

Evaluation of predator-exclusion cages used in turtle conservation: cost analysis and effects on nest environment and proxies of hatchling fitness

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Abstract

Context. A main goal of conservation is to mitigate anthropogenic impacts on natural ecosystems, thus conservation tools themselves should not negatively affect target species. Predator-exclusion cages are effectively used to reduce predation of turtle nests; however, their effects on nest environment and developing hatchlings have not been examined.

Aims. Our study had the following four goals: (1) to examine effects of cages on the nest environment, (2) determine whether nest caging affects proxies for hatchling fitness, (3) evaluate whether nest predators preferentially interact with certain cage types, and (4) assess the cost-effectiveness of different nest caging designs.

Methods. In 2010 and 2011 in Algonquin Provincial Park, Ontario, painted turtle (*Chrysemys picta*; $n = 93$) and snapping turtle (*Chelydra serpentina*; $n = 91$) nests were assigned to one of three treatments (wooden-sided cages, above- and below-ground wire cages) or a control (no nest cage) and outfitted with a data logger to record incubation temperature. After emergence, hatching success and proxies of hatchling fitness were measured.

Key results. Nest temperature, hatching success, frequency of hatchling deformities and locomotor performance did not differ among cage treatments. However, hatchling body condition differed among treatments; wooden-sided and below-ground cages had the most positive influence on body condition in painted and snapping turtles, respectively. Predator interactions did not differ among treatments, and wooden-sided cages were the most inexpensive to construct.

Conclusions. Nest cages did not alter the nest environment from natural conditions but did alter hatchling body condition, and nest caging affected species differently.

Implications. Nest cages are known to reduce nest depredation, and our data indicated that, in general, nest cages also do not affect the nest environment or proxies for hatchling fitness. Thus, our findings indicated that cages are effective conservation tools that do not present secondary deleterious effects on potential recruitment.

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Introduction

An increasingly important priority in the field of conservation biology is the mitigation of human impacts on natural ecosystems. Thus, conservation techniques themselves should not incur negative, secondary impacts on target species (Frazer 1992). A global threat to marine and freshwater turtles and tortoises is increased nest predation as a result of subsidised predators. Predators of turtle eggs (e.g. raccoons, skunks, foxes) increase in abundance because of an increase in food resources associated with human presence, which in turn causes nest predation to occur at higher-than-natural levels (Christiansen and Galloway 1984; Garber and Burger 1995; Mitchell and Klemens 2000; Engeman *et al.* 2003; Barton and Roth 2008; Kurz *et al.* 2011; Spotila 2011; Smith *et al.* 2013). In addition, decreases in populations of top predators in urban areas (e.g. wolves, large cats) have increased the numbers of mesopredators, again leading to increases in depredation of turtle eggs (Prugh *et al.* 2009; Ritchie and Johnson 2009). In some turtle populations, subsidised

mesopredators annually depredate 100% of nests, resulting in chronic prevention of recruitment (Spinks *et al.* 2003; Fordham *et al.* 2008). Substantial, repeated reductions in recruitment (e.g. 50% or more decrease in egg survival) have been found to exacerbate population declines (Crouse *et al.* 1987; Crowder *et al.* 1994; Heppel 1997; Tomillo *et al.* 2008; Reed *et al.* 2009). The conservation tool used worldwide to eliminate nest predation and promote recruitment is nest caging. Nest caging reduces nest predation from as high as 100% depredation of nests without cages to as low as 0% depredation of nests with cages (Addiston 1997; Ratnaswamy *et al.* 1997; Yerli *et al.* 1997; Gillingwater 2001; Engeman *et al.* 2006; Kornaraki *et al.* 2006; Kurz *et al.* 2011; Perez-Heydrich *et al.* 2012; Smith *et al.* 2013). This tool has been in use since the 1960s (Breckenridge 1960), and specific methods vary considerably among studies, many of which are unpublished stewardship projects. Shortcomings of the technique have been noted, namely, wire cages can entrap hatchlings (Adamany *et al.* 1997), wire cages alter the magnetic field

around the nest, which may affect sea turtle orientation and navigation (Irwin *et al.* 2004), and cages with materials above ground level may alter the nest environment (e.g. shading which would reduce incubation temperature; Breckenridge 1960; Rahman and Burke 2010). Female turtles select nest sites on the basis of environmental characteristics that maximise hatching success (Wilson 1998; Refsnider and Janzen 2010). Evidence that nest caging may alter those characteristics suggests that this technique requires evaluation.

Nest environment (e.g. incubation temperature, soil moisture) greatly influences hatchling performance and morphology. Reduced incubation temperature results in a decline in hatching success (Correa-H *et al.* 2010; Garrett *et al.* 2010). In northern temperate regions (e.g. Ontario, Canada), if incubation temperatures are reduced, turtle embryos may not complete development or emerge from the nest cavity before the onset of cool fall temperatures (Yntema 1968; Choo and Chou 1987; Bobynd and Brooks 1994; Du and Ji 2003). In addition, incubation may be extended into a time period when habitat conditions are unfavourable (Bobynd and Brooks 1994; Matsuzawa *et al.* 2002). Furthermore, embryos exposed to low temperatures during incubation have reduced body condition, a higher frequency of deformities and reduced locomotor performance (Diaz-Paniagua *et al.* 1997; Packard *et al.* 1999; Hewavisenthi and Parmenter 2001; Steyermark and Spotila 2001; Reece *et al.* 2002; Du and Ji 2003; Booth *et al.* 2004). Many turtle species exhibit temperature-dependent sex determination (TSD), and altered nest temperatures could result in skewed sex ratios (Schwarzkopf and Brooks 1985; Janzen and Paukstis 1991; Hanson *et al.* 1998; Wibbels 2003), in turn leading to reduced population viability (Steen and Gibbs 2004). More broadly, hatchling survival, growth rates, behaviours and habitat selection have all been related to incubation temperature (McKnight and Gutzke 1993; O'Steen 1998; Rhen and Lang 1999; Booth *et al.* 2004). In general, a substantial portion of a turtle's individual characteristics are determined by the environment during embryogenesis.

The goal of the research presented here was to evaluate nest caging types in terms of their impacts on nest environment, proxies for hatchling fitness, predator attraction and logistical considerations. The following three types of nest cages currently used in the recovery of at-risk turtles were evaluated: above- and below-ground hardware cloth cages, and wooden-sided cages. Data were also collected from natural nests (e.g. non-caged control nests). The first objective of the study was to compare the effect different nest cage types may have on the environment of the nest. We tested the hypothesis that if nest cage materials block solar radiation, then temperature within the nest cavity of above-ground and wooden-sided cages will be reduced. The second objective was to determine the effect different nest cages may have on hatching success, incubation duration, hatchling body condition and performance. Following the prediction from objective one, if temperature within the nest cavity is reduced, this will have negative effects on nest success and hatchling fitness. We tested the prediction that above-ground and wooden-sided cages would experience lower hatching success and longer incubation durations. Also, we predicted that above-ground and wooden-sided cages would have a negative effect on proxies of hatchling fitness, measured as a higher frequency of deformities

(Mast and Carr 1989; Türkozan *et al.* 2001), reduced body condition (Shine *et al.* 2001) and reduced locomotor performance (Freedberg *et al.* 2004; Delmas *et al.* 2007). Nest predators may use research markers as visual cues for predation (Burke *et al.* 2005; Rollinson and Brooks 2007; Spotila 2011); thus, the third objective was to determine whether predators preferentially interacted with, and depredated nests with protective cages. If nest cages were used as visual cues, then we predicted that predators would preferentially interact with, and depredate above-ground and wooden-sided nest cages. As conservation initiatives are often constrained by funding, the final objective of our study was to perform a cost analysis of each nest cage type.

Methods

Study area

The study took place in Algonquin Provincial Park, Ontario, Canada, near the Wildlife Research Station (WRS; 45°35'N, 78°30'W). Elevations on the western side of Algonquin Park (370–570 m above sea level) are higher than the surrounding landscape and the area experiences a colder and wetter climate as a result (Ontario Ministry of Natural Resources 1998). This climate is reflective of the northern range limits of both turtle species studied. The study area is within the Algonquin–Lake Nipissing ecoregion, and is a rugged landscape underlain by Precambrian Shield outcrops (Ontario Ministry of Natural Resources 1998). Forest cover dominates, including predominantly mixed upland forests of sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis*) and eastern hemlock (*Tsuga canadensis*). Field sampling was concentrated in two study sites, namely, WRS and Arowhon. Nesting habitat in the WRS site varies from natural sand dunes to gravel embankments along access roads and Highway 60. The Arowhon site main nesting area is the sand and gravel embankment of a public hiking trail (Schwarzkopf and Brooks 1985).

Snapping and painted turtle populations are substantial at the study sites, and nesting has been consistently documented over the past 35+ years (R. J. Brooks, University of Guelph, unpubl. data). By using these two relatively common species, we could achieve large sample sizes and robust statistical analyses, leading to findings about nest caging applicable to turtle species-at-risk. Examining two species with different nest characteristics, namely, shallow nest with a small number of eggs (painted turtles) vs deep nest with a large number of eggs (snapping turtles), allowed us to capture variation among species.

Field methodology

Nest-site monitoring occurred from 20 May to 20 June 2010, and 5 June to 4 July 2011. Nest monitoring commenced when females of either species started to congregate in aquatic habitats adjacent to nest sites (i.e. staging), and/or terrestrial nest searching behaviour was observed. Nest sites were monitored visually by researchers from dawn (~0500 hours) to ~1000 hours, and in the afternoon from just before dusk (~1700 hours) until after dark, as long as nesting activity was occurring. This timing captured peak nesting for snapping and painted turtles (Ernst and Lovich 2009). Nest monitoring ceased when 3 days elapsed continuously without any observations of nesting activity.

Nests were excavated after females completed oviposition and covering of their nests. Nest locations were marked with metal stakes and flagging tape. Eggs were removed, and placed in plastic bins lined with moistened vermiculite. As eggs were removed, they were numbered using a pencil, to ensure that they were returned to the nest cavity in the same order and orientation as they were found. Depth to the top and bottom of the nest cavity was measured to the nearest 0.1 cm by using a ruler. After excavation, nest cavities were filled with soil removed during egg retrieval, to reduce desiccation of the nest chamber. Eggs were transported back to the WRS laboratory, where data were collected for the long-term study. Eggs were returned to the nest cavity within 24 h post-oviposition, before the vitelline membrane adhered to the inner shell surface to form a 'white spot' on the egg (Yntema 1968; Rafferty and Reina 2012), ensuring no trauma to developing embryos (Samson *et al.* 2007).

Each nest was randomly assigned to one of the treatments (Table 1). In 2010, there were two treatments, above- and below-ground hardware cloth cages, and a control (no cage). In 2011, an additional treatment, namely wooden-sided cages, was added. The cages were all open-bottomed cubes. The above-ground cage was made with 1-cm-mesh hardware cloth; dimensions were 30 × 30 × 40 cm, with 8-cm flaps, and they were installed with 20 cm above and 20 cm below ground surface (Fig. 1; Addiston 1997; Irwin *et al.* 2004). Below-ground cages were also made of 1-cm hardware cloth; dimensions were 30 × 30 × 20 cm and did not have any flaps, and they were installed so that the top of the cage was just below ground level (Fig. 1; Hughes and Brooks 2006; Bolton *et al.* 2008). The wooden-sided cages consisted of a wooden square frame (made of boards 14 × 3.8 × 35 cm) with a 1-cm hardware-mesh top. The dimensions were 35 × 35 × 9 cm, and the cages were installed over the top of the nest, with none of the cage extending below the ground, and with either rocks or stakes holding the cage securely against the soil (Fig. 1; Standing *et al.* 1999). Eggs were reburied in the original nest cavity, at the original depths and order, with a temperature data logger in the centre of the clutch. The temperature data loggers were either a waterproofed iButton® (accuracy of ±1°C or 0.5°C; Thermochron DS1921G; Dallas Semiconductor, Sunnyvale, CA, USA), or a HOBO StowAway® (accuracy of ±0.2°C; TidbiT TBI32-05+37; Onset Computer Corporation, Bourne, CA, USA). Temperature readings from data loggers of different types and with and without waterproofing did not

differ ($F_{3,2480}=2.01$, $P=0.94$; Roznik and Alford 2012). Data loggers recorded temperature hourly. Cages were installed immediately after egg reburial and data-logger deployment. For the control nests, a second data logger was buried beside the nest at the same depth as the logger within the nest cavity. This was done so that if a control nest was depredated and the 'in-nest' data logger was consumed or excavated by the predator, incubation-temperature data would still be available. In late August 2010 and 2011, close to the estimated date of hatchling emergence (and after embryogenesis), above-ground cages were installed on the control nests so that emerging hatchlings could be collected for measurement and fitness tests.

Nest environmental variables were measured for all cage treatments. Vegetation cover was estimated by placing a 1-m² quadrat on the ground with the nest in the centre, and the percentage of bare ground, herbaceous and woody plants, and leaf litter were visually estimated (Wilson 1998). In 2010 and 2011, vegetation cover was sampled monthly and bi-weekly, respectively, post-egg reburial until hatchling emergence. Additionally, on 16 August 2011, soil moisture (%) was measured at a depth of 10 cm within the area enclosed by the nest cage, or for control nests, beside the stake marking the nest site. Soil moisture was measured using a VG-METER-200 (Vegetronix, Bluffdale, UT, USA).

In 2010 and 2011, predator interactions with nest cages were recorded daily throughout nesting and hatching seasons. Outside of those time periods in 2010, nests were surveyed opportunistically. In 2011, nests were surveyed for predator interactions once per week. A 'predator interaction' was recorded when substrate was cleared away from the nest cage, and/or the cage was dug up. After recording a predator interaction, soil was replaced around the nest so that multiple interaction events could be recorded. If a nest was found with the eggs dug up and/or eaten, the nest was recorded as depredated. If tracks and scat were discernible, the predator species was identified and recorded. Additionally, four trail cameras (119456C; Bushnell Corporation, Overland Park, KS, USA) were set up from 1 July to 1 October 2011 at four different locations to capture interactions of predators with nest cages.

Daily monitoring of nests began just before the estimated period of hatchling emergence. The emergence time for nests was estimated using incubation durations reported in literature for snapping (63–104 days) and painted turtles (89–99 days; Ernst and Lovich 2009). The first nests of both snapping and painted turtles emerged on 25 August in 2010 and, in 2011, on 27 August for painted turtles and 1 September for snapping turtles. Once a nest emerged, all hatchlings and unhatched eggs were collected and transported to the WRS laboratory for processing. Hatchling carapace and plastron lengths were measured to the nearest 0.01 mm by using digital calipers (3148, Traceable Digital Calipers, Control Co., Friendswood, TX, USA). Hatchling mass was measured to the nearest 0.1 g by using a digital scale (SP202, Scout Pro, Ohaus Corporation, Pine Brook, NJ, USA). Any deformities (e.g. curly tails, additional scutes) were recorded. Deformities were considered to be any deviation from the normal body plan (Ernst and Lovich 2009) that did not appear to be caused by injury (Davy and Murphy 2009).

Each hatchling underwent a righting test. Each hatchling was placed on its carapace on a cloth-covered board (30 × 15 cm) and

Table 1. Number of nests per treatment and number of hatchlings per treatment (in parentheses, italic font) sampled during the 2010 and 2011 field seasons in Algonquin Park, Ontario, Canada

For the control treatment, the first number before the comma represents the number of nests sampled for both environmental and hatchling data, and the number after the comma represents the number of nests sampled only for environmental data

| Treatment | <i>Chrysemys picta</i> | | <i>Chelydra serpentina</i> | |
|-------------------|------------------------|------------|----------------------------|-------------|
| | 2010 | 2011 | 2010 | 2011 |
| Control (no cage) | 9, 5 (31) | 12, 6 (43) | 8, 6 (193) | 12, 3 (288) |
| Below-ground cage | 12 (63) | 13 (56) | 12 (291) | 12 (352) |
| Above-ground cage | 12 (29) | 12 (61) | 12 (294) | 14 (312) |
| Wooden-sided cage | – | 12 (66) | – | 12 (272) |
| Total | 38 (123) | 55 (226) | 38 (778) | 53 (1224) |

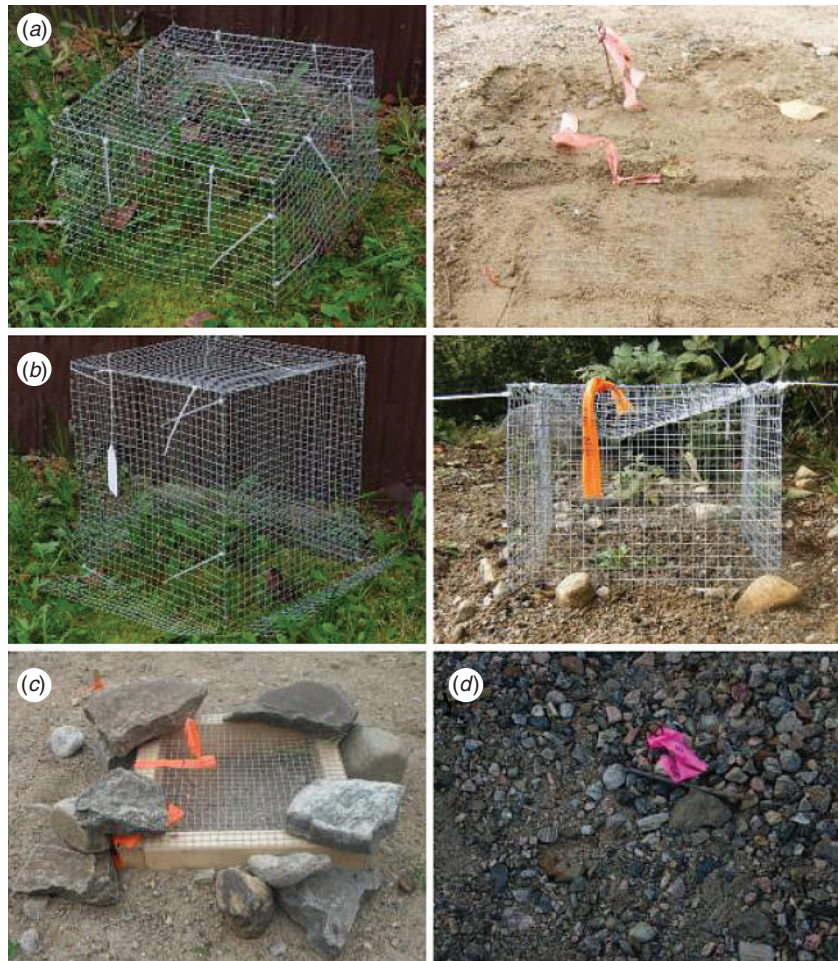


Fig. 1. Three nest-cage types were tested. In 2010, nests were randomly assigned to (a) below-ground cages, (b) above-ground cages (shown before and post-installation from left to right), or (d) uncaged nests (controls). In 2011, the 2010 treatments were repeated with the addition of (c) wooden-sided nest cages.

attempts to flip over onto its plastron were observed for 15 min. The following two variables were timed to the nearest 0.01 s by using a digital stopwatch: (1) latency period (LP), the time from placement on carapace until the first righting attempt; and (2) righting period (RP), the time from the first righting attempt until successful righting (Freedberg *et al.* 2004; Delmas *et al.* 2007; Rasmussen and Litzgus 2010). Each trial was recorded with a digital camera (Photosmart R742, Hewlett-Packard Development Co., Mississauga, ON, USA), and LP and RP were confirmed from the recordings. The number of hatchlings that failed to right themselves within 15 min was compared among treatments; these hatchlings were removed from the comparison of RP among treatments. A turtle that cannot quickly right itself is more likely to be depredated, or to succumb to desiccation and drowning (Finkler and Claussen 1997). Thus, performance in righting tests is thought to reflect a hatchling's future survival, and therefore was used as a proxy for fitness (Freedberg *et al.* 2004; Delmas *et al.* 2007). After data collection, hatchlings were released at their nests within 24 h. The unhatched eggs were candled to assess fertility (Yntema 1964).

Emergence ceased on 30 September 2010 and 28 September 2011. Once daily mean air temperature dropped below 5°C for three consecutive days, any remaining nests were dug up to assess hatching success. In 2010, unemerged nests were dug up from 1 to 4 October, and in 2011 from 2 to 7 October. Any hatchlings were processed as described above.

We undertook a cost analysis of each caging design. First, we calculated the monetary cost of equipment for each design, and second, we calculated the effort (salary cost) to make and install each cage by a technician. Time (to nearest min) was recorded as technicians constructed and installed cages. The times for all actions were then averaged, and multiplied by the technician's salary cost (CAN\$10.25 per h, the minimum wage in Ontario, Canada) to estimate the effort per cage design.

Analyses

Nest environmental variables, except temperature, were averaged for each treatment. Because these data were non-normal, mean soil moisture and vegetation cover were compared among

treatments separately for each turtle species, using a non-parametric Kruskal–Wallis ANOVA test. Depth to the bottom of the nest cavity was compared between species using an ANOVA.

The temperature data were extracted from data loggers, and data from ‘in-nest’ data loggers were preferentially used for the control treatments. No difference was found in temperature between ‘in-nest’ and ‘out-of-nest’ data loggers (2011 data: $t_{44} = 2.02$, $P = 0.89$), so the ‘out-of-nest’ temperature data were used when a control nest was predated.

Temperature data were analysed three ways. (1) Mean nest temperature from the date of the last nest reburied to the first nest emerged (a 44- and 57-day period in 2010 and 2011, respectively), and daily temperature variance during this time period, were calculated for each nest. Mean daily temperature variance describes daily temperature fluctuations above and below mean nest temperature (Paitz *et al.* 2010; Neuwald and Valenzuela 2011), and is known to affect hatchling development (Schwarzkopf and Brooks 1985; Doody 1999; Ashmore and Janzen 2003; Du and Ji 2003; Mullins and Janzen 2006; Les *et al.* 2007; Paitz *et al.* 2010). (2) Mean temperature for each third of incubation (Packard and Packard 1998), and daily mean temperature and mean temperature variance for the second third of incubation, the time period when sex is determined (Mahmoud *et al.* 1973; Yntema 1979; Bull and Vogt 1981; Bull 1985; Paitz *et al.* 2010; Neuwald and Valenzuela 2011), were calculated. (3) Heat units (degree days, °D) were calculated for each nest by using the hourly trapezoid method (Holt 2000). Degree days represent the number of heat units over a 24-h period above a threshold temperature (T_0). Below T_0 , no development occurs, but above it, heat units stimulate development (Holt 2000). The threshold temperature is 14°C for painted turtles (Les *et al.* 2007), and 20°C for snapping turtles (Holt 2000). We used hourly temperatures to capture fluctuations in temperature that affect development. The hourly trapezoid method uses hourly temperatures ($T_0, T_1, T_2, \dots, T_{23}$) to calculate heat units (HU) above a threshold temperature (T_0):

$$\text{HU} = [((T_0 + T_1)/2) + ((T_1 + T_2)/2) + \dots + ((T_{22} + T_{23})/2)] / (24 - T_0)$$

Temperature, temperature variance, temperatures for each third of incubation, and heat units were compared among treatments using ANOVAs that also included the additional factors of species and year as necessary.

Hatching success (%) for each nest was calculated as the number of live hatchlings divided by the number of eggs laid. Hatching success was compared among treatments, species and years by using a Kruskal–Wallis ANOVA. Incubation duration was the number of days between oviposition and hatchling emergence for each nest and was compared among treatments using an ANOVA that included the additional factors of species and year. Deformities were quantified as the proportion of deformed turtles out of the number of hatchlings in a clutch (Davy and Murphy 2009). Proportion of deformed turtles in each clutch was transformed using an arcsine-square-root transformation to ensure normality (Gotelli and Ellison 2004). Mean number of deformities per turtle in each clutch was also calculated (de Solla *et al.* 2008). To control for the random effect

of mother’s identity, a linear mixed-effects model (LMEM) using a restricted maximum likelihood estimation of variance was used to examine whether deformities (proportion of hatchlings with deformities in each clutch, and mean number of deformities per hatchling per clutch) varied among treatments. In this model, additional fixed factors of species and year were included. Body condition was tested separately for each species using a LMEM to compare mass against the fixed effects of treatment and carapace length, and the random effect of clutch nested in mother’s identity (García-Berthou 2001; Litzgus *et al.* 2008; Rasmussen and Litzgus 2010). The number of hatchlings that failed to right themselves within 15 min was compared among treatments using a Pearson’s Chi-squared test of association. Performance variables (LP and RP) were transformed using $\log(y + 1)$ to ensure normality. LP and RP were tested separately for each species among treatments using a LMEM that included the fixed effect of processing temperature (room temperature during righting tests), which is linearly related to ectotherm performance (Hutchison *et al.* 1966), and the random effect of clutch nested in mother’s identity (to control for genetic effects).

Pearson’s Chi-squared tests of association were used to test whether count data from predation interactions and depredation events differed among treatments. A one-way ANOVA was used to test whether installation time (s) differed among treatments.

In all statistical tests, assumptions of normality and heterogeneity of variance were verified. Measures were transformed for normality as needed. An *a posteriori* Tukey HSD test, which adjusted for multiple comparisons, was used to identify differences among sample means when significant differences were found among treatments (Logan 2010). If a significant interaction was found in models with multiple fixed factors, it was reported, and if no significant interaction was found, 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. Statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria).

Results

Nest environment

Mean soil moisture (%) in the nests of painted and snapping turtles did not differ among treatments (Table 2). For painted turtle nests, percentage bare ground, leaf litter and herbaceous plants did not differ among treatments (Table 2). Mean percentage woody plants differed among treatments; nests with wooden-sided cages had a greater percentage of woody plants than did those in other treatments (Table 2). None of the vegetation characters differed among treatments for snapping turtle nests (Table 2). Nest depth differed between species ($F_{1,177} = 726.1$, $P < 0.01$); snapping turtles had deeper nests (20.7 ± 0.4 cm) than painted turtles (9.6 ± 0.1 cm).

Nest thermal environment

Mean incubation temperature (°C) did not differ among cage treatments ($F_{3,155} = 1.05$, $P = 0.37$) or between species ($F_{1,155} = 0.01$, $P = 0.91$), but differed between years ($F_{1,155} = 22.17$, $P < 0.01$; Tables 3, 4). Mean daily temperature variance in nests of painted turtles varied more than in those of

Table 2. Summary of soil moisture and vegetation types quantified in 1-m² quadrats centred on turtle nests in four nest-caging treatments

Data are means ± s.e. (%). Number of nests (*n*) is provided for each treatment. Test statistics from the Kruskal–Wallis ANOVA test (*H*), degrees of freedom, and *P*-values are given for each nest characteristic. Significant test results are indicated in bold font. Data were analysed separately for each species to determine whether nest-environment characteristics differed among nest-cage treatments

| Nest-environment characteristics | <i>Chrysemys picta</i> | | | | <i>Chelydra serpentina</i> | | | | Test statistics | |
|----------------------------------|-----------------------------|-----------------------------|-----------------------------|------------------|-----------------------------|-----------------------------|-----------------------------|------------------|---|---|
| | Above-ground cages (n = 24) | Below-ground cages (n = 24) | Wooden-sided cages (n = 12) | No cage (n = 31) | Above-ground cages (n = 25) | Below-ground cages (n = 24) | Wooden-sided cages (n = 12) | No cage (n = 29) | | |
| Soil moisture | 9.2 ± 1.0 | 6.1 ± 0.5 | 6.7 ± 0.8 | 8.5 ± 1.1 | 5.3 ± 0.6 | 6.9 ± 1.0 | 6.4 ± 1.5 | 8.1 ± 1.8 | <i>H</i> ₃ = 7.04, <i>P</i> = 0.07 | <i>H</i> ₃ = 3.35, <i>P</i> = 0.34 |
| Bare ground | 77.1 ± 2.4 | 81.1 ± 3.3 | 67.9 ± 5.2 | 70.5 ± 3.3 | 87.9 ± 2.7 | 86.3 ± 2.4 | 89.2 ± 2.6 | 84.0 ± 2.6 | <i>H</i> ₃ = 7.74, <i>P</i> = 0.06 | <i>H</i> ₃ = 1.13, <i>P</i> = 0.77 |
| Herbaceous plants | 13.2 ± 1.9 | 12.2 ± 2.6 | 13.6 ± 3.7 | 17.8 ± 2.6 | 7.5 ± 1.5 | 7.8 ± 1.7 | 6.1 ± 1.8 | 12.6 ± 2.3 | <i>H</i> ₃ = 2.93, <i>P</i> = 0.40 | <i>H</i> ₃ = 0.43, <i>P</i> = 0.94 |
| Woody plants | 6.5 ± 1.3 | 3.6 ± 0.9 | 13.6 ± 3.7 | 7.0 ± 1.4 | 1.3 ± 0.6 | 1.8 ± 0.7 | 3.2 ± 1.9 | 1.3 ± 0.5 | <i>H</i>₃ = 8.24, <i>P</i> = 0.04 | <i>H</i> ₃ = 1.50, <i>P</i> = 0.68 |
| Leaf litter | 3.2 ± 0.5 | 3.1 ± 0.9 | 6.8 ± 2.2 | 4.3 ± 0.8 | 3.3 ± 1.7 | 4.0 ± 1.8 | 1.6 ± 0.5 | 2.1 ± 0.6 | <i>H</i> ₃ = 5.74, <i>P</i> = 0.13 | <i>H</i> ₃ = 3.37, <i>P</i> = 0.34 |

snapping turtles ($F_{1,159} = 203.93, P < 0.01$; Tables 3, 4). Mean daily temperature variance did not differ among treatments for painted turtles ($F_{3,75} = 1.42, P = 0.24$; Table 3), but did differ among treatments for snapping turtles ($F_{3,81} = 3.42, P = 0.02$; Table 4). Snapping turtle nests with wooden-sided cages experienced less variation than did nests with below-ground cages ($P = 0.02$ for the comparison between wooden-sided and below-ground cages, $P > 0.05$ for all other cases; Table 4).

Mean temperature in each third of incubation did not differ among treatments ($F_{3,67} = 1.09, P = 0.36$; $F_{3,67} = 1.98, P = 0.13$; $F_{3,67} = 2.36, P = 0.08$, respectively), or between species ($F_{1,67} = 1.82, P = 0.18$; $F_{1,67} = 0.01, P = 0.94$; $F_{1,67} = 0.78, P = 0.38$, respectively), but differed between years ($F_{1,67} = 39.14, P < 0.01$; $F_{1,67} = 13.99, P < 0.01$; $F_{1,67} = 18.17, P < 0.01$, respectively; Tables 3, 4).

In the second third of incubation, mean daily temperature did not differ among treatments ($F_{3,67} = 2.09, P = 0.11$) or between species ($F_{1,67} = 0.003, P = 0.95$), but differed between years ($F_{1,67} = 12.76, P < 0.01$; Tables 3, 4). For the same period, mean daily temperature variance did not differ among treatments ($F_{3,67} = 1.30, P = 0.28$) or between years ($F_{1,67} = 1.70, P = 0.20$), but differed between species ($F_{1,67} = 300.45, P < 0.01$); mean daily temperature variance was greatest in painted turtle nests (Tables 3, 4).

Heat units (°D) were tested separately for each species, because the threshold temperature differs between them. Mean heat units did not differ among treatments or between years for painted turtles ($F_{3,13} = 0.51, P = 0.68$; $F_{1,13} = 0.15, P = 0.71$, respectively; Table 3) or snapping turtles ($F_{3,47} = 0.66, P = 0.58$; $F_{1,49} = 2.36, P = 0.13$, respectively; Table 4).

Clutch and hatchling characteristics

Mean incubation duration did not differ among treatments ($F_{3,81} = 0.44, P = 0.73$), or between species ($F_{1,81} = 0.74, P = 0.39$), but it was shorter in 2011 than in 2010 ($F_{1,81} = 4.27, P = 0.04$; Tables 5, 6). Hatching success did not differ among treatments in painted ($H_3 = 5.25, P = 0.15$; Table 5) or snapping turtles ($H_3 = 2.93, P = 0.40$; Table 6).

Mean proportion of deformed turtles in each clutch did not differ among treatments ($F_{3,130} = 1.56, P = 0.20$), between species ($F_{1,130} = 0.28, P = 0.60$) or between years ($F_{1,130} = 2.25, P = 0.14$; Tables 5, 6). Mean number of deformities per hatchling in each clutch also did not differ among treatments ($F_{3,131} = 2.56, P = 0.06$) or between species ($F_{1,131} = 3.15, P = 0.08$), but there were on average more deformities per hatchling in 2011 than in 2010 ($F_{1,131} = 5.11, P = 0.03$; Tables 5, 6).

Body condition of painted turtles differed among treatments ($F_{3,345} = 3.09, P = 0.03$). Mass was greater at all carapace lengths measured for hatchlings from wooden-sided cages. For body condition of snapping turtles, a significant interaction was found between treatment and carapace length ($F_{3,1988} = 107.09, P < 0.01$). Mass was slightly greater at all carapace lengths measured for hatchlings from below-ground cages than that for those from the control and above-ground cages. Hatchlings from above-ground cages had the lowest mass at carapace lengths less than 27 mm. At carapace lengths less than ~28 mm, hatchlings from wooden-sided cages had the greatest mass, but at carapace lengths greater than ~28 mm, they had the lowest masses.

Table 3. Summary of *Chrysemys picta* nest-temperature data
Data are means \pm s.e. and sample sizes (n = number of nests) are given in parentheses

| Temperature variable | Nest-cage treatment | | | | | | | |
|---|--------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------|--------------------------------|--------------------------------|--------------------------------|
| | Above-ground cages | | Below-ground cages | | Wooden-sided cages | | Control (no cage) | |
| | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 |
| Summer hourly temperature ($^{\circ}\text{C}$) | 23.8 \pm 0.15 (n = 11) | 24.8 \pm 0.26 (n = 9) | 23.7 \pm 0.45 (n = 9) | 25.3 \pm 0.25 (n = 9) | – | 24.4 \pm 0.31 (n = 12) | 23.9 \pm 0.24 (n = 14) | 24.3 \pm 0.30 (n = 16) |
| Heat units ($^{\circ}\text{D}$) | 803.4 (n = 1) | 815.8 \pm 0.5 (n = 4) | – | 819.6 \pm 0.3 (n = 2) | – | 781.8 \pm 0.6 (n = 4) | 849.6 \pm 0.3 (n = 5) | 812.9 \pm 0.02 (n = 2) |
| Temperature variance ($^{\circ}\text{C}$) | 15.0 \pm 3.1 (n = 11) | 15.7 \pm 1.6 (n = 9) | 15.6 \pm 2.0 (n = 9) | 19.2 \pm 1.2 (n = 9) | – | 15.0 \pm 1.9 (n = 12) | 15.9 \pm 1.5 (n = 14) | 20.8 \pm 1.5 (n = 16) |
| First third of incubation hourly temperature ($^{\circ}\text{C}$) | 20.3 (n = 1) | 22.7 \pm 0.9 (n = 4) | – | 22.2 \pm 0.5 (n = 2) | – | 22.5 \pm 0.6 (n = 4) | 21.9 \pm 0.2 (n = 5) | 22.2 \pm 0.2 (n = 2) |
| Second third of incubation hourly temperature ($^{\circ}\text{C}$) | 24.4 (n = 1) | 25.7 \pm 0.6 (n = 4) | – | 27.5 \pm 0.5 (n = 2) | – | 25.4 \pm 0.7 (n = 4) | 24.8 \pm 0.3 (n = 5) | 25.4 \pm 0.06 (n = 2) |
| Final third of incubation hourly temperature ($^{\circ}\text{C}$) | 23.0 (n = 1) | 21.0 \pm 0.6 (n = 4) | – | 22.5 \pm 0.02 (n = 2) | – | 20.4 \pm 1.1 (n = 4) | 22.8 \pm 0.3 (n = 5) | 20.2 \pm 0.3 (n = 2) |
| Second third of incubation daily temperature ($^{\circ}\text{C}$) | 24.4 (n = 1) | 25.7 \pm 0.6 (n = 4) | – | 27.5 \pm 0.5 (n = 2) | – | 25.4 \pm 0.7 (n = 4) | 24.8 \pm 0.3 (n = 5) | 25.4 \pm 0.06 (n = 2) |
| Second third of incubation temperature variance ($^{\circ}\text{C}$) | 22.6 (n = 1) | 20.4 \pm 2.1 (n = 4) | – | 21.1 \pm 5.4 (n = 2) | – | 20.39 \pm 3.8 (n = 4) | 19.0 \pm 2.9 (n = 5) | 19.9 \pm 2.4 (n = 2) |

Table 4. Summary of *Chelydra serpentina* nest temperature data
Data are means \pm s.e. and sample sizes (n = number of nests) are given in parentheses

| Temperature variable | Nest-cage treatment | | | | | | | |
|---|-------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------|-------------------------------|-------------------------------|--------------------------------|
| | Above-ground cages | | Below-ground cages | | Wooden-sided cages | | Control (no cage) | |
| | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 | 2010 | 2011 |
| Summer hourly temperature ($^{\circ}\text{C}$) | 23.5 \pm 0.3 (n = 12) | 24.7 \pm 0.4 (n = 9) | 24.1 \pm 0.2 (n = 11) | 25.0 \pm 0.3 (n = 10) | – | 24.2 \pm 0.4 (n = 11) | 24.2 \pm 0.2 (n = 14) | 24.5 \pm 0.3 (n = 14) |
| Heat units ($^{\circ}\text{D}$) | 291.2 \pm 0.2 (n = 6) | 322.1 \pm 0.3 (n = 8) | 296.6 \pm 0.2 (n = 8) | 326.6 \pm 0.3 (n = 10) | – | 310.5 \pm 0.3 (n = 7) | 284.2 \pm 0.1 (n = 5) | 292.0 \pm 0.3 (n = 10) |
| Temperature variance ($^{\circ}\text{C}$) | 4.1 \pm 0.4 (n = 12) | 4.6 \pm 0.6 (n = 9) | 6.3 \pm 1.7 (n = 11) | 6.7 \pm 1.0 (n = 10) | – | 3.1 \pm 0.5 (n = 11) | 6.0 \pm 1.0 (n = 14) | 5.1 \pm 0.7 (n = 14) |
| First third of incubation hourly temperature ($^{\circ}\text{C}$) | 21.5 \pm 0.4 (n = 7) | 23.0 \pm 0.3 (n = 8) | 21.1 \pm 0.2 (n = 8) | 23.1 \pm 0.2 (n = 10) | – | 22.9 \pm 0.4 (n = 7) | 21.8 \pm 0.4 (n = 5) | 23.0 \pm 0.3 (n = 10) |
| Second third of incubation hourly temperature ($^{\circ}\text{C}$) | 24.6 \pm 0.2 (n = 7) | 26.4 \pm 0.5 (n = 8) | 25.0 \pm 0.3 (n = 8) | 26.3 \pm 0.5 (n = 10) | – | 26.1 \pm 0.4 (n = 7) | 24.6 \pm 0.2 (n = 5) | 25.3 \pm 0.6 (n = 10) |
| Final third of incubation hourly temperature ($^{\circ}\text{C}$) | 22.4 \pm 0.6 (n = 7) | 21.4 \pm 0.6 (n = 8) | 23.1 \pm 0.3 (n = 8) | 20.9 \pm 0.5 (n = 10) | – | 21.1 \pm 0.5 (n = 7) | 21.3 \pm 0.6 (n = 5) | 20.2 \pm 0.7 (n = 10) |
| Second third of incubation daily temperature ($^{\circ}\text{C}$) | 24.6 \pm 0.2 (n = 7) | 26.4 \pm 0.5 (n = 8) | 25.1 \pm 0.4 (n = 8) | 26.3 \pm 0.5 (n = 10) | – | 26.1 \pm 0.4 (n = 7) | 24.6 \pm 0.2 (n = 5) | 25.3 \pm 0.6 (n = 10) |
| Second third of incubation temperature variance ($^{\circ}\text{C}$) | 4.1 \pm 0.6 (n = 7) | 4.7 \pm 0.4 (n = 8) | 4.4 \pm 1.0 (n = 4) | 6.7 \pm 2.7 (n = 2) | – | 2.7 \pm 0.5 (n = 7) | 3.4 \pm 0.4 (n = 5) | 4.6 \pm 0.7 (n = 10) |

The number of hatchlings that failed to right themselves in 15 min did not differ among treatments for painted ($\chi^2_3 = 0.52$, $P = 0.92$; Table 5) or snapping turtles ($\chi^2_3 = 3.60$, $P = 0.31$; Table 6). LP and RP of painted turtles did not differ among treatments ($F_{3,327} = 1.79$, $P = 0.15$; $F_{3,326} = 0.17$, $P = 0.92$; Table 5), and LP and RP of snapping turtles did not differ among treatments ($F_{3,1853} = 1.81$, $P = 0.14$; $F_{3,1850} = 2.08$, $P = 0.10$; Table 6). A significant interaction between treatment and processing temperature was found for snapping turtle RP ($F_{3,1850} = 2.93$, $P = 0.03$). Hatchlings from above-ground cages had the slowest RP across all processing temperatures. The slope of RP versus processing temperature was similar for the wooden-sided and control treatment; over most processing temperatures,

hatchlings from wooden-sided and control nests had the fastest RP. However, RP did not vary by more than half a second between any treatments.

Predators and nest cages

Over the course of incubation, the number of predator interactions with above-ground cages was 14, with below-ground cages 16, and with wooden-sided cages it was 2. The number of caged nests with which predators interacted did not differ among treatments ($\chi^2_2 = 0.06$, $P = 0.97$). The number of successful depredation events was 3, 1 and 3 for above-ground, below-ground and wooden-sided cages, respectively. The number of

Table 5. Summary of *Chrysemys picta* clutch and hatchling data

Data are means \pm s.e. For the first four variables (1–4), samples size (n) represents the number of nests, and for the last three variables (5–7), n represents the number of hatchlings. If data were analysed between years, means are shown separated by commas, and in some instances, year data were pooled. LP, latency period; RP, righting period

| Clutch and hatchling variable | Nest-cage treatment | | | |
|--|--|---|---|---|
| | Above-ground cages | Below-ground cages | Wooden-sided cages | Control (no cage) |
| (1) Incubation duration (days) | 97.0, $n=1$ (2010); 89.3 \pm 4.5, $n=4$ (2011) | – (2010); 83.5 \pm 3.5, $n=2$ (2011) | – (2010); 94.0 \pm 5.8, $n=4$ (2011) | 94.2 \pm 3.4, $n=6$ (2010); 97.3 \pm 3.1, $n=3$ (2011) |
| (2) Hatching success (%) | 69.3 \pm 8.0, $n=24$ | 79.1 \pm 6.7, $n=25$ | 76.4 \pm 4.4, $n=12$ | 59.7 \pm 8.8, $n=20$ |
| (3) Proportion of deformed hatchlings per clutch | 0.23 \pm 0.1, $n=6$ (2010); 0.18 \pm 0.06, $n=10$ (2011) | 0.41 \pm 0.11, $n=10$ (2010); 0.30 \pm 0.08, $n=10$ (2011) | – (2010); 0.36 \pm 0.06, $n=12$ (2011) | 0.37 \pm 0.12, $n=8$ (2010); 0.33 \pm 0.05, $n=9$ (2011) |
| (4) Number of deformities per turtle per clutch | 0.30 \pm 0.12, $n=6$ (2010); 0.38 \pm 0.14, $n=10$ (2011) | 1.23 \pm 0.41, $n=10$ (2010); 0.55 \pm 0.2, $n=10$ (2011) | – (2010); 0.63 \pm 0.1, $n=12$ (2011) | 0.73 \pm 0.27, $n=8$ (2010); 0.59 \pm 0.12, $n=9$ (2011) |
| (5) Number of hatchlings that failed to right | 3, $n=91$ | 5, $n=115$ | 3, $n=63$ | 9, $n=77$ |
| (6) LP (s) | 73.9 \pm 11.6, $n=86$ | 126.0 \pm 13.9, $n=114$ | 106.0 \pm 17.6, $n=62$ | 128.6 \pm 17.1, $n=70$ |
| (7) RP (s) | 11.6 \pm 4.1, $n=86$ | 23.1 \pm 8.0, $n=114$ | 11.0 \pm 5.1, $n=62$ | 12.4 \pm 4.0, $n=70$ |

Table 6. Summary of *Chelydra serpentina* clutch and hatchling data

Data are means \pm s.e. For the first four variables (1–4), samples size (n) represents the number of nests, and for the last three variables (5–7), n represents the number of hatchlings. If data were analysed between years, means are shown separated by commas, and in some instances, year data were pooled. LP, latency period; RP, righting period

| Clutch and hatchling variable | Nest-cage treatment | | | |
|--|---|---|--|--|
| | Above-ground cages | Below-ground cages | Wooden-sided cages | Control (no cage) |
| (1) Incubation duration (days) | 93 \pm 2.2, $n=10$ (2010); 88.1 \pm 3.0, $n=10$ (2011) | 92.7 \pm 1.8, $n=9$ (2010); 89.8 \pm 1.9, $n=12$ (2011) | – (2010); 89.4 \pm 2.0, $n=8$ (2011) | 93.4 \pm 2.7, $n=7$ (2010); 89.8 \pm 2.7, $n=11$ (2011) |
| (2) Hatching success (%) | 82.2 \pm 6.9, $n=23$ | 85.2 \pm 4.2, $n=21$ | 73.2 \pm 12.0, $n=10$ | 73.0 \pm 7.0, $n=20$ |
| (3) Proportion of deformed hatchlings per clutch | 0.27 \pm 0.06, $n=10$ (2010); 0.24 \pm 0.07, $n=10$ (2011) | 0.36 \pm 0.06, $n=11$ (2010); 0.28 \pm 0.06, $n=12$ (2011) | – (2010); 0.25 \pm 0.07, $n=8$ (2011) | 0.28 \pm 0.08, $n=8$ (2010); 0.14 \pm 0.04, $n=11$ (2011) |
| (4) Number of deformities per turtle per clutch | 0.47 \pm 0.12, $n=10$ (2010); 0.41 \pm 0.14, $n=10$ (2011) | 0.72 \pm 0.2, $n=11$ (2010); 0.53 \pm 0.2, $n=12$ (2011) | – (2010); 0.41 \pm 0.1, $n=9$ (2011) | 0.54 \pm 0.2, $n=8$ (2010); 0.19 \pm 0.06, $n=11$ (2011) |
| (5) Number of hatchlings that failed to right | 53, $n=599$ | 42, $n=643$ | 21, $n=271$ | 19, $n=481$ |
| (6) LP (s) | 75.2 \pm 6.0, $n=546$ | 67.1 \pm 5.1, $n=634$ | 108.3 \pm 9.6, $n=250$ | 93.7 \pm 7.1, $n=429$ |
| (7) RP (s) | 3.9 \pm 0.9, $n=546$ | 6.7 \pm 1.9, $n=634$ | 5.5 \pm 1.8, $n=250$ | 11.6 \pm 2.8, $n=429$ |

Table 7. Summary of the cost analysis for each nest cage design

The costs shown are per single nest cage. Salary cost was CAN\$10.25/h, the minimum wage in Ontario, Canada

| Nest-cage type | Material cost (\$) | Construction time (min) | Installation time (min) | Total time (min) | Salary cost (CAN\$) | Total cost (CAN\$) |
|--------------------|--------------------|-------------------------|-------------------------|------------------|---------------------|--------------------|
| Wooden-sided cages | 2.20 | 15 ($n=25$) | 6 ($n=7$) | 21 | 3.60 | 5.80 |
| Below-ground cages | 4.90 | 82 ($n=5$) | 13 ($n=14$) | 95 | 16.20 | 21.10 |
| Above-ground cages | 9.10 | 100 ($n=15$) | 10 ($n=7$) | 110 | 18.80 | 27.90 |

nests successfully depredated did not differ among treatments ($\chi^2_2=0.22$, $P=0.90$). The number of nests successfully depredated after being interacted with by a predator one or more times was 1 for above-ground cages, 0 for below-ground cages, and 1 for wooden-sided cages.

Images of predators investigating the nest cages (either sniffing or looking at the cage) were captured 23 times by the trail cameras, and included *Vulpes vulpes*, *Canis lycaon* and *Corvus corax*. Predators interacting with nest cages (e.g. digging) were captured 18 times by the cameras and included

V. vulpes and *C. lycaon*; these interactions were verified by visual inspections of the nests.

Cost analysis of nest-cage designs

Wooden-sided cages were made of the least expensive materials, and above-ground cages required the most expensive materials (Table 7). Construction time was shortest for wooden-sided cages and longest for above-ground cages (Table 7). Installation time did not differ among cage types ($F_{2,24}=2.11$, $P=0.14$;

Table 7). The total cost, including materials and salary, was lowest for wooden-sided cages (Table 7).

Discussion

Nest environment

Most environmental characteristics did not differ among nest-cage types. Around painted turtle nests with wooden-sided cages, the percentage of woody plants was greater than that in all other treatments. Installing wooden-sided cages did not involve digging through plant roots, whereas installation of the other cages involved digging 20 cm below ground through plant roots. Thus, differences in cage-installation requirements may alter the vegetation around the nest from what was maternally selected.

Most thermal-environment characteristics did not differ among treatments, which did not support our prediction that nest-cage materials would shade and reduce the temperature within the nest. Nest temperature, temperature during each third of embryo development, and heat units did not differ among treatments. Daily temperature variance in snapping turtle nests was lower in the wooden-sided than below-ground cage treatment, but not different among any other treatments for snapping or painted turtles. Wooden-sided cages appear to reduce temperature fluctuations in snapping turtle nests. Overall, the thermal environment of the nest was not altered greatly from natural conditions by any of the nest-cage types. These findings indicated that nest cages preserve most of the thermal characteristics that females select to maximise hatching success, and turtle researchers who use above- and below-ground nest cages in their studies are recording temperature data reflective of natural conditions.

None of the nest-cage types tested in our study altered temperature from natural conditions during the TSD thermo-sensitive period. During the thermo-sensitive period, painted turtle embryos across all treatments were incubated at a mean of 25°C, with a daily temperature variance of 20°C. According to the variable degree model of TSD in painted turtles, if development in the thermo-sensitive period occurs between 22°C and 28°C, then that clutch will be entirely male (Bull and Vogt 1981; Neuwald and Valenzuela 2011). However, both male and female painted turtles occur at our study site, which may be attributable to temperature fluctuations during the thermo-sensitive period having a feminising effect on the embryos (Les *et al.* 2007; Paitz *et al.* 2010; Neuwald and Valenzuela 2011), or as a result of prior higher annual temperatures. Snapping turtles across all treatments were incubated at a mean of 26°C, with a daily temperature variance of 5°C; this mean temperature is known to result in development of males (Wilhoft *et al.* 1983), but does not account for an effect of thermal variance, which has also been found to affect sex ratio in *C. serpentina* (Freedberg *et al.* 2011). In recent years, conservation concerns have grown over road mortality of females that results in male-biased populations, and global warming which also may skew sex ratios (Mrosovsky and Provanča 1989; Steen and Gibbs 2004; Hawkes *et al.* 2007). As cages were not found to alter nest temperature, wildlife managers could not use nest cages to promote equal sex ratios in turtle populations where sex ratio is skewed. More importantly, nest caging does not appear to alter nest temperatures in a way that will affect TSD in turtles.

Clutch and hatchling characteristics

Incubation duration did not differ among treatments, which was expected because incubation duration is inversely related to incubation temperature and there were no differences in temperature among cage treatments. In central Ontario, near the northern range limits of turtles, this is critical knowledge as conservation strategies must take into account a short active season. Nest cages did not extend incubation into colder months that could prevent development, and/or force over-wintering within the nest (Bobyne and Brooks 1994). Also, nest cages did not alter hatching success from natural levels. Thus, nest cages are a conservation tool that maintains natural levels of hatching success within the natural emergence period, while in turn increasing the amount of nest success by protecting nests from predators.

Some proxies of hatchling fitness did not differ among treatments. Deformity rates and locomotor performance (LP and RP) did not differ among treatments, which is not surprising because incubation temperature affects both of these proxies (McKnight and Gutzke 1993; Díaz-Paniagua *et al.* 1997; Hewavisenthi and Parmenter 2001; Steyermark and Spotila 2001) and we found no differences in temperature among cage treatments. Hatchling body condition, another proxy for fitness, varied among treatments and the effects of cages on body condition were not consistent between species. For painted turtles, wooden-sided cages, and for snapping turtles, below-ground cages had positive effects on body condition. Above-ground cages negatively affected body condition of snapping turtles. Species-specific differences may be due to differences in nest environment. Nest-site preferences differ between painted and snapping turtles (Schwarzkopf and Brooks 1987; Weisrock and Janzen 1999; Kolbe and Janzen 2002; Hughes and Brooks 2006). We found that the amount of woody plants around nests was different among treatments in painted turtles but not snapping turtles. Also, nest-temperature variance was much greater in the nests of painted turtles than in the nests of snapping turtles, which may relate to the fact that snapping turtles lay nests about two times deeper than do painted turtles (Ernst and Lovich 2009); the greater depth buffers the nest from temperature fluctuations (Spotila 2011). The differences found between species highlighted the importance of a multi-species approach to the evaluation of conservation techniques. Additional species-specific evaluation of this conservation tool is necessary for species with nest characteristics that differ greatly from the species studied here (e.g. sea turtles; Spotila 2011).

Differences in body condition among treatments did not appear to be driven by the hypothesised mechanism (shading by cage material) because incubation temperature did not differ among treatments. In contrast to our predictions, hatchlings incubated in wooden-sided and above-ground cages (the cages with the potential for shading) did not have the poorest body conditions. So, what is the mechanism behind the differences found? Temperature variance differed significantly among treatments in snapping turtles. Nests with below-ground cages had the most variable temperatures of all the treatments (Table 4) and produced hatchlings that were heavier across all carapace lengths. Painted turtles incubated in wooden-sided cages were heavier than hatchlings at the same carapace length in other

treatments, and even though a difference was not found among treatments, nest temperature was less variable than in the other treatments (Table 3). Perhaps temperature variation, and in turn hatchling body condition, are affected by nest-cage type, and the effect differs between species. Shallow painted turtle nests experience more temperature variance, so reducing the variance by using wooden-sided cages may be beneficial, whereas in snapping turtles, increasing the variance is not harmful (and may even be beneficial) because they generally experience low variance in their deep nests. It would appear that a moderate amount of temperature variance ($\sim 10^{\circ}\text{C}$) may improve body condition, and this is achieved by lowering variance in painted turtle nests and increasing variance in snapping turtle nests. Furthermore, temperature variation influences the development of turtles and has been found to affect body size, locomotor performance and growth in various species (Doody 1999; Ashmore and Janzen 2003; Du and Ji 2003; Booth 2006; Les *et al.* 2007; J. L. Riley, S. Freedberg and J. D. Litzgus 2013, unpubl. data). Alternatively, other measures of nest environment that were not measured in our study may have been affected by nest-cage type, and may have driven the differences in hatchling body condition among cage types. Overall, more research is needed to uncover the mechanism driving the differences in hatchling body condition among treatments.

Predators and nest cages

Predator interactions with nest cages and successful depredation events did not differ among nest-cage types. This indicates that predators are not attracted to one nest-cage type over another, and that all nest-caging types are equivalent in protecting turtle nests. Similarly, Burke *et al.* (2005) and Strickland *et al.* (2010) found that marking nests did not increase raccoon depredation rates. Additionally, Kurz *et al.* (2011) found that an enlarged version of the above-ground cage in our study did not attract foxes over other, less conspicuous, cage types. In contrast, Mroziak *et al.* (2000) found that nest caging may cue raccoons to the presence of a nest and attract depredation attempts. In an 8-year study, Rollinson and Brooks (2007) found that *Corvus* sp. used nest markers as visual cues of nests. Within a short-term study period (2 years), nest cages or nest flags are not likely to be a 'learned' cue for nest predators; however, over the long term, certain predator species may learn to associate nest markers with a food source. In our study, above-ground and wooden-sided cages both had instances where after repeated predator interactions, the nest was successfully depredated. Cages, such as these, that extend above ground level may attract multiple predation interactions, which increase both the probability of nest predation, and opportunities to learn that nest cages cue a food source. It is also important to note that our study was conducted in an area where predation pressures were relatively low. In areas where subsidised predators are abundant, or where burrowing predators (like snakes and rodents) are present, cages that extend underground around the nest cavity may be desirable (Rodríguez-Robles 1998; Converse *et al.* 2002; Plummer 2010).

Cost analysis of nest-cage designs

Often, the biggest constraint for conservation actions is funding. Our cost analysis showed that wooden-sided cages were the most

cost-effective design. Wooden-sided cages are also simple for volunteers to construct (Mersey Tobeatic Research Institute and Parks Canada 2009). However, there are other factors to consider. For instance, nest caging that extends above the ground (potentially paired with an educational sign) could be used to increase public awareness of the threats facing turtle populations (Newbury *et al.* 2002). At study sites in Algonquin Park, many park visitors took interest in our nest cages. Signs were posted around the study sites, which often prompted the public to engage in discussions regarding actions an individual could take to restore turtle populations. But, if other concerns are driving decisions about nest caging, for example maximising crypsis of nest cages in areas where disturbance from the public is prevalent (Bolton *et al.* 2008), or if threats of poaching exist, then other nest-caging types, such as below-ground cages, should be employed. The highest priority in a management program should be to maximise nest success and hatchling fitness, but it is understandable that wildlife managers need to also consider logistical, funding and goal-based concerns when choosing a nest-cage type for conservation programs.

Conclusions

Overall, nest cages did not substantially alter the nest environment from natural conditions. Above-ground cages negatively affected body condition in one species, but the other cage types did not negatively affect hatchling fitness, indicating that they are effective conservation tools that do not present secondary deleterious effects on potential recruitment. In conservation of long-lived species, such as turtles, it is important to pay attention to indicators of survival in early life stages because it is difficult to see the population-level outcomes of recovery actions for decades, which delays evaluation and adaptive management (Spencer and Janzen 2010). The effects of nest-cage types on hatchling body condition differed between species, perhaps due to differences in nest depth and thermal regimes. Selection of a nest-cage type for conservation strategies should be based on maximising nest success, hatchling fitness, and on logistical concerns (e.g. ease and efficiency of construction, installation and material costs). Our evaluation of nest caging provides essential knowledge to researchers, wildlife managers, and conservationists for use globally in at-risk turtle-management strategies and research.

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