

Nesting sites in agricultural landscapes may reduce the reproductive success of populations of Blanding's Turtles (*Emydoidea blandingii*)

A.B. Mui, C.B. Edge, J.E. Paterson, B. Caverhill, B. Johnson, J.D. Litzgus, and Y. He

Abstract: Almost all turtle species nest in terrestrial environments and maternal site selection represents a critical component of nest success. Females use cues in the current environment to predict the future conditions for embryo development. However, in disturbed landscapes, current and future conditions may not be correlated. We compared selection of nest sites by Blanding's Turtles (*Emydoidea blandingii* (Holbrook, 1838)) in a (relatively undisturbed) park and a (heavily disturbed) agricultural landscape in Ontario, Canada, using field measurements and satellite imagery. Environmental variables were compared using logistic regression and Akaike's information criterion (AIC) based on data measured at nest (presence) and random (pseudo-absence) locations. Specific environmental variables associated with site selection differed between study areas. Most notably, NDVI (normalized difference vegetation index, a proxy for vegetation cover) increased significantly during the year at the agricultural locale, corresponding with the growth of planted fields. No parallel change was observed at the park locale where canopy cover remained more consistent. An increase in vegetation cover may alter nest temperatures and soil moisture. Combined with the unpredictability in timing of crop sowing and harvesting, findings suggest that nests in agricultural fields may act as ecological sinks and that other species nesting in similarly altered habitats may be subjected to the same threats.

Key words: Blanding's Turtle, *Emydoidea blandingii*, nest-site selection, disturbance, agricultural landscape, sink, normalized difference vegetation index, remote sensing.

Résumé : Presque toutes les espèces de tortues nidifient dans des milieux terrestres et la sélection du site par la mère constitue une composante clé du succès du nid. Les femelles utilisent des signaux dans le milieu actuel pour prédire les conditions futures pour le développement des embryons. Il se peut toutefois que, dans des paysages perturbés, les conditions présentes et futures ne soient pas corrélées. Nous avons comparé la sélection de sites de nidification par des tortues mouchetées (*Emydoidea blandingii* (Holbrook, 1838)) dans un parc (relativement non perturbé) et un paysage agricole (relativement perturbé) en Ontario (Canada) en utilisant des mesures de terrain et des images satellites. Des variables du milieu ambiant ont été comparées en utilisant la régression logistique et le critère d'information d'Akaike (CIA) à la lumière de données mesurées au nid (présence) et dans des emplacements aléatoires (absence). Les variables du milieu ambiant associées à la sélection du site variaient selon la région étudiée. L'IVDN (indice de végétation par différence normalisée, une variable substitutive du couvert végétal) augmentait notamment de manière significative durant l'année au site agricole, reflétant la croissance des cultures dans les champs. Aucun changement parallèle n'a été observé au site de parc, où le couvert végétal demeurait plus constant. Une augmentation de ce dernier pourrait modifier la température et l'humidité du sol aux nids. Combinés au caractère imprévisible du moment des semences et des récoltes, les résultats donnent à penser que les nids dans les champs agricoles pourraient agir comme puits écologiques, et d'autres espèces nidifiant dans des habitats présentant le même type de changements pourraient être exposées aux mêmes menaces. [Traduit par la Rédaction]

Mots-clés : tortue mouchetée, *Emydoidea blandingii*, sélection du site de nidification, perturbation, paysage agricole, puits, indice de végétation par différence normalisée, télédétection.

Introduction

Almost all turtles, whether they are marine, terrestrial, riverine, or semiaquatic, undergo migrations of varying distances to construct their nests in terrestrial habitats (Steen et al. 2012). Aside from the selection of a suitable nest environment, turtles provide no further parental care. Because incubation time for freshwater turtle eggs ranges from 60 to 90 days (Packard et al. 1987; Cagle et al. 1993), female turtles likely use contemporary environmental cues as predictors of the future nest-site condi-

tions (Kolbe and Janzen 2002; Hughes and Brooks 2006; López et al. 2013). Site selection therefore is an important determinant of offspring success and females must select a location that provides appropriate conditions for embryo development, minimizes the risk of flooding, and is within relatively close proximity to suitable overwintering sites for hatchlings (Kolbe and Janzen 2002; López et al. 2013). Typical characteristics of successful nests include well-drained, and sparsely vegetated, loose sandy soil with a low canopy cover (Hughes and Brooks 2006; Dowling et al. 2010).

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Variables such as slope, elevation, and soil composition may remain stable throughout an active season; however, others such as soil temperature and moisture, which are related to the amount of vegetation cover, can change from the time of nest construction to hatchling emergence, particularly in human-altered environments.

Obtaining accurate data on vegetation cover across different locations and temporal scales can be challenging; however, satellite-derived imagery can provide this information. The normalized difference vegetation index (NDVI) is an indirect measure of surface vegetation that has provided numerous benefits for ecological research. By exploiting the multitemporal feature of satellite imagery, NDVI time-series data have been applied to questions investigating the reproductive success of herbivores with the onset and duration of vegetation growth (e.g., [Pettorelli et al. 2005](#)) and to predict species occurrence across time ([Osborne et al. 2001](#); [Mueller et al. 2008](#)). In multitemporal studies of crop growth, NDVI shows obvious benefits for providing information on canopy closure over turtle nests as an indication of altered understory temperature.

Thermal nest environments are especially important to turtles because they exhibit temperature-dependent sex determination and vegetation cover has been correlated with changes to below canopy soil temperature and moisture ([Carlson et al. 1990](#)). Eggs incubated at lower temperatures have been correlated with poor development in Snapping Turtles (*Chelydra serpentina* (L., 1758)), as well as a higher incidence of egg mortality due to mould ([Yntema 1978](#)). Studies on early development of Chinese Soft-shelled Turtles (*Pelodiscus sinensis* (Wiegmann, 1835)) found that hatchlings of eggs incubated in lower thermal environments were smaller and performed poorly in locomotor tests compared with those incubated at higher temperatures ([Du and Ji 2003](#)).

In human-altered areas, turtles have selected nest sites in plowed fields, backyard flowerbeds, hydro line right-of-ways, abandoned railroad beds, and roadsides ([Joyal et al. 2001](#); [Grgurovic and Sievert 2005](#); [Beaudry et al. 2010](#); [Dowling et al. 2010](#); [Edge et al. 2010](#); [Paterson et al. 2013](#)). While these anthropogenic nest sites can provide habitats that are similar to natural sites, development and overall success of eggs and hatchlings may be negatively impacted ([Kolbe and Janzen 2002](#)), depending on the characteristics of the disturbed area and the frequency and extent of ongoing disturbance. Across much of the world, conversion of the landscape to agriculture represents a dominant type of land-cover disturbance and southern Ontario, the location of our study, is no exception with significant wetland losses occurring since the 1960s ([Bardecki 1982](#)). With the increase in row crops adjacent to turtle habitat, female turtles have been found to preferentially select agricultural areas as nesting sites. Previous work on Snapping Turtles found that variation in temperature associated with agricultural practices has an impact on the sex ratios of hatchlings ([Freedberg et al. 2011](#)). Yet few studies have compared nesting preferences across different landscapes nor examined the change in overstory vegetation growth from the time of nesting to after emergence.

In this study, we tested two hypotheses related to nest-site selection across a relatively undisturbed landscape located in a provincial park and a landscape heavily impacted by agricultural practices. We postulated that female Blanding's Turtles (*Emydoidea blandingii* (Holbrook, 1838)) from the two different study areas select nest sites based on similar environmental conditions. Secondly, we hypothesized that environmental cues used by turtles in altered landscapes to select nest locations are not reliable indicators of future nest conditions. We tested this by comparing vegetation cover (as a proxy for substrate temperature) over nest locations at the park locale and the agricultural locale in the spring and late summer.

Materials and methods

Study sites

We examined nest-site selection by female Blanding's Turtles from a protected area in Algonquin Provincial Park (hereafter park locale) and an intensive agricultural region of Brant County (hereafter agricultural locale) in Ontario, Canada ([Figs. 1A, 1B](#)). The park locale contains areas of formerly disturbed but currently abandoned landscape elements such as an unused railway bed, old paved areas (e.g., decommissioned helicopter pad, radio observatory), and logging and visitor roads that receive seasonal traffic, while the agricultural locale is dominated by active agricultural fields, a population of <400 people/km², and a relatively dense network of roads.

Nest characteristics

To locate nests, 24 female Blanding's Turtles were radio-tracked nightly during the nesting period. All animals were handled in accordance with protocols approved by institutional animal care committees at Laurentian University (park locale) and the Ontario Ministry of Natural Resources (agricultural locale). Nest construction occurred between 26 May and 24 June at the park site and between 29 May and 30 June at the agricultural site. Hatchlings emerged from the nest between mid-August and early October at both sites. To characterize selection of a nest location by gravid females, we compared biophysical and proximity variables between nest sites and random locations. Substrate properties of nests (composition, moisture), distance to nearest wetland, and canopy cover were measured for 12 nests and for 12 paired random locations at each locale to characterize alternate habitat availability in the surrounding area. Random locations were selected by choosing an arbitrary direction and distance within 90 m (± 10 m) of a nest location. The 90 m constraint represents the mean daily movement of Blanding's Turtles ([Gibbs and Shriver 2002](#)) and ensured that random locations represented areas that were accessible to the turtles and not remote locations where the species would not reasonably be found. If variables between nests and random locations differed significantly, then it was accepted that females were nonrandomly selecting the location for oviposition. In this case, significant variables relating to maternal choice of nest location were compared between the park and the agricultural locales.

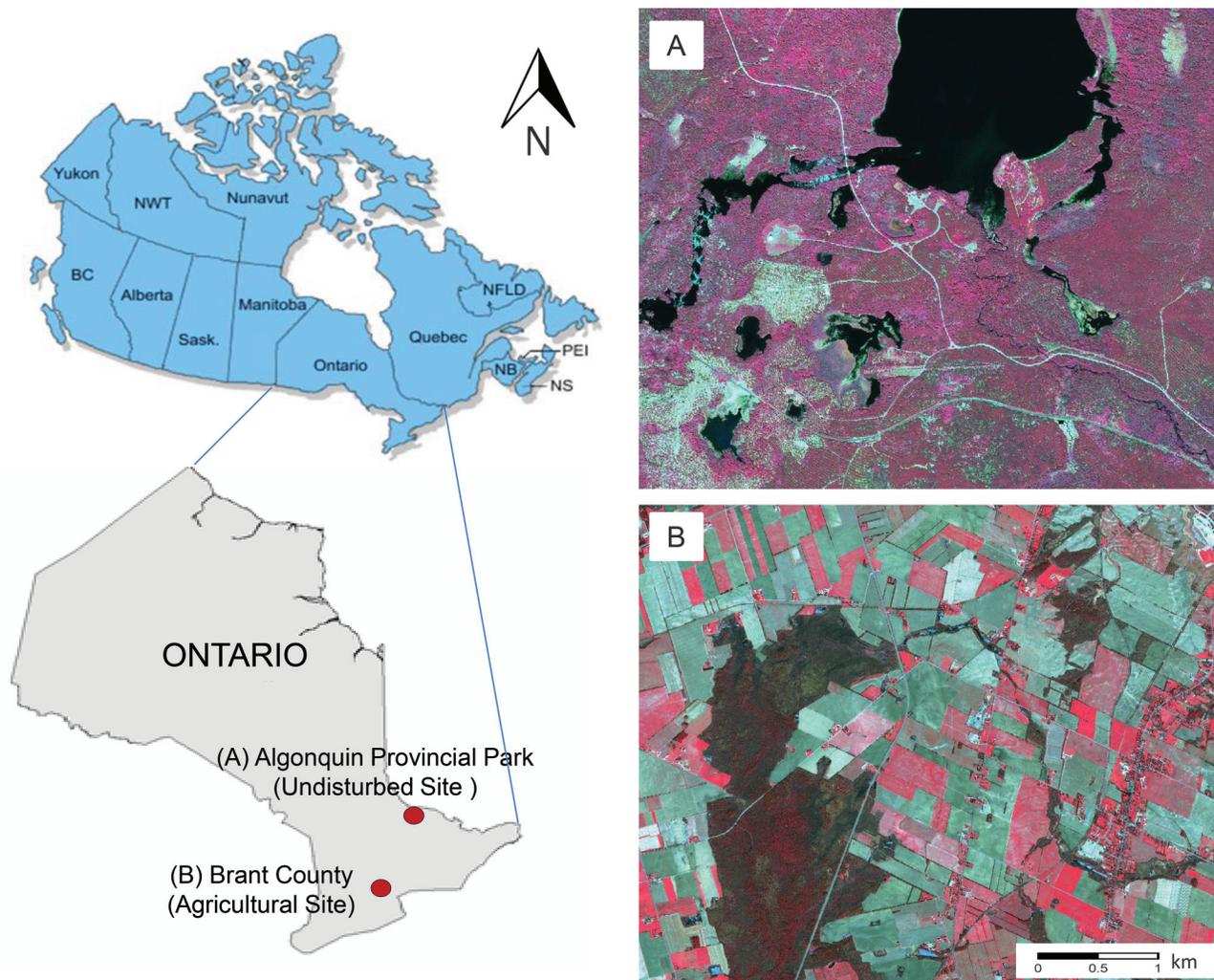
At each nest and random location, a 15 cm deep soil sample was collected using a 2 cm diameter soil corer. This depth corresponds to approximate nest chamber depth in this species ([Standing et al. 1999](#)). Samples were analyzed for moisture content, organic matter, and particle composition (percent sand, percent silt, percent clay). Soil moisture was measured as the percent change in mass of samples after drying at 105 °C for 24 h. Organic matter and particle composition was determined following the methods of [Bledsoe et al. \(1999\)](#). Distance to nearest wetland was measured with ArcGIS (ArcMap version 10.2; Environmental Systems Research Institute (ESRI), Inc., Redlands, California, USA).

To characterize vegetation canopy cover during the nesting season, NDVI (normalized difference vegetation index) was calculated from satellite imagery. NDVI is a widely used indicator of live green vegetation that uses the reflectance (ρ) of the visible (red) and near-infrared (NIR) bands of the electromagnetic spectrum to estimate vegetation characteristics ([Rouse et al. 1974](#)).

$$\text{NDVI} = \frac{\rho_{\text{NIR}} - \rho_{\text{red}}}{\rho_{\text{NIR}} + \rho_{\text{red}}}$$

NDVI values range from -1 to 1, where higher values indicate a greater coverage of photosynthetically-active vegetation, while values less than zero typically do not have any ecological meaning. Numerous studies have shown a strong relationship between

Fig. 1. Location of the two study locales in southern Ontario, Canada: (A) Algonquin Provincial Park, representing a relatively undisturbed landscape, and (B) Brant County, representing a highly disturbed landscape dominated by agriculture. Images collected from the GeoEye-1 satellite on (A) 25 May 2013 and (B) 9 April 2012 and shown in false colour composite (RGB: near infrared – red – green). Colour online.



NDVI and green leaf area (e.g., leaf area index (LAI)), which is defined as the amount of green foliage area per unit ground surface area (Chen and Cihlar 1996). Plants are known to both absorb and reflect solar radiation, and the logical assumption is that increasing canopy cover will reduce the amount of solar radiation incident upon the substrate below. Previous work has established an inverse relationship between NDVI and soil-surface temperature (Carlson et al. 1990). Other factors related to increased vegetation cover include an alteration of soil moisture level surrounding the nest, which may affect gas exchange in the eggs, and changes to soil permeability due to rapid growth of belowground root systems.

While the exact timing of nesting and hatchling emergence is variable, the acquisition of satellite data in the spring and late summer encapsulates the majority of the incubation period.

NDVI was calculated from a set of satellite imagery obtained in the spring (agricultural locale: 9 April 2012; park locale: 25 May 2012) and late summer (agricultural locale: 5 September 2012; park locale: 12 September 2012), which coincided with the general period of nesting and hatching at both study sites. Satellite imagery was acquired from the high spatial resolution (1.84 m) GeoEye-1 sensor, which collects data across four multispectral bands (blue, green, red, and near infrared). Images were processed

for geometric and radiometric corrections using PCI Geomatica software (version 10.3; PCI Geomatics, Markham, Ontario, Canada).

Statistical analyses

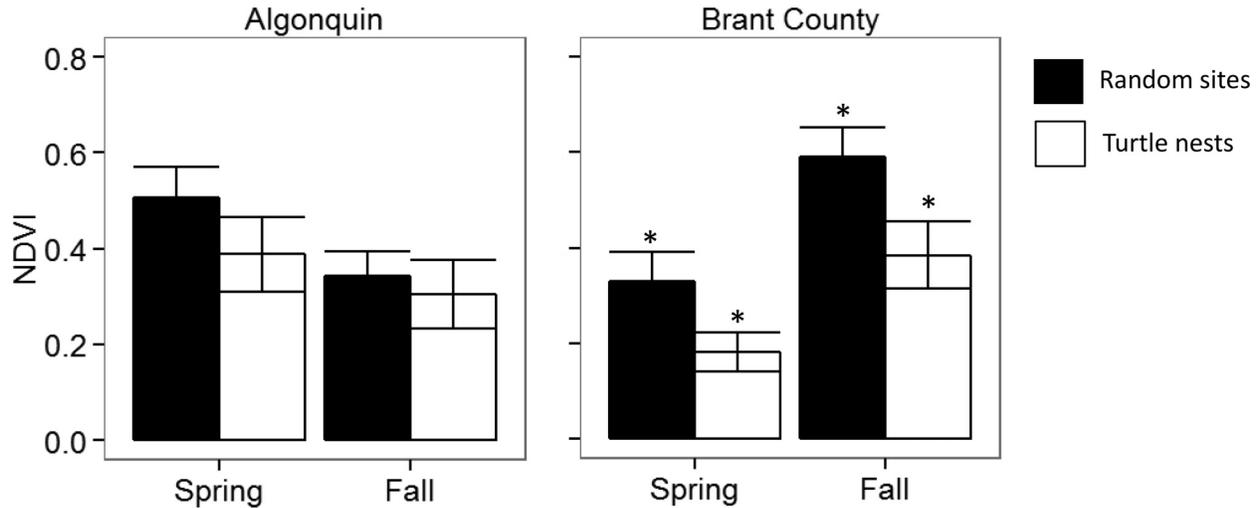
Comparison of nest-site characteristics

To compare measured environmental variables at turtle nests between locales, we used univariate analysis of variance (ANOVA). At each locale, a logistic regression model and Akaike's information criterion corrected for small sample size (AIC_c) were used to determine the set of predictor variables that best discriminated between nest and random locations. A correlation matrix for all variables was constructed and correlations >0.5 were reviewed. Percent silt and percent moisture were removed from analyses because they were correlated with other variables. Model averaging was performed on all models with $\Delta AIC_c < 2$ and top models from both study areas were compared.

Comparison of change in vegetation cover

We compared NDVI during the two sampling periods (spring and late summer) and between locales with a nested ANOVA, with type (turtle or random) nested within population, and nest ID as a random factor. All statistics were performed with R version 3.0.1 (R Core Team 2013).

Fig. 2. Mean (\pm SE) normalized difference vegetation index (NDVI, which is a proxy for vegetation cover) in the spring and fall at the two study locales: Algonquin, which is a relatively undisturbed park locale, and Brant County, which is a disturbed agriculture locale. Solid bars represent random sites and open bars represent nest locations of female Blanding's Turtles (*Emydoidea blandingii*) (*, $p < 0.01$).



Results

Nest-site location

Blanding's Turtles in the park locale nested in areas of open canopy created through anthropogenic alteration such as gravel logging roads, dirt shoulders of paved areas, and abandoned railway beds. All radio-tracked female Blanding's Turtles in the agricultural locale nested in early-season bare crop fields.

Nest characteristics

Data for two nests at the park locale and one nest from the agricultural locale were not used due to missing data or measurement errors. All nests ($n = 10$) in the park locale were characterized as sand (>85% sand, <10% clay, <15% silt) mixed with rocks and pebbles of varying size, while nests in the agricultural locale were characterized as loamy sand (>70% sand, <15% clay, <30% silt; $n = 7$) or sand ($n = 4$). Mean organic matter in nests differed between locales; it was 1.77% for park locale nests and 2.13% for agricultural locale nests ($p < 0.05$). Soil moisture was considerably lower in turtle nests at the park locale (1.65%) than at the agricultural locale (10.07%) ($p < 0.05$).

Results of the seasonal comparison of vegetation cover showed that NDVI over nests at the park locale did not differ between spring and late summer ($p > 0.05$) nor between nests and random locations in either season ($p > 0.05$) (Fig. 2). These results indicate relatively stable canopy conditions and no preferential selection for vegetation cover. At the agricultural locale, NDVI over turtle nests increased significantly ($p < 0.01$) from the spring to the fall, which suggests an increase in vegetation growth (and cover) from the time of nest construction to the end of the incubation period. NDVI also differed significantly between turtle nest and random locations ($p < 0.01$). Higher NDVI values at random locations suggest that turtles at the disturbed agricultural site preferentially selected nest sites with lower vegetation, cover but this variable did not remain static throughout the incubation period.

Nest-site selection model

Model fitting found that seven models had some support for predicting turtle nest sites at the agricultural locale (Table 1). Models at the agricultural locale included four variables: organic matter was found in all seven models; sand, distance to nearest wetland, and NDVIs were each found in three models. Turtles primarily preferred locations that had lower organic content, had more sand, had lower NDVI (vegetation cover) in the spring, and were closer to wetlands than random location (Table 2). At the

Table 1. Comparison of the top seven logistic regression models for nest-site selection by female Blanding's Turtles (*Emydoidea blandingii*) at the two study locales (all models with $\Delta AIC_c < 2$ were included in model averaging).

Population	Variables	K	ΔAIC_c	Cumulative ω
Agricultural locale	OrgMat + NDVIs	3	0.00	0.28
	Sand + OrgMat	3	1.36	0.42
	OrgMat	2	1.59	0.55
	OrgMat + Dist	3	1.74	0.67
	OrgMat + NDVIs + Dist	4	1.76	0.78
	Sand + OrgMat + NDVIs	4	1.81	0.90
	Sand + OrgMat + Dist	4	1.98	1.00
Park locale	Sand + OrgMat	3	0.00	0.3
	OrgMat	2	0.06	0.59
	OrgMat + NDVIs	3	2.27	0.69
	OrgMat + Dist	3	2.45	0.77
	Clay + OrgMat	3	2.47	0.86
	Sand + OrgMat + NDVIs	4	2.89	0.93
	Clay + Sand + OrgMat	4	2.91	1.00

Note: Models with $\Delta AIC_c < 2$ are set in boldface type. K, the number of parameters; ω , Akaike weight; OrgMat, organic matter; NDVIs, normalized difference vegetation index calculated from the spring satellite image (April–May).

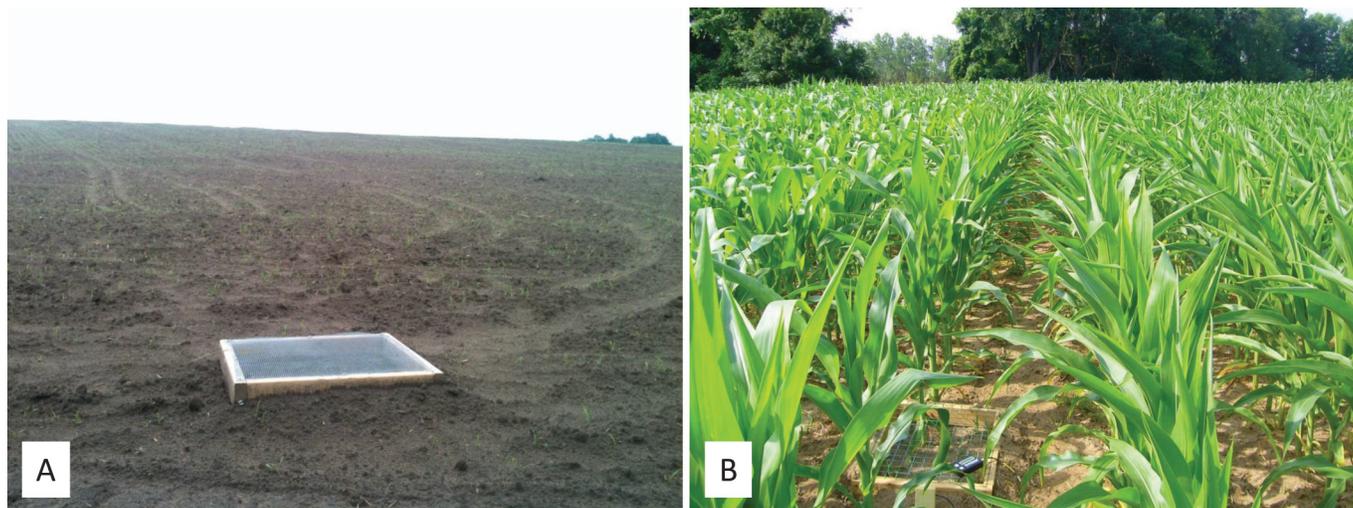
Table 2. Result of model averaging all models within ΔAIC_c of 2 of the top model to predict nest of female Blanding's Turtles (*Emydoidea blandingii*) or random locations.

Population	Variable	No. of models	Coefficient	SE
Agricultural locale	OrgMat	7	-108.51	63.59
	Sand	3	18.50	15.19
	Dist	3	-0.015	0.012
	NDVIs	3	-10.11	8.33
Park locale	OrgMat	2	-21.44	10.31
	Sand	1	-20.22	13.7

Note: OrgMat, organic matter; Dist, distance to the nearest wetland; NDVIs, normalized difference vegetation index calculated from the spring satellite image (April–May).

park locale, only two models consisting of two variables showed support for distinguishing between nest and random locations (Table 1). Females at the park locale primarily preferred sites with less organic matter and secondarily preferred sites with less sand than what was available at random sites (Table 2). Common variables correlated with nest-site preference at both locales were

Fig. 3. Change in vegetation cover over nest sites (under nest protector) of female Blanding's Turtles (*Emydoidea blandingii*) at the disturbed agricultural locale in Brant County, Ontario, Canada, in (A) June 2011 after nesting and in (B) July 2011 during incubation (note the nest protector at bottom left of image). Colour online.



sand and organic matter content. At both study locales, turtles selected nest locations with less organic content. However, females at the agricultural locale also utilized sites with more sand than random locations.

Discussion

Historically, Blanding's Turtles likely relied on shifting riverine sandbars, sand plateaus, and natural disturbances to open the forest canopy in areas for nesting, but now humans have become the dominant agents of landscape disturbance (Beaudry et al. 2010). At both of the study locales, radio-tracked females nested in locations altered by human disturbance. At the park locale, nests were located along logging roads, an abandoned railway bed, and adjacent to the few paved areas that provided loose substrate and open canopy. At the agricultural locale, females nested exclusively in bare agricultural fields, which were adjacent to wetlands and comprised a large proportion of the total study area.

With regards to substrate composition at nests between study areas, females from both locales chose sites with less organic matter that may be related to avoidance of canopy cover (Hughes and Brooks 2006). Females in the agricultural locale also selected sites that had more sand, lower NDVI, and were closer to wetlands than random sites. Comparing model selection across the two study areas suggests that female nest-site selection differs between the two study locales and that a universal model for predicting nest-site preference may not be possible. It is important to note that the use of paired locations implies that preference is relative to availability and this affects the interpretation of the results.

Nesting in early-season bare crop fields was preferentially selected by all radio-tracked females in the agricultural locale, indicating a high level of attraction to these altered areas; a behaviour also documented in Snapping Turtles (Freedberg et al. 2011). Naturally-occurring nest sites such as sand banks and an artificial nest site were also present in the study area but were not preferentially selected. A major difference between nesting sites at the agricultural and park locales is that considerable and rapid vegetation growth occurred in crop fields at the agricultural locale from spring to fall (Figs. 3A, 3B), while limited vegetation growth occurred in nesting habitats used in the park locale. NDVI (a proxy for vegetation cover) did not differ at nest sites between spring and late summer at the park locale, suggesting that the cues turtles used to select nest locations are good predictors of future

canopy conditions at this less-disturbed site. However, in the agricultural locale, NDVI over nests was higher in the late summer than in the spring, demonstrating that the cues turtles used to select nest sites are not a good predictor of canopy cover during the incubation period. Early-season crop fields consist of bare open soil with high sand content, preferred characteristics for nesting turtles; however, the rapid growth of crops can change critical environmental variables (temperature and moisture) that can determine whether eggs hatch and develop successfully. While female turtles may select nesting sites with lower organic content to avoid significant vegetation growth after nesting (Hughes and Brooks 2006), this cue may not accurately predict future conditions at agricultural sites because plant matter is removed from active crops before planting.

At higher latitudes, temperature is a major determinate of nesting success and the length of the embryonic period (Obbard and Brooks 1981; Bohn and Brooks 1994). Low temperatures decrease development rate and can dramatically reduce nesting success. Lower nest temperatures also have the potential to affect sex ratios because Blanding's Turtles display temperature-dependent sex determination: males are typically produced at temperatures below 26.5 to 30 °C, whereas females are produced at higher temperatures (Ewert and Nelson 1991). Nest shading would lower incubation temperatures and can result in biased sex ratios (Kolbe and Janzen 2002; Freedberg et al. 2011), which could impact population stability because sexually mature females are the most important cohort for maintaining a stable population (Congdon et al. 2003). Painted Turtles (*Chrysemys picta* (Schneider, 1783)) were found to repeatedly select nest sites based on similar quantities of overstory vegetation cover, which was considered a reasonable predictor of nest thermal environments (Janzen and Morjan 2001). Similarly, female Blanding's Turtles likely also select sites based in part on overstory vegetation, though in agricultural landscapes this important variable does not remain static throughout the incubation period of the nest.

Row crops also affect posthatching dispersal, as vision has been identified as the primary orientation cue used by hatchling Blanding's Turtles (Pappas et al. 2009) and row crops restrict the view of both near and far horizons. Previous research has shown that hatchling turtles in row crops orient randomly or in the direction of crop rows (Pappas et al. 2013). The inability to orientate effectively could impact the ability of hatchling turtles to find suitable

overwintering locations (Paterson et al. 2012) and may increase overall dispersal time and distance.

If emergence from nests is delayed due to low temperatures, then the probability of nest destruction by harvesting equipment increases because agricultural machinery can kill both hatchlings and entire nests (Tingley et al. 2009; Saumure et al. 2006). Other effects of prolonged incubation include delayed development and reduced hatchling fitness (Yntema 1978; Du and Ji 2003). Chemical additions to the crop fields such as pesticides and fertilizers may result in developmental abnormalities or affect sexual development (Bishop et al. 1991; de Solla et al. 1998). Unfortunately, the presence of chemical contaminants, rapid crop growth, and the timing of heavy farming machinery are variables that females are unlikely to detect or predict when choosing nest sites in regions altered for farming.

Early-season bare crop fields emulate the conditions of natural nesting habitats for female Blanding's Turtles, but are likely acting as population sinks, or ecological traps, as conditions rapidly change throughout the season. Furthermore, embryos incubating in agriculture sites are exposed to pesticides, nests may be disturbed or destroyed by planting or harvesting, hatchlings and adults can be killed by agricultural machinery, and the rapid growth of crops alters environmental characteristics of nests. Because no maternal care is provided beyond nest-site selection and construction, there is no mechanism that allows female turtles to learn from and respond to these human-induced changes, resulting in potential recurrent dead-end or low-quality nest-site selection for the reproductive life of that female. Overall, this indicates that agricultural oviposition sites may act as ecological sinks and that other turtle species nesting in similarly-altered habitats may be subjected to the same threat.

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