Canadian Science Publishing

ARTICLE

Effects of landscape composition on wetland occupancy by Blanding's Turtles (*Emydoidea blandingii*) as determined by environmental DNA and visual surveys

Vincent K. Fyson and Gabriel Blouin-Demers

Abstract: Habitat loss and degradation have led to the extinction of many species worldwide. The endangered Blanding's Turtle (*Emydoidea blandingii* (Holbrook, 1838)), a semi-aquatic freshwater turtle, occupies a wide range of wetlands and landscapes primarily in southeastern Canada and the Great Lakes region of the United States. We explored whether the probability of wetland occupancy by Blanding's Turtles is affected by the surrounding landscape. We used visual surveys, environmental DNA, and Atlas data to document the presence of Blanding's Turtles in wetlands in Ottawa, Ontario, Canada. We tabulated landscape composition at multiple scales surrounding the wetlands to determine whether landscape composition can predict wetland occupancy. Generally, wetlands surrounded by forest and other undisturbed lands were most likely to harbour Blanding's Turtles, whereas those surrounded by more human-disturbed lands were least likely to harbour Blanding's Turtles. Larger wetlands and a high proportion of wetlands in the surrounding landscape also increased the probability of occupancy by Blanding's Turtles. Finally, older wetlands were more likely to be occupied by Blanding's Turtles. The ability to estimate a species' probability of occupancy can aid in conservation efforts, such as critical habitat delineation.

Key words: Blanding's Turtle, boosted regression tree, *Emydoidea blandingii*, habitat selection, modelling, scale of maximum effect, spatial occurrence.

Résumé : La disparition et la dégradation des habitats ont mené à la disparition de nombreuses espèces à l'échelle mondiale. La tortue mouchetée (*Emydoidea blandingii* (Holbrook, 1838)), une espèce de tortue d'eau douce semi-aquatique en voie de disparition, occupe un vaste éventail de milieux humides et de paysages principalement dans le sud-est du Canada et la région des Grands Lacs des États-Unis. Nous vérifions si le paysage environnant a une incidence sur la probabilité d'occupation de milieux humides par des tortues mouchetées. Nous utilisons des relevés visuels, l'analyse d'ADN environnemental et des données d'atlas pour documenter la présence de tortues mouchetées dans des milieux humides à Ottawa (Ontario, Canada). Nous tabulons la composition du paysage à différentes échelles autour des milieux humides afin de déterminer si elle peut permettre de prédire l'occupation de milieux humides. En général, les milieux humides entourés de forêts et autres terres non perturbées sont les plus susceptibles d'abriter des tortues mouchetées, alors que ceux entourés par des terres plus perturbées par la présence humaine sont les moins susceptibles d'en abriter. Des milieux humides plus grands et une grande proportion de milieux humides dans le paysage environnant sont également associés à une plus grande probabilité d'occupation par des tortues mouchetées. Enfin, les milieux humides plus vieux sont plus susceptibles d'être occupés par des tortues mouchetées. La capacité d'estimer la probabilité d'occupation par une espèce peut contribuer aux efforts de conservation, tels que la délimitation d'habitats essentiels. [Traduit par la Rédaction]

Mots-clés : tortue mouchetée, arbre de régression intensifié, *Emydoidea blandingii*, sélection d'habitats, modélisation, échelle d'effet maximum, présence dans l'espace.

Introduction

Habitat loss and degradation, climate change, and other unsustainable practices threaten biodiversity and have led to the extinction of many species worldwide (Sisk et al. 1994; Thomas et al. 2004; Tilman et al. 2017). Currently, an estimated 1 million species are at risk of extinction due to anthropogenic threats, and even populations of some common and widespread species are declining (IPBES 2019; Rosenberg et al. 2019). Reptiles are no exception in the current biodiversity crisis: over one-fifth of reptile species worldwide are threatened with extinction (IUCN 2020). Threats to reptiles are diverse and include agricultural practices, urban development, and collection for food, pets, and medicine (Klemens and Thorbjarnarson 1995; da Nóbrega Alves et al. 2008; IUCN 2020). Included in legal protections offered to species at risk in many jurisdictions is the designation of critical habitat: habitat deemed essential for the persistence of a species (e.g., ESA 1973; Species at Risk Act 2002). Identifying critical habitat properly is important not only for ensuring a species' persistence, but also to ensure scientific credibility for those who have economic interest in the protected habitat (Rosenfeld and Hatfield 2006). To identify critical habitat properly, determining how a species associates with habitat from a local patch to a landscape scale is required (Rosenfeld and Hatfield 2006).

Within a landscape, a species' occurrence and abundance is dictated by the availability of suitable habitat and of resources

Received 4 January 2021. Accepted 13 April 2021.

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Published at www.cdnsciencepub.com/cjz on 28 April 2021.

necessary for survival (Johnson 1980). When there are strong links between landscape composition and the probability of occurrence of a species, landscape composition can be used to estimate the probability of occupancy of a species. For example, Mazerolle et al. (2005) found that probability of pond occupancy by Green Frogs (*Lithobates clamitans* (Latreille, 1801)) in New Brunswick, Canada, was significantly correlated with landscape features such as wetland cover and forest cover around the pond. Interestingly, probability of occupancy by Green Frogs depended on forest cover at scales up to 1000 m from the focal pond, indicating that landscape features well outside the 60 m^2 mean home-range area of Green Frogs (Martof 1953) can influence site occupancy. Similarly, probability of site occupancy by Eastern Newts (*Notophthalmus viridescens* (Rafinesque, 1820)) in Vermont, USA, was positively correlated with forest and wetland cover in the surrounding landscape and negatively correlated with developed area (Rinehart et al. 2009). These studies exemplify how threats like habitat fragmentation and habitat loss at the landscape scale can act negatively on a local population (Burkey 1995; Fahrig 2003; Cushman 2006).

In freshwater turtles, habitat fragmentation, habitat loss, and road mortality are often the most significant threats (Gibbons et al. 2000; Turtle Conservation Fund 2002; Steen and Gibbs 2004). The Blanding's Turtle (*Emydoidea blandingii* (Holbrook, 1838)), a semi-aquatic freshwater turtle, is considered at risk across most of its range in southeastern Canada and the northeastern United States (COSEWIC 2016). For instance, the Great Lakes and St. Lawrence population in Ontario and Québec is estimated to have been reduced by >60% in the past three generations owing largely to habitat loss and road mortality (COSEWIC 2016). Blanding's Turtles inhabit wetlands such as swamps, ponds, and marshes (Ross and Anderson 1990; Edge et al. 2010) and individual turtles can use numerous wetlands over the course of a year (Beaudry et al. 2009). Blanding's Turtles are vagile and also use upland habitat for nesting and inter-wetland travel (Edge et al. 2010; Millar and Blouin-Demers 2011; Markle and Chow-Fraser 2014), making them particularly susceptible to road mortality. Apart from the direct effect of roadways increasing wildlife mortality (Trombulak and Frissell 2000; Row et al. 2007), roads can also degrade habitat connectivity by acting as barriers to movement (Attum et al. 2008; Robson and Blouin-Demers 2013; Proulx et al. 2014). As road mortality and habitat loss are putative drivers of Blanding's Turtle population decline, landscape features such as road density and human development could potentially be used to estimate the probability of turtle presence. Landscape features such as the density of surrounding roads, docks, and cottages are good predictors of the probability of site occupancy by the Eastern Musk Turtle (*Sternotherus odoratus* (Latreille, 1801)) in Ontario, Canada (Markle et al. 2018). Also in Ontario, Northern Map Turtles (*Graptemys geographica* (Le Sueur, 1817)) prefer natural shorelines over developed shorelines (Carrière and Blouin-Demers 2010). Literature on Blanding's Turtle site occupancy is largely focused on microhabitat selection and on habitat suitability modelling at large spatial scales (Ross and Anderson 1990; Edge et al. 2010; Millar and Blouin-Demers 2012; Markle and Chow-Fraser 2014).

Detection of rare species in the field can be challenging. Species detection with environmental DNA (eDNA) is a relatively new methodology that is based on the collection and detection of persistent DNA shed from a target species into the environment (Ficetola et al. 2008). eDNA has been used with mixed success for the detection of aquatic and semi-aquatic species (Jerde et al. 2011; Thomsen et al. 2012; Raemy and Ursenbacher 2018). For example, eDNA was used successfully to detect an invasive carp in the Lake Michigan watershed before its detection by traditional survey methods (Jerde et al. 2011). eDNA was also determined to be superior to visual surveys, but inferior to trap surveys, for the detection of the European Pond Turtle (*Emys orbicularis* (Linnaeus, 1758)) (Raemy and Ursenbacher 2018). Due to mixed success and to a lack of previous studies on the use of eDNA to survey for Blanding's Turtles, we used visual surveys and existing Blanding's Turtle occurrence records to validate eDNA data, thus increasing the robustness of our study.

We explore the effects of landscape composition on the probability of occurrence of Blanding's Turtles. More precisely, we test the hypothesis that the probability of wetland occupancy by Blanding's Turtles can be estimated from boosted regression tree models based on the composition of the landscape surrounding that wetland. Specifically, we predicted that wetlands surrounded by more anthropogenic land covers should be less likely to harbour Blanding's Turtles.

Materials and methods

Study sites

We conducted visual surveys and eDNA sampling for Blanding's Turtles from May to August 2018 and 2019 in Ottawa, Ontario, Canada (Supplementary Fig. S1).[1] The study area was approximately 2800 km^2 of a low-lying and predominantly flat region of the mixedwood plains ecozone. The study area comprised 48% agriculture, 18% wetlands, 16% forests, 15% anthropogenic lands (which included urban developments, roadways, waste facilities, and quarries), and 4% open water. Road density across the study area was 2.3 km/km^2. We studied 137 wetlands: 80 wetlands were surveyed for Blanding's Turtles by visual surveys, 89 wetlands were sampled for eDNA (70 wetlands overlapped between visual surveys and eDNA sampling), and the remaining 38 wetlands were included based on Blanding's Turtle sightings obtained from the Ontario Reptile and Amphibian Atlas (Ontario Nature 2018). We deemed a wetland to be occupied if Blanding's Turtles were documented by at least one sampling method. Wetlands ranged in size from 0.1 to 2411 ha (mean = 60.1 ha, median = 5.3 ha). Of the 99 wetlands surveyed by at least one of the methods, 59 were included in the study based on contractual obligations because they were situated in the National Capital Commission's Greenbelt. The remaining 40 wetlands were selected in the same region in locations with underrepresented landscape configurations and in locations with known Blanding's Turtle populations to ensure enough occupied sites for modelling. Ephemeral wetlands were not included in the study.

Blanding's Turtle occupancy

Visual surveys

We conducted visual surveys for Blanding's Turtles from late April to mid-June of 2018 and 2019 when Blanding's Turtles are most likely to be basking and thus easiest to detect (Millar and Blouin-Demers 2011; OMNRF 2015). Visual surveys for Blanding's Turtles were done in accordance with the "Survey Protocol for Blanding's Turtle (*Emydoidea blandingii*) in Ontario" (OMNRF 2015). We visited 80 wetlands and used a spotting scope and binoculars to detect Blanding's Turtles (26 wetlands in 2018 and 54 wetlands in 2019). We visited wetlands from mid-morning to late afternoon on days without precipitation and spent approximately 1 h per wetland per visit. As per the Ontario Ministry of Natural Resources and Forestry guidelines, we visited wetlands a minimum of five times per year, unless Blanding's Turtles were detected before the fifth visit, in which case we no longer visited that wetland.

[1]Supplementary tables, figure, AutoBufferCorrelation code, and supporting data files are available with the article at https://doi.org/10.1139/cjz-2021-0004.

Environmental DNA

During the summer of 2019, we collected 445 water samples from 89 wetlands to survey for Blanding's Turtles with eDNA. We collected water samples from late April to mid-June, usually on the second or third visual survey. We sampled wetlands by collecting five 1 L samples of water per wetland, with samples randomly dispersed in each wetland, but with one sample taken at the outflow and one at the inflow (if present). Samples were taken within the first 10 cm from the surface. Water samples were filtered by vacuum through Whatman GC/F glass microfiber filters, with one filter used per sample. We sterilized equipment between wetlands by soaking in a solution of sodium hypochlorite for 10 min then rinsing with tap water. Sample bottles were also flushed with water from the target wetland before sampling. Additionally, we collected 25 negative control samples from distilled water (10), municipal water (10), and a wetland certain not to contain Blanding's Turtles (5). DNA was extracted using a QIAgen DNeasy kit. Samples were diluted by a factor of 10 and amplified using qPCR with three replicates per sample.

Ontario Reptile and Amphibian Atlas

We used the 522 Blanding's Turtle sightings submitted to the Ontario Reptile and Amphibian Atlas within the study area between 2008 and 2018 to identify additional occupied wetlands. We retained sightings that could be associated with a specific wetland (i.e., within or on the edge of a wetland).

Landscape composition

We used land cover data (28 classes) from the Ontario Land Cover Compilation v.2.0 with 15 m resolution (OMNRF 2014). We assumed that the landscape composition of the study area did not change substantially in the 6 years before publication and the 5 years after publication of the land-cover data. Consultation of aerial photographs of the study area over the full duration of the study indicated that this was a reasonable assumption. We merged land-cover classes into the following six categories: (1) open water, (2) wetlands, (3) forest, (4) anthropogenic (buildings, roadways, gravel pits and quarries, and other human-disturbed sites), (5) agriculture, and (6) other (for instance, alvar and bedrock). Wetlands in the study area were mostly marshes, while ponds, swamps, bogs, and fens were much less common. We used road information from OpenStreetMap (OpenStreetMap contributors 2019) and included motorways, primary, secondary, tertiary, and residential roads. We delineated wetlands at a scale of 1:5000 using aerial photographs taken in the spring of 2014 (National Capital Commission 2014) and ground-truthed delineations for accuracy. Because wetlands in the study area include naturally occurring wetlands, storm water ponds, and wetlands formed due to changes in land use and development, we included wetland age determined (in years) from historical aerial photographs (University of Toronto 1954; City of Ottawa 1958, 1976, 1991, 1999, 2008; National Capital Commission 1965, 2001, 2014) as the mean of the age of the aerial photograph in which the wetland first appeared and the age of the next oldest aerial photograph. The earliest aerial photographs covering our study site were taken in 1954, so the wetlands already present in 1954, regardless of actual age, were binned as 65 years old.

Because it is difficult to know a priori at which spatial scales landscape features will affect the probability of occupancy, we determined a scale of maximum effect for each of the landscape variables (the proportion of open water cover, the proportion of wetland cover, the proportion of forest cover, the proportion of anthropogenic land cover, the proportion of agricultural land cover, and road density (km/km^2)). The scale of maximum effect is determined as the buffer size surrounding a wetland in which the landscape variable has the highest correlation with wetland occupancy. Buffers ranged from 100 to 4000 m (Fortin et al. 2012; Fyson et al. 2020) in 100 m increments. We calculated land-cover variables as a percentage of the buffer area, excluding the focal wetland, and road density (km/km^2) within each buffer. We then calculated a point biserial correlation between each variable and Blanding's Turtle occupancy, as determined by the visual surveys, the eDNA sampling, and the Atlas data, at all buffer scales to determine the variables' scale of maximum effect (Fyson et al. 2020; Čapkun-Huot et al. 2021; AutoBufferCorrelation code and supporting data files in the Supplementary material[1]). We retained each variable at its scale of maximum effect for model building. We completed all geospatial analyses using ArcGIS version 10.4.1 (ESRI, Inc. 2016) and Python version 2.7.10 (Python Software Foundation 2015).

Modelling

We used boosted regression tree (BRT) modelling to test whether the probability of wetland occupancy by Blanding's Turtles between 2008 and 2019 could be predicted from landscape composition in 2014. BRT is a machine learning method used to model ecological interactions and to assess landscape effects on organisms (Elith et al. 2008; Ruso et al. 2019). A distinct advantage of BRT modelling for our study is that, unlike other modelling methods, the final model predictions are little affected by outliers and by collinearity among predictor variables (Elith et al. 2008; Main et al. 2015). Collinearity is almost inevitable in landscape studies because increased cover of a given habitat necessarily means less cover of the other habitats. To assess collinearity, we created a correlation matrix for the predictor variables.

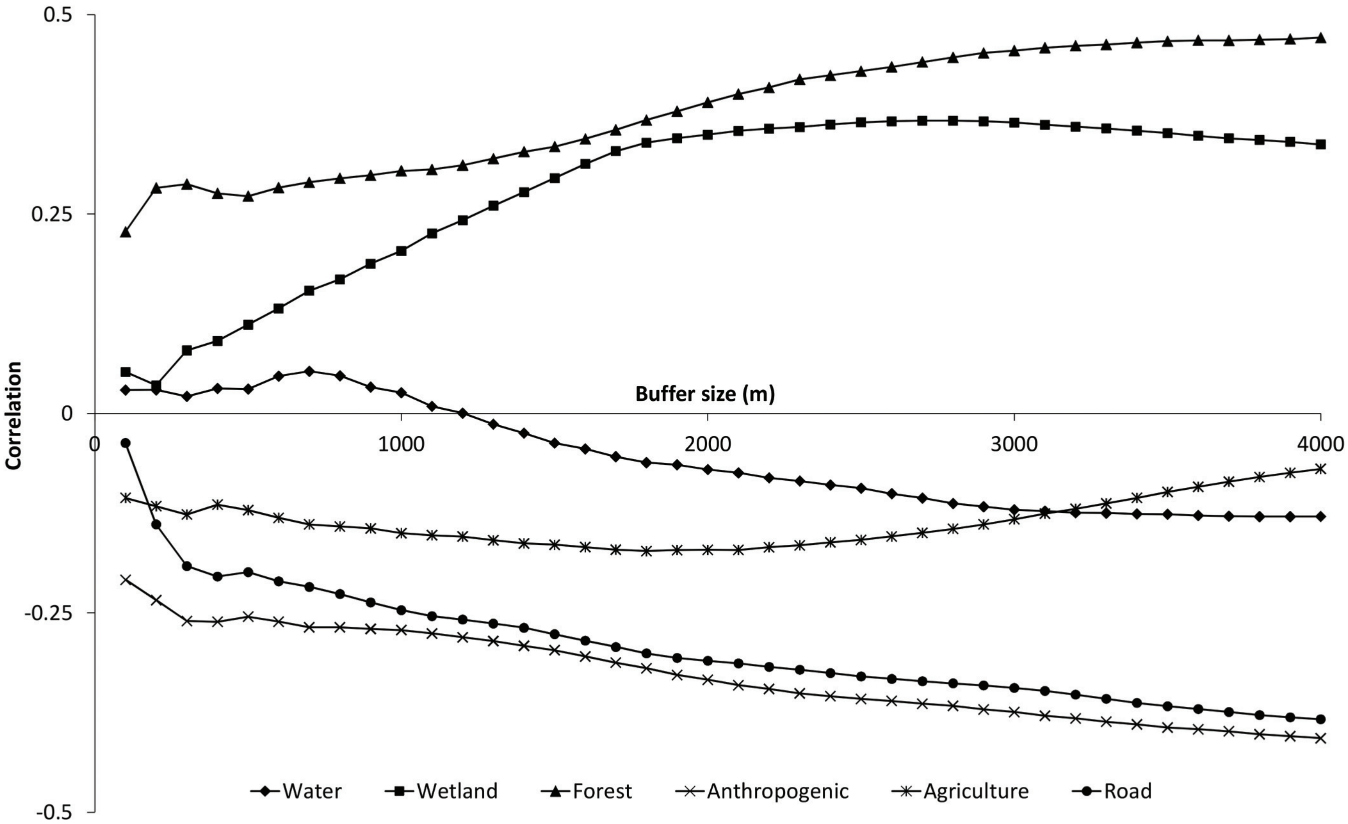
Using the dismo (Hijmans et al. 2017) and gbm (Greenwell et al. 2019) packages in R version 3.5.2 (R Core Team 2018), we built a model using the 137 wetlands that resulted from pooling the visual survey data, the eDNA data, and the Atlas data. The model included eight explanatory variables: the proportion of open water cover, the proportion of wetland cover, the proportion of forest cover, the proportion of anthropogenic land cover, the proportion of agricultural land cover, road density (km/km^2), wetland age (years), and wetland area (ha). The buffer size within which each land-cover class and road density were tabulated was determined based on the scale of maximum effect (Fig. 1).

BRTs are optimized using tree complexity (the number of splits in each tree), learning rate (the scaling rate of each tree), and bag fraction (the proportion of the data randomly selected to build the trees). Optimization is evaluated based on the cross-validation deviance, the number of trees in the model, and the area under the receiver operating curve (Elith et al. 2008). First, we set the tree complexity to five. Next, we built models with decreasing learning rates from 0.01 to 0.001 to determine the optimal value. Similarly, we tested bag fractions of 0.5, 0.6, and 0.7 using the retained learning rate (Elith and Leathwick 2017; Ruso et al. 2019). Finally, once the optimal learning rate and bag fraction were determined, we tested tree complexity with values of two, three, and four, which are considered suitable for small to modest sample sizes (Elith et al. 2008).

BRT model performance is primarily evaluated based on cross-validation (CV) deviance and cross-validation area under the receiver operating curve (CV AUC), which are more reliable than self-statistics such as residuals (Elith et al. 2008; Elith and Leathwick 2017). Percent deviance explained, calculated as (null deviance − CV deviance)/(null deviance × 100) (Buston and Elith 2011), gives a goodness-of-fit measure equivalent to the coefficient of determination (R^2) of a linear regression (Leyk and Zimmermann 2004). We also considered the number of trees because models are ideally fit with at least 1000 trees (Elith et al. 2008).

To test for model overfit and the model's ability to make predictions on external data, we selected a subset of 80 wetlands at random from the original 137 which we used as training data to build a new model and the remaining 57 wetlands were retained as validation data. We repeated this process 100 times with new

Fig. 1. Point biserial correlations between wetland occupancy by Blanding's Turtles (*Emydoidea blandingii*) between 2008 and 2019 in Ottawa, Ontario, Canada, and six landscape variables measured in buffers of increasing size (m). The landscape variables are open water proportion (Water), wetland proportion (Wetland), forest proportion (Forest), anthropogenic land proportion (Anthropogenic), agricultural land proportion (Agriculture), and road density (Road; km/km^2). Water had the highest correlation at 3900 m, Wetland at 2700 m, Forest at 4000 m, Anthropogenic at 4000 m, Agriculture at 1800 m, and Road at 4000 m.



random subsets of training and validation data for each model. We used the subset models to estimate wetland occupancy by Blanding's Turtles for the training wetlands and the validation wetlands. Occupancy estimates from all 100 models were grouped into four categories: (1) unoccupied training wetlands, (2) occupied training wetlands, (3) unoccupied validation wetlands, and (4) occupied validation wetlands. Welch's *t* tests were used to compare the means of the four categories of estimated probabilities of occupancy and a kernel density estimation was performed for a visual comparison of the categories. Similarly, we used the final BRT model built using all data to make estimations of wetland occupancy for all 137 wetlands included in our study.

Results

Blanding's Turtle occupancy

Visual surveys

We confirmed the presence of Blanding's Turtles at 24 of the 80 wetlands surveyed visually in 2018 and 2019. Of the 24 wetlands occupied by Blanding's Turtles, we confirmed presence on the first visit for 18 wetlands, on the second visit for three wetlands, on the fourth visit for one wetland, and on the fifth visit for two wetlands. No wetlands were found to be occupied by Blanding's Turtles beyond the fifth visit, even though some wetlands were visited up to 10 times.

Environmental DNA

Three of the 25 negative controls (4 of 75 replicates) tested positive for Blanding's Turtle DNA due to contamination either during the sampling, filtering, or DNA extraction. For this reason, we deemed sites to be occupied based on strict criteria to avoid possible false positives due to contamination. We filtered samples by quantification cycle (Cq) values and number of positive replicates to the point where all contaminated negative controls were eliminated. Based on the Cq values from positive control replicates, we determined that values between 8 and 16 represent values that are unlikely to be contamination. Additionally, we also eliminated sites where only 1 of the 15 replicates was positive. After eliminating possible contamination, 26 of the 89 sites tested positive for Blanding's Turtle DNA. As positive controls, we took samples for eDNA from 23 wetlands within a day after confirming the presence of Blanding's Turtles based on our visual surveys. Eleven of the 23 positive control wetlands indeed tested positive for Blanding's Turtle DNA, while the other 12 resulted in false negatives.

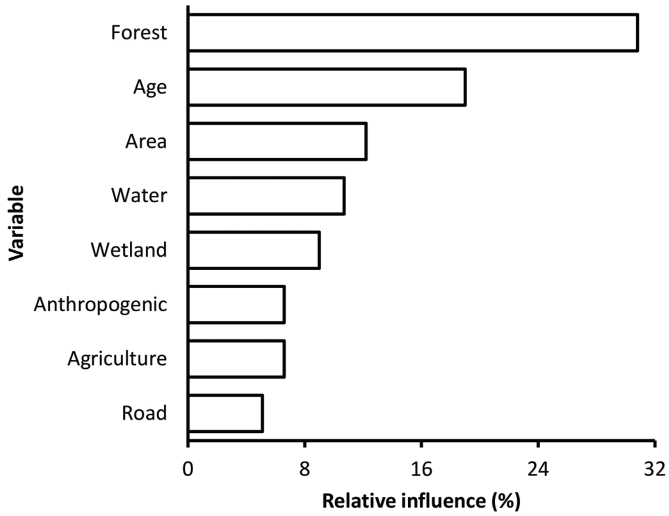
Ontario Reptile and Amphibian Atlas

We used Blanding's Turtle sightings from the Ontario Reptile and Amphibian Atlas to confirm occupancy at an additional 48 wetlands. Of these wetlands, we had surveyed 10 by either eDNA or visual surveys and the remaining 38 had not been surveyed, bringing the total number of wetlands with confirmed Blanding's Turtle presence to 89 out of the 137 wetlands included in the study. Of the 41 wetlands determined to be occupied by eDNA or visual surveys, 11 also had observations in the Ontario Reptile and Amphibian Atlas.

Landscape composition

The scale of maximum effect for each landscape composition variable varied between 1800 and 4000 m. The scale of maximum

Fig. 2. Relative influence (%) of the explanatory variables used to predict the probability of wetland occupancy by Blanding's Turtles (*Emydoidea blandingii*) between 2008 and 2019 in Ottawa, Ontario, Canada, with a boosted regression tree model. The explanatory variables are open water proportion (Water), wetland proportion (Wetland), forest proportion (Forest), anthropogenic land proportion (Anthropogenic), agricultural land proportion (Agriculture), road density (Road; km/km^2), and wetland area (Area; ha).



effect for open water cover was 3900 m, wetland cover was 2700 m, forest cover was 4000 m, anthropogenic land cover was 4000 m, agricultural land cover was 1800 m, and road density was 4000 m (Fig. 1). Within the buffer sizes determined as the scales of maximum effect, the proportions of the various land covers and road density were comparable with those of the study area (Supplementary Table S1[1]). Summary statistics of the landscape variables used for modelling are available in Supplementary Table S2[1].

Modelling

We fit a BRT model to the data using a tree complexity of five. We determined that a learning rate of 0.001 and a bag fraction of 0.6 resulted in the best performing model. The model explained 28.9% of the deviance in Blanding's Turtle occupancy and had a CV AUC of 0.845.

The model ranked forest cover as the most important variable (30.8% relative influence) followed by wetland age (19.0% relative influence), and wetland area (12.2% relative influence) (Fig. 2). Marginal effects showed increased forest cover, wetland age, wetland area, water cover, and wetland cover have positive relationships with occupancy, while anthropogenic land cover and road density have negative relationships with occupancy (Fig. 3). Agricultural land cover was unclear in its effect on wetland occupancy. The model, when used to predict occupancy for all 137 wetlands, predicted wetlands where Blanding's Turtles were indeed present to have a significantly higher ($p < 0.001$) probability of occupancy than wetlands where Blanding's Turtles were absent (Fig. 4; occupied wetlands: mean = 84.3%, SE = 1.5%; unoccupied wetlands: mean = 29.6%, SE = 2.6%). The subset models, built using 80 randomly selected wetlands, performed less well than the full model (mean deviance explained = 22.7%; mean CV AUC = 0.817). Welch's *t* test determined there was a significant difference ($p < 0.001$) between the mean probabilities of occupancy when comparing the occupied training wetlands (mean = 0.80) and the unoccupied training wetlands (mean = 0.38), the occupied validation wetlands (mean = 0.75) and the unoccupied validation wetlands (mean = 0.50), the occupied training wetlands and the occupied validation wetlands, and the unoccupied training wetlands and the unoccupied validation wetlands (Fig. 5).

Discussion

Landscape effects on occupancy

We tested the hypothesis that wetland occupancy by Blanding's Turtles (data collected between 2008 and 2019) is affected by landscape composition (data collected in 2014) around the wetland. We assumed that there were no local extinctions or colonizations during our study. We found that landscape composition in 2014 did indeed affect whether a wetland was occupied by Blanding's Turtles between 2008 and 2019, with the BRT model explaining over a quarter of the deviance in occupancy. Wetlands in less disturbed landscapes with a higher proportion of natural land-cover types, such as forest cover and wetland cover, had a higher probability of harbouring Blanding's Turtles. By contrast, wetlands located in more human-influenced landscapes with a high proportion of urban land cover and a high road density were less likely to be occupied by Blanding's Turtles.

Although the predictions of BRT models are unaffected by collinearity (Elith et al. 2008; Main et al. 2015), the determination of the relative importance of the predictor variables is not immune to collinearity. As a result, in our case, the exact importance of each landscape variable was difficult to determine due to high collinearity among some of the predictor variables. The proportion of forest cover, for example, had a very high correlation with anthropogenic land cover (Table 1). Therefore, it was difficult to determine the exact dynamics of the relationship between forest cover, anthropogenic land cover, and Blanding's Turtle occupancy: are Blanding's Turtles influenced positively by forest cover, are they influenced negatively by anthropogenic land cover, or both?

Road mortality is a leading cause of Blanding's Turtle population decline (COSEWIC 2016), so landscape features like roadways and urban areas should negatively affect Blanding's Turtle populations in nearby wetlands. We indeed found that increased road density and urban land cover reduced the probability of wetland occupancy by Blanding's Turtles. Roadways and urban areas, in addition to causing direct mortality, also decrease habitat connectivity (Underhill and Angold 2000), which may result in reduced recruitment from neighbouring wetlands. By contrast, an increase in wetland cover increased the probability of occupancy. In addition to being the preferred habitat (Edge et al. 2010; Millar and Blouin-Demers 2011), wetlands in close proximity may increase Blanding's Turtle immigration, which reduces the likelihood of local extinction. Forest cover also increased the probability of wetland occupancy. Although forest is not the preferred habitat of Blanding's Turtles, forest is used for inter-wetland travel and for travel to nesting sites (Markle and Chow-Fraser 2014). Forest is also a natural landscape with few anthropogenic threats; thus, it may increase the probability of occupancy simply by merit of not being heavily influenced by humans.

Agricultural land cover did not have a strong effect on the probability of wetland occupancy by Blanding's Turtles. The land-cover data that we used did not distinguish between agricultural lands that were currently in use and lands that had been fallow for as many as 50 years. Although it is difficult to get an exact proportion of agricultural lands that were fallow versus that were active, our estimate based on aerial photographs is that about 10% of the agricultural lands across the study area were fallow. Our estimate of fallow lands increased to over 50% in conservation and wilderness areas where most of the surveyed wetlands were located. The lack of distinction between fallow and active agricultural lands may have contributed to the weak effects of agriculture in our BRT models because fallow lands may provide suitable habitat for Blanding's Turtles given that they provide

Fig. 3. The marginal effects of the eight explanatory variables used to predict the probability of wetland occupancy by Blanding's Turtles (*Emydoidea blandingii*) between 2008 and 2019 in Ottawa, Ontario, Canada, with a boosted regression tree model. The explanatory variables are open water proportion (Water), wetland proportion (Wetland), forest proportion (Forest), anthropogenic land proportion (Anthropogenic), agricultural land proportion (Agriculture), road density (Road; km/km^2), wetland age (Age; years), and wetland area (Area; ha).

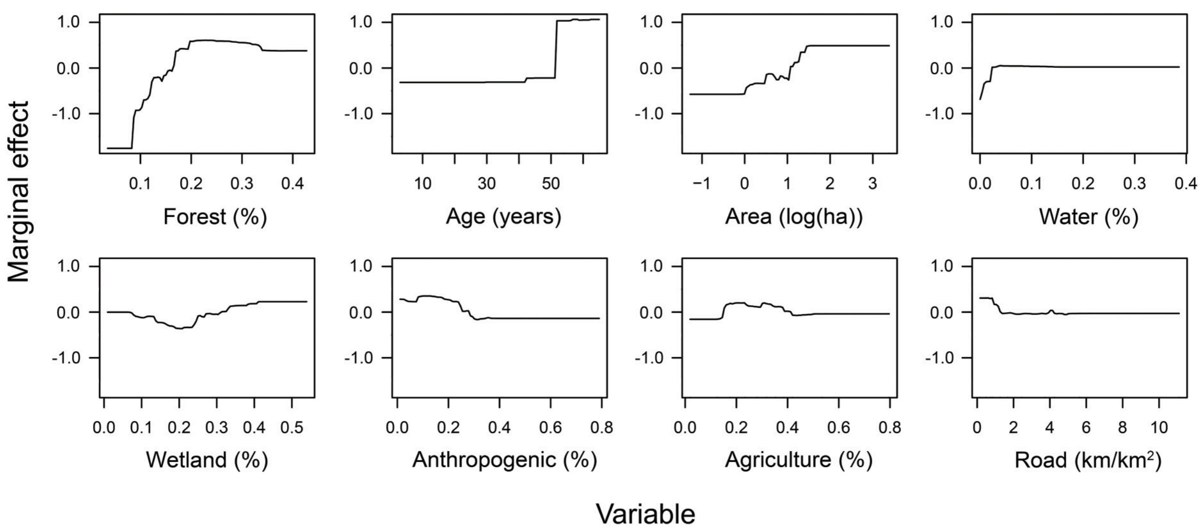


Fig. 4. Predicted probability of wetland occupancy by Blanding's Turtles (*Emydoidea blandingii*) for 137 wetlands in Ottawa, Ontario, Canada, as predicted with a boosted regression tree model. Wetlands are sorted by Blanding's Turtle occupancy. Solid circles represent the mean values (unoccupied wetlands: mean = 0.30, SE = 0.03; occupied wetlands: mean = 0.84, SE = 0.02).

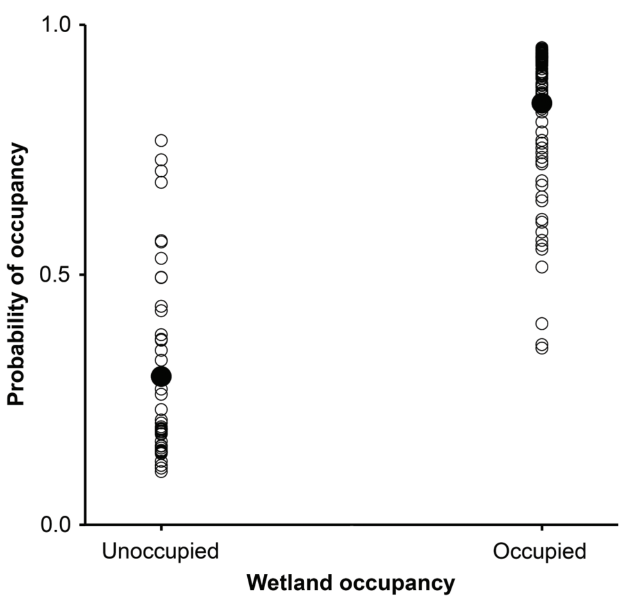
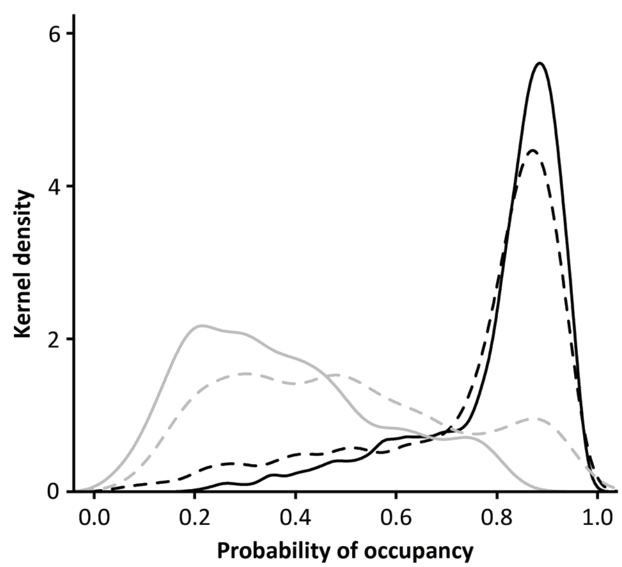


Fig. 5. Kernel density plots for the probability of wetland occupancy by Blanding's Turtles (*Emydoidea blandingii*) as predicted by 100 boosted regression tree models, each built using a different random subset of 80 wetlands from the 137 wetlands. Wetlands are sorted by Blanding's Turtle occupancy (unoccupied (grey lines), occupied (black lines)) and data source (training wetlands (solid lines), validation wetlands (broken lines)). Sample sizes are n = 2764 (unoccupied training), n = 5236 (occupied training), n = 2036 (unoccupied validation), and n = 3664 (occupied validation).



vegetative cover during the entire active season and there are no threats such as pesticides and agricultural machinery (Mui et al. 2016).

Older wetlands had a higher likelihood of being occupied by Blanding's Turtles. Although Blanding's Turtles are relatively mobile compared with other freshwater turtles, populations in close geographic proximity can have low gene flow, which is indicative of isolation (Mockford et al. 2005). Combined with the low recruitment rate of Blanding's Turtles (Refsnider 2009), low gene flow indicates that wetland colonization must happen slowly. Our results may provide an estimation of wetland colonization rates by Blanding's Turtles because marginal effects plots suggest a large increase in the probability of occupancy at approximately 50 years of wetland age and little difference in the probability of occupancy for wetlands younger than 50 years. The majority of the 30 wetlands younger than 50 years of age are wetlands formed by beaver activity or other changes to drainage (12) and storm water ponds (7). Although colonization rates of Blanding's Turtles have not been well studied, the estimate of 50 years is consistent with a study on freshwater turtle colonization in Tommy Thompson Park in Toronto, Ontario, Canada, where Blanding's Turtles were first

Table 1. Correlations (Pearson's correlation coefficient) between the explanatory variables of the boosted regression tree model used to predict the probability of wetland occupancy by Blanding's Turtles (*Emydoidea blandingii*) between 2008 and 2019 in Ottawa, Ontario, Canada.

	Wetland	Forest	Anthropogenic	Agriculture	Road	Age	Area
Water	−0.31*	−0.27*	0.06	0.01	0.04	0.12	−0.06
Wetland	—	0.63*	−0.55*	−0.40*	−0.54*	0.38*	0.10
Forest		—	−0.71*	−0.31*	−0.71*	0.45*	0.03
Anthropogenic			—	−0.20*	0.98*	−0.48*	−0.10
Agriculture				—	−0.20*	−0.26*	0.11
Road					—	−0.44*	−0.10
Age						—	0.11
Area							—

Note: Explanatory variables are open water proportion (Water), wetland proportion (Wetland), forest proportion (Forest), anthropogenic land proportion (Anthropogenic), agricultural land proportion (Agriculture), road density (Road; km/km^2), wetland age (Age; years), and wetland area (Area; ha). An asterisk (*) indicates a significant correlation ($p < 0.05$).

observed between 40 and 50 years after habitat creation (Dupuis-Desormeaux et al. 2018). Colonization should occur more rapidly in our study area because the closest known Blanding's Turtle population to Tommy Thompson Park is 15 km away (Dupuis-Desormeaux et al. 2018), whereas the mean distance between unoccupied wetlands and the nearest occupied wetland in our study area is 3.9 km.

Larger wetlands were more likely to be occupied by Blanding's Turtles. Although it is possible that this relationship was due to a higher abundance of turtles in larger wetlands and thus an increased likelihood of detection during visual surveys, similar relationships have been observed in previous studies on Blanding's Turtles (Piepgras and Lang 2000; Attum et al. 2008). For example, in Minnesota, USA, radio-tagged Blanding's Turtles spent more time in larger wetlands (Piepgras and Lang 2000).

Model performance

The BRT model indicated that landscape composition explained over a quarter of the deviance in Blanding's Turtle occupancy, which is higher than a similar study in the Pontiac region of Québec, Canada (Fyson et al. 2020). The model's CV AUC score of 0.845, which is lower than the training AUC of 0.977, suggests that the model is overfit to the data. However, overfitting of BRT models is not necessarily an issue (Elith et al. 2008). Our model evaluation with training and validation subsets confirmed that models were indeed overfit. Regardless of overfit, however, there was a significant difference between the estimated probabilities of occupancy for occupied versus unoccupied validation wetlands indicating that our model has the ability to predict wetland occupancy by Blanding's Turtles for external data.

Survey method comparison

The predicted probability of wetland occupancy by Blanding's Turtles for all 137 wetlands provides some insight into possible survey errors. Three occupied wetlands had a probability of occupancy below 50% (35% to 40%; mean predicted probability of occupancy for occupied sites is 84.3% and SE is 1.5%), but they had Blanding's Turtles present. Blanding's Turtles were detected in two of these wetlands based on eDNA, but we did not find Blanding's Turtles at those two wetlands by visual surveys and there were no sightings in the Ontario Reptile and Amphibian Atlas. These three wetlands may thus represent false-positive eDNA detections, although it is impossible to verify. The third occupied wetland was a seasonal downtown pond that is drained each fall. It is thought that the records from this pond, obtained from the Ontario Reptile and Amphibian Atlas, may be from released Blanding's Turtles that were kept as pets. Our subsequent visual surveys did not allow us to confirm the presence of Blanding's Turtles despite the pond's ease of surveying. Sites with a high predicted probability of occupancy in which we did not find Blanding's Turtles may be false-negative survey results, a common issue with rare and elusive species (Zhou and Griffiths 2007; Miller et al. 2011). Seven unoccupied wetlands had a predicted probability of occupancy greater than 50% (53% to 77%; mean predicted probability of occupancy for unoccupied sites is 29.6% and SE is 2.6%), but they were not found to be occupied by Blanding's Turtles. Five of the seven wetlands are located in areas that have documented Blanding's Turtle populations.

The visual surveys and the eDNA samples both allowed the detection of Blanding's Turtles, but with differing effectiveness. Of the 70 wetlands surveyed by both methods, we confirmed Blanding's Turtles to be present in 20 wetlands based on visual surveys, 9 of which were also determined to be occupied by Blanding's Turtles based on eDNA. By contrast, eDNA indicated Blanding's Turtle occupancy in 23 wetlands of the 70, of which 9 were found to be occupied by Blanding's Turtles based on visual surveys. Between the two methods, we found 34 of the 70 wetlands to be occupied by Blanding's Turtles. Although the reasons for the discrepancy in detection between the two methods are not entirely clear, there are some possible explanations. The 14 wetlands in which Blanding's Turtles were detected with eDNA, but not with visual surveys, are typically wetlands with high cattail (*Typha* sp.) cover that may have hampered detection. There is also the possibility that some sites where eDNA, but not visual surveys, detected Blanding's Turtles are false positives or sites with DNA persisting from individuals that had since dispersed. There is no fully satisfying explanation for why DNA was not detected at sites where we located Blanding's Turtles by visual surveys, although low concentration of DNA in the samples, DNA degradation, and the presence of inhibitors are all possibilities (Jane et al. 2015; Strickler et al. 2015). Generally, eDNA did not perform as well as we anticipated, and we advise that eDNA should not be relied on solely to determine the presence of Blanding's Turtles. Data from visual surveys can be improved with additional site visits, whereas improvements to eDNA data are more difficult to achieve.

Conclusion

We demonstrated that probability of wetland occupancy by Blanding's Turtles can be predicted from boosted regression trees by using landscape composition variables. Although there are certainly many untested factors influencing whether a wetland is occupied by Blanding's Turtles, we showed the importance of the surrounding landscape composition in determining occupancy. The finding that human-influenced landscapes are generally less suitable for Blanding's Turtles was not surprising given what we know about the impacts of humans on biodiversity. Our findings also provide valuable insight into the complicated relationships between landscape features and species that inhabit those landscapes. The ability to define critical habitat properly for species at risk is one of the most difficult aspects of species conservation, and determining critical habitat requires knowledge of a species' interactions with the biotic and abiotic environments at multiple scales (Rosenfeld and Hatfield 2006).

Our study provides information at the landscape scale that can be applied directly to critical habitat delineation and other conservation efforts for Blanding's Turtles and other species with similar habitat requirements.

Acknowledgements

We thank the National Capital Commission (especially E. Katic and A. Stone) for their participation in fieldwork, access to their lands, and for providing funding. We also thank the City of Ottawa for access to their lands. We are grateful to Ontario Nature and all the citizen scientists who contributed their Blanding's Turtle observations to public databases. Finally, we thank the laboratory of Y. Surget-Groba at the Université du Québec en Outaouais for processing the eDNA samples.

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