.
- Paitz, R.T., Gould, A.C., Holgersson, M.C. and Bowden, R.M. 2010. Temperature, phenotype, and the evolution of temperature-dependent sex determination: how do natural incubations compare to laboratory incubations? *J. Exp. Zool. B: Mol. Devel. Evol.*, **314**: 86–93.
- Paterson, J.E., Steinberg, B.D. and Litzgus, J.D. 2012. Revealing a cryptic life history stage: differences in habitat selection and survivorship between hatchlings of two turtle species at risk (*Glyptemys insculpta* and *Emydoidea blandingii*). *Wildl. Res.*, **39**: 408–418.
- Paterson, J.E., Steinberg, B.D. and Litzgus, J.D. 2014. Effects of body size, habitat selection, and exposure on hatchling turtle survival. *J. Zool.*, **294**: 278–285.
- Pieau, C., Dorizzi, M. and Richard-Mercier, N. 1999. Temperature-dependent sex determination and gonadal differentiation in reptiles. *Cell. Mol. Life Sci.*, **55**: 887–900.
- Rafferty, A.R. and Reina, R.D. 2012. Arrested embryonic development: a review of strategies to delay hatching in egg-laying reptiles. *Proc. R. Soc. Lond. B*, **279**: 2299–2308.
- Rasmussen, M.L. and Litzgus, J.D. 2010. Patterns of maternal investment in spotted turtles (*Clemmys guttata*): implications of trade-offs, scales of analyses, and incubation substrates. *Écoscience*, **17**: 47–58.
- Refsnider, J.M. 2013. High thermal variance in naturally incubated turtle nests produces faster offspring. *J. Ethol.*, **31**: 85–93.
- R Development Core Team. 2012. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing [<http://www.R-project.org/>].
- Rhen, T. and Lang, J.W. 1998. Temperature during embryonic and juvenile development influences growth in hatchling snapping turtles, *Chelydra serpentina*. *J. Therm. Biol.*, **24**: 33–41.
- Riley, J.L. and Litzgus, J.D. 2013. Evaluation of predator-exclusion cages used in turtle conservation: cost analysis, and effects on nest environment and proxies for hatchling fitness. *Wildl. Res.*, **40**: 499–511.
- Riley, J.L., Tattersall, G.J. and Litzgus, J.D. 2014. Potential sources of intra-population variation in painted turtle (*Chrysemys picta*) hatchling overwintering strategy. *J. Exp. Biol.*, **217**: 4174–4183.
- Roosenburg, W.M. 1996. Maternal condition and nest site choice: an alternative for the maintenance of environmental sex determination? *Am. Zool.*, **36**: 157–168.
- Roosenburg, W.M. and Kelley, K.C. 1996. The effect of egg size and incubation temperature on growth in the turtle, *Malaclemys terrapin*. *J. Herpetol.*, **30**: 198–204.
- Roznik, E.A. and Alford, R.A. 2012. Does waterproofing Thermochron iButton dataloggers influence temperature readings? *J. Therm. Biol.*, **37**: 260–264.
- Samson, J. 2003. The life history strategy of a northern population of midland painted turtle, *Chrysemys picta marginata*. Unpublished MSc thesis, University of Guelph, Guelph, Ontario.

- Samson, J., Hughes, E.J. and Brooks, R.J. 2007. Excavation is a nondeleterious method for obtaining fecundity and morphometric data from small-sized eggs of freshwater turtles. *Chelonian Conserv. Biol.*, **6**: 255–259.
- Schwanz, L.E., and Janzen, F.J. 2008. Climate change and temperature dependent sex determination: can individual plasticity in nesting phenology prevent extreme sex ratio? *Physiol. Biochem. Zool.*, **81**: 826–834.
- Schwarzkopf, L. and Brooks, R.J. 1985. Sex determination in northern painted turtles: effect of incubation at constant and fluctuating temperatures. *Can. J. Zool.*, **63**: 2543–2547.
- Shine, R. 1999. What is sex determined by nest temperature in so many reptiles? *Trends Ecol. Evol.*, **14**: 186–189.
- Shine, R., Elphick, M.J. and Harlow, P.S. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology*, **78**: 2559–2568.
- Spencer, R.J. and Janzen, F.J. 2014. A novel hypothesis for the adaptive maintenance of environmental sex determination in a turtle. *Proc. R. Soc. Lond. B*, **281**: 20140831.
- Standora, E.A. and Spotila, J.R. 1985. Temperature dependent sex determination in sea turtles. *Copeia*, **1985**: 711–722.
- St. Juliana, J.R., Bowden, R.M. and Janzen, F.J. 2004. The impact of behavioral and physiological maternal effects on offspring sex ratio in the common snapping turtle, *Chelydra serpentina*. *Behav. Ecol. Sociobiol.*, **56**: 270–278.
- Telemeco, R.S., Abbott, K.C. and Janzen, F.J. 2013. Modeling the effects of climate change-induced shifts in reproductive phenology on temperature-dependent traits. *Am. Nat.*, **181**: 637–648.
- Tucker, J.K. 2000. Annual variation in hatchling size in the red-eared slider turtle (*Trachemys scripta elegans*). *Herpetologica*, **56**: 8–13.
- Valenzuela, N. 2004. Evolution and maintenance of temperature-dependent sex determination. In *Temperature Dependent Sex Determination in Vertebrates* (N. Valenzuela and V.A. Lance, eds.), pp. 131–147. Washington, DC: Smithsonian Books.
- Van Damme, R., Bauwens, D., Brana, F. and Verheven, R.R. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica*, **48**: 220–228.
- Warner, D.A. and Shine, R. 2008. The adaptive significance of temperature-dependent sex determination in a reptile. *Nature*, **451**: 566–568.
- Weisrock, D.W. and Janzen, F.J. 1999. Thermal and fitness-related consequences of nest location in painted turtles (*Chrysemys picta*). *Funct. Ecol.*, **13**: 91–101.
- Wilimovsky, N.J. 1990. Misuses of the term ‘Julian Day’. *Trans. Am. Fish. Soc.*, **119**: 162.
- Wilson, D.S. 1998. Nest-site selection: microhabitat variation and its effects on the survival of turtle embryos. *Ecology*, **79**: 1884–1892.
- Yntema, C.L. 1968. A series of stages in the embryonic development of *Chelydra serpentina*. *J. Morphol.*, **125**: 219–251.