Canadian Journal of Zoology

Nesting in close quarters: Causes and benefits of high-density nesting behaviour in Painted Turtles

Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2021-0159.R1
Manuscript Type:	Article
Date Submitted by the Author:	09-Dec-2021
Complete List of Authors:	Kell, S.J.; Laurentian University, Department of Biology Rollinson, N.; University of Toronto, Department of Ecology and Evolutionary Biology Brooks, R.J.; University of Guelph, Integrative Biology Litzgus, Jacqueline; Laurentian University, Department of Biology
Is your manuscript invited for consideration in a Special Issue?:	Not applicable (regular submission)
Keyword:	communal nesting, nesting synchrony, nesting behaviour, reptile sociality, reproductive biology, Painted Turtle, <i>Chrysemys picta</i>

SCHOLARONE™
Manuscripts

Canadian Journal of Zoology - Article

Nesting in close quarters: Causes and benefits of high-density nesting behaviour in Painted Turtles

S.J. Kell¹, N. Rollinson², R.J. Brooks³ and J.D. Litzgus¹

¹ Department of Biology, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON P3E 2C6, Canada

² Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, ON M5S 3B2, Canada

³ Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada

Corresponding author: J.D. Litzgus (e-mail: jlitzgus@laurentian.ca)

Running head: Turtle nesting

Nesting in close quarters: Causes and benefits of high-density nesting behaviour in Painted Turtles

S.J. Kell, N. Rollinson, R.J. Brooks and J.D. Litzgus

Abstract: Many oviparous reptiles nest in aggregations and with temporal synchrony. We hypothesized that these traits reflect attraction by conspecifics rather than limiting suitable habitat. We quantified whether Painted Turtles (*Chrysemys picta* (Schneider, 1783)) in Algonquin Park, Ontario, were nesting communally, identified cues females used to select nest sites, and tested whether hatching success was higher in spatially-clustered nests. We found that nests were closer to one another than expected by chance (i.e., were clustered), but that individual nest site selection was only weakly influenced by micro-habitat characteristics. Survival of clustered nests (49%) was not significantly higher than that of solitary nests (39%). When turtle models were placed on the nesting embankment, females nested most often with the highest density of models. Given that reproductive lifespan is the major axis of fitness and that there was little benefit to nest survival in clustered nests, we suggest that clustering is related to females cueing to conspecific nests to expedite the nesting process and gain a good-quality nest site (chosen by the first nesting female in the cluster) while investing little energy in nest-site selection. This strategy may reduce time spent on land, thereby minimizing chances of dehydration, temperature stress, and adult depredation.

Key words: *Chrysemys picta*, communal nesting, egg clustering, nesting synchrony, nesting behaviour, Painted Turtle, reptile sociality, reproductive biology

Introduction

Although the study of social behaviour in turtles is in its infancy, recent work has indicated that conspecific interactions are much more complex than once thought (Doody et al. 2021). Communal nesting (i.e., many females nesting in close proximity at the same time), which is well-recognized among sea turtles (Eckrich and Owens 1995; Spotila 2004) and other reptiles (e.g., Rand 1968; Doody et al. 2009), may also occur in freshwater turtles, thus facilitating social interactions (Colbert et al. 2010; Ferrara et al. 2013; Jennings et al. 2020). Turtle eggs in wild nests experience high rates of depredation (Snow 1982; Riley and Litzgus 2014; Oddie et al. 2015; Lovich et al. 2018), which suggests that communal nesting might merely reflect lack of suitable nesting habitat. However, some research suggests that depredation rates may be reduced when females partake in communal nesting (Robinson and Bider 1988; Eckrich and Owens 1995), perhaps through prey saturation or confusion when scattered eggs and hatchling remains obscure remaining intact nests, which could subsequently reduce offspring mortality and increase fitness. Peaks in nesting activity can be predicted from ambient temperatures (Obbard and Brooks 1987) and often occur during rainfall events (Burke et al. 1998; Spencer and Thompson 2003), resulting in temporal nest clusters, and females may use olfactory and visual cues to locate other females' nests (Iverson et al. 2016); thus, mechanisms exist that may promote synchronous nesting at high-densities in turtles.

Turtles are quintessential bet-hedgers whose slow life histories include high adult survival that is critical to population persistence (Congdon et al. 1987, 1993; Heppell 1998; Enneson and Litzgus 2008). High levels of embryo and early juvenile mortality, and low levels of recruitment, generally will not result in population decline, as long as most adult females survive to reproduce in subsequent years (Clutton-Brock 1984; Congdon et al. 1993; Harms et al.

2005), and females accrue fitness benefits from increased offspring survival within a reproductive season, and from minimization of harm to themselves during nesting (Spencer 2002). In turtles, a model taxon for such slow life histories, females invest substantial energy into their offspring via the energy-rich yolk (Wilhoft 1986), and by selecting nesting sites that increase offspring survival (Hughes and Brooks 2006; Rasmussen and Litzgus 2010). Nest site characteristics such as canopy density, vegetation cover, aspect ratio, and slope are selected by females and significantly increase offspring survival (Janzen 1994; Kolbe and Janzen 2001; Hughes and Brooks 2006). These characteristics promote embryonic development (Wilson 1998), hatchling locomotor ability (Christens and Bider 1987), hatching success (Janzen and Morjan 2001), and ensure adequate heat-unit accumulation during incubation that can be limiting in cooler environments and varies depending on yearly weather conditions (Schwarzkopf and Brooks 1985, 1987; Bobyn and Brooks 1994; Edge et al. 2017).

We examined causes and potential benefits of nest-site choice by female Painted Turtles (*Chrysemys picta marginata* (Schneider, 1783)) in a population where nest-site selection, reproductive synchrony, and nest clustering are suspected. We hypothesized that spatial nesting aggregations and temporal nesting synchrony reflect attraction by conspecifics rather than limiting suitable habitat. We explored whether females nest in non-random spatial and temporal clusters, and whether females consider visual and olfactory cues from conspecifics when choosing a nest site. We also tested whether nests in high density clusters receive a survival benefit from lower rates of depredation.

Materials and methods

Study site description

Our study took place in Algonquin Provincial Park, Ontario, at Wolf Howl Pond (WHP) (45°34'40''N, 78°41'22''W; Fig. 1), a black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) bog bisected by a decommissioned gravel and sand railway embankment (built ca. 1895), now part of the Mizzy Lake Hiking Trail. The embankment is 230 m long, and has a flat top (6-7 m wide) with minimal vegetation. The northeast side is shaded by mature trees (white pine, *Pinus strobus* (L.), balsam fir, *Abies balsamea* ((L.) Mill.), and tamarack, *Larix laricina* ((Du Roi) K. Koch)), and is rarely used by nesting turtles. The southwest side is sloped (ca. 30°), 3-4 m wide, and is the main nesting site for turtles at WHP. The embankment is divided into 23, 10-m² grid sections (Fig. 1), established in 1985 (Schwarzkopf and Brooks 1985) that are used to re-locate nest cavities and for recording the location of events in field note books. The embankment is used annually as an oviposition site by 100-150 Painted Turtles. Demographic and reproductive monitoring of the Painted Turtle population at WHP has taken place annually since 1978 (Samson 2003; Moldowan et al. 2020).

Ethics approval

All work involving animals was carried out under approved Laurentian University Animal Care protocols (AUP #2017-02-01 and #2008-12-02), conforms to the guidelines of Canadian Council on Animal Care, and was authorized by permits from Ontario Parks.

Data collection

Nest location data from 2015, 2016 and 2017 were used to calculate nest densities, and experiments took place during the 2017 nesting season. The duration of the nesting season varies slightly year to year but usually starts within the first week of June and runs until the end of

June/start of July, lasting 3-4 weeks. The 2017 nesting season had a late start (June 14) and ran into the second week of July. Throughout the spring pre-nesting period, we palpated the hind leg pockets of females, feeling for shelled eggs to mark the start of nesting season and the start of embankment monitoring. Embankment monitoring consisted of arriving each day between 1200-1400 h to start patrols before turtles began nesting, and walking the embankment back and forth at relatively consistent intervals (~15-20 min) until dawn. Each nest was marked with a flagged stake (see below). At the start of each field season in 2015, 2016 and 2017, we raked the entire nesting embankment, so that all soil was freshly and consistently turned across the embankment. When nests were depredated, we marked the date on their stakes, holes were re-filled with substrate, and egg shells were removed and discarded. Any invested test pits were also re-filled at the end of each day to reduce confusion for researchers during nest detection. An invested test pit (ITP) was defined as a partially excavated nest chamber that the female abandoned without laying eggs.

Visual cues

Turtle models were used to determine if females use visual cues from other females to choose nesting locations. Turtle models were carved out of Styrofoam, coated in a layer of tile adhesive and burlap, and painted to resemble an adult female Painted Turtle (Fig. 2). For this experiment, three areas at WHP were chosen based on their annually high numbers of nesting females (Brooks et al., unpubl. data). Vegetation at the water's edge was cut in all three locations so females in the water could easily see the embankment and models. Models were set and monitored near the end of nesting season (June 30 - July 7), after an olfaction experiment was complete (see below), and after the first large peak in nesting. There were three treatments:

control (no models), low density of females (1 model), and high density of females (3 models). On a given day, treatments were randomly assigned to one of the three locations and were at least 8 m apart. At the end of each nesting day, the number of nests and the number of ITPs within 3 m of models were counted. The models were rotated among the three locations each day, allowing each treatment to be in each area three times. We examined whether females nested more often with other females already present (i.e., the models) or by themselves. Three Pearson's Chi-square tests of Independence were used to determine if there were differences in number of nests laid, number of ITPs dug, and number of nests + ITPs among high density, low density, and control treatments. Tests were performed in R v3.3.1 using contingency tables in package *Rcmdr*.

Olfactory cues

To determine whether females use olfactory cues to select nest sites, we constructed artificial nests scented with turtle urine or water. Female turtles hold urine in their bladders, which they release while digging nests (Iverson et al. 2016; pers. obs.). On the first day of the nesting season, we captured all females observed staging and at the water's edge. We collected urine from these females by placing them in a clean container immediately after capture until they released their urine. Urine from all females (n=23) was mixed together (~800 ml) to reduce any maternal effects. No females were allowed to nest on the first day of the nesting season. At the start of the following day (second day of nesting season), 16 locations spaced >1 m apart were selected in the five most popular nesting areas (based on long-term study; Brooks et al., unpubl. data) and based on site characteristics described by Hughes and Brooks (2006), so that artificial nests could be created in locations in which turtles would likely nest (Marchand et al.

2002). In each of these locations, a pair of artificial nests was constructed 1 m apart (Fig. 3). Each nest in the pair was then randomly assigned to one of two treatments: urine or distilled water (control). Each nest received 50 ml of liquid; 25 ml into the excavated cavity and 25 ml onto the substrate used to refill the cavity (no eggs were present in these artificial nests). Nests were dug using a spoon by researchers wearing latex gloves to lessen the transfer of human scent. These artificial nests were the only “nests” present at WHP when females re-initiated nesting on the second day of the nesting season.

We examined whether females chose to nest more often near the urine or distilled water scented nests by counting the number of nests within a 0.5 m radius circle of each treatment. We applied these counts to all nests laid over the course of the nesting season, and to nests laid within only the first 2 days of artificial nest creation. We examined these two time periods because we did not know if the artificial nests would be detectable by females for the whole season, or only for the first couple days until many other real nests were laid, obscuring the artificially-scented nests. We also recorded all instances (nests or ITPs) in which females nested on top of or touching another nest, nest cavity, or recently depredated nest. Two Pearson’s Chi-square tests of Independence, one for the entire season and one for within 2 days of artificial nest creation, were used to compare the number of real nests laid to the number expected within a 0.5 m radius circle of each artificial nest treatment (urine or distilled water). Tests were performed in R v3.3.1 using contingency tables in package *Rcmdr*.

Nest clustering

To test whether nests were spatially clustered, we conducted nearest neighbour analyses in Whitebox Geospatial Analysis Tools (GAT) 3.3. The location of nests at the end of each

season were recorded using a high accuracy Tremble GPS (± 30 cm). Nests were marked using 50 cm pigtail stakes with flagging tape labeled with nest ID and lay date. Stakes were located at the stake's length up the bank so that predators could not use the stakes as cues to locate nests (Rollinson and Brooks 2007). We compared the nearest neighbour distances among nests within each year (2015, 2016, 2017) to determine the level of clustering relative to random nest distributions, and we visually compared the distribution of nests between years by overlaying maps created in Whitebox GAT. We compared nest distributions among years to test whether females were choosing nest sites based on individual annual site fidelity (Rowe et al. 2005), and to test whether females chose nest sites based on where other females nested that year. Nearest neighbour analyses were completed with an edge effect strategy by tiling the data. Tiling the data subdivides the data points into gridded sections, increases accuracy by taking into account smaller areas rather than the entire distribution of points, which can bias the results when points are already in large groups or along linear distributions (Zhang et al. 2004).

Environmental characteristics

We tested whether females chose nest sites based on environmental characteristics measured for each nest. At the time the nest was laid, soil (2-3 cm below ground surface), air and water (in closest waterbody) temperatures were measured using a digital thermometer. At the end of the nesting season, after all nests had been laid, we measured soil surface temperature at noon, overstory vegetation density, understory vegetation density, and slope; these characteristics tend to remain fairly consistent throughout the nesting season. Soil surface temperature at noon at known nests, measured between 12:00 pm and 1:00 pm with a digital thermometer, was quantified as an indication of heat retention along the embankment. Overstory vegetation density

was measured using a spherical densitometer at breast height for the four cardinal directions, then averaged. Understory vegetation (plants under 1 m height) density was measured by placing a 1 m x 1 m grid (containing 100 squares) centered on the nest and counting how many squares contained vegetation. Slope was measured using a clinometer mounted to a piece of wood so slope could be measured at ground level. Soil moisture, substrate type, and aspect ratio were not measured for each nest, as the embankment is a decommissioned railway bed constructed from sand, thus all nests point in the same cardinal direction and have consistently similar substrate.

Euclidean distances (a measure of density based on a nest's relative distance to all other nests) for each nest in the 2017 field season were measured using Whitebox GAT by extracting raster data. Linear regressions were then used to examine the effect of each environmental characteristic on nest density (i.e., Euclidian distance).

Nest predation and survival

To determine if nest clustering affected nest depredation, we examined depredation rates of both natural (WHP) and artificial nests (created at Sand Dunes; Figs. 1, 4). More than a month after the end of the nesting season, and nearing the end of summer (28 August 2017), we did a final check of nests at WHP to determine how many had been excavated by predators. All nests at WHP were grouped into one of two categories: solitary (>20 cm from another nest) or clustered (<20 cm from another nest). A distance of 20 cm was used as this appeared to be the minimum distance a female could nest beside another female without touching and interfering with her during the nesting process. The predation status of nests (partially or wholly excavated = predated; undisturbed = not predated) was recorded and compared between the two groups (solitary, clustered). Nests were not monitored or excavated to determine success rate after this

date.

We also performed a manipulative experiment at Sand Dunes (45°34'31"N, 78°42'28"W; Figs. 1, 4), another nesting site that is monitored as part of the long-term study. Sand Dunes is a large open area (~30 m wide and 200 m long) about 2 km from WHP; it has few trees and the substrate is loose sand. We created two artificial nest densities: low (one solitary nest) and high (3 clustered nests). Each density was within a 0.5-m radius circle, with the solitary nest located in the middle and the clustered nests located in the middle but in a triangular pattern spaced 20 cm apart. Each nest was dug to a depth of 5-10 cm with a spoon and filled with 5 Snapping Turtle (*Chelydra serpentina* (Linnaeus, 1758)) eggs collected from turtles that nested at another known nesting site on the embankment, Joe Lake (45°34'32"N, 78°42'50"W; Fig. 1). Snapping Turtle eggs were used because a large number of eggs could be collected from fewer adults compared to Painted Turtles (i.e., Snapping Turtles lay substantially larger maximum clutch sizes than Painted Turtles, up to 60 eggs vs. 14 eggs, respectively; Ernst and Lovich 2009). There were 15 nest pairs assembled, creating two parallel rows (A and B) with one density from each treatment in each row. The 0.5-m radius circles were placed 2 m from their apposing density treatment in the opposite row and spaced 2 m from the neighbouring pair (Fig. 4). Densities were randomly assigned to row A or B. These artificial nests were all created on the same day and monitored every 5 days up to 15 days after creation. At the end of the field season (28 August 2017), all nests were excavated to check final survival status. All nests were flagged 0.5 m from their circle to mark their locations and so that predators would not cue in on the flags (Rollinson and Brooks 2007). Any nest that was depredated was left as found, with sand and eggshells scattered around.

Separate Pearson's Chi-square tests compared survival between solitary and clustered nests at WHP and Sand Dunes. The expected depredation frequency for all tests was based on the observed depredation rate (57%) of nests at WHP in the 2017 season. Each nest at WHP was considered its own treatment unit and recorded as depredated or survived, but then additionally separated into solitary or clustered treatment groups based on the 20-cm spacing. Observed depredation frequency was calculated from the actual survival of all clustered nests and survival of all solitary nests at WHP. At Sand Dunes, nest survival between clustered and solitary nests was also compared using expected depredation frequencies from WHP and observed depredation frequencies from clustered and solitary treatment groups at Sand Dunes. Tests were performed in R v3.3.1 using contingency tables of package *Rcmdr*.

Results

Visual cues

Females displayed significantly more nesting activity near the turtle models (Fig. 5). Over the 9 days of data collection, female turtles nested most often among models (i.e., within 3 m of the models) at high density ($n = 9$ females), less often in the low density of models ($n = 5$), and least often in the control ($n = 3$). The number of ITPs differed significantly among nest densities (high density = 31, low density = 20, control = 9; $\chi^2 = 6.55$, $df = 2$, $P = 0.04$). Similarly, overall nesting activity (ITPs + nests) differed significantly among model densities ($\chi^2 = 8.14$, $df = 2$, $P = 0.02$), with the most nesting activity occurring with models at high density ($n = 40$), moderate activity with models at low density ($n = 25$), and the least activity in the control ($n = 12$).

Olfactory cues

The mean number of real nests within a 0.5 m radius of urine-scented artificial nests ($N_{\text{urine}} = 20$, mean = 1.25 ± 0.38) and distilled water-scented nests ($N_{\text{water}} = 18$, mean = 1.13 ± 0.36) did not differ ($X^2 = 0.05$, $df = 1$, $P = 0.82$). Of the 9 real nests laid within the first two days after artificial nests were constructed, again, the number of nests within a 0.5 m radius of urine-scented nests ($N_{\text{urine}} = 6$, 0.38 ± 0.15) and distilled water-scented nests ($N_{\text{water}} = 3$, 0.19 ± 0.10) did not differ ($X^2 = 1.20$, $df = 1$, $P = 0.27$). Of the 165 nests recorded during the 2017 nesting season, 14 (8%) ITPs were found on top of other nests or depredated nests (i.e., <20 cm away), and 27 (16%) nests were laid on top of another nest or depredated nest. In 8 of these 27 cases (30%), a female dug up another turtle's nest while excavating her own. Looking at total nesting activity (ITPs + nests), 41 (25%) of the 165 nests recorded in 2017 at WHP had another nest or nest chamber constructed on top, or directly beside them, resulting in the adjacent nest chambers merging into a single chamber.

Nest clustering

We investigated whether Painted Turtles were nesting synchronously by virtue of temporal nest clustering. In each study year, there are generally 1-2 peaks in nesting activity that result in a large majority of turtles nesting within 1-3 days, with the first larger peak usually consisting of first clutches, and the second smaller peak consisting of late first clutches and early second clutches (Brooks et al., unpubl. data). The numbers of nests per day in each field season were visualized graphically to examine the possibility of nesting synchrony. In each study year (2015, 2016, 2017), nests were significantly clustered (i.e., not randomly distributed) on the WHP embankment (Table 1, Fig. 6). Clustering increased as the number of nests increased. More

than 80% of nests were confined to about 40% of the WHP embankment (Fig. 6), and this area comprises 6 nesting sections in the study grid (Fig. 1). Examination of each of these sections revealed that there were differences in locations of nest clusters between years. For example, in 2015, in sections 2 and 4 (Fig. 6), females nested at relatively low densities compared to 2016 and 2017, whereas the highest density was in section 6. In section 2 in 2017, females nested closer to the water and in 2016 nested farther up the embankment. In sections 3 and 5 in 2017, females nested to the east side whereas in 2016, they nested to the west side. Along with the clustering on the embankment, turtles also appeared to cluster temporally with the majority of their nesting concentrated in two short time windows: 4 days in a large peak at the start of the nesting season, and 3 days during a smaller peak at the end of the nesting season (Supplemental Fig. S1).

Environmental characteristics

Environmental factors had limited influence on nest clustering (Table 2). Only understory vegetation ($F = 6.25$, $R^2 = 0.029$, $P = 0.013$) and slope ($F = 4.32$, $R^2 = 0.019$, $P = 0.039$) were significantly related to nest proximity as measured by Euclidean distances. As embankment slope increased and understory vegetation decreased, the proximity to neighbouring nests increased.

Nest depredation and survival

At WHP, clustered nests ($n = 65$) had higher survivorship (49%) than solitary nests ($n = 101$, 39%), but the difference was not statistically significant ($X^2 = 0.51$, $df = 1$, $P = 0.48$). Predators of nests at WHP consisted primarily of Common Raven (*Corvus corax* (Linnaeus,

1758)) and Red Fox (*Vulpes vulpes* (Linnaeus, 1758)) based on depredation characteristics (corvids: small hole, no egg shells; fox: large hole, eggs shells scattered and still remaining).

At Sand Dunes, the manipulation experiment revealed that clustered nests had marginally higher survival (16%) than solitary nests (13%), but the difference between treatments was not significant ($X^2 = 0.012$, $df = 1$, $P = 0.91$). When we considered each treatment unit's area as a location, the clustered treatment had more locations with un-depredated nests (clustered = 7/15 (47% survivorship); solitary = 2/15 (13% survivorship); $X^2 = 3.65$, $df = 1$, $P = 0.058$), as the predator(s) of the clustered treatments often missed at least one nest in the cluster before apparently moving on. All nests that were depredated in the Sand Dunes experiment appeared to be depredated by a Red Fox, possibly a single individual.

Discussion

Our study provides evidence that female Painted Turtles choose to nest together. When given options to nest with different densities of turtle models, which provided a visual cue, females most often chose to nest near the highest density of models, suggesting that turtles are cueing to conspecific females to choose their nest site. Further, across multiple nesting seasons, females placed their nests in spatial and temporal clusters, suggesting that they may be participating in communal nesting. Chemical cues are unlikely to be involved in putative communal nesting, as turtles did not appear to discriminate among nesting locations saturated with turtle urine versus distilled water. Finally, our experiments suggest that communal nesting is unrelated to probability of nest depredation; specifically, both observational and manipulative studies revealed equivocal evidence of lower depredation rates in clustered nests.

Perhaps the most compelling evidence of communal nesting in our study is that female turtles preferred to nest in areas with the highest density of turtle models. Indeed, if turtles are cueing to conspecific females to choose nest sites, then we expect that the spatial distribution of nests will be significantly clustered. This is precisely what we observed, where nests were significantly aggregated in all three years of the study. Although our analysis does not preclude the possibility that clusters are driven by variation in nest-site quality, we found that high nest density was not strongly associated with environmental characteristics of the site. Specifically, the only characteristics associated with nest location (slope and reduced understory vegetation) coincidentally occupy a large majority of the WHP embankment. Thus, good quality nesting habitat (e.g., suitable for proper embryonic development) is not limiting at WHP, such that environmental characteristics are important in a female's choice of general location, but are not a significant driver of precise nest location (Schwarzkopf and Brooks 1987). This is consistent with many species of communal nesting reptiles that cluster in areas where nesting sites are not limiting, therefore refuting the saturated habitat hypothesis (Doody et al. 2009). Doody et al. (2009) summarized all cases of communal nesting in *Chelonia*, concluding that 16% of species have been observed nesting communally, and Davis and Burghardt (2011) showed evidence to support that turtles can learn from visual cues and conspecifics. Vogt (pers. com.) also described nesting aggregations of *Podocnemis expansa* (Schweigger, 1812) that arrive and leave nesting areas together. These observations shed light on the role that sociality may play in turtle reproduction.

Our experimental data did not support the use of olfactory cues in nest detection. However, other observational evidence may indicate olfaction in detecting nests laid by other females. For instance, many females (15-25%) in our study placed their nests close to, or on top

of, another female's nest, suggesting that females may be able to detect nests that have already been laid. Similarly, during sea turtle *arribada*, thousands of females will nest on the same beach and over 40% of nests can be dug up by females that nest subsequently (Wen pers. comm.). It is difficult to envision how such a behaviour could be adaptive, and perhaps clustering is a byproduct of selecting high-quality nest locations, and only by chance do females nest on each other's nests. Yet, females may be using scent to locate nests, as suggested by the "head-ploughing" or "ground-nuzzling" behaviour observed while females are searching for a nest location (Morjan and Valenzuela 2001; Iverson et al. 2016; pers. obs.), so it is difficult to believe that females cannot detect locations of previous nests. For example, Iverson et al. (2016) found that female Painted Turtles nested more often near nests scented with urine versus water, which is contrary to our findings, but supports the idea that olfactory cues from other females are important in nest site selection. One weakness of our design is that we pooled all urine samples before deploying them to experimental locations, such that any unappealing agent present in the urine of one turtle (e.g., blood from infection or injury) may have caused aversion to all replicates in our study; future tests of this hypothesis should keep urine samples separate before application to maintain replicate independence. Further, our experiment may have placed treatments (scented and unscented nests) too close together for turtles to distinguish and therefore prevented a proper control, as Iverson et al. (2016) did find evidence of olfactory cues when treatments were separated by distances 3x greater than those in our study. Clearly, more work is needed to understand the role of olfaction in nest-site choice, and whether females can indeed detect the specific location in which other females have previously nested.

The observed peaks in nesting activity (Supplemental Fig. S1) underline that a non-trivial proportion of nesting activity is also temporally synchronous, although the peak in activity could

easily arise for reasons unrelated to any adaptive benefit of temporal synchrony. Nesting synchrony has been observed in sea turtles (*Lepidochelys olivacea* (Eschscholtz, 1829), Eckrich and Owens 1995; Spotila 2004) and has been suggested for Snapping Turtles (Robinson and Bider 1988). These nesting peaks seem to be related to weather. Nesting peaks tend to coincide with or occur shortly after warm rainfall (Burke et al. 1998; Spencer and Thompson 2003; Kell pers. obs.). Nesting during rainfall may improve malleability of the soil, reducing the need for females to carry large amounts of liquid in their bladders, in turn reducing the risk of overheating, and covering scents used as cues by predators (Bowen and Janzen 2005; although rain may increase nest scent and therefore predation; Brooks pers. obs.). It is of course possible that temporal synchrony arises because follicular maturation is approximately normally distributed, such that many turtles reach nest readiness at the same point during the nesting season. Timing of oviposition is closely related to thermal environment, and turtles in similar habitats oviposit at the same time (Obbard and Brooks 1987). Regardless of the underlying reason, Painted Turtle nesting tends to be temporally synchronous, in addition to spatially aggregated.

We tested whether clustered nests result in higher nest survivorship than solitary nests. Clustered nests may cause prey saturation, supporting the attack abatement hypothesis (*sensu* Turner and Pitcher 1986) that predicts that predators are less likely to find a single group of many eggs than many scattered small groups. This hypothesis also encompasses a dilution effect: as the number of eggs increases, the potential for depredation for each mother, neonate, or egg, is reduced (Doody et al. 2009). Prey saturation or predator swamping is hypothesized to reduce mortality among animals (Sweeny and Vannote 1982). Red Foxes (scent-oriented predator) and Common Ravens (visual-oriented predator), the two main predators at our sites, may be

befuddled by clustered nests. This swamping strategy may be useful for deterring predators that do not alter foraging behaviour after encountering nests (Wirsing et al. 2012). A “scent saturation” strategy may also be possible, as nests may become camouflaged by surrounding depredated nests once scent (i.e., from eggshells and sand) are scattered over top of neighbouring intact nests, and visually by looking non-rewarding to predators, as the open hole of the depredated nests may mask the presence of the intact nest.

Despite well-developed theory on the benefits of communal nesting, our study did not demonstrate any fitness benefit from nesting in clusters. Specifically, survivorship for the clustered nests at WHP during 2017 was not higher than that of solitary nests, and our manipulative experiment revealed no benefit to nest clustering, whether analyzed on a per-nest basis or a per-location basis. Although this provides evidence that nest clustering is not advantageous, there are methodological limitations that may limit the scope of our inference. For instance, our maximum nest cluster was 3 nests, but this value was arbitrary, and natural nest clusters may be larger, on average, and may confer a benefit. Indeed, a more fine-grained analysis that included the sequence of nests laid in the season, for example, may have indicated an advantage to later nests in a cluster that were more difficult to locate after all nests were present and depredation had begun. Nevertheless, our results suggest that nest clustering, and hence predator swamping, does not occur in our population of turtles.

There are mechanisms other than predator swamping that could explain why turtles in the present study nested together. First, annual egg survival has little impact on fitness of turtles, but adult survival is paramount (Congdon et al. 1993; Heppell 1998; Enneson and Litzgus 2008). Thus, turtles may be cueing to other turtles during nesting to reduce time, energy, and risk involved in nest searching (Kell 2016). If turtles generally exhibit some degree of nest site

choice, then constructing a nest near a turtle that is already nesting forgoes the long process of nest site choice, thereby saving energy and reducing time out of the water where adults are susceptible to depredation and dehydration. This explanation is consistent with the life-history strategy of turtles and subsumes the idea of nest-site choice; any small benefit of putative predator swamping (e.g., a 10% decrease in mortality of clustered nests in our study) would be an ancillary benefit of maximizing the number of reproductive events in the adult's lifetime.

In sum, we provide evidence that female Painted Turtles choose nesting locations near nests of other females. We also provide evidence to suggest that female Painted Turtles choose nest sites based on visual cues provided by other females and their nests. These novel findings reveal a potential sociality in turtle nesting behaviour that warrants further investigation. This nest clustering behaviour may be driven by benefits that the mothers receive by reducing nest searching time, and may also slightly increase nest survivorship through predator swamping or prey saturation mechanisms.

Acknowledgements

This work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to J.D.L. (grant #311994), the Ontario Ministry of Natural Resources and Forestry, and Ontario Parks. We wish to thank the Algonquin Wildlife Research Station and Ontario Parks for support of the long-term turtle studies in Algonquin Provincial Park. We thank Taylor Wynia, Nicole Brunet, Carter Rouleau, and Patrick Moldowan for assistance with field work.

References

- Bobyn, M.L., and Brooks, R.J. 1994. Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling turtles (*Chelydra serpentina*). *J. Zool.* **233**: 233-257.
- Bowen, K.D., and Janzen, F.J. 2005. Rainfall and depredation of nests of the painted turtle, *Chrysemys picta*. *J. Herpetol.* **39**: 649-652.
- Burke, V.J., Rathburn, S.L., Bodie, J.R., and Gibbons, J.W. 1998. Effect of density on predation rate for turtle nests in a complex landscape. *Oikos*, **83**: 3-11.
- Christens, E., and Bider, J.P. 1987. Nesting activity and hatchling success of the painted turtle (*Chrysemys picta marginata*) in southeastern Quebec. *Herpetologica*, **43**: 55-65.
- Clutton-Brock, T.H. 1984. Reproductive effort and terminal investment in iteroparous animals. *Am. Nat.* **123**: 212-229.
- Colbert, P.L., Spencer, R.J., and Janzen, F.J. 2010. Mechanism and cost of synchronous hatchling. *Funct. Ecol.* **24**: 112-121.
- Congdon, J.D., Breitenbach, G.L., van Loben Sels, R.C., and Tinkle, D.W. 1987. Reproduction and nesting ecology of snapping turtles (*Chelydra serpentina*) in South-eastern Michigan. *Herpetologica*, **43**: 39-54.
- Congdon, J.D., Dunham, A.E., and van Loben Sels, R.C. 1993. Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): Implications for conservation and management of long-lived organisms. *Conserv. Biol.* **7**: 826-833.
- Davis, K.M., and Burghardt, G.M. 2011. Turtles (*Pseudemys nelson*) learn about visual cues indicating food from experienced turtles. *J. Comp. Psychol.* **125**: 404-410.
- Doody, J.S., Diets, V., and Burghardt, G.M. 2021. *The Secret Social Lives of Reptiles*. Johns Hopkins University Press, Baltimore, Md.

- Doody, J.S., Freedberg, S., and Keogh, J.S. 2009. Communal egg-laying in reptiles and amphibians: Evolutionary patterns and hypotheses. *Quart. Rev. Biol.* **84**: 229-252.
- Eckrich, C.E., and Owens, D.W. 1995. Solitary versus arribada nesting in the olive ridley sea turtles (*Lepidochelys olivacea*): A test of the predator-satiation hypothesis. *Herpetologica*, **51**: 349-354.
- Edge, C.B., Rollinson, N., Brooks, R.J., Congdon, J.D., Iverson, J.B., Janzen, F.J., and Litzgus, J.D. 2017. Phenotypic plasticity of nest timing in a post-glacial landscape: How do reptiles adapt to seasonal time constraints? *Ecology*, **98**: 512-524.
- Enneson, J.J., and Litzgus, J.D. 2008. Using long-term data and a stage-classified matrix to assess conservation strategies for an endangered turtle (*Clemmys guttata*). *Biol. Conserv.* **141**: 1560-1568.
- Ernst, C.H., and Lovich, J.E. 2009. *Turtles of the United States and Canada*. 2nd ed. Johns Hopkins University Press, Baltimore, Md.
- Ferrara, C.R., Vogt, R.C., and Sousa-Lima, R.S. 2013. Turtle vocalizations as the first evidence of posthatching parental care in chelonians. *J. Comp. Psychol.* **127**: 24-32.
- Harms, H.K., Paitz, T.R., Bowden, R.M., and Janzen, F.J. 2005. Age and season impact resource allocation to eggs and nest behaviour in the painted turtle. *Physiol. Biochem. Zool.* **78**: 996-1004.
- Heppell, S.S. 1998. Application of life-history theory and population model analysis to turtle conservation. *Copeia*, **1998**: 367-375.
- Hughes, E.J., and Brooks, R.J. 2006. The good mother: Does nest-site selection constitute parental investment in turtles? *Can. J. Zool.* **84**: 1545-1554.
- Iverson, J.B., Klondaris, H., Angell, C.S., and Tori, W.P. 2016. Olfaction as a cue for nest-site

- choice in turtles. *Chelonian Conserv. Biol.* **15**: 206-213.
- Janzen, F.J. 1994. Vegetation cover predicts the sex ratio of hatchling turtles in natural nests. *Ecology*, **75**: 1593-1599.
- Janzen, F.J., and Morjan, C.L. 2001. Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination. *Anim. Behav.* **62**: 73-82.
- Jennings, N.D., Gati, E.V., Ramirez, G.A., Cole, J., Mason, B., Briggs-Gonzalez, V., and Mazzotti, F.J. 2020. A communal nesting site of the Central American river turtle (*Dermatemys mawii*) along the New River, Belize. *Herpetol. Bull.* **152**: 7-10.
- Kell, S.J. 2016. Nest-site fidelity, search time and nest predation in painted turtles (*Chrysemys picta*). B.Sc. Thesis, University of Guelph, Guelph, Ont.
- Kolbe, J.J., and Janzen, F.J. 2001. The influence of propagule size and maternal nest-site selection on survival and behavior of neonate turtles. *Funct. Ecol.* **15**: 772-781.
- Lovich, J.E., Ennen, J.R., Agha, M., and Gibbons, J.W. 2018. Where have all the turtles gone, and why does it matter? *BioScience*, **68**: 771-781.
- Marchand, M.N., Litvaitis, J.A., Maier, T.J., and DeGraaf, R.M. 2002. Use of artificial nests to investigate predation on freshwater turtle nests. *Wildl. Soc. Bull.* **30**: 1092-1098.
- Moldowan, P.D., Brooks, R.J., and Litzgus, J.D. 2020. Sex, shells and weaponry: Coercive reproductive tactics in the painted turtle (*Chrysemys picta*). *Behav. Ecol. Sociobiol.* **74**: 142.
- Morjan, C.L., and Valenzuela, N. 2001. Is ground-nuzzling by female turtles associated with soil surface temperatures? *J. Herpetol.* **35**: 668-672.
- Obbard, M.E., and Brooks, R.J. 1987. Prediction of the onset of the annual nesting season of the common snapping turtle, *Chelydra serpentina*. *Herpetologica*, **43**: 324-328,

- Oddie, M.A.Y., Coombes, S.M., and Davy C.M. 2015. Investigation of cues used by predators to detect snapping turtle (*Chelydra serpentina*) nests. *Can. J. Zool.* **93**: 299-305.
- Rand, A.S. 1968. A nesting aggregation of iguanas. *Copeia*, **1968**: 552-561.
- Rasmussen, M.L., and Litzgus, J.D. 2010. Patterns of maternal investment in spotted turtles (*Clemmys guttata*): Implications of trade-offs, scales of analyses, and incubation substrates. *Écoscience*, **17**: 47-58.
- Riley, J.R., and Litzgus, J.D. 2014. Cues used by predators to detect freshwater turtle nests may persist late into incubation. *Can. Field-Nat.* **128**: 179-188.
- Robinson, C., and Bider, J.R. 1988. Nesting synchrony: A strategy to decrease predation of snapping turtle (*Chelydra serpentina*) nests. *J. Herpetol.* **22**: 470-473.
- Rollinson, N., and Brooks, R.J. 2007. Marking nest increases the frequency of nest depredation in a northern population of painted turtles (*Chrysemys picta*). *J. Herpetol.* **41**: 174-176.
- Rowe, J.W., Coval, K.A., and Dugan, M.R. 2005. Nest placement, nest-site fidelity and nesting movements in midland painted turtles (*Chrysemys picta marginata*) on Beaver Island, Michigan. *Am. Midl. Nat.* **154**: 383-397
- Samson, J. 2003. Growth, maturity and survivorship patterns of the Wolf Howl Pond population of midland painted turtles, *Chrysemys picta marginata*. M.Sc. Thesis, University of Guelph, Guelph, Ont.
- Schwarzkopf, L., and Brooks, R.J. 1985. Sex determination in northern painted turtles: Effect of incubation at constant and fluctuating temperatures. *Can. J. Zool.* **63**: 2543-2547.
- Schwarzkopf, L., and Brooks, R.J. 1987. Nest-site selection and offspring sex ratio in painted turtles, *Chrysemys picta*. *Copeia*, **1987**: 53-61.

- Snow, J.E. 1982. Predation on painted turtle nests: nest survival as a function of nest age. *Can. J. Zool.* **60**: 3290-3292.
- Spencer, R.J. 2002. Experimentally testing nest site selection: Fitness trade-offs and predation risk in turtles. *Oikos*, **8**: 2136-2144.
- Spencer, R.J., and Thompson, M.B. 2003. The significance of predation in nest site selection of turtles: An experimental consideration of macro- and microhabitat preferences. *Oikos*, **102**: 592-600.
- Spotila, J.R. 2004. *Sea Turtles: A Complete Guide to Their Biology, Behavior, and Conservation*. Johns Hopkins University Press, Baltimore, Md.
- Sweeney, B.W., and Vannote, R.L. 1982. Population synchrony in mayflies: A predator satiation hypothesis. *Evolution*, **36**: 810-821.
- Turner, G.F., and Pitcher, T.J. 1986. Attack abatement: A model for group protection by combined avoidance and dilution. *Am. Nat.* **128**: 228-240.
- Wilhoft, D.C. 1986. Eggs and hatchling components of the snapping turtle (*Chelydra serpentina*). *Comp. Biochem. Physiol.* **84**: 483-486.
- Wilson, D.S. 1998. Nest-site selection: microhabitat variation and its effect on the survival of turtle embryos. *Ecology*, **79**: 1884-1892.
- Wirsing, A.J., Philips, J.R., Obbard, M.E., and Murray, D.L. 2012. Incidental nest predation in freshwater turtles: Inter- and intraspecific differences in vulnerability are explained by relative crypsis. *Oecologia*, **168**: 977-988.
- Zhang, J., Papadias, D., Mamoulis, N., and Tao, Y. 2004. All-nearest-neighbors queries in spatial databases. *Proceedings. 16th International Conference on Scientific and Statistical Database Management* 297-306.

Figure Captions

Fig. 1. (Top) Google Map showing locations of all field sites used or sampled during the 2017 turtle nesting season in Algonquin Park, Ontario. Blue pins represent the main wetlands that are monitored as part of the long-term turtle study. Wolf Howl Pond (WHP) is the main site where most experiments in the current study took place. Green pins represent sites that were used for the scent saturation experiment, allowing access to Snapping Turtle (*Chelydra serpentina*) nests and a sandy location for artificial nests. The pale line running alongside the wetlands is the decommissioned railway embankment, now part of the Mizzy Lake hiking trail, upon which the turtles nest. (Bottom) WHP embankment, where ~100-150 Painted Turtles (*Chrysemys picta*) nest each season, divided into 23 grid sections, indicated by the blue rectangles. Yellow dots represent turtle nests from the 2015 season as an example of nest distribution.

Fig. 2. (Top) Female Painted Turtle (*Chrysemys picta*) models on the nesting embankment at Wolf Howl Pond (WHP), Algonquin Park, Ontario during the 2017 nesting season. Three different model densities (i.e., high = 3 models, low = 1 model, control = no models) were positioned at WHP to test the influence of visual cues from other females in nesting preference. Models were created from a combination of shaped foam covered in burlap and tile adhesive, then painted. (Bottom) Female Painted Turtle nesting amongst the turtle models. Flagging indicates the location of other nests.

Fig. 3. (Top) Layout of 8/16 artificial nest pairs created at the start of nesting season at Wolf Howl Pond (WHP), Algonquin Park, Ontario. Red markers refer to urine-scented nests and blue markers refer to distilled water-scented nests. Numbers on the markers correspond with nest pair. (Bottom) Layout of 11/16 nest pairs at WHP with distribution of natural nest locations in 2017

(green dots). Red circles refer to the urine treatment and blue circles refer to the distilled water treatment. The black box shows the area of the embankment shown in the above photo.

Fig. 4. Picture of the Sand Dunes illustrating approximately $\frac{3}{4}$ of the linear stretch used for the egg clustering manipulation experiment. Flags in photos represent each treatment (A). Layout of 10/15 pairs of solitary (red) and clustered (blue) nest treatments, randomly assigned to each side (B). Illustration of the approximate layout of treatment pairs for solitary (red) and clustered (blue) nest treatments (C).

Fig. 5. Nesting activity by female Painted Turtles (*Chrysemys picta*) at Wolf Howl Pond (WHP), Algonquin Park, Ontario during the 2017 nesting season in response to different densities of model turtles. Densities consisted of 3 models (high), 1 model (low), and 0 models (control). We considered a nest as a cavity containing eggs, and an invested test pit (attempted nest) as a cavity close-to or fully-dug but not containing eggs. Data were collected over a 9-day period near the end of nesting season, with each treatment in each location 3 times. There was a significant difference in the number of invested test pits among treatments, and in the number of invested test pits + nests among treatments, as indicated by the asterisks.

Fig. 6. Distribution of Painted Turtle (*Chrysemys picta*) nests among years at Wolf Howl Pond (WHP), Algonquin Park, Ontario. (Top) All nests from the 2015, 2016, and 2017 nesting seasons. The black box represents the area shown in the zoomed-in map below. (Bottom) Nests from a section of the WHP embankment (~40%) that receives >80% of nests every year. Black boxes divide the nesting embankment into 6 major clusters, based on the most open areas of the embankment to facilitate visual comparison of nest clustering and numbers of nests among the 3 study years.

Table 1. Nearest neighbour (NN) analysis output examining probability of nest clustering by Painted Turtles (*Chrysemys picta*) in nesting seasons 2015, 2016 and 2017 in Algonquin Park, Ontario. In all years, the z -score is < -1.96 indicating that nests were significantly clustered.

Statistic	2015	2016	2017
Observed mean NN distance (m)	0.893	1.58	0.625
Expected mean NN distance (m)	1.896	3.216	1.64
Standard error	0.084	0.13	0.064
NN ratio	0.471	0.491	0.381
z -score	-11.978	-12.579	-15.805
P -value	<0.0001	<0.0001	<0.0001

Table 2. Relationships between environmental characteristics and Euclidean distances for all Painted Turtle (*Chrysemys picta*) nests on the embankment at Wolf Howl Pond in Algonquin Park, Ontario during the 2017 field season. Understory vegetation and embankment slope were significantly related to Euclidean distance (bold font). The nesting area is a linear, human-constructed embankment of sandy substrate (decommissioned railway berm now used as a hiking trail), so cardinal direction, substrate type, and soil moisture were generally consistent. Values in bold font indicate significant differences.

Characteristic	<i>F</i> -stat	DF	Adjusted R ²	<i>P</i> -value
Understory vegetation	6.25	1, 172	0.029	0.013
Overstory density	0.92	1, 172	-0.0004	0.338
Slope	4.32	1, 172	0.019	0.039
Nesting day	0.31	1, 173	-0.004	0.581
Air temperature	1.56	1, 164	0.0033	0.213
Soil temperature	0.006	1, 160	-0.0062	0.937
Water temperature	0.40	1, 139	-0.004	0.528
Soil temperature (noon)	1.52	1, 172	0.002	0.219

Maps Data: (Top) Google Earth imagery ©2011 CNES / Airbus, Maxar Technologies; (Bottom) Scholars GeoPortal ©2011-2022 Ontario Council of University Libraries.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6