



Anthropogenic nest sites provide warmer incubation environments than natural nest sites in a population of oviparous reptiles near their northern range limit

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Abstract

Oviposition site choice affects a host of offspring phenotypes and directly impacts maternal fitness. Recent evidence suggests that oviparous reptiles often select nest sites where the landscape has been altered by anthropogenic activity, whereas natural nest sites are less often used. We leverage a long-term study of snapping turtle (*Chelydra serpentina*) to identify natural nest sites and anthropogenic nest sites and to compare habitat variables among nest site types. Natural and anthropogenic nest sites did not differ in average canopy closure, distance to nearest water, substrate composition, or aspect. However, anthropogenic nest sites had less ground-level vegetation and greater soil brightness, and were 3.3 °C warmer than natural nests during incubation. We used the Schoolfield model of poikilotherm development to assess differences in development rate between natural and anthropogenic nests. Because of the difference in temperature, embryos in anthropogenic nests were predicted to have undergone nearly twice as much development as embryos in natural nests during incubation. We outline why the evolution of fast embryonic development rate cannot compensate indefinitely for the low temperature incubation regimes that become increasingly prevalent at northern range margins, thereby underlining why maternal nest site choice of relatively warm anthropogenic sites may help oviparous reptiles persist in thermally constrained environments. Future research should aim to quantify both the thermal benefits of anthropogenic nest sites, as well as associated fitness costs (e.g., increased adult mortality) to elucidate whether anthropogenic disturbance of the landscape can be an ecological trap or serve a net benefit to some reptiles in northern environments.

Keywords *Chelydra serpentina* · Ecological trap · Embryonic development · Nest microhabitat · Thermal performance

Introduction

Offspring survival is a major component of parental fitness. In organisms that do not demonstrate parental care or post-natal provisioning, greater offspring survival is often achieved through maternal effects such as energy allocation to eggs (Congdon and Gibbons 1985; Bernardo 1996; Rollinson and Hutchings 2013) and nest site selection (Hughes and Brooks 2006). In oviparous organisms that lack parental care, nest site selection is particularly important, since the parent does not have direct control over the microenvironmental conditions during embryonic development or the environment faced by newly emerged hatchlings. Hence, females may adaptively manipulate the outcome of their nests by selecting nest sites based on microhabitat characteristics that are conducive to embryonic development, that minimize depredation, or confer favourable offspring phenotypes (Refsnider and Janzen 2010).

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Our findings of a thermal trade-off between anthropogenic and natural nests explains one of the most pervasive threats to turtle populations globally: road mortality and roads as an ecological trap.

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The thermal environment of the nest, in particular, exerts a strong influence on a variety of hatchling traits, and mothers should be able to exert some control over incubation temperatures via nest site selection. In many reptiles, for instance, incubation temperature is an important determinant of sex ratio in a given clutch (Bull and Vogt 1979), and female choice of microhabitat characteristics, such as vegetative cover, is predictive of the thermal environment of nests, and hence the sex ratio (Janzen 1994; Janzen and Morjan 2001; St. Juliana et al. 2004). In addition to affecting sex and early life traits such as hatching success (Wilson 1998; Kolbe and Janzen 2002), incubation temperature can also affect traits later in life, such as post-hatch growth, body size, and locomotor performance (Boby and Brooks 1994; Booth et al. 2004; Mitchell et al. 2013; Riley et al. 2014).

Interestingly, in areas with significant anthropogenic presence, females often choose to nest on human-altered sites (Loncke and Obbard 1977; Obbard and Brooks 1980). The use of anthropogenic nest sites, such as roadsides, quarries, borrow pits, backyards, mulch piles, hydro-line right-of-ways, railway embankments, and dams (Beaudry et al. 2010; Joyal et al. 2001; Thompson et al. 2017; de Solla and Gugelyk 2018), is becoming increasingly prevalent among freshwater turtles. In fact, studies done in human-disturbed areas over the past few decades have reported that around 80% of Blanding's turtle (*Emydoidea blandingii*) nests, 75% of spotted turtle (*Clemmys guttata*) nests, and 90% of snapping turtle nests were located in anthropogenic sites (Beaudry et al. 2010; Joyal et al. 2001; Thompson et al. 2017). The general implications of anthropogenic nest sites on fitness and population growth are unclear, and likely vary with the type of human alteration and the climate. For instance, Kolbe and Janzen (2002) showed that female painted turtles (*Chrysemys picta*) in the northern USA use microhabitat characteristics to choose nest sites, and that incubation temperatures were correlated with these microhabitat characteristics in natural nest sites, but uncorrelated in anthropogenic nest sites (i.e., residential areas consisting of isolated trees, houses, sand roads, and mowed lawns). In fact, incubation temperature in residential areas was 2 °C lower than in natural sites due to a higher percentage of canopy cover caused by human alterations (Kolbe and Janzen 2002), and it is possible that the lack of correlation between microhabitat characteristics assessed by females and realized incubation conditions may result in an ecological trap that depresses fitness and population growth rate (Thompson et al. 2017; Kolbe and Janzen 2002). An ecological trap occurs when animals mistakenly prefer habitats (often those brought about by human-induced rapid environmental change) where their fitness is depressed compared to other available habitats (Hale and Swearer 2016).

On the other hand, it is widely assumed that temperature and length of the growing season limit the geographic

range of many ectothermic species (Bleakney 1958a, b). One mechanism by which this occurs is a limitation on the amount of thermal energy for embryonic development, which ultimately limits reproductive success (Boby and Brooks 1994; Kearney and Porter 2004; Brooks 2007; Lesbarrères et al. 2014). Many temperate squamates have overcome this ecological constraint by evolving viviparity (Mesquita et al. 2016), thereby allowing limited thermal energy to be behaviourally exploited by the mother. However, other reptiles such as turtles do not exhibit viviparity (Andrews and Mathies 2000), and selection of warm nest environments by the mother is important, as behavioural modification of body temperature is not possible at the embryonic stage (Telemeco et al. 2016). In thermally constrained environments, like those toward species range limits (Lesbarrères et al. 2014), anthropogenic constructions such as dams, walking trails, dykes, and embankments may provide suitable nest sites with thermal regimes that do not resemble natural regimes.

Anthropogenic land modification can have variable influence on nest site thermal characteristics. For example, some residential areas may result in cooler, more shaded habitats relative to natural nest sites (Kolbe and Janzen 2002), whereas increasingly urbanized landscapes, with large areas of thermally absorptive and impervious surfaces, are proposed to serve as heat sinks (Bowne et al. 2018). As such, anthropogenic land modification may increase incubation temperatures relative to natural nest sites, which may improve average nest success in these environments where warm incubation regimes are, otherwise, uncommon (Edge et al. 2017). For instance, the northernmost record of a successful snapping turtle nest (48.2°N) occurred on a rural property that had been cleared of vegetation that would, otherwise, provide shade, and where the nest was protected from cool northerly winds due to its close proximity to a house (Lapointe 2018). Indeed, nests of the snapping turtle tend to be laid in shaded areas in the southern portion of this species range, whereas nests are more often laid in full sunlight toward the northern extent of the range, reflecting adaptive maternal behaviour that may both balance sex ratios in warm climates and ensure hatching success in cool climates (Ewert et al. 2005). In relatively northern environments, then, it is possible that alterations of the landscape by humans may affect the quality of nest sites by virtue of altering the potential thermal regime of nests, thereby impacting the persistence of oviparous reptiles in these landscapes.

In the present study, we examine how nest characteristics differ in anthropogenic versus natural nest sites of the snapping turtle (*Chelydra serpentina*), in a population near its northern range limit. Nest site selection has been extensively documented in the snapping turtle (Kolbe and Janzen 2002; Obbard and Brooks 1980). Female snapping turtles often migrate out of their home ranges, and travel significant

distances both in water and on land to find a nesting site (Obbard and Brooks 1980) and may dig multiple nests at a given site before finding a suitable location (Ernst and Lovich 2009). This particular maternal effect is likely to have important ramifications for offspring phenotype and fitness in this species, since snapping turtles undergo temperature-dependent sex determination (Yntema 1976), and many nest microhabitat characteristics are correlated with temperature (Janzen 1994; Janzen and Morjan 2001; Valenzuela 2001; St. Juliana et al. 2004). Despite the breadth of research on human-altered nest sites of freshwater turtles, anthropogenic nest sites of snapping turtles across a wide range of human disturbance have yet to be characterized, and microhabitats of snapping turtle nests in relatively pristine habitats also remain poorly understood.

Materials and methods

Study site

The present work is part of a long-term study (established 1972) on turtles at the Algonquin Wildlife Research Station, Algonquin Provincial Park, Ontario, Canada. The focal population consists of approximately 250 female snapping turtles, which typically nest from early June to early July. Although not directly quantified, the overwhelming majority of turtle nests in our study area appear to occur in locations where the landscape has been directly altered by humans (hereafter termed ‘anthropogenic sites’), such as dams, railway embankments, unpaved roadways, roadside culverts/

shoulders, portage trails, and sand dunes with frequent human disturbance. It is less common to detect nests in locations devoid of direct human impact (‘natural sites’), such as river and lake embankments, rock outcroppings with naturally sparse vegetation, and North American beaver (*Castor canadensis*) dams and lodges.

In June and July 2017, nests were located by patrolling known nest sites daily from 05:00 to ~10:00 and from ~17:00 to ~23:00 or until nesting activity ceased. Additional nests in both natural and anthropogenic sites were located by searching outside of our usual monitoring areas, in places that looked suitable for snapping turtle nesting. For this study, a total of 13 nests were located, 6 in anthropogenic sites, and 7 in natural sites (Table 1). Natural nest sites were those devoid of direct human influence, and included a remote, sparsely vegetated rocky peninsula on a lake, a sandy and slumping river embankment, a rocky island outcrop, a beaver dam, a beaver lodge, and natural lakeside sand dunes (Fig. 1, Table 1). Anthropogenic nest sites included a biking trail, a road shoulder, lakeside dams, a railway embankment, and a high human activity sand dune. Notably, sand dunes appear in both natural and anthropogenic treatments, but were characterized as natural or anthropogenic based on whether or not the site was human-modified. Nest site A.5 (Table 1, Fig. 1) was treated as an anthropogenic sand dune, because it had been heavily modified by an old railway bed, and because of frequent human disturbance in the area (hiking, horseback riding, and nearby motorized vehicle use). Lakeside sand dunes N.1 and N.2 (Table 1) were treated as natural nest sites, because the dunes are naturally occurring; the location is relatively remote (e.g.,

Table 1 Description of each snapping turtle (*Chelydra serpentina*) nest in the study, anthropogenic (A) and natural (N), with corresponding habitat data

Nest	Description	CC	VC	DFS	DTW	FineS	Temp	Depth	RSB
A.1	Gravel biking trail	0.12	0.19	16	4.55	0.633	24.1	14	0.948
A.2	Gravel road shoulder	0.01	0.13	44	2.47	0.532	24.0	21.25	0.719
A.3	Dam on lake 1	0.30	0.12	123	5.59	0.588	24.4	19.5	0.775
A.4	Dam on lake 2	0.14	0.01	111	11.3	0.353	24.4	16.0	1.00
A.5 ^a	Sand dune	0.04	0.2	74	319	0.784	21.9	16.5	0.732
A.6 ^a	Railway embankment	0.11	0.03	45	2.9	0.747	22.5	16.5	0.827
N.1	Natural lake sand dune 1	0.00	0.42	52	7.38	0.974	23.4	16.5	0.645
N.2	Natural lake sand dune 2	0.02	0.25	167	14.2	0.895	20.1	15.5	0.691
N.3	Rocky lake peninsula	0.05	0.195	99	4.28	0.527	20.4	14.5	0.258
N.4	Forested river bank	0.41	0.295	37	1.50	0.674	NA ^b	16.5	0.609
N.5	Rocky island outcrop	0.61	0.555	73	2.57	0.434	18.1	16.5	0.533
N.6 ^a	Beaver lodge on river	0.21	0.43	33	0.770	0.240	18.7	16.5	0.431
N.7 ^a	Beaver dam on pond	0.06	0.99	50	0.390	0.372	20.6	16.5	0.369

CC proportion of canopy cover, VC proportion ground vegetation cover, DFS degrees from south (°), DTW distance to water (m), FineS proportion of substrate ≤0.5 mm diameter by weight, Temp average temperature (°C) during incubation (July 2 and September 5, 2017), Depth intermediate nest depth (cm), position of temperature data logger (i.e., depth at centre of clutch, when eggs present), RSB relative soil brightness

^aSimulated nest

^bThermal logger lost



Fig. 1 **a** Map of snapping turtle (*Chelydra serpentina*) nest sites in the western uplands of Algonquin Provincial Park, Ontario, Canada. Natural nests (denoted with “N”) marked in grey, anthropogenic nests (“A”) marked in black. Examples of sampled nest sites: **b** rocky peninsula (N.3) with shallow soil and blueberry (*Vaccinium cyanococcus*) on Lake Sasajewun; **c** slumping forested riverbank (N.4)

along the North Madawaska River; **d** North American beaver (*Castor canadensis*) lodge (N.6); **e** gravel shoulder of high traffic volume provincial highway (A.2), Highway 60, through southern Algonquin Provincial Park; **f** man-made water retention dam and log chute (A.3 and A.4) on Lake Sasajewun; **g** former railway bed and contemporary hiking trail (A.6), Mizzy Lake Trail, at West Rose Lake

cannot be accessed directly by road) and not part of a trail or recreational area.

Although we principally sought confirmed turtle nest locations within the 2017 sampling year (“true nests”), we also drew on nest site locations from the recent past (2010–2016) to sample a diversity of anthropogenic and natural nest sites. Specifically, four nests in the present study were former nest cavities or simulated cavities (hereafter “simulated nest”) excavated by E.A. Francis at an exact

previous nest location, detected either through primary observation of nesting females or secondary observation of depredated nests. In total, two of six anthropogenic nests were simulated, and two of seven natural nests were simulated (Table 1). We emphasise that all nests (true nests and simulated nests) were from exact known snapping turtle nest locations presently or in the recent past. To explore the impact of simulated nests on our main findings, we re-analyzed all significant results herein while excluding simulated

nests, thereby retrospectively estimating whether simulated nests were an important contributor to the patterns which we uncovered.

Nest site characteristics

For each nest, the following microhabitat characteristics were measured in July 2017: incubation temperature at intermediate nest depth (defined below), canopy cover, understory vegetation, distance to nearest water, and slope aspect. Below-ground metal nest cages were buried at each nest to protect the nests from depredation. These predator-exclusion cages do not interfere with nest temperature or mean soil moisture and, because they were applied to each nest, are expected to have consistent effects on understory vegetation, if any (Riley and Litzgus 2013). Temperatures inside each nest were measured at hourly intervals from 02-Jul-2018 until 05-Sept-2018, by placing an iButton® (Dallas Semiconductor/Maxim Integrated Products, Sunnyvale, CA, USA) in each nest cavity. Temperature data loggers were enclosed in latex balloons (for waterproofing) and deployed at intermediate nest depths (i.e., depth at centre of clutch), which was calculated using the formula:

$$I = \frac{b - t}{2} + t,$$

where I is intermediate nest depth, b is depth to bottom of clutch from soil surface, and t is depth to top of clutch from soil surface. To inform temperature data logger placement in simulated nests, mean intermediate nest depth (16.5 cm) was calculated from seven true nests; this average value was used for temperature data logger placement in all simulated nests. Temperature data loggers in nest cavities containing eggs were placed at the calculated intermediate nest depth, but 5 cm from the edge of the clutch to eliminate the possibility of reading variation in temperature caused by metabolically generated heat in the egg chamber, or variation in temperature caused by thermal inertia of eggs.

Understory vegetation was measured using a 1 × 1-m quadrat-containing a hundred 10-cm² squares, centred over the nest cavity. Percentage of detritus, woody plants, herbaceous plants, lichen, moss and exposed rocks, and plant roots were visually estimated by counting the number of 10-cm² squares occupied by each. Percent canopy cover was measured using a spherical densiometer (Robert E. Lemmon, Forest Densiometers, Bartlesville, OK) held 1 m over the nest. At each nest, canopy cover measurements were recorded facing due North, West, South, and East, and averaged.

Slope aspect was measured to the nearest degree using a standard compass, and distance from nest to nearest body of water was measured to the nearest tenth of a metre using a 60-m fibreglass tape measure. Google Maps software was used to calculate distance to water for nests farther than 60 m

from water. Soil samples (100 g) were obtained from each nest site and transported to the Algonquin Wildlife Research Station for analysis. Soil samples were placed in a drying oven (Hotpack, Waterloo, ON) at ~40 °C until dry prior to sieving. Following a method similar to Hughes and Brooks (2006), fine substrate was separated using a 1-mm sieve, and weighed. The proportion of fine substrate was calculated as the mass of ≤0.5-mm-diameter substrate over the total mass of the sample. We calculated relative soil brightness (which we assume is a proxy of relative soil albedo) as a function of soil colour using a digital image of dried soil samples from each nest site (Fig. 2). Using the ImageJ software, mean pixel intensity was measured at six randomly selected areas (40 × 40 pixels) in each soil sample, all in the same image (Fig. 2), and averaged. Using mean pixel intensity values of a white surface in the same image as a reference sample, relative brightness values were calculated as a ratio of the mean reference value to the mean brightness value from each soil sample. The resultant value for each sample reflects a ratio of how white the soil sample is, and we call this value relative soil brightness.

Statistical analyses

Data were analyzed in R statistical software (version 3.5.0). All nests were considered independent data points, as each

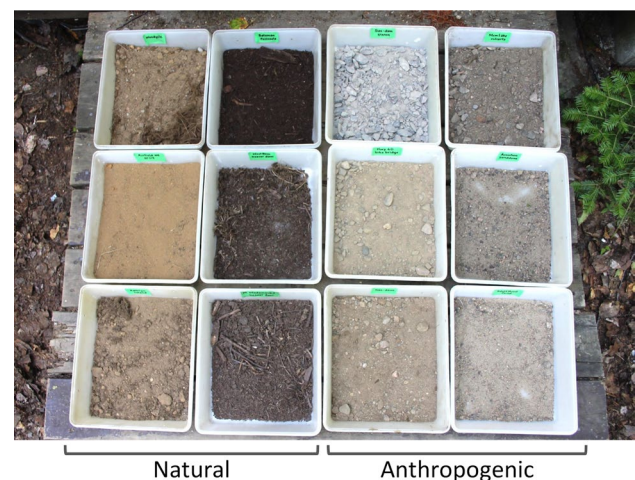


Fig. 2 Substrate samples from natural (left) and anthropogenic (right) snapping turtle (*Chelydra serpentina*) nest sites in Algonquin Provincial Park, Ontario. Natural nest sites, clockwise from upper left: slumping forested riverbank (N.4); rocky peninsula on lake (N.3); North American beaver (*Castor canadensis*) dam (N.7); North American beaver lodge (N.6); rocky island outcrop on lake (N.5); and natural lakeside sand dune (N.1 and N.2). Anthropogenic nest sites, clockwise from centre left: man-made water retention dam and log chute (A.3); gravel shoulder of highway (A.2); former railway bed and contemporary sand dune recreational area (A.5); former railway bed and contemporary hiking trail (A.6); man-made water retention dam and log chute (A.4); and biking trail (A.1)

nest was selected and laid by a different individual, and all were used in analyses. All proportional data were logit transformed prior to analysis, but means are reported on original scale. All habitat variables passed a Levene's Test for homogeneity of variance, except nest distance to water ($F_{1,11}=6.78$, $P=0.025$). Shapiro–Wilk tests revealed that all variables were normally distributed, except for nest distance to water ($w=0.350$, $P<0.001$). We, therefore, applied a Mann–Whitney U test to analyze distance to water, and ANOVA was applied to test for treatment effects (anthropogenic vs. natural) for other variables. Following initial analysis of nest microhabitat and thermal data, we probed the data set in an attempt to uncover the mechanism responsible for lower mean nest temperature in natural nests. We used backward elimination to uncover significant predictors of mean nest temperature, testing the effects of factors that could conceivably affect nest temperature: Canopy Closure, Degrees from South, Vegetation Cover, Fine Substrate, Burial Depth, and Relative Soil Brightness. All statistical analyses were conducted in R statistical software, version 3.4.4 and 3.5.0 (R Core Team 2018).

Modeling thermal performance and embryonic growth

Rollinson et al. (2018) modeled embryonic development of snapping turtles in the focal population as a function of temperature. In brief, Rollinson et al. (2018) estimated development rate at 14 constant temperatures (14–38 °C), resulting in 42 individual estimates of development rate. Rollinson et al. (2018) then used median development rate per temperature treatment ($n=14$ estimates in total) and fit the development rate–temperature reaction norm using cubic splines. In the present study, we use the full data set from Rollinson et al. (2018) ($n=42$ estimates of development rate) to estimate the parameters of the Schoolfield et al. (1981) model of poikilotherm development, which assumes that developmental rates are limited by the activity of a particular enzyme. That enzyme is assumed to be maintained at a constant concentration, but the relative proportion of activated enzyme (and hence the developmental rate) varies with temperature. We fit the model following Eq 4. in Schoolfield et al. (1981):

$$r(T) = \frac{\rho_{(25\text{ }^{\circ}\text{C})} \frac{T}{298} \exp \left[\frac{\Delta H_A^{\ddagger}}{R} \left(\frac{1}{298} - \frac{1}{T} \right) \right]}{1 + \exp \left[\frac{\Delta H_L}{R} \left(\frac{1}{T_{1/2L}} - \frac{1}{T} \right) \right] + \exp \left[\frac{\Delta H_H}{R} \left(\frac{1}{T_{1/2H}} - \frac{1}{T} \right) \right]}$$

The developmental rate r is a function of the temperature (T) in Kelvin. In addition to the universal gas constant ($R=1.987$ cal deg⁻¹ mol⁻¹), there are six parameters that must be fit to the data: (1) $\rho_{(25\text{ }^{\circ}\text{C})}$, the developmental rate at 25 °C; (2) $\Delta H_A^{\ddagger}$, the enthalpy of activation; (3) $T_{1/2L}$, the temperature at which half of the enzyme is inactivated by

cold; (4) ΔH_L , the change in enthalpy from inactivation by cold; (5) $T_{1/2H}$, the temperature at which half the enzyme is inactivated by heat; and (6) ΔH_H , the change in enthalpy from heat inactivation. We used a differential evolution algorithm (Price et al. 2006; Ardia et al. 2016) to minimize the sum squared error between expected and measured developmental rates. We minimized the weighted difference according to the reciprocal of the developmental rate, since low developmental rates may be measured more accurately (Schoolfield et al. 1981). Specifically, we used the DEoptim package (Ardia et al. 2016) in R statistical software (version 3.4.4), and ran the differential evolution algorithm for 500 generations using the default population size of ten times the number of fitted parameters (giving a total of 60). The process was repeated ten times to increase the odds of locating a global optimum.

After optimizing model fit, we used the model to estimate the amount of development that occurred in anthropogenic vs. natural nests between 02-Jul-2017 and 05-Sep-2017, which was when data loggers were present and recording in all nests. The units of development used by Rollinson et al. (2018) are expressed in equivalent development at 20 °C, where a developmental increment of 1.0 is equivalent to the amount of morphological development that occurs after 1 week of incubation at a constant temperature of 20 °C. Using the incubation profile specific to each nest, we summed the developmental increments over time, as in Rollinson et al. (2018), to estimate the average developmental age achieved in each nest by September 05, 2017.

Results

Understory vegetation in nest sites was predominantly composed of herbaceous and woody vegetation, moss, lichen, detritus, and exposed roots. Herbaceous vegetation included grasses and young speckled alder (*Alnus rugosa*). Woody vegetation was dominated by blueberry (*Vaccinium* sp.). Detritus was largely composed of pine (*Pinus* spp.) and spruce (*Picea* spp.) needles. Notably, moss, lichen, and exposed rock were absent in all anthropogenic sites. Mean ($\pm$ SE) proportion of vegetative cover differed significantly between natural (0.414 ± 0.117) and anthropogenic (0.112 ± 0.0322) nest sites (Table 2). Overstory was predominantly composed of mature coniferous trees, including black spruce (*Picea mariana*), white spruce (*Picea glauca*), white pine (*Pinus strobus*), tamarack (*Larix laricina*), balsam fir (*Abies balsamea*), and speckled alder. Mean canopy cover in anthropogenic sites (0.120 ± 0.0414) was not significantly different from natural (0.196 ± 0.0877) nest sites (Table 2).

There was no significant difference in degrees from south between natural and anthropogenic nest sites (Table 2). Similarly, there was no statistical difference in distance to

Table 2 Results for habitat variables associated with natural and anthropogenic nests of snapping turtles (*Chelydra serpentina*) in Algonquin Provincial Park, Ontario

Habitat variable	Natural		Anthropogenic		<i>F</i>	<i>P</i>
	Mean	SE	Mean	SE		
Proportion canopy closure	0.196	0.0877	0.120	0.0414	0.067	0.80
Proportion vegetation cover	0.414	0.117	0.112	0.0322	7.08	0.022
Degrees from south	73.0	17.8	68.8	17.0	0.028	0.87
Distance to water (m)*	57.6	52.3	4.44	1.87	–	0.23
Proportion fine substrate	0.588	0.103	0.606	0.0638	0.13	0.79
Mean temperature (°C)	20.2	0.755	23.5	0.431	14.6	0.003
Relative soil brightness	0.505	0.0599	0.834	0.0480	17.5	0.002

Summary statistics and ANOVA analysis, or *Mann–Whitney *U* test, presented for comparison of microenvironment characters between natural and anthropogenic nest sites. Nest character considered significantly different between natural and anthropogenic sites at $\alpha = 0.05$

nearest water between natural (4.44 ± 1.87 m) and anthropogenic nests (57.6 ± 52.3 m), nor was the proportion of substrate ≤ 0.5 -mm-diameter different between anthropogenic (0.606 ± 0.0638) and natural (0.588 ± 0.103) nests (Table 2). The temperature data logger for site N.4 was lost during a flooding event, so our comparison of incubation temperatures consisted of six nests in each treatment. Mean incubation temperature was significantly lower in natural (20.2 ± 0.755 °C) versus anthropogenic (23.5 ± 0.431 °C) nests (Table 2). The loss of the temperature datalogger at site N.4 is unlikely to have biased this result, as site N.4 is known to be an unusually cool incubation environment, and since 1972 not a single nest has been known to complete development at site N.4 (R.J.Brooks personal communication; N. Rollinson personal observation). Thus, the thermal profile of site N.4 is expected to have been consistent with the cool incubation profiles at other natural sites. Finally, relative soil brightness was significantly greater in anthropogenic compared to natural nests (Table 2). Notably, the significant differences between natural and anthropogenic nests presented above (vegetation cover, temperature, and soil brightness) remained statistically significant even when all simulated nests were excluded from the analysis despite the reduced sample size (Table S1, Online Resource 1).

We estimated the Schoolfield et al. (1981) model for poikilotherm development (Fig. 3). We used the model to explore how variation in incubation temperatures resulted in variation in embryonic age over time, estimating the hourly development rate of embryos under fluctuating conditions, and summing development over time (Rollinson et al. 2018). Over the 65-day interval between 02-Jul-2017 and 05-Sep-2017 (9.3 weeks), anthropogenic nests experienced an equivalent of (mean $\pm$ SD) 14.7 ± 1.06 weeks of development at 20 °C; natural nests experienced the equivalent of 7.41 ± 1.56 weeks of development at 20 °C (Fig. 4).

We used backward elimination to uncover whether Canopy, Degrees from South, Vegetation Cover, Fine Substrate, Burial Depth, and Relative Soil Brightness can

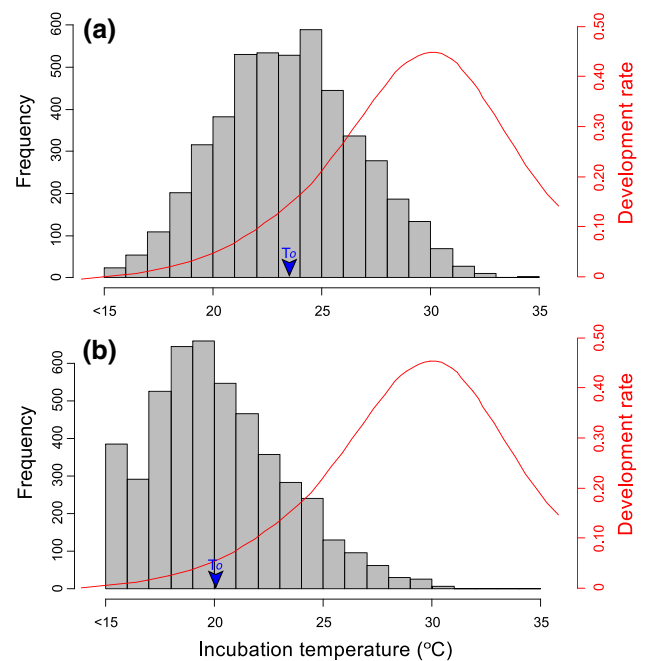


Fig. 3 Histogram of snapping turtle (*Chelydra serpentina*) nest temperatures from 02-Jul-2017 to 05-Sep-2017, collapsed across all nests in **a** anthropogenic sites and **b** natural sites. Mean incubation temperature across all nests in a given site type (anthropogenic or natural) is T_o , in blue. The Schoolfield et al. (1981) model is parameterized in red (identical in both panels) and overlaid on incubation temperatures. Parameters for the Schoolfield equation are: $\rho_{(25\text{ °C})} = 0.273$, $\Delta H_A = 37,153.37$, $T_{1/2L} = 290.40$, $\Delta H_L = -32,047.45$, $T_{1/2H} = 303.76$, and $\Delta H_H = 99,995.61$

help to explain variation in mean nest temperature. Of these variables, only relative soil brightness was formally retained in the final model, explaining 53% of the variation in nest temperature (Fig. 5). Notably, canopy closure was negatively related to nest temperature, as expected, but it was not retained in the final model as the parameter estimate was non-significant (canopy closure controlling for soil brightness, $\beta \pm \text{SE} = -0.658 \pm 0.394$, $P = 0.13$).

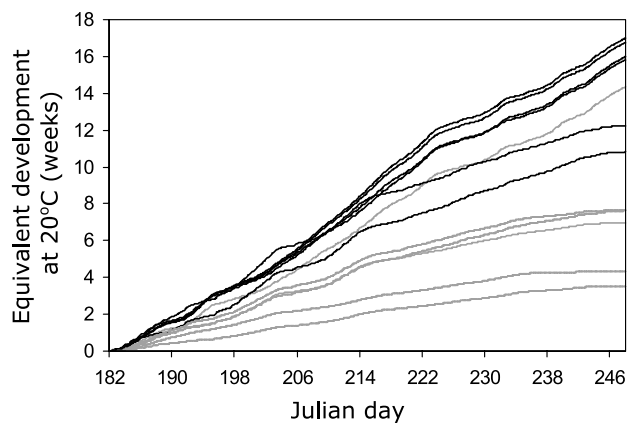


Fig. 4 Estimated developmental progression of snapping turtle (*Chelydra serpentina*) embryos in anthropogenic (black) and natural nests (grey) between 02-Jul-2017 and 05-Sep-2017 (Julian day 182–248). For the purposes of this exercise, we assumed that all nests were laid on Julian day 182, so that development is comparable among nests. Development is expressed as the estimated embryonic stage that is reached after y weeks of development at a constant temperature of 20 °C. In other words, on Julian day 214 (03 August 2017), the slowest developing nest is estimated to be at a developmental stage equivalent to about 2 weeks of development at a constant temperature of 20 °C, whereas the fastest-developing nest is at a developmental stage equivalent to about 9 weeks of development at 20 °C. By Julian day 248, anthropogenic nests had reached an equivalent age of ($\mu \pm \text{SE}$) 14.4 ± 1.20 weeks of development at 20 °C, whereas natural nests had reached only 7.50 ± 1.91 weeks of development at 20 °C

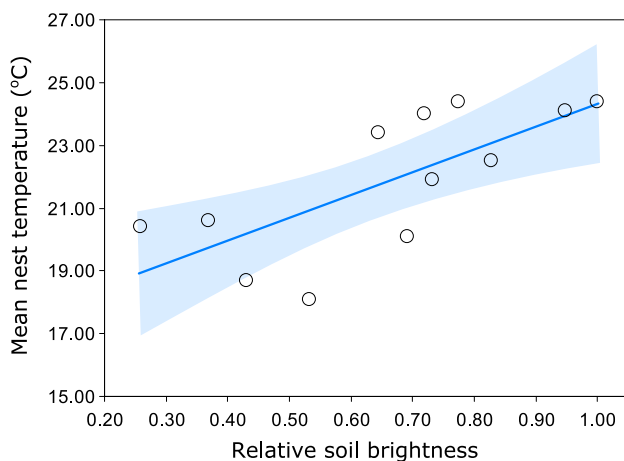


Fig. 5 Mean nest temperature as a linear function of relative soil brightness ($y = 17.1 + 7.28x$, $r^2 = 0.53$, $P = 0.007$)

Discussion

Our results indicate that, on average, the microenvironments of natural and anthropogenic nest sites of snapping turtles were similar for the majority of measured microhabitat variables (canopy cover, distance to water, slope

aspect, and substrate grain size), with three major exceptions. Natural and anthropogenic sites differed substantially in temperature, understory vegetation, and relative soil brightness. Furthermore, soil brightness explained a majority of variation in nest temperature (Fig. 5). Although it is clear that some natural nest sites do provide a high-quality thermal environment (Fig. 4), anthropogenic nest sites tended to be more uniform in providing a high-quality thermal environment, likely in part because human alteration of the landscape in Algonquin Park tends to result in a relative absence of vegetation cover and homogenization of soil properties across sites, as reflected in our measure of soil brightness (Fig. 2, Table 2). Below, we explore why these findings are important with respect to performance theory, organismal fitness, and conservation near this species' northern range limit.

Oviparous reptiles with long incubation durations may persist in thermally constrained environments in two complementary ways. First, embryonic development rate may evolve to be very fast, compensating for the low incubation temperatures and short growing seasons; second, mothers may choose high-quality thermal environments for their nests. Indeed, rate of embryonic development in a common environment increases with latitude in turtles, at least at some set-point temperatures (Ewert 1985), suggesting selection on development rate. As we explain below, however, maternal nest site choice is likely a key component of nest success in thermally constrained environments, because theory suggests that the evolution of development rate cannot indefinitely compensate for the decrease in mean temperature and the increase in temperature variation associated with short growing seasons.

Theory and data suggest that thermal performance of development rate, and other traits, is limited by a trade-off between the range of temperatures to which development responds (response breadth) and maximum development rate (Martin and Huey 2008; Richter-Boix et al. 2015; Vickers et al. 2017; but see Angilletta 2009). The trade-off ultimately constrains development rate, so that it is, for example, high over a narrow range of temperatures, or modest across a larger range of temperatures (Fig. S1, Online Resource 1; Gilchrist 1995). The evolution of a relatively large thermal response breadth, then, comes at the cost of maximum performance, but, in seasonal environments, a large response breadth allows the organism to leverage the wide range of temperatures to which it is exposed, thereby maximizing cumulative development over the season (Gilchrist 1995; Amarasekare and Johnson 2017). A limit to the evolution of development rate likely occurs when the environment becomes very cool and highly seasonal, such as at relatively extreme latitudes, as the trade-off between performance breadth and height may dictate that there is no dimension in which the thermal

performance curve can evolve to support the development of embryos to term (Fig. S1, Online Resource 1).

Although thermal performance curves cannot evolve indefinitely to compensate for low and variable incubation temperatures, there is a second and complementary trait that may help reptiles persist in thermally constrained environments: maternal nest site choice of warm incubation environments. In the present study, we found that anthropogenic nest sites resulted in an average of two times more embryonic development than natural nest sites (Fig. 4), and that greater soil brightness was associated with higher nest temperatures (Fig. 5). Indeed, low incubation temperatures in the focal population are associated with low hatching success and low hatchling growth rates under laboratory conditions (Bobyne and Brooks 1994). Furthermore, the focal population exhibits extremely stochastic bouts of recruitment (Brooks et al. 1988; Edge et al. 2017), and the previous studies on this population have concluded that insufficient thermal energy for incubation plays a significant role in constraining annual reproductive success, even in anthropogenic nest sites (Obbard and Brooks 1981; Bobyne and Brooks 1994; Edge et al. 2017). The high-quality thermal environments that are associated with anthropogenic nest sites may, therefore, be an important component of reproductive success in this thermally constrained population of turtles.

Although our long-term study of snapping turtles (1972—present; R.J. Brooks, J.D. Litzgus, and N. Rollinson) has never directly quantified the proportion of nests laid in natural vs. anthropogenic sites, personal observations spanning decades suggest that anthropogenic sites are overwhelmingly selected as nesting locations, and that females will migrate considerable distances (up to 16 km roundtrip) to nest on anthropogenic structures (R.J. Brooks, personal communication; N. Rollinson, personal observation; Obbard and Brooks 1980). What is not clear, however, is whether the thermal benefits of anthropogenic sites outweigh other fitness costs that may be associated with these sites. For instance, relatively high densities of nesting females may attract egg predators, resulting in higher nest depredation rates than might, otherwise, be seen in natural nest sites where the spatial distribution of suitable nesting habitat is less predictable and the nesting habitats themselves smaller. More importantly, some forms of anthropogenic sites, such as roadsides, may facilitate human-induced mortality of adult turtles. At the population level (Haxton 2000), the death of even a few adult turtles may outweigh the thermal benefits of anthropogenic sites (e.g., Heppel 1998). In effect, anthropogenic nest sites, especially roadsides, could serve as ecological traps (Hale and Swearer 2016) for turtles by attracting reproductive females and elevating mortality by even small levels (2–5%; Brooks et al. 1988, 1991; Congdon et al. 1993, 1994; Midwood et al. 2015; Keevil et al. 2018), such that population-level fitness is depressed, leading to

steady population decline. Therefore, while the present study has demonstrated that anthropogenic nest sites in our study area may represent high-quality thermal environments, further research is needed to explore whether anthropogenic sites result in a net benefit to population growth, or whether increases in nest depredation and/or adult mortality (e.g., road mortality) outweigh any thermal benefit of anthropogenic sites.

In the present study, we attempted to identify the proximate factors associated with lower mean nest temperature in natural nests, finding a positive association between soil brightness and temperature. Thermal conductivity of material can be attributed in part to colour, which affects the absorption and reflectance of solar radiation, or the albedo. Typically, surfaces with a relatively high albedo (roughly corresponding to brighter substrates) absorb relatively little thermal energy, and should warm relatively slowly compared to surfaces with relatively low albedo (i.e., absorptive, often dark-coloured surfaces). Yet, the relationship between soil brightness and nest temperature was in the opposite direction of the expected relationship between soil albedo and thermal absorbance (Fig. 5). Variation in soil brightness may, instead, be associated with temperature, because brightness reflects the properties of the soil, such as organic content and moisture retention potential, which may affect thermal conductivity. This speculation remains to be confirmed, however. It is, nevertheless, notable that anthropogenic nest substrates are visually similar and homogenous in composition to each other (Fig. 2), likely owing to similar origins (e.g., quarried rock and sand). Soil moisture, a possible consequence of substrate consistency, grain size, and/or soil organic content, may account for differences in hatching success between the anthropogenic and natural nest sites (Paterson et al. 2013). This relationship between soil characteristics and temperature has ramifications for the increasingly common conservation practice of artificial nest mound construction as supplementary nest habitat for imperiled turtle species (Buhlmann and Osborn 2011; Paterson et al. 2013; Quinn et al. 2015).

In sum, reptiles face unique challenges in seasonal environments, as many populations face cool temperatures coupled with time constraints on growth and development, constraints that cannot easily be overcome by evolving faster development rates. The present study suggests that snapping turtles may be nesting on human-altered nest sites for several reasons, most notably temperature. Natural nest sites appear to be limited in this region, and nest site characteristics such as slope aspect, distance to water, substrate particle size, and canopy cover are comparable between natural and anthropogenic nest sites. Moreover, incubation temperatures are significantly higher in anthropogenic nest sites, which is especially favourable in their northern range limit where snapping turtles are thermally constrained

(Bobyne and Brooks 1994; Holman and Andrews 1994). It is important to consider the ramifications of anthropogenic nest site choice by turtles. Females may choose to nest at heavily disturbed sites, such as roadsides, over natural sites that are more thermally limited. While the anthropogenic site may improve thermal conditions in the nest, nests may be more heavily depredated, and adults may face a greater probability of mortality during nesting migration. As turtles are long-lived, field studies may not be able to explore this problem. However, a simulation approach may prove fruitful, where the model could be informed by field studies such as this one; fitness could be expressed in terms of population growth, and the costs and benefits of anthropogenic nest sites could be explored over a broad parameter space.

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Author contribution statement PDM conceived and designed the experiment. EAF and PDM conducted fieldwork. EAF, MAG, and NR analyzed the data. EAF, PDM, MAG, and NR wrote the manuscript.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

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